SOCIETÀ ITALIANA DELLE SCIENZE VETERINARIE

In collaborazione con:

Università degli Studi di Perugia

Dipartimento di Medicina Veterinaria di Perugia

IZS dell'Umbria e delle Marche

XV Convegno S.I.C.V.

XIII Convegno S.I.R.A.

II Convegno RNIV

XI Convegno So.Fi.Vet

XII Convegno AIPVet

ATTI DEL LXIX CONVEGNO SISVET

Perugia, 15-17 Giugno 2015

Università degli Studi di Perugia

Dipartimento di Medicina Veterinaria

Via S. Costanzo, 4 - 06126 Perugia

SOCIETÀ ITALIANA DELLE SCIENZE VETERINARIE
Joint meeting

LXIX Convegno S.I.S.Vet
XV Convegno S.I.C.V.
XIII Convegno S.I.R.A.
XII Convegno A.I.P.Vet
XI Convegno So.Fi.Vet.
II Convegno R.N.I.V.
PERUGIA 15-17 GIUGNO 2015

Dipartimento di Medicina Veterinaria
Via S. Costanzo, 4 - 06126 Perugia

ATTI 2015

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Saluto e relazione del Presidente al

69° Convegno SISVet

Cari amici e colleghi,

a nome del Consiglio Direttivo, del Comitato Scientifico e del Comitato Organizzatore vi dò il benvenuto al 69° convegno della SISVet. Quest'anno il Simposio accoglie i convegni della SICV (Società Italiana di Chirurgia Veterinaria), AIPVet (Associazione Italiana dei Patologi Veterinari), SIRA (Società Italiana di Riproduzione Animale), SOFIVet (Società di Fisiologia Veterinaria) e RNIV (Rete Nazionale di Immunologia Veterinaria).

Sono in programma 279 lavori scientifici sotto forma di comunicazioni orali e posters, oltre a quattro workshops, una conferenza e cinque *main lectures*. Inoltre siete tutti invitati alla "*Mistery Case Evening*" del 15 Giugno, in cui saranno presentati, in modo del tutto informale e interattivo, casi misteriosi di patologia, clinica e parassitologia. Durante l'evento verrà offerto un buffet ai partecipanti e sarà l'occasione per trascorrere insieme una gradevole serata.

Anche quest'anno abbiamo incoraggiato la partecipazione dei più giovani e dei non strutturati mediante agevolazioni per l'iscrizione al Convegno e premi in denaro. Il Consiglio Direttivo della SISVet ha deliberato di bandire, per il Convegno 2015, 20 premi da 500 € ciascuno, destinati alle comunicazioni e poster che saranno successivamente pubblicati su riviste indicizzate. L'ESCCAP ha bandito una Borsa di Studio da 500 € e la RNIV ha bandito due borse da 500 € ciascuna per comunicazioni presentate al Convegno SISVet 2015.

La cena sociale si svolgerà presso il Palazzo Bernabei, di fronte alla magnifica Basilica di Assisi.

Desidero pertanto ringraziare i membri del Comitato Organizzatore, del Consiglio Direttivo, del Comitato Scientifico e in particolare la Presidente del Comitato Scientifico, Prof.ssa Adriana Ferlazzo, che con il loro impegno hanno garantito la buona riuscita del Convegno.

Un doveroso ringraziamento va agli Sponsor e agli Enti patrocinatori: in primo luogo all'Università di Perugia e al Magnifico Rettore, nostro collega, prof. Franco Moriconi, al Dipartimento di Medicina Veterinaria, al personale tutto e al Direttore, Prof. Piero Ceccarelli per aver messo a disposizione la sede del Convegno e il supporto logistico. Altrettanta gratitudine va alla Conferenza dei Direttori dei Dipartimenti di Medicina Veterinaria, ai rappresentanti del CUN, all'Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche e al suo Direttore Generale Dott. Silvano Severini.

Colgo infine l'occasione per augurare a tutti i partecipanti una buona e proficua permanenza al Convegno e invitarvi a visitare nel tempo libero la città di Perugia e i magnifici tesori d'arte da essa custoditi.

Benvenuti a Perugia

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Presidente SISVet

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SOCIETÀ DELLE SCIENZE VETERINARIE

In collaborazione con

PROGRAMMA GENERALE

Lunedì 15 giugno

14.30 – 16.30 **Workshop 1**: La peste suina africana: una minaccia globale

14-15 **Sessione scientifica** SICV

16.30 – 17.00 *Coffee break*

17.00 – 19.00 **Sessioni scientifiche parallele**: SISVet, AIPVet, SoFiVet, SICV, SIRA, RNIV

17.00 - ***Main lecture*** SOFIVET**:** "New biomarkers monitoring animal welfare" - Knight CH.

17.00 - ***Conference*** RNIV: "Immune correlates of protection against microbial infections of farm animals"

19.00-20.00 Assemblee delle Società Scientifiche

20.30-23-30 ***Mistery Case Evening*** con buffet

Martedì 16 Giugno

08.30 – 10.00 **Sessioni scientifiche parallele**: SISVet, AIPVet, SoFiVet, SICV, SIRA, RNIV

8.30 - 9.00 ***Main lecture*** AIPVet: "Le dermatiti atopiche nel cane e nel gatto" - Marsella R.

8.30 – 9.00 ***Main lecture*** SICV: "Insufficienza funzionale del legamento crociato: prospettive attuali di trattamento" – Pozzi A.

10.00 - 10.30 *Coffee break*

10.30 - 13.00 Conferenza dei Direttori di Dipartimento, incontri con rappresentanti CUN

10.30 - 13.00 **Workshop 2**: Ricerca e benessere animale alla luce del DL 4 marzo 2014, n. 26

13.00 – 14.30 *Pausa pranzo*

14.30 – 16.30 **Workshop 3**: Sicurezza alimentare e sostenibilità delle produzioni zootecniche

16.45 - 17.45 Sessioni scientifiche parallele: SISVet, AIPVet, SoFiVet, SICV, SIRA, RNIV

17.45 - 18.15 *Coffe break*

18.15-18.45 **Inaugurazione del LXIX Convegno SISVet**

18.45-19.15 **Assemblea soci SISVet**

20.30 *Cena Sociale*

Mercoledì 17 Giugno

8.30 – 10.00 Sessioni scientifiche parallele: SISVet, AIPVet, SoFiVet, SICV, SIRA, RNIV

8.30-9.00 ***Main lecture*** AIPVet: "Osteochondrosis: a serious problem in livestock breed cattle bulls in Andalusia" - Méndez Sánchez A.

10.00 – 10.30 *Coffee break*

10.30 – 13.00 **Workshop 4**: Aggiornamenti sulla leishmaniosi

13.00 – 14.30 *Pausa pranzo*

14.30 – 16.00 Sessioni scientifiche parallele: SISVet, AIPVet, SoFiVet, SICV, SIRA, RNIV

16.00 – 16.30 *Coffee break*

16.30 - 18.00 Sessioni scientifiche parallele: SISVet, AIPVet, SoFiVet, SICV, SIRA, RNIV

WORKSHOP

Workshop 1

African Swine Fever: a global threat

PESTE SUINA AFRICANA: UNA MALATTIA RIEMERGENTE

Gian Mario De Mia

Istituto Zooprofilattico Sperimentale Umbria e Marche, Perugia (Italy)

La peste suina africana (PSA) è stata descritta per la prima volta nel 1921 in Kenya e per molto tempo è rimasta confinata nel territorio Africano. L'infezione è presente in forma endemica nella fascia sub-sahariana, ma non ha risparmiato aree più a sud, tra le quali anche l'isola del Madagascar. A partire dagli anni '50, si sono registrati eventi epidemici anche al di fuori del continente africano: prima in Europa (nel 1957 in Portogallo, nel 1960 in Spagna, nel 1964 in Francia, nel 1967 in Italia, nel 1985 in Belgio, nel 1986 in Olanda) e successivamente, a cavallo degli anni '80 in Brasile, Cuba, Repubblica Dominicana e Haiti. Negli anni '90, si sono avute nuove segnalazioni in Portogallo ed Olanda, classificate però come eventi sporadici. La situazione endemica in Sardegna, dove la PSA è presente in forma continuata dal 1978, è stata a lungo considerata a sé stante perché, grazie alle misure restrittive intraprese, l'infezione è rimasta sempre confinata nell'isola. A partire dal 2007 la PSA è stata introdotta nelle repubbliche Caucasiche e in Russia e, attualmente, è segnalata anche all'interno dell'Unione Europea (Polonia, Lituania, Lettonia, Estonia) ove ha destato grande preoccupazione. Per tali ragioni la PSA viene attualmente considerata una vera e propria malattia riemergente. Sino ad ora tutti i tentativi di vaccinazione per proteggere gli animali dalla malattia hanno avuto esito negativo. Da ciò scaturisce la necessità di una diagnosi rapida ed accurata che è determinante per un controllo efficace della malattia attraverso l'applicazione delle misure di eradicazione.

La PSA è una malattia virale che colpisce suino domestico e suidi selvatici, caratterizzata da elevata morbilità e mortalità. L'agente causale è un virus DNA dotato di involucro. Può essere albergato e trasmesso da zecche del genere *Ornithodorus*. In particolare, *Ornithodorus moubata*, presente in Africa, costituisce un serbatoio inesauribile d'infezione. In Europa, quando la malattia è stata presente nella penisola Iberica, il virus si è diffuso invece attraverso zecche del genere *Ornithodorus erraticus*. In Sardegna la presenza di zecche *Ornithodorus* non è mai state segnalata. Il virus è molto resistente e molto stabile soprattutto alle variazioni di pH. Nelle carcasse e nei prodotti a base di carne i virioni permangono infettanti per mesi. Il genoma virale, costituito da DNA bicatenario della lunghezza di circa 170-180 kb, e può presentare addizioni o delezioni dell'ordine di 10-20 kb grazie alle quali si è evidenziata una grande variabilità genetica

tra stipiti. Prima della sua comparsa in Europa, la PSA era presente in Africa sotto forma di infezione subclinica nei suidi selvatici locali (facoceri e potamoceri) e nelle zecche, nelle quali il virus può permanere anche per anni. I facoceri sono considerati i principali portatori del virus nelle regioni ove il loro numero è consistente, potendo contrarre l'infezione molto precocemente ed in forma del tutto asintomatica. In Sardegna, in assenza di zecche, la trasmissione si realizza esclusivamente per contatto diretto per via oro-nasale, oppure per contatto indiretto ad esempio, attraverso la somministrazione di residui di cucina infetti o tramite oggetti o veicoli da trasporto contaminati. Nella patogenesi della malattia, le tonsille e i linfonodi mandibolari costituiscono il primo sito di replicazione virale quando l'infezione viene contratta per via oro-nasale. Successivamente il virus viene reperito nel sangue (fase viremica) dove raggiunge in pochi giorni titoli elevati replicando attivamente nei monociti e nei polimorfonucleati. Con il sangue perviene quindi in tutti gli organi e tessuti a partire dal midollo osseo, dalla milza e dai linfonodi. Cellule bersaglio sono rappresentate dai macrofagi, dalle cellule reticolari ed endoteliali.

Clinicamente la PSA si manifesta come una malattia febbrile della quale sono possibili forme diverse: iperacuta, acuta e cronica, oppure asintomatica. Il decorso dipende dal tipo di virus; è però determinato anche dall'età e dalla razza dei suini colpiti. Il periodo d'incubazione è compreso tra 2 giorni e 2 settimane. Il decorso acuto è caratterizzato da febbre alta persistente e da casi di morte improvvisa con tasso di mortalità anche vicino al 100%. La cute, i reni, le sierose e i linfonodi possono presentare emorragie puntiformi o più estese. Spesso la milza è molto gonfia, di colore rosso scuro e di consistenza friabile. I linfonodi gastroepatici e quelli renali possono essere fortemente ingrossati e presentare anch'essi una colorazione rosso scura. Nel decorso cronico, il quadro clinico è caratterizzato da sintomi aspecifici e, talvolta, il dimagramento è l'unico sintomo rilevabile. Altre volte si sono manifestate lesioni articolari (tumefazioni a carico del carpo e del tarso) o cutanee (processi ulcerativo necrotici). Le forme croniche non sono mai state osservate in Sardegna e sono state descritte solo nella penisola Iberica.

Un ruolo epidemiologico importante viene rivestito dai portatori sani. Si tratta di soggetti che, contratta l'infezione, non hanno presentato una sintomatologia clinica manifesta oppure, sopravvissuti alla malattia, continuano ad albergare il virus nel proprio organismo. Le modificazioni della virulenza subite dal virus lungo decenni in Europa, hanno portato ad un incremento delle forme sub-acute e croniche caratterizzate da mortalità ridotta. Conseguentemente è andata aumentando la comparsa di soggetti portatori, il cui ruolo nella diffusione della PSA è di notevole importanza. Infatti si è visto che questi soggetti possono andare incontro a fenomeni di riattivazione dell'infezione con sintomi di malattia più o meno manifesti e comunque, in grado di disseminare il virus nell'ambiente per periodi anche lunghi. Sembra che la riacutizzazione possa avvenire a seguito di eventi stressanti o essere indotta da farmaci, come i cortisonici. La risposta immunitaria è protettiva e duratura ed è correlata ad una serie di fenomeni assai complessi ed ancora non del tutto conosciuti. Sta di fatto che, ad oggi, non esistono ancora presidi immunizzanti validi ed efficaci nei confronti di questa malattia. Nella resistenza nei confronti del virus sembra inoltre che giochi un ruolo importante oltre

all'immunità umorale anche l'immunità cellulare. Singolare è anche il fatto che gli anticorpi protettivi non appartengono alla classe degli anticorpi neutralizzanti. Il virus infatti non ne induce la formazione e questo fa sì che per la diagnosi sierologica di questa malattia non possa essere impiegata la prova di sieroneutralizzazione. In sede di diagnosi differenziale devono essere prese in considerazione tutte le cosiddette "malattie rosse" del suino, a cominciare dalla Peste Suina Classica che è praticamente indistinguibile e per la quale si rende quindi necessaria la diagnosi di laboratorio. La conoscenza degli aspetti fondamentali dell'infezione è di fondamentale importanza per una formulazione veloce del "sospetto" e per l'adozione delle conseguenti misure di eradicazione.

La Peste suina africana in Europa, situazione attuale e prospettive di controllo

Laddomada Alberto

IZS della Sardegna

La evoluzione della situazione della Peste suina africana in Europa in questi ultimi anni ha causato grandissime preoccupazioni, anche al di fuori dell'Europa. Dopo la sua introduzione in suini domestici in Georgia nel 2007, probabilmente tramite rifiuti contententi carni suine contaminate dal virus provenienti da una nave approdata dall'Africa al porto di Poti, il virus si è diffuso nelle Repubbliche Caucasiche e successivamente nella confinante Russia. In questo paese la diffusione geografica della malattia è stato particolarmente allarmante. Al momento si ritiene che la malattia sia presente allo stato endemico negli allevamenti di suini domestici e nei cinghiali sia nel sud che nel centro della Russia Europea, dove la situazione non è chiara e appare spesso fuori controllo.

Dalla Russia, la malattia si è successivamente diffusa in Bielorussia e Ucraina. Dalla Bielorussia e probabilmente dalla stessa Russia il virus si è successivamente diffuso tramite i cinghiali, in Lituania, Bielorussia, Lettonia ed Estonia, probabilmente a seguito di ripetute introduzioni indipendenti l'una dall'altra. In questi ultimi paesi della Unione Europea si sono verificati un certo numero di focolai negli allevamenti suinicoli, quasi esclusivamente a carattere familiare, quale conseguenza della infezione dei cinghiali. Tuttavia, con l'eccezione della Polonia, si è anche verificata una certa progressione geografica della malattia nel cinghiale stesso che al momento viene studiata con attenzione e preoccupazione dagli esperti della UE.

Nella Unione Europea sono state applicate le misure di controllo previste dalla Direttiva del Consiglio 2002/60/CE e dalla Decisione della Commissione 2003/422/CE. Queste misure prevedono, nel caso di focolai nei suini domestici, le classiche misure di controllo delle malattie contagiose: stamping-out dei suini nelle aziende infette, sospette di infezione e di contaminazione, indagini epidemiologiche, bocco delle movimentazioni, sorveglianza e controlli specifici nelle aziende attorno ai focolai, etc. In caso di malattia nel cinghiale, specifici piani di eradicazione devono essere attuati con l'aiuto di gruppi di esperti, dopo un adeguato studio della situazione epidemiologica. Le misure di controllo della malattia nel cinghiale non escludono il diradamento della popolazione di cinghiali, ma non prevedono detta misura come obbligatoria, nell'assunto che l'infezione nei cinghiali possa essere self-limiting, come osservato in passato in Spagna e Portogallo e che il depopolamento possa anche comportare di rischi di ulteriore diffusione e/o di endemizzazione della malattia. Tale tendenza alla auto-limitazione non è però ancora stata osservata, in particolare nelle Repubbliche Baltiche, mentre in Polonia la malattia è

rimasta confinata in un area molto vicina al confine con la Bielorussia, dove la situazione sembra estremamente preoccuante.

Le misure di "regionalizzazione" messe in piedi nella UE, che consistono in severe restrizioni della commercializzazione di suini vivi, loro materiale gentico, carni suine fresche e preparate, sottoporodotti di origine suina dalle zone in cui la malattia si è diffusa, sono tuttavia state attuate con successo: la malattia si è sempre presentata all'interno di zone già sottoposte a restrizioni commerciali, e la ulteriore diffusione geografica del virus è stata costantemente associato alla infezione dei cinghiali (mediata o meno da comportamenti dell'uomo) e non a pratiche commerciali.

La Commissione Europea e l'Autorità Europea per la Sicurezza Alimentare (European Food Safety Authority, EFSA) hanno intrapreso numerose inziative tese a migliorare le conoscenze relative alla presenza, persistenza e diffusione del virus PSA, in particolare nei cinghiali selvatici. L'EFSA ha prodotto numerosi opinioni scientifiche sul ruolo epidemiologico del cinghiale e sulle possibili misure atte a contrastare la possibile persistenza e/o diffusione del virus in popolazioni di cinghiali. Il contatto con carcasse di cinghiali o con il loro sangue infetto è considerato il fattore di rischio principale in relazione alla ulteriore diffusione del virus tra i selvatici, tenuto anche conto della elevata persistenza del virus nell'ambiente, in particolare durante i lunghi inverni del nord e centro Europa. Anche il contatto tra cinghiali, in particolare attorno ai luoghi di alimentazione, rappresenta un fattore di rischio specifico che deve essere adeguatamente controllato, sebbene il ruolo epidemiologico dei cinghiali sopravvissuti alla malattia quali possibili portatori di virus e diffusori sia ancora da definire. L'aumento della densità delle popolazioni di cinghiali verificatosi negli ultii decenni in molte aree dell'Europa centrale ed orientale può favorire il perdurare della malattia. Affinchè siano efficaci, le strategie di diradamento delle popolazioni di cinghiali basate sugli abbattimenti e sul prelievo venatorio devono essere accuratemente studiate e attuate per almeno due/cinque anni e focalizzate al diradamento delle femmine adulte e subadulte.

La PSA persiste ancora in Sardegna, dove il principale serbatoio di malatia sono i suini tenuti illegalmente allo stato brado, che convivono a contatto con i cinghiali selvatici. Questo problema persiste in particolare in alcune aree interne dell'Isola dove la sua soluzione si scontra con forti resistenze da parte dei detentori di suini che devono essere affrontate tenendo in considerazione gli aspetti socio-culturali di quelle zone della Sardegna. Un nuovo Piano di eradicazione è stato elaborato dalle autorità regionali, che presenta, rispetto ai piani precedenti, alcuni importanti elementi di novità, quali un sistema di incentivi atto a premiare economicamente gli allevatori/detentori di suini in regola con le norme per la sanità ed il benessere degli animali e di penalizzazione di coloro che non rispettano tali regole, con interventi di abbattimento coercitivo dei suini tenuti illegalmente. Al momento sono in atto misure finlizzate a far emergerere dalla illegalità il maggior nuemro possibile di allevatori di suini e limitare dunque la necessità degli interventi più risoluti. Il programma si prefigge di eradicare la malattia nel giro di tre anni.

INTERNATIONAL INITIATIVES FOR ASF CONTROL

Beltran Alcrudo D.

AGAH, FAO, Roma

Given the current status of African swine fever (ASF), with the disease spreading steadily despite all efforts in Africa and Europe, there are many national and international initiatives to create awareness, build capacity, coordinate efforts and, in general, to assist affected and at risk countries. FAO has a particularly active role in many of these efforts, which include activities in the following fields:

1. Coordination and Global Initiatives: The FAO-lead Global platform for ASF and other important diseases of swine aims to coordinate all stakeholders involved in the prevention and control of the disease. Similarly, the Global ASF Research Alliance (GARA) focuses specifically on the coordination of research. ASF Regional Sub-Networks, together with Ad hoc coordination meetings, can be very valuable to coordinate at the sub-regional level. These are best exemplified by the Standing Group of Experts (SGE) on ASF in the Baltic and Eastern Europe Region (SGE) and the Eastern Africa ASF Working Group (EA-ASF-WG). Finally, the ASF Strategy for Africa developed by FAO, AU-IBAR and ILRI will help to progressively control and eradicate the disease when feasible.
2. Emergency & Assessment missions, mainly those conducted by the Crisis Management Center for Animal Health (CMC-AH) of FAO to Georgia, Armenia, Belarus, Tanzania and Côte d'Ivoire, and by EMPRES to Ukraine. These 1-2 week missions aim to rapidly assess the situation and identify gaps and steps forward in countries facing an ASF emergency.
3. Technical Cooperation Program (TCPs) projects are a very useful tool to assist countries over 1-2 years to fill the most immediate gaps in the control of ASF. There are ongoing TCPs in Belarus, Ukraine and China, and others recently finalized in Georgia, Armenia and Cape Verde.
4. Capacity building trainings and workshops on laboratory diagnosis, field activities, epidemiology and tick collection and identification. These may be part of TCPs or Ad hoc.
5. FAO Publications: primarily the regular updates and early warning messages (EMPRES Watch, EMPRES Bulletin and Focus on) as well as manuals (on pig biosecurity, development of ASF contingency plans or the diagnosis of ASF).
6. Research, such as the EC-funded consortium ASFORCE, as well as IAEA's Coordinated Research Project (CRP) for Early and Rapid Diagnosis and Control of ASF.
7. ASF Reference Centers, which can assist countries on epidemiology, laboratory and other technical aspects.

Workshop 2

Ricerca e Benessere Animale alla luce del D.L. N.26/2014

RICERCA E BENESSERE ANIMALE: UN APPROCCIO OLISTICO ALLE ESIGENZE FUNZIONALI DI SPECIE

Adriana Ferlazzo

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La condivisione del principio che riconosce gli animali quali "*esseri senzienti*" (Trattato di Lisbona, 2007) impegna gli Stati Membri dell'Unione Europea a garantire la tutela e il benessere degli animali quali valori comuni, imponendo il rispetto dei bisogni fisiologici ed etologici delle singole Specie, nonché la prevenzione di danni, dolore, sofferenza e angoscia in tutte le pratiche che le vedono coinvolte. Il D.L. n. 26/2014, attuando la Direttiva 2010/63/EU sull'utilizzo degli animali nella ricerca, ha recepito i principi di "*Replacement, Reduction, Refinement*", regolamentando la valutazione delle finalità e delle modalità di esecuzione delle procedure sperimentali e definendo i requisiti indispensabili degli àmbiti e delle caratteristiche sperimentali. Ha inoltre introdotto ruoli e competenze per la valutazione tecnico-scientifica e il monitoraggio dei progetti di ricerca e, più in generale, della sperimentazione. Nella ricerca veterinaria, lo studio di patologie multifattoriali, in carenza di metodi alternativi validati, può richiedere l'utilizzo di Specie con strutture nervose più evolute dei comuni modelli animali, con risvolti che impegnano il medico veterinario nel doppio ruolo di ricercatore e di soggetto garante del benessere animale.

Lo stato di benessere o *welfare* di un animale rappresenta il risultato dell'adeguata integrazione del soggetto con il proprio mezzo interno e con l'ambiente; cioè la possibilità di soddisfare i propri bisogni essenziali, gerarchicamente organizzati, e di evocare un'attivazione fisiologica generalizzata dell'organismo *(arousal*) a stimoli positivi o negativi e manifestazioni comportamentali e adattamenti biochimici e funzionali finalizzati al mantenimento dell'omeostasi funzionale ed emozionale. Tale "*capacità allostatica*" si esprime attraverso meccanismi dinamici di adattamento fisiologico (*coping*) con caratteristiche specie-specifiche e individuali in funzione dei "fattori collativi" che incidono sui moduli di attivazione della condizione stimolante (es., grado di novità, supporto sociale, ecc.), avvalendosi di specifici meccanismi neurofunzionali e di biomodulatori dei processi nervosi, funzionali, metabolici e immunitari. Il concetto di "*senzienza animale*" implica, quindi, che all'animale da esperimento si debba garantire la possibilità di esprimere la proprie capacità di adattamento funzionale e emozionale anche

in situazioni in grado di provocare dolore, sofferenza e angoscia. In termini neurofunzionali, il dolore è una sensazione percepita negativamente e le emozioni sono stati mentali e fisiologici, associati a modificazioni psicofisiologiche di risposta a stimoli naturali o appresi, espresse anche attraverso il comportamento. In tutte le Specie è documentabile la possibilità di controllo del dolore e l'associazione a tratti di temperamento di emozioni primarie (es., paura), che sono espresse con tipiche espressioni facciali o posturali. Le emozioni si attivano prevalentemente a livello inconscio in rapporto a determinati stimoli e risultano funzionali sotto il profilo adattativo. La risposta di adattamento, espressa attraverso numerosi indicatori che consentono di individuare un eventuale stato di stress, dà contenuto e forma alle capacità di un animale di adattarsi e mantenere uno stato di benessere psico-fisico. Le emozioni sono pertanto delle transazioni con l'ambiente, a cui sono associate modificazioni fisiologiche, esperienziali (sensazione esperita) e comportamentali, processate dal sistema nervoso animale in funzione della propria specificità, delle caratteristiche individuali e delle esperienze pregresse.

Dal punto di vista neurofunzionale, l'amigdala **–**parte del sistema limbico, cioè della paleocorteccia comune ai Rettili e all'Uomo **-** rappresenta il mediatore centrale delle emozioni, e svolge un ruolo equivalente nelle differenti Specie, presentando un'interfaccia agli stimoli sensoriali e un'interfaccia di controllo delle risposte emotive, attuate attraverso sottosistemi specifici per le diverse emozioni. Il circuito talamo- amigdala, comune agli esseri umani e agli animali che non hanno sviluppato la neocorteccia, è responsabile dell'elaborazione di stimoli emozionali grezzi e risposte immediate, attribuendo significato emotivo alla sensazione esperita. L'ippocampo elabora i complessi input provenienti dall'ambiente, costruendo una rappresentazione configurazionale della situazione contestuale su reti strutturate di associazioni reciproche tra molteplici stimoli, elaborando risposte efficaci di adattamento e evitamento degli stimoli ansiogeni. Il complesso ippocampo-amigdala rende conto, pertanto, dell'influenza reciproca tra valutazione emotiva e elaborazione cognitiva, il cui livello è peculiare nelle differenti Specie animali e nei differenti individui, coinvolgendo numerose strutture centrali (corteccia prefrontale e orbitofrontale, insula, giro del cingolo, nucleo accumbens, area del tegmento ventrale, grigio periacqueduttale). L'equilibrio tra i segnali eccitatori dell'amigdala e i segnali inibitori dell'ippocampo inviati al Nucleo Paraventricolare (NPV) determina, infatti, la condizione di stress e la quantità di CRF, di ACTH e infine di CORT rilasciata.

Tutte le Specie domestiche manifestano capacità cognitive, ma con diverso grado di consapevolezza, e risposte emozionali (*emotion*) che, in funzione del grado di consapevolezza specie-specifica e individuale, possono variare in termini di valenza e intensità, generando o meno un'emozione consapevole (*feeling*), quali la paura o la sofferenza, che può modificare il comportamento o agire da rinforzo nei processi di apprendimento. La risposta individuale allo stress risulta, in definitiva, funzione del "carico allostatico" individuale, cioè della capacità di percezione dello stress e del relativo

controllo. I meccanismi adattativi si avvalgono di strutture esecutive (ipotalamo, prosencefalo basale, nuclei del tegmento mesencefalico) e di numerosi biomodulatori centrali e periferici (citochine, endorfine, dopamina, serotonina, prostaglandine), che garantiscono adattamenti funzionali e comportamentali adeguati ai livelli di stress fisici ed emozionali sperimentati. E' ampiamente documentato, ad esempio, che un'aumentata percezione dello stress o la sua durata producono un'alterata attività citochinica con conseguenti alterazioni funzionali e comportamentali e insorgenza di patologie. La scoperta dei neuroni specchio, e le più recenti teorie neurobiologiche integrate sulle emozioni confermano l'importanza che potranno dare contributi di ricerche di base alla migliore comprensione dei rapporti strutturali e funzionali che sottendono ai meccanismi di genesi delle emozioni nelle differenti Specie, sebbene sia stato ipotizzato che le Specie con maggiori capacità cognitive siano in grado di migliorare le strategie di adattamento al dolore.

Su tali basi, una adeguata applicazione del principio di "*refinement*" sollecita il rispetto delle esigenze funzionali e comportamentali di Specie in termini di "arricchimento" delle condizioni ambientali e, soprattutto, la prevenzione del disagio e del discomfort mentale eventualmente connesso alla sperimentazione. Atti medici e tecnici consapevoli -dalle tecniche di manipolazione degli animali all'attenzione alle procedure gestionali della sperimentazione, con focalizzazione sui "*bisogni etologici*" dei soggetti in esperimento- incidono positivamente sulla valutazione cognitiva delle potenziali condizioni di stress connesse alla sperimentazione, promuovendo l'adozione di strategie fisiologiche di *coping.* Gli studi di condizionamento dimostrano che l'apprendimento emotivo dell'esperienza può essere indirizzato in senso positivo o negativo. E' noto infatti che l'attivazione del sistema di ricerca e ricompensa, con specifici meccanismi appetitivi di attivazione (annusare, toccare, leccare, esplorare, giocare) e successivi eventi consumatori, favorisce i meccanismi di apprendimento positivo dell'esperienza (emozione positiva), con la mediazione del sistema dopamina-endorfine; le emozioni negative, generate dai sottosistemi della paura e dell'angoscia, alterano l'equilibrio tra la "lotta" e la " fuga". Poiché, in termini neurali, la condizione emozionale deve presentarsi alle strutture nervose coinvolte, che sono la causa immediata dello stato emozionale, si tratta in definitiva di intervenire correttamente nello stadio di presentazione degli stimoli emozionali.

Le competenze intrinseche all'aspetto professionale caratterizzante del medico veterinario, precipuamente dedicate alla tutela del benessere animale e atte ad applicare in maniera compiuta il concetto di "*refinement*", risulteranno, pertanto, indispensabili per garantire il più corretto approccio alla sperimentazione sugli animali di tutti i ricercatori coinvolti.

NORMATIVA NAZIONALE ED INTERNAZIONALE SULLA PROTEZIONE DEGLI ANIMALI UTILIZZATI AI FINI SCIENTIFICI

Silvio Borrello

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In Italia la protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici è disciplinata dal D.lvo 4 marzo 2014, n. 26 (Direttiva 2010/63/UE).

Il D.lvo 26/2014 si applica agli animali vertebrati vivi non umani, comprese le forme larvali capaci di alimentarsi autonomamente; ovvero alle forme fetali di mammiferi a partire dall'ultimo terzo del loro normale sviluppo e infine ai cefalopodi vivi.

Le Autorità competenti sono il Ministero, le regioni, le province autonome, i comuni e le ASL.

Gli stabilimenti utilizzatori sono autorizzati dal Ministero previa verifica ispettiva dei requisiti strutturali, gestionali e sanitari ivi compreso la competenza professionale del personale ed è in corso di perfezionamento un D.M. riguardante tutti gli aspetti relativi alla formazione professionale degli operatori del settore.

Ciascun utilizzatore istituisce un organismo preposto al benessere degli animali (OPBA) che assolve al rilascio di un parere sui progetti di ricerca che invia al Ministero per l'autorizzazione via PEC (dgsa@postacert.sanita.it.), insieme alla proposta del progetto, la sintesi non tecnica e le informazioni dettagliate sulle procedure sperimentali.
I progetti di ricerca che prevedono l'utilizzo di animali devono essere in possesso della preventiva autorizzazione del Ministero.

Per il rilascio dell'autorizzazione, il Ministero richiede una valutazione tecnico-scientifica all'Istituto superiore di sanità o ad altri Enti tecnico-scientifici ovvero al Consiglio superiore di sanità in caso di utilizzo di primati non umani, cani, gatti ed esemplari di specie in via di estinzione.

Il Ministero, rilascia l'autorizzazione entro 40 gg. lavorativi se la richiesta è completa e conforme.
L'autorizzazione ha una durata non superiore a cinque anni
La DGSAF per facilitare gli utilizzatori e gli organi di valutazione scientifica ha emanato le Linee Guida per una corretta formulazione dei progetti di ricerca.
Ogni richiesta di informazione sull'iter autorizzativo dei progetti deve essere indirizzata a: animaliericercascientifica@sanita.it,
Esiste incompatibilità tra le funzioni di responsabile del benessere animale negli stabilimenti e dei progetti di ricerca.
Il Ministero effettua la valutazione retrospettiva del progetto, se prevista.

La sintesi non tecnica del progetto compilata dal responsabile del progetto è pubblicata sul portale del Ministero.

Il Ministero promuove lo sviluppo e la ricerca di approcci alternativi, nelle procedure che usano animali, che non prevedono l'uso di animali o utilizzano un minor numero di animali o che comportano procedure meno dolorose.

Presso il Ministero è istituito, il Comitato nazionale per la protezione degli animali usati a fini scientifici che svolge funzioni di consulenza alle autorità competenti ed agli organismi preposti al benessere degli animali su questioni relative all'acquisizione, all'allevamento, alla sistemazione, alla cura e all'uso degli animali nelle procedure e assicura la condivisione delle migliori pratiche.

Per quanto riguarda le tariffe spettanti al Ministero per l'esame delle domande di autorizzazione è in corso di emanazione apposito decreto ministeriale.
Le informazioni statistiche sull'uso degli animali nelle procedure, sono raccolte e pubblicate dal Ministero conformemente alla Decisione 2012/707/UE.

ASPETTI CULTURALI, VALUTAZIONE TECNICO SCIENTIFICA DEI PROGETTI E RUOLO DELL'ISTITUTO SUPERIORE DI SANITÀ ALLA LUCE DEL DL.VO N. 26/2014. ANALISI E CONSIDERAZIONI DOPO IL PRIMO ANNO DI ATTIVITÀ.

Rodolfo Nello Lorenzini

Direttore, Servizio Biologico e per la Gestione della Sperimentazione Animale, Istituto Superiore di Sanità, Roma

Il recepimento della direttiva 2010/63/UE sulla "protezione degli animali utilizzati a fini scientifici", avvenuto con il DL.vo n.26 del 4/3/2014, ha cambiato, in modo fondamentale in Italia, molti aspetti del sistema della ricerca scientifica che prevede l'utilizzo degli animali; sistema che si era stabilizzato, negli ultimi 22 anni, con l'applicazione del DL.vo 116/92.

In particolare, dal punto di vista delle procedure operative, finalizzate ad ottenere la prevista autorizzazione da parte del Ministero della Salute, si possono identificare due aspetti cruciali di diversità con la precedente legislazione. Aspetti che, non solo si presentano come un approccio culturale diverso al problema della sperimentazione animale, ma che determinano ripercussioni dirette sul sistema della tutela degli animali utilizzati nelle procedure sperimentali.

Il primo è costituto dal fatto che tutta la ricerca sperimentale che avviene utilizzando animali da esperimento è soggetta alla procedura autorizzativa. Il secondo è costituito dal fatto che non è più il responsabile della ricerca a presentare ed a sottoporre direttamente la documentazione per ottenere la autorizzazione dall'autorità competente. Ma l' Organismo Preposto al Benessere degli Animali (OPBA), codificato e costituito con specifiche professionalità tecniche e scientifiche ai sensi dell'articolo 25 del DL.vo 26/2014, e che, di fatto, oltre la competenza generale su tutte le attività connesse alla sperimentazione animale, assume un ruolo strategico dal punto di vista culturale ed operativo e che, meglio sottolinearlo, è fortemente influenzato dal livello di formazione professionale delle figure da cui è composto e dal grado di ampiezza della competenza generalista che è in grado di mettere in campo in contesti applicativi molto diversificati.

L'Istituto Superiore di Sanità, organo del Ministero della Salute e del Servizio Sanitario Nazionale, nonché Ente di Ricerca con altissime e differenziate competenze, è specificatamente individuato all'articolo 31 del 26/2014 tra gli enti che possono espletare la Valutazione Tecnico Scientifica (VTS) dei progetti che vengono presentati al Ministero per ottenere l'autorizzazione alla sperimentazione con animali.

Nel lavoro che viene presentato allo Workshop "Ricerca e Benessere Animale", viene analizzata, in modo dettagliato, l'attività svolta dall'Istituto Superiore di Sanità nel primo anno di applicazione del nuovo DL.vo 26/2014. E sono anche analizzati ed illustrati

quantitativamente e qualitativamente gli aspetti operativi da cui dipende il processo di Valutazione Tecnica e Scientifica dei progetti.

Si osserva in particolare che, come primo effetto, nel 2015, l'attività è aumentata circa del 1000% (mille percento) annuo passando da circa 200 progetti nell'ultimo anno di attività con il vecchio decreto 116/92, agli oltre 1200 progetti nel primo anno di applicazione del 26/2014. Tale mole di lavoro, in Istituto Superiore di Sanità, investe circa 100 persone tra tecnici, ricercatori, primi ricercatori, tecnologi e primi tecnologi, dirigenti di ricerca, suddivisi per competenza nelle più importanti aree tematiche della ricerca biomedica nazionale.

Nella presentazione vengono individuati e discussi in modo approfondito sia i compiti dell'Organismo Preposto al Benessere Animale sia i ruoli ed i livelli di responsabilità delle sue componenti professionali. Vengono proposte anche alcune riflessioni culturali comparando gli OPBA ed i loro compiti rispetto a Comitati Etici, Comitati scientifici e Comitati interni per l'uso sperimentale degli animali da laboratorio (IACUC).

Una volta delineati gli aspetti funzionali ed i contesti operativi degli OPBA si passa poi all'analisi di come le varie componenti (Università, Enti Pubblici di Ricerca ed Operatori Privati tra cui l'industria farmaceutica) hanno interpretato, definito e previsto, sul piano pratico ed applicativo, la costituzione ed il funzionamento dei propri OPBA.

Infine si esamina il meccanismo della elaborazione del parere motivato da parte degli OPBA e la sua importante valenza di garanzia, ai fini della Valutazione Tecnico Scientifica che viene svolta dall'istituto Superiore di Sanità. E, non ultimo, si osserva che la collocazione delle attività che svolgono le OPBA non può che essere considerata di tipo Welfarista. Considerando questa posizione culturale come un superamento pragmatico e pratico, mediato dal diritto, di una antitesi, generalmente mai dialettica, tra le istanze degli sperimentisti e degli animalisti. Di questa posizione, non nuova, ma mai neanche completamente affermata ed accettata, viene evidenziato che devono essere valorizzati al massimo gli aspetti legati alla tutela del benessere degli animali in sperimentazione.

Vengono poi presentati in modo sintetico ma approfondito i dati relativi alla attività vera e propria di Valutazione Tecnico Scientifica dei progetti, che è svolta presso l'Istituto superiore di Sanità. Con particolare attenzione alle specifiche esigenze della valutazione dei diversi aspetti dell'impianto sperimentale così come anche riportati e richiesti dall'articolo 31 del nuovo decreto.

In dettaglio vengono anche presi in considerazione gli aspetti più importanti correlati ad una corretta Valutazione Tecnico Scientifica dei progetti e le relative criticità. Queste vengono valutate sia su un piano di analisi descrittiva sia conferendo, ad alcuni aspetti della valutazione, il significato di indicatori della funzionalità degli OPBA. In particolare

vengono riportati ed analizzati i dati relativi alla valutazione del grado di sofferenza (lieve, moderato e grave) così come è stato stimato dagli OPBA stessi.

Nelle conclusioni si vuole porre in evidenza la grande differenza delle matrici culturali oggi sul campo. Ed in particolare porre attenzione alla necessaria crescita che tutto il sistema deve compiere. Sia per disporre di Organismi Preposti al Benessere Animale che siano sempre più omogenei nei comportamenti e nella composizione; ed anche indipendenti e consapevoli del loro ruolo di terzietà previsto dalla legge. Sia per disporre di operatori competenti e preparati, tra quelli individuati nei vari articoli del DL.vo 26/2014, a qualsiasi livello essi operino.

In questo saranno importanti sicuramente due fattori. Il primo sarà costituito dal decreto del Ministro della Salute che stabilirà i criteri con cui il personale sarà considerato idoneo a svolgere le diverse funzioni operative. Il secondo sarà il ruolo dell'Università e dell'Accademia che dovranno essere in grado di cogliere le istanze che provengono dalla società civile in termini di tutela del benessere degli animali utilizzati in sperimentazione ed attivare idonei ed adeguati corsi di insegnamento e di formazione in questo importante settore strategico.

3 R E RICERCA DI BASE: UN CONNUBIO INSCINDIBILE

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La storia della scienza ci insegna che le grandi innovazioni sono frutto della ricerca "*curiosity driven*", ovvero la "*ricerca di base*", quella che avendo come fine l'aumento delle conoscenze, non è direttamente connessa ad obiettivi industriali o commerciali, e rappresenta, quindi, "l'humus su cui si innestano tutte le altre ricerche". In particolare, nella ricerca di base in ambito biomedico e veterinario, ancora oggi il modello animale rappresenta la condizione ideale per lo studio di fondamentali processi biologici e/o patologici come riprodurre i sintomi di una certa patologia ("face validity"), o replicare i meccanismi inerenti a una certa malattia ("construct validity"), o studiare come curare un particolare disturbo ("predictive validity").

Nel corso degli ultimi anni è stata data sempre maggiore importanza al concetto di benessere animale ed il principio delle "3R", Rimpiazzare (*Replacement*), Ridurre (*Reduction*) e Rifinire (*Refinement*), è la spina dorsale della Direttiva europea 2010/63 sulla protezione degli animali utilizzati a fini scientifici e del recente decreto legislativo 26/2014, sospirato recepimento da parte del Governo italiano della Direttiva. Il Principio delle "3R" si ritrova in diversi articoli e diversi allegati del testo non come raccomandazione, come nella precedente normativa, ma come obbligo di legge. Tuttavia, si potrebbe quasi affermare che tale principio sia insito già nel concetto "ricerca di base". A scopo esemplificativo, vale la pena ricordare alcuni esempi di ricercatori che, grazie a ricerche esclusivamente su modelli posizionati in basso nella scala evolutiva, hanno ricevuto il Premio Nobel: A. Fire e C. Mello, hanno studiato la cosiddetta "interferenza da RNA", o RNAi, un sistema di controllo universale dell'espressione genica in un organismo apparentemente insignificante, il nematode *Caenorhabditis elegans*; E. Lewis, C. Nüsslein-Volhard e E. Wieschaus hanno scoperto i geni omeotici nel moscerino della frutta *Drosophila melanogaster*; O. Shimomura, M. Chalfie e R. Tsien, hanno scoperto e studiato la proteina fluorescente GFP nella medusa *Aequorea victoria.* Tutte queste scoperte hanno rivoluzionato la biologia, hanno avuto un impatto dirompente anche sulla ricerca applicata, e sono state sviluppate nel pieno rispetto dei principi di "sostituzione" e "riduzione". Tuttavia, dopo che M.R. Capecchi, M.J. Evans e O. Smithies (Premi Nobel 2007 per la Medicina) hanno scoperto il principio per introdurre modificazioni gene- specifiche nei topi a partire da cellule staminali embrionali, il topo è diventato il modello animale preferito nella stragrande maggioranza degli esperimenti di laboratorio, e topi geneticamente modificati, transgenici e topi knockout, ovvero con un gene soppresso, oggi sono dei validissimi strumenti in quasi tutti i campi della ricerca medica. Non bisogna però trascurare l'impiego e la rilevanza scientifica di animali di

interesse veterinario come modelli nella ricerca biomedica di base. Ne sono un esempio i maiali, tra le più antiche specie di animali domestici, che, oltre a costituire una delle più importanti fonti di alimenti, con una fisiologia e morfologia molto simili a quelle umane, sono una miniera di risorse mediche industriali. Infatti, l'utilizzo di questa specie come modello animale si è consolidato nella ricerca di base così come nei più importanti campi di ricerca, dal cardiovascolare all' obesità, stress, dermatologia, tossicologia, immunologia, emodinamica, fisiologia renale, chirurgia sperimentale, gastroenterologia ed altri. Inoltre è un utilissimo modello da utilizzare nell'ambito dell'alta formazione pratica sia veterinaria che medica.

In conclusione, nel contesto di un complessivo miglioramento delle condizioni di vita degli animali da laboratorio sono stati compiuti importanti passi in avanti negli ultimi anni. Il principio delle 3R ha ispirato profondamente tali miglioramenti. In particolare i punti ritenuti principali: il riconoscimento dell'importanza, nell'ambito della ricerca di base, della ricerca di alternative alla sperimentazione animale o di una razionale e scientificamente ben ponderata scelta del modello da utilizzare; una globale e consistente riduzione del numero di animali utilizzati in ricerca; l'attenzione dedicata al miglioramento delle tecniche utili a controllare la sofferenza degli animali sperimentali; la creazione di solidi criteri e processi di validazione, volti all'implementazione di metodi alternativi, e lo sforzo verso una generale armonizzazione di tali azioni; l'impegno dedicato a creare protocolli che risultino più efficaci, più predittivi e più attenti al grado di sofferenza degli animali sperimentali.

RICERCA APPLICATA E PROTEZIONE DEL BENESSERE ANIMALE

Eugenio Scanziani

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L'utilizzo dei modelli animali rappresenta uno dei più accurati e validi mezzi sperimentali per lo studio delle molteplici patologie umane oggetto di ricerca biomedica. Studi in vivo sono ancora necessari nella ricerca di base ed applicata, in particolare prima che qualsiasi terapia innovative venga applicata all'uomo. Attualmente si assiste ad una aumentata e diffusa sensibilità dell'opinione pubblica nei confronti dei temi legati al benessere animale, sensibilità spesso legata all'emotività personale, ad una scarsa preparazione scientifica specifica, all'informazione spesso superficiale e non riconducibile a parametri scientifici ed obiettivi. Si impone quindi la necessità di valutare con metodiche precise, da parte di professionisti preparati, parametri oggettivi correlabili allo stato di benessere.

Nella relazione vengono proposti alcuni casi relativi a patologie spontanee o sperimentalmente indotte in animali di laboratorio con specifico riferimento ai riflessi sul benessere animale.

Dagli esempi proposti emerge come gli aspetti anatomo-patologici possono essere utilizzati come indice oggettivo del benessere animale che possono essere contrapposti alle valutazioni soggettive legate alla sensibilità personale. Il veterinario, per la sua specifica competenza professionale, rappresenta la figura più qualificata nello stabilire le norme riferite al benessere animale e al loro controllo. Per quanta riguarda la formazione è urgente creare una classe di veterinari qualificati e specializzati. E' necessario procedere ad una valutazione integrata *ex ante* (autorizzazione), durante (vigilanza) ma soprattutto *ex post* degli aspetti legati al benessere animale. Si auspica un aggiornamento costante ed uno scambio di informazioni tra le diverse figure professionali coinvolte nella sperimentazione animale ed infine una esauriente informazione e confronto con i non addetti ai lavori (opinione pubblica).

METODI ALTERNATIVI: POSSIBILITÀ APPLICATIVE IN AMBITO VETERINARIO

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L'interesse e le aspettative rivolte allo sviluppo, validazione ed accettazione normativa dei Metodi Alternativi (MA), nell' accezione del termine definita dal principio delle 3R formulato da Russel e Burch nel 1959, sono oggi molto forti sia all'interno della comunità scientifica che nell'intera comunità sociale, così come ampio ed articolato è il dibattito che accompagna il loro impiego. Tra i motivi principali di tale attenzione sono da segnalare da un lato la maggiore considerazione nei confronti del benessere animale, ormai parte integrante del codice genetico del ricercatore, ma, soprattutto, la maggiore disponibilità di metodi e modelli alternativi scientificamente più affidabili ed innovativi che portano ad una migliore capacita predittiva nei confronti della salute umana e dell'ambiente.

E' importante, infatti, sottolineare che il miglioramento della vita degli animali da laboratorio e l'uso di metodi scientificamente avanzati, migliora la qualità del dato scientifico, la sua estrapolabilità e la condivisione dei risultati. Possiamo quindi affermare che i MA rappresentano un importante esempio di percorso che coniuga interessi scientifici ed economici ad aspetti più specificatamente etici quali l'eliminazione, o quanto meno la riduzione, del sacrificio e/o della sofferenza animale.

E' necessario inoltre evidenziare che l'uso di MA, se disponibili, è esplicitato richiesto in numerosi dei testi legislativi recentemente emanati dalla Comunità Europea, come i regolamenti REACH (2006/1907/EC) e CLP (1272/2008/EC) per le sostanze chimiche, il regolamento cosmetico (2009/1223/EC) o la recente Direttiva sulla protezione degli animali usati nella sperimentazione scientifica (2010/63/EU), recepita nel nostro paese attraverso il Decreto Legislativo n. 26 del 4 marzo 2014.

In ambito veterinario, dove il numero di animali usati a scopo scientifico nell'Unione Europea (in base ai dati riportati nella 7a relazione sulle statistiche del numero di animali utilizzati a fini sperimentali e/o scientifici nella UE, 2013) è di circa 2 milioni il settore della ricerca e sviluppo e di circa 300.000 unità per il settore produttivo e dei controlli di qualità, alcune delle più promettenti aree applicative per i MA sono;

- l'uso di metodi immunoenzimatici su campioni biologici per la valutazione dello stato sanitario di animali da laboratorio,

- l'implementazione di MA (sulla base delle indicazioni della Farmacopea Europea) a sostituzione dei saggi relativi all'accertamento della potenza/efficacia e innocuità /sicurezza dei vaccini per uso veterinario,

 l'impiego di simulatori chirurgici virtuali e video didattici, oltre all'utilizzo di ethically-sourced cadavers e metodi di indagine non invasivi nella didattica veterinaria,

 la disponibilità in biobanche veterinarie dedicate (si veda a questo proposito il sito www.biowarehouse.net sulla rete nazionale di biobanche veterinarie) da utilizzare a scopo di ricerca e diagnostico,

 la messa a punto di metodologie innovative, come ad esempio l'uso di cellule staminali in medicina regenerativa e terapia cellulare, in medicina veterinaria

IMPATTO DEL D.L. N. 26/2014 SULLA RICERCA BIOMEDICA E CLINICA

Antonio Crovace

Dipartimento delle Emergenze e dei Trapianti d'Organo

Università di Bari - Aldo Moro

L'autore valuta criticamente l'impatto che il decreto legge N. 26/2014 ha avuto sulla ricerca biomedica e su quella clinica .La valutazione parte dalla direttiva UE 2010/63 e del consiglio UE del 22 Settembre 2010 che aveva come tema La Protezione degli animali utilizzati ai fini scientifici. Questa direttiva era nata per eliminare le disparità tra le disposizioni legislative, regolamentari e amministrative degli Stati Membri relative alla protezione degli animali utilizzati a fini sperimentali sorte in seguito alle attuazioni della direttiva 86/609/CEE. Alcuni Stati membri avevano adottato misure nazionali di attuazione che garantivano un elevato livello di protezione degli animali utilizzati a fini scientifici, mentre altri si erano limitati ad applicare i requisiti minimi. Tali disparità rischiavano di costituire degli ostacoli agli scambi di prodotti e sostanze per lo sviluppo dei quali erano stati effettuati esperimenti su animali.

Per cui la necessità di una direttiva che prevedesse norme più dettagliate al fine di ridurre tali disparità e per garantire il corretto funzionamento del mercato interno.

L'autore ha analizzato le procedure consentite o che possono essere autorizzata dal nuovo decreto legge ed anche quelli che non possono essere autorizzate, con le loro ripercussioni e con le conseguenze che tali articoli di legge hanno avuto. Il testo originale della direttiva EU era stato ottenuto dal confronto tra scienziati e animalisti, e rappresenta il giusto compromesso tra le necessità della ricerca e il benessere animale. Questo ha comportato per gli operatori italiani alcune criticità quali l'aver introdotto il divieto di allevamento di cani, gatti e primati non umani sul territorio nazionale. Non essendo vietata la Sperimentazione, questi animali dovranno compiere lunghi viaggi per raggiungere il nostro Paese, aumentando lo stress degli stessi ed i costi. (REGOLATORIE). Sono vietati gli xenotrapianti, le ricerche sulle sostanze d'abuso e le esercitazioni didattiche con gli animali nella maggior parte degli indirizzi universitari. Questi divieti sono una peculiarità della legge italiana, dato che non sono presenti nella Direttiva UE e non sono adottati dagli altri componenti dell'Unione che, al contrario dell'Italia, alla direttiva si sono strettamente attenuti. Il risultato di questa disparità rispetto all'Europa, creata dal decreto 2014/26, ha come conseguenza l'impossibilità dei nostri ricercatori di prendere parte a progetti di ricerca Europei ed utilizzare risorse che l'Italia comunque versa all'UE come contributo nazionale ai progetti di ricerca.

Viene in questo modo negata la scelta della miglior terapia per il paziente, viene ostacolata la ricerca di terapie contro il cancro e contro le dipendenze patologiche, si formano dei professionisti impreparati e non competitivi a livello europeo.

Si rendono obbligatorie anestesia o analgesia per procedure anche banali, quali un prelievo di sangue o una semplice iniezione ma contemporaneamente non si specifica nell'art. 23 chi deve compiere atti medici ne' chi e' abilitato a compiere atti chirurgici quando previsti nella sperimentazione.

L'autore ha affrontato poi il problema delle pubblicazioni scientifiche cliniche che queste direttive europee hanno conseguentemente determinato.

In particolare le "ARRIVE GUIDELINES" (Animals in Research: Reporting In Vivo Experiments) e le determinazioni della "International Association of Veterinary Editors" che ha sviluppato delle linee guida sull'etica e sul benessere degli animali che devono essere utilizzate ed adottate dagli autori che intendono pubblicare su riviste scientifiche veterinarie e nelle riviste che pubblicano articoli che coinvolgono animali. Allo stesso tempo indica le azioni che gli editori devono attuare per garantire che i lavori pubblicate sulle loro riviste rispettino i requisiti di una buona pratica etica e di benessere animale.

Workshop 3

Sicurezza alimentare e sostenibilità delle produzioni zootecniche

COMPETENZA IMMUNITARIA E MALATTIE DA PRODUZIONE NEGLI ANIMALI DI INTERESSE ZOOTECNICO

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Il raggiungimento di alti livelli di produzione in zootecnia determina una maggiore difficoltà di numerosi animali ad adattarsi all'ambiente. Questo si traduce in aumento dei tassi di rimonta, riduzione della speranza di vita, una maggiore frequenza di malattie variegate e multifattoriali e maggior uso di farmaci veterinari. I due modelli più studiati sono quelli dei suini nelle fasi post svezzamento e messa a terra e quello delle vacche da latte nel periodo post-parto. In queste fasi, una maggiore incidenza di problemi clinici sono per lo più riferibili a malattie condizionate, che costituiscono una parte significativa delle perdite economiche legate a morbilità, mortalità e rimozione anticipata dalla mandria. L'insorgenza di malattie condizionate è effettivamente facilitata dalla presenza di fenotipi animali ad alta produzione che richiedono elevate competenze tecniche e gestionali, strutture adeguate e controlli intensivi dell'allevatore. Pertanto, un divario evidente può sorgere tra le esigenze degli animali e ambiente in cui questi vengono allevati. Questa fondamentale condizione di rischio può coesistere con elevati livelli di prestazioni produttive, che poi diminuiscono e infine cessano quando si verificano malattie clinicamente conclamate e/o gravi disfunzioni metaboliche; entrambi i casi portano allo stesso risultato: rimozione precoce degli animali dal processo produttivo e aumento complessivo dei tassi di rimonta. L'aumento delle produzioni ottenuta per selezione genetica non è di per sé causa di riduzione del benessere animale, bensì un fattore cui una parte della popolazione animale non è in grado di rispondere con strategie di adattamento adeguate. La ridotta risposta surrenalica degli attuali fenotipi suini è un chiaro esempio di questo principio. I fenotipi suini a carne magra e rapido accrescimento presentano uno squilibrio strutturale tra sviluppo delle masse muscolari e dell'apparato cardio-circolatorio, con conseguente ipossia tissutale cronica e alti livelli di stress ossidativo. Studi immunologici recenti dimostrano che la risposta all'ipossia tissutale genera un profondo squilibrio nell'omeostasi dei linfociti T e quindi della risposta immunitaria adattativa nel suo complesso. Inoltre, stress ossidativo e risposta infiammatoria si stimolano a vicenda, con indubbie ricadute sulla capacità di controllo di tali risposte. Inoltre, un vero e proprio stress metabolico può portare ad attivazione del sistema immunitario innato e a malattie "da produzione". Il fondamento scientifico di tale concetto risiede

nella capacità del sistema immunitario di rispondere sia ad agenti microbici (stressori infettivi) che a *noxae* non infettive, anche metaboliche, con i medesimi tipi di risposta, ancorché differenziata per entità e durata temporale. Il sistema immunitario è pertanto in grado di rispondere a DAMPs (Damage-Associated Molecular Patterns) e a neo-antigeni da stress infettivo o non infettivo. La correlazione provata tra somministrazione di ormone della crescita nelle bovine e insorgenza di mastite delinea chiaramente la correlazione tra stress metabolico e insorgenza di malattia. Alcuni fenotipi animali che mostrano livelli di produzione elevata possono presentare maggiore suscettibilità a virus e batteri (vedi la correlazione, negli anni Ottanta, tra PRRS nei suini e la presenza di ibridi a carne magra). In una prospettiva globale, il maggiore uso di farmaci veterinari associato a malattie da produzione costituisce una grave minaccia per i livelli generali di sicurezza alimentare. A questo proposito, la correlazione critica tra benessere degli animali, salute animale e sicurezza alimentare può essere definita dalla seguente catena di eventi: stress cronico → immunosoppressione → malattie condizionate → maggiore necessità di antibiotici → problemi di sicurezza alimentare. In pratica, una sovrastimolazione dei meccanismi fisiologici di omeostasi può indurre uno stato di immunosoppressione, che a sua volta predispone a insorgenza di malattia. Lo stress cronico può derivare da diversi fattori ambientali, compresa l'interazione negativa con l'allevatore (Stockman's effect). Un ulteriore problema è lo squilibrio persistente del microbioma intestinale in alcune specie animali allevate. Per il pollo, il problema deriva dall'assenza di "imprinting" da parte della flora batterica dei soggetti adulti nei primi giorni di vita dopo la schiusa in incubatoio. Per il suino, il problema deriva dalla persistente pratica di trattamenti antibiotici di massa allo svezzamento. E' un problema assai grave, poiché la composizione corretta del microbioma intestinale condiziona la sensibilità a molti agenti microbici (vedi *Salmonella spp*), lo sviluppo e la funzionalità del sistema immunitario nel suo insieme. Le risposte infiammatoria, immunitaria e da stress rappresentano un complesso ancestrale e sovrapposto atto alla neutralizzazione di *noxae* che possono alterare l'omeostasi dell'ospite. Le funzioni immunitarie rappresentano pertanto un sistema "reporter" cruciale del processo di adattamento ambientale a causa delle connessioni funzionali e anatomiche tra cervello e organi linfoidi. I modelli di stress cronico sono molto più pertinenti a questo concetto di base dei modelli di stress acuto, in quanto gli effetti dello stress sul sistema immunitario sono generalmente adattativi a breve e dannosi nel medio e lungo periodo. I due circuiti principali, "stimoli psico-sensitivi / risposta comportamentale" e "stimoli antigenici / risposta immunitaria" sono sottosistemi di un complesso integrato unitario volto a fornire le condizioni ottimali per la sopravvivenza dell'ospite e l'adattamento. Pertanto, la risposta infiammatoria è innescata nell'ospite per ottenere una migliore capacità di superare stress infettivi e non infettivi. Allo stesso tempo, questa risposta deve essere controllata per evitare danni ai tessuti e inutili sprechi di energia metabolica. In effetti, gli episodi di malattia condizionata sono spesso preceduti da alterazioni infiammatorie che possono essere valutate mediante saggi di laboratorio quali formula leucocitaria, elettroferogramma del siero, parametri umorali dell'immunità innata e, in particolare, della risposta di fase acuta. Indipendentemente dall'effettivo verificarsi di malattie condizionate, il benessere degli animali è scadente se questi sono costretti a uno sforzo notevole e prolungato di adattamento ambientale (anche evidenziato dai test immunologici). Si possono in tale contesto adottare saggi immunologici che evidenziano fasi pre-cliniche dei problemi

e consentono possibili trattamenti farmacologici precoci e sicuramente più efficaci (ad esempio i test delle proteine di fase acuta nelle bovine da latte post parto). Si possono infine adottare saggi di immunologia clinica a finalità predittiva, quali il test del lisozima e dell'interleuchina-6 nelle bovine in asciutta. Questi ultimi si basano su un effetto di "imprinting" del sistema immunitario innato da parte di stressori ambientali che condiziona la risposta successiva dell'ospite allo stress metabolico.

STATO ATTUALE DELL'USO DI ANTIBIOTICI NELLE DIVERSE FILIERE ZOOTECNICHE

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Antibiotics must be used judiciously in humans and animals because both uses contribute to the emergence, persistence, and spread of resistant bacteria. Considerable attention is being given to antibiotic resistance (AMR) regarding Public and animal Health and, now more than ever, is essential that all stakeholders involved in the problem promotes effective actions under an integrated approach "one Health". In this regard, on 12th May of 2011, the European Parliament adopted a Resolution about antibiotic resistance, that strongly emphasizes the rapid rise of this risky situation and seeks to establish a strategy at EU level to counteract the problem. Consequently, many Member States (Denmark, France, Netherlands, etc.) adopted specific action plans and strategies relating this important issue, but Italy didn't.

The main limit for our country, to address AMR, is the lack of knowledge about the real quantities and qualities of drugs used in animal. Moreover, the absence of computerized prescriptions of veterinary medicinal products (VMPs) does not permit to quantify the exact volumes of antimicrobial used and the target species which are destined. This could seriously limit the evaluation of the strategies adopted at national level. At this moment only "monitoring plans", to test antibiotic resistance and evaluate correct use of VMPs in food-producing animal, are active in Italy by the use of specific ministerial checklists.

In this regard, in absence of strong national initiatives and instruments to address AMR, Emilia Romagna Region (E.R.) has activated a specific regional Project to evaluate the use of antibiotics for the prevention of resistance in animals (food-producing animals and Pets) and drafting guidelines about prudent and rational use of antimicrobials in animals. Specifically to the zootechnical sector, from 2013 to 2014, every year about 30% of the farms present in E.R. were checked by Public Veterinaries from Local Health Authority (AUSL) to evaluate the drug management by use specific "regional checklists".

In this study, are considered the data collected in the years 2013 and 2014. Globally, 2825 farms were screened (1998 bovine; 544 swine; 257 poultry and 26 rabbit) , and finally, data from 477 checklists have been aggregated. For all the farms included in the study, were considers as much as possible its location in order to represent, all the provinces of E.R. Different types of farms were

considerate: dairy cows (212) and beef cattle (44); pig farms were divided into fattening pigs (88) and swine reproduction (49); poultry herds were formed by layer flocks (24), broiler (8) and breeding turkey (26) and finally were considerate 26 rabbit farms.

The "regional checklist", used to evaluate the drug management, consists of two sections, one about pharmacovigilance (that we don't considerate in this study) and the second focused to set up a risk analysis in order to disclose the potential impact of the antibiotic management to develop antibiotic resistance at farm level. For this purpose a questionnaire with 23 points were used. The main questions were about: how to make diagnosis, use of antimicrobial administered to treat (therapy) or prevent (prophylaxis) disease, rational antimicrobial use (dose level and treatment duration), stimulation of immunity by vaccination, use of medicated feed or water, etc…For each, point a score was assigned and the final results indicated the risk level for the considered farms. to develop AMR.

The results of checklists provide a first picture about farm management in Emilia Romagna and antimicrobial use in food-producing animal; the final score (useful for AMR risk analysis) was not taken into consideration, but individual questions were aggregated in order to carry out a re-evaluation of the same.

Preliminary aggregation show that some important aspects of drug management need to be further investigated or improved. Despite empirical use of antimicrobials should be avoided whenever possible and antimicrobials should be preferably prescribed on the basis of laboratory diagnosis and antimicrobial susceptibility testing. Most of the farms investigated use only clinical diagnosis before administering an antimicrobial. Irrational use of antibiotic (discrepancies in dose and/or treatment duration) it seems not to be a serious problem, in the regional farms investigated, and also the choice of antibiotics was not as function of withdrawal periods but correctly on the basis of the real therapeutic effect. However prophylactic and metaphylactic use of the antimicrobials to a group of animals is a widespread practice. Depending on the farm system, antimicrobials are usually supplied to animals by feed or water, as therapy, but checklists show that sometime we also assist to an extralabel use of medicated feedingstuffs. Without the data on the real consumption of antibiotic, the presented preliminary data are useful to deeply understand and try to address the AMR problem at regional level. One of the purpose of the E.R. project is to share the obtained results with the colleagues involved in this topic. A series of training days to Veterinarians (AUSL and large animal veterinarians) and farmers will be organized. The final aim is to evaluate the usefulness to improve the checklist in order to improve its effectiveness. Finally these data will be a starting point for the drafting of the guidelines relating the reduction antimicrobial use in farm animals.

The prudent and rational antimicrobial use are a part of good veterinary practice, the need to preserve their efficacy and limit the spread of AMR has become an important aspect not only of the

veterinary profession but also for human medicine and for the agricultural sector. An holistic and multidisciplinary approach is needed to improve health and prevents the risks of AMR in a common view of the “One Health Perspective” taking into account the interaction between humans, animals and environment.

USO PRUDENTE E RAGIONATO DEGLI ANTIBIOTICI NEGLI ANIMALI DI INTERESSE ZOOTECNICO

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Il ruolo degli antibiotici nella produzione del latte

Gli antibiotici hanno rappresentato un importante fattore nello sviluppo della zootecnia moderna. Infatti, quantità e qualità delle produzioni attuali sono il frutto non solo del miglioramento genetico e delle tecniche di gestione degli allevamenti, ma anche dalla possibilità di curare e, soprattutto, di prevenire le malattie delle bovine da latte. Come esempio si pensi agli effetti positivi legati all'adozione della terapia antibiotica in asciutta che permette sia di eliminare le infezioni esistenti, sia di prevenire quelle nuove.

Purtroppo, come spesso accade, a fronte di evidenti benefici, con il tempo si sono evidenziati anche alcuni problemi. Innanzitutto si è visto che, dopo i primi anni in cui la terapia antibiotica sembrava essere l'arma risolutiva, non tutti gli animali guarivano. Questi insuccessi erano dovuti a diversi fattori tra cui l'incompleta conoscenza dei meccanismi patogenetici, ma soprattutto allo sviluppo dell'antibioticoresistenza. Questo problema non riguarda solo la mancata guarigione degli animali, ma è ancora più importante per i riflessi sulla salute umana nel caso in cui tale resistenza sia trasmessa a patogeni pericolosi per l'uomo (due esempi su tutti *Salmonella* e *S.aureus* MRSA). Quest'ultimo problema è risultato particolarmente evidente per gli antibiotici che erano usati come promotori di crescita negli alimenti per gli animali e che ha portato al loro bando prima in Europa e quindi in molti altri Paesi.

Nuovi approcci terapeutici e antibiotico-resistenza

Gli insuccessi terapeutici osservati nel tempo hanno indotto lo sviluppo di nuove molecole e successivamente, quando lo sviluppo di tali molecole non era più remunerativo, di nuovi approcci terapeutici che contribuiscono a mantere e, in alcuni casi ad aumentare il problema dell'antibiotico resistenza. Ad esempio, per quanto riguarda i nuovi approcci terapeutici, dobbiamo pensare alla somministrazione di farmaci per tempi maggiori rispetto alle indicazioni terapeutiche (terapia prolungata) oppure la somministrazione di più antibiotici contemporaneamente (terapia combinata). Questo approccio terapeutico, molto diffuso soprattutto in questi ultimi 10-15 anni, attualmente si sta riducendo molto per due motivi: il costo elevato di tali protocolli senza che vi sia un reale miglioramento dell'efficiacia terapeutica da un lato e, dall'altro, una pressione da parte della veterinaria pubblica per un uso prudente dei farmaci che porta quindi alla repressione di tali pratiche. Non a caso, in alcuni paesi europei, come ad esempio Scandinavia e Olanda, tali terapie sono oggi proibite.

Come ridurre il rischio

Nell'immediato è comunque possibile intervenire riducendo i rischi connessi all'uso di antibiotici nella filiera produttiva, e tale intervento sarà tanto più efficace quanto sarà di filiera e non legato all'azione di singoli individui. Infatti, l'utilizzo di antibiotici è strettamente legato alla presenza di problemi a livello di allevamento (mastite, patologie podali, problemi riproduttivi) che vengono gestiti per via farmacologica, spesso in modo impropri. In queste condizioni, inoltre, il latte proveniente da tali allevamenti non solo è a rischio per la presenza di residui, ma generalmente ha anche una qualità inferiore a quella di allevamenti ben gestiti.

L'obiettivo comune sia dell'allevatore sia del trasformatore dovrebbe essere quello di produrre prodotti di qualità e, attraverso questi, aumentare il proprio profitto. Proprio questo obiettivo comune dovrebbe essere la leva che permette di affrontare il problema in modo proattivo e non solo attraverso un sistema basato su multe e penalità.

Un approccio di filiera prevede una condivisione esplicita degli obiettivi da parte delle componenti principali (allevatore, veterinario e latteria) e la messa in atto di strumenti che possano favorire la riduzione dei problemi a livello di allevamento, aumentando di conseguenza la qualità e la quantità di latte. Il miglioramento della sanità dell'allevamento si ottiene quindi mettendo in atto un programma di gestione sanitaria centrato sull'identificazione dei fattori di rischio per le diverse patologie, sulla loro eziologia, sul management aziendale e sull'implementazione di azioni volte a ridurre e, possibilmente, eliminare tali problemi.

Conclusioni

La terapia antibiotica è tuttora un utile strumento per il veterinario e l'allevatore che non può essere eliminato, ma è necessario un approccio più razionale ed efficiente per il controllo delle patologie. D'altra parte, anche quando si utilizzano in modo corretto gli antibiotici, possiamo avere la presenza di loro residui e di loro metaboliti nel latte con concreti rischi di aumentare la pressione selettiva sulla flora lattica. La qualità del latte non è quindi solo grasso e proteine, ma anche l'assenza di sostanze che ne alterano la composizione e quindi il valore dei prodotti. Da qui l'importanza di una corretta e puntuale gestione sanitaria degli allevamenti e un monitoraggio altrettanto costante della presenza di sostanze antimicrobiche nel latte.

Va sottolineato come quasi sessant'anni fa un noto esperto del settore diceva: "A questo stadio dello sviluppo dell'industria lattiero-casearia, due sono le cose più importanti: migliorare l'efficienza produttiva dell'allevamento in modo da ridurre i costi di produzione e aumentare la qualità del latte prodotto. Prodotti di buona qualità non possono essere prodotti da latte di bassa qualità e, in un mondo competitivo, l'obiettivo è avere la qualità più alta al prezzo più basso. E' giunto il tempo che quanto detto finalmente diventi la "mission" della filiera latte italiana.

IMPIEGO DELLE INFORMAZIONI SULLA CATENA ALIMENTARE (ICA) PER RICONOSCERE E QUANTIFICARE IL RISCHIO FARMACO NELLE FILIERE ZOOTECNICHE.

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Dipartimento di Medicina Veterinaria

Introduzione

L'obbligo di trasmettere le informazioni sulla catena alimentare (ICA) ai titolari dei macelli sussiste dal 1° gennaio 2006 per il settore avicolo, dal 1° gennaio 2008 per il settore suinicolo, dal 1° gennaio 2009 per il settore equino e del vitello di età non superiore a 8 mesi. Dal 1° gennaio 2010 l'obbligo è esteso a tutte le altre specie e alle restanti categorie della specie bovina. Per un approfondimento sui contenuti, sulle modalità di trasmissione, sul controllo e sulla verifica delle ICA si rimanda ai Regolamenti (CE) n° 852/2004 del Parlamento europeo e del Consiglio del 29 aprile 2004 sull'igiene dei prodotti alimentari: Allegato I, parte A, punti 7 e 8; Regolamento (CE) n° 853/2004 del Parlamento europeo e del Consiglio del 29 aprile 2004 che stabilisce norme specifiche in materia di igiene per gli alimenti di origine animale: Allegato II, sezione III; Regolamento (CE) n°854/2004 del Parlamento europeo e del Consiglio del 29 aprile 2004 che stabilisce norme specifiche per l'organizzazione di controlli ufficiali sui prodotti di origine animale destinati al consumo umano: Allegato I, sezione I, cap. II, A e sezione II, cap. II; Regolamento (CE) n°2074/2005 della Commissione del 5 dicembre 2005 recante modalità di attuazione relative a taluni prodotti di cui al Regolamento (CE) n° 853/2004 del Parlamento europeo e del Consiglio e all'organizzazione di controlli ufficiali a norma dei regolamenti del Parlamento europeo e del Consiglio (CE) n°854/2004 e (CE) n°882/2004, deroga al Regolamento (CE) n°52/2004 del Parlamento europeo e del Consiglio e modifica dei regolamenti (CE) n°853/2004 e (CE) n°854/2004: articolo 1 e Allegato I; Regolamento (CE) n°2076/2005 della Commissione del 5 dicembre 2005 che fissa disposizioni transitorie per l'attuazione dei regolamenti del Parlamento europeo e del Consiglio (CE) n°853/2004, (CE) n°854/2004 e (CE) n°882/2004 e che modifica i regolamenti (CE) n°853/2004 e (CE) n°854/2004: articolo 8.

Considerazioni sull'analisi del rischio

Spesso nella nostra vita prendendo delle decisioni ci troviamo a valutare i vantaggi e gli svantaggi e le nostre scelte finali sono basate sui benefici che riteniamo più importanti per noi e sugli svantaggi che pensiamo di poter accettare. Lo stesso avviene nelle scelte che facciamo su ciò che mangiamo, anche se, quando si tratta della nostra sicurezza alimentare, tendiamo a fare molta più attenzione e richiediamo un maggior numero di informazioni sull'origine del cibo, sul suo contenuto, sulle modalità con le quali gli animali sono stati allevati o i prodotti vegetali coltivati ed infine su come il

nostro governo decide quale cibo è sicuro per noi (Cenci-Goga e Clementi, 2010; Poeta *et al.*, 2013; Salamano *et al.*, 2013; Salamano e Cenci Goga, 2015). Oggi come oggi, nonostante le nostre numerose ed aggiornate conoscenze nel settore alimentare le malattie trasmesse attraverso il cibo rappresentano ancora il più grande problema sanitario nel mondo contemporaneo ed una causa molto importante di riduzione della produttività economica (Cenci-Goga e Clementi, 2002; Cenci-Goga e Clementi, 2010). E' difficile stabilire se un cibo è sano o meno, anche perché non si può provare che sia interamente pericoloso o altrettanto sicuro: sarà al massimo possibile stabilirne il grado di pericolosità in determinate condizioni. Del resto come risulterebbe impossibile richiedere un alimento completamente sicuro, potrebbe invece essere plausibile la richiesta di alimenti nei quali siano stai ridotti potenziali pericoli (Adams e Moss, 2000). Il fatto che per anni il concetto di sicurezza alimentare sia stato studiato separatamente dal concetto di prevenzione del deterioramento, non può essere una giustificazione, anche perché le due tematiche, da un punto di vista microbiologico ed ecologico sono indistinguibili. Al di là di considerevoli sforzi, l'assicurazione sulla sicurezza microbiologica del cibo rimane ancora molto lontana, persino nei paesi più industrializzati. La morte, la sofferenza, le perdite economiche e i diritti civili nell'interesse delle vittime di malattie trasmesse dal cibo, sono messe a confronto con le perdite economiche causate dal deterioramento del cibo (Mossel *et al.*, 1995).

Gli sforzi per raggiungere il controllo

L'uso di alcuni termini da parte degli scienziati della sicurezza alimentare spesso risulta ingannevole o fuorviante. Il significato in inglese del termine «*to control*» nella scienza degli alimenti è di assicurare buona qualità e sicurezza. In italiano invece il termine controllo corrisponde al termine inglese «inspection» o «*monitoring*». Perciò, tra studiosi, la parola inglese «*control*» è stata ampiamente adottata a significare «*management*», come viene usata in medicina. La giusta strategia è il cosiddetto «controllo avanzato per garantire la sicurezza alimentare». In passato il controllo della sicurezza e della qualità del cibo era raggiunto attraverso un sistema di controllo retroattivo, repressivo e tardivo, consistente nel prelevare campioni dopo l'ingresso del cibo nella catena alimentare, nel ricercare agenti patogeni, deterioramenti e microrganismi e in un secondo momento prendere appropriate decisioni. Sistema fallimentare per due motivi: in primis perché l'approccio retroattivo è in definitiva un'ispezione che può solo misurare gli effetti, ignorando il meccanismo e quindi incapace di portare alla gestione del rischio. In secondo luogo perché i campioni dovevano essere scelti casualmente, facendo riferimento alla distribuzione di Poisson; tutto ciò avrebbe portato ad un numero elevatissimo di campioni da analizzare e ad un inutile riscontro per il produttore o per il consumatore. L'approccio di intervento invece, esteso lungo tutta la catena alimentare, sulle linee di distribuzione e di stoccaggio, conduce ad una adeguata protezione per il consumatore. Ciò implica l'adesione a quello che viene definito il «codice di buona produzione e pratiche di distribuzione». La normativa vigente si affida all'analisi quantitativa del rischio (il sistema HACCP- hazard analysis critical control point); l'attuazione di un necessario intervento su tutte le linee di produzione, distribuzione e preparazione gastronomiche (il concetto

LISA - longitudinally integrated safety assurance) e la meticolosa codifica delle procedure alle quali attenersi completamente (il concetto GMDPs- – good manufacturing and distribution practices). Inoltre il presupposto fondamentale è che le regole generali d'igiene siano estese affinché assicurino l'igiene anche al livello di imprese agricole. Nel far questo,la legislazione Comunitaria sull'igiene alimentare è dotata di strumenti che coprono l'intera catena alimentare. Per raggiungere i richiesti livelli di igiene al livello di azienda agricola, viene suggerito che i possibili rischi che possono accadere nella produzione primaria e i metodi per controllarli, siano considerati guide di buona pratica (Cenci-Goga *et al.*, 2005; Rossitto *et al.*, 2012; Sechi *et al.*, 2014). Sebbene questo sistema di di sicurezza alimentare proposto, al livello agricolo sia basata sul rischio, una formale attuazione del sistema HACCP, non è prevista. Tale sistema potrebbe essere introdotto in un secondo momento allorché l'esperienza con le nuove normative di igiene dimostri che questo possa essere applicato praticamente anche alla produzione primaria (Cenci-Goga e Clementi, 2002).

Ruolo del medico veterinario

Il veterinario ufficiale attua controlli ufficiali sui prodotti di origine animale destinati al consumo umano, secondo il Reg. 854/2004 CE, sia nei macelli che commercializzano carni fresche, sia nei centri di lavorazione della selvaggina e nei laboratori di sezionamento. I sei punti fondamentali dell'attività ispettiva di carni fresche sono: 1) raccolta delle informazioni sulla catena alimentare, considerando: - i registri tenuti presso l'azienda di provenienza degli animali destinati alla macellazione e i risultati delle analisi e dei controlli effettuati in azienda; - i certificati ufficiali che accompagnano gli animali e le eventuali dichiarazioni fatte dai veterinari che effettuano i controlli a livello della produzione primaria; - le misure aggiuntive atte a garantire la sicurezza degli alimenti nella catena alimentare, qualora gli operatori del settore alimentare le adottino. 2) ispezione ante mortem: eseguita in tutti gli animali prima della macellazione entro 24 ore dall'arrivo al macello e meno di 24 ore prima della macellazione e un esame clinico accurato di tutti gli animali scartati dall'OSA o dall'assistente. In caso di macellazione d'emergenza fuori dal macello o di selvaggina cacciata, il veterinario ufficiale non potendo effettuare l'ispezione ante mortem, esamina la dichiarazione di accompagnamento della carcassa dell'animale rilasciata dal veterinario che ha effettuato il controllo sanitario in azienda. In altri casi (suini domestici, pollame, selvaggina d'allevamento), su decisione dell'autorità competente, l'ispezione ante mortem può essere effettuata nell'azienda di provenienza; 3) verifica del benessere degli animali applicando le norme comunitarie e nazionali; 4) ispezione post mortem; 5) gestione del materiale specifico a rischio e di altri sottoprodotti animali: in conformità alle specifiche norme comunitarie, verificando che siano messe in atto le misure necessarie per evitare di contaminare le carni durante la macellazione; 6) campionamento per le prove di laboratorio: previste dalla normativa vigente o necessarie e controllare che i materiali prelevati siano adeguatamente identificati, manipolati e inviati al laboratorio idoneo.

Le sezione II del regolamento 854: Provvedimenti successivi ai controlli, Capo I: Comunicazione dei risultati delle ispezioni, al punto 1 recita: "Il veterinario ufficiale registra e valuta i risultati delle attività ispettive" Al punto 2. a): "Se dalle ispezioni emerge la presenza di una malattia o condizione che potrebbe ripercuotersi sulla salute pubblica o degli animali, oppure una situazione che compromette il benessere degli animali, il veterinario ufficiale ne informa l'operatore del settore alimentare". Al capo II: decisioni relative alle informazioni sulla catena alimentare, punto 1: "Il veterinario ufficiale accerta che gli animali siano macellati soltanto se l'operatore del macello ha ottenuto e verificato le pertinenti informazioni sulla catena alimentare." Questo concetto sta alla base di una grossa rivoluzione culturale, che responsabilizza fortemente la produzione primaria. In questo ambito è da porre l'attenzione anche sul benessere degli animali che, considerato un prerequisito, è un passo imprescindibile per allevamento, trasporto e macellazione, oltre che per la qualità delle produzioni. Un alimento è migliore dal punto di vista nutritivo e della conservabilità se ottenuto da animali trattati in modo degno; per cui il benessere degli animali rappresenta un'opportunità per l'OSA. Il benessere alla macellazione, con il Regolamento 1099/2009, rappresenta un'ulteriore opportunità per l'OSA di diretto controllo di processo e quindi di verifica effettiva, misurata, sugli effetti di una corretta attenzione a determinati aspetti etologici e di conseguenza a manovre migliori in tutte le fasi delle filiera produttiva.

Valutazione e gestione del rischio

Il cibo contiene per natura sostanze chimiche e può venire a contatto con molte sostanze naturali o artificiali durante la raccolta, la produzione o la preparazione. Sono incluse tra queste, le sostanze chimiche presenti naturalmente o prodottesi durante i processi lavorativi, i microrganismi, i contaminanti ambientali ed i pesticidi. Sin quando la possibilità di essere danneggiati da questi potenziali pericoli viene chiamata rischio, l'analisi dei rischi potrebbe essere meglio definita come la scienza della sicurezza, dato che la gestione dei rischi ne è una parte essenziale. Una importante discussione viene portata avanti a livello nazionale ed internazionale sul ruolo che la precauzione dovrebbe avere nel guidare le decisioni politiche. Questa tematica sulla sicurezza alimentare riflette la necessità di trovare un equilibrio più valido tra il raccogliere i benefici della tecnologia e l'innovazione da un lato, e l'evitare o minimizzare i rischi di effetti avversi inaccettabili del progresso tecnologico dall'altro. E' stata proprio l'esperienza con gli inaspettati effetti avversi dei nuovi prodotti chimici, vissuta nella prima metà del secolo, che ha portato ad un crescente supporto per l'applicazione del «principio di precauzione». Tale approccio cautelativo richiede lo sviluppo di metodi migliori per la prevenzione degli effetti contrari delle nuove tecnologie e di riesaminare le tecnologie più attentamente, esplorando vie alternative per trarre benefici e nel contempo, minimizzare gli effetti collaterali, prima che qualsiasi altra innovazione venga largamente adottata (Groth, 2000). I dati sugli effetti delle singole sostanze non possono prevedere gli effetti dell'interazione di una molteplicità di sostanze chimiche alle quali i consumatori sono quotidianamente esposti. I metodi di valutazione per i pericoli associati al cibo, come i contaminanti microbiologici e gli organismi geneticamente modificati (OGM), sono in realtà meno sviluppati

rispetto a quelli per i prodotti chimici. E' tuttavia possibile talvolta, usando gli strumenti disponibili per la valutazione del rischio, essere ragionevolmente certi che il cibo sia "*sicuro*". L'essenza della «valutazione cautelativa del rischio» è quella di trattare questioni scientifiche in maniera «scientifica», invece che trattarla in maniera «politica» (Groth, 2000). La distinzione concettuale tra la valutazione del rischio (comprensione) e la gestione dello stesso (azione) risulta utile per varie finalità di rilievo, come isolare l'attività scientifica dalle pressioni politiche e mantenere la distinzione tra la dimensione del rischio ed il costo per fronteggiarlo. Per la finalità di perfezionare la comprensione delle decisioni attinenti al rischio e rendere questa comprensione più ampiamente accettata, una rigida distinzione di questo tipo davvero non aiuta. Questo perché le attività analitiche generalmente considerate parte della valutazione del rischio non sono sufficienti da sole a garantire la necessaria comprensione.

Tuttavia la gestione del rischio, pur dipendendo dalla scienza, non è un'attività esclusivamente scientifica: è piuttosto un processo di decisioni che implica considerazioni politiche, sociali ed economiche per poter sviluppare, analizzare e confrontare le varie possibilità normative. Tutto ciò allo scopo di scegliere la risposta normativa più adeguata per un potenziale pericolo per il consumatore (Cenci-Goga e Clementi, 2002).

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SICUREZZA ALIMENTARE E SOSTENIBILITÀ DELLE PRODUZIONI ZOOTECNICHE: QUALE RUOLO DEGLI ALLEVATORI E DEI SISTEMI DI QUALITÀ

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Negli ultimi decenni i temi della sicurezza alimentare, della conservazione dell'ambiente e dello sviluppo sostenibile sono arrivati all'attenzione dei consumatori e di tutti gli operatori delle filiere agroalimentari. La presa di coscienza di tali problematiche nasce come tentativo di risposta ad una serie di segnali, diventati ormai non più trascurabili, quali le continue emergenze del settore agroalimentare; il peggioramento dello stato dell'ambiente e il lento ma progressivo cambiamento climatico. l'Unione Europea e l'Italia, proprio per far fronte a questi segnali, hanno adottato delle strategie globali di intervento per la sicurezza e la sostenibilità dai campi alla tavola dei consumatori. In questa formula è racchiuso lo spirito dell'intervento normativo e di controllo degli ultimi anni: affrontare la sfida per garantire cibi sani e sicuri lungo tutta la filiera produttiva; predisporre un controllo integrato e abbandonare l'approccio settoriale e verticale. Tale sfida si basa su una combinazione di requisiti elevati per la sicurezza alimentare e la sostenibilità delle aziende agricole e quelle zootecniche, in particolare. Le prime valutazioni sul tema della sicurezza alimentare risalgono all'anno 1997 con il "Libro verde della Commissione sui principi generali della legislazione in materia alimentare dell'Unione Europea" e hanno trovato la formulazione condivisa nel "Libro Bianco sulla sicurezza alimentare" del 2000. Mentre, per la sostenibilità aziendale, nel 2009 la UE ha emanato la Direttiva n. 28/EC sulla promozione dell'uso di energia da fonti rinnovabili, che prescrive, entro il 2020, la riduzione delle emissioni di gas serra del 20%, l'utilizzo del 20% di fonti rinnovabili e la riduzione del 20% dei consumi energetici mediante aumento dell'efficienza energetica. Questi fondamentali documenti hanno ispirato l'impianto normativo, comunitario e nazionale, in materia di sicurezza alimentare e sostenibilità. Anche nel mondo allevatoriale italiano è cresciuta la consapevolezza dell'importanza di questi segnali sull'economia delle filiere e sulla società nella sua interezza, pertanto, alla luce di queste evidenze è nata l'esigenza di delineare delle azioni volontarie per certificare gli interventi sia sulla sicurezza e tracciabilità alimentare sia sulla sostenibilità ambientale, economica e sociale degli aziende zootecniche. La zootecnia italiana è un settore chiave dell'agricoltura nazionale. Il valore delle produzioni animali, infatti, seppure in flessione in questi ultimi anni per il calo dei prezzi, si attesta intorno a 34 miliardi di euro. In assoluto, il comparto delle carni costituisce la seconda voce del valore della produzione agricola, dopo il settore ortofrutticolo, con circa 21 miliardi, mentre il settore latte ha un valore che si attesta intorno agli 11 miliardi di euro. L'attività di allevamento sviluppa, inoltre, un indotto rilevante, che va dalla trasformazione dei prodotti di origine animale, alla commercializzazione e ai servizi connessi. I prodotti della zootecnia costituiscono, quindi, la

materia prima per dar vita alle specialità più importanti della tradizione agroalimentare italiana, con un forte aggancio al territorio e sostengono la differenziazione del made in Italy alimentare. L'allevamento italiano, così come viene riportato nel Libro bianco del Ministero delle Politiche Agricole Alimentari e Forestali "*Sfide ed opportunità dello sviluppo rurale per la mitigazione e l'adattamento ai cambiamenti climatici*", rappresenta una delle realtà zootecniche più complesse sia nel contesto della zootecnia europea sia in quella mondiale dei Paesi a economia avanzata, inoltre, è dotato di particolarità produttive di enorme rilievo tecnico ed economico. L'Associazione Italiana Allevatori (AIA), in collaborazione con il Dipartimento Qualità Agroalimentare (DQA), ente terzo di importanti filiere agroalimentari Italiane, ha implementato e sviluppato dei sistemi di qualità e di certificazione per garantire l'origine, la tracciabilità e la sicurezza alimentare e per misurare l'impatto ambientale del comparto zootecnico, in termini di emissioni e di riduzioni del carbon footprint. In particolare, per la sicurezza alimentare è stato predisposto uno specifico disciplinare di produzione (ITALIALLEVA), che ha come scopo la valorizzazione delle produzioni zootecniche nazionali, mediante un sistema di certificazione, che permette di garantire e tracciare l'origine italiana dei prodotti agroalimentari, la sicurezza alimentare ed il benessere degli animali in allevamento. Le produzioni a marchio ITALIALLEVA, pertanto, sono ottenute dal latte e dalla carne di animali allevati, esclusivamente, in aziende ricadenti nel territorio Italiano. Il marchio Italialleva apposto su un prodotto garantisce che il latte e la carne, tal quali o come ingredienti di un formaggio o di un prodotto a base di carne, sono di origine italiana, sono tracciati in ogni fase del processo produttivo e sono sicuri. Il percorso di garanzia Italialleva inizia negli allevamenti, là dove nasce la sicurezza alimentare grazie al lavoro quotidiano e impegnativo degli allevatori, e prosegue nelle fasi successive di trasformazione del prodotto per far sì che le garanzie create in allevamento vengano mantenute. Il marchio porta dentro di sé il valore fondamentale della trasparenza nei confronti dei consumatori che devono poter scegliere in maniera consapevole ciò che mangiano. Grazie al marchio Italialleva, il patrimonio di conoscenze e di esperienza del sistema allevatori viene messo a disposizione della società e, partendo dagli allevamenti, giunge fino alle tavole dei consumatori finali per garantire la sicurezza e l'italianità dei prodotti. Per la sostenibilità degli allevamenti zootecnici, partendo dai risultati presentati nel Libro Bianco per il Settore Zootecnico, è stato definito da AIA e DQA un sistema di misurazione per la valutazione delle emissioni con effetto climalterante derivanti dalle attività riferite alla fase di allevamento. Con tale sistema sono valutate le emissioni riconducibili direttamente all'animale, come peraltro previsto dalla metodologia IPCC (Intergovernmental Panel on Climate Change), per la stesura degli inventari Nazionali delle emissioni. In particolare, sono state definite le metodiche per il calcolo delle emissioni di CH_4 derivanti da fermentazione enterica e di CH_4 e N_2O derivanti dalle deiezioni. Tali metodiche, inoltre, sono comparate e certificate per la loro standardizzazione. Per quanto riguarda le emissioni a monte e a valle delle fasi di allevamento sono calcolate secondo la metodologia del Life Cycle Assessment (LCA). Successivamente alla determinazione delle emissioni di CO_2 equivalente, le aziende si impegnano a mettere in atto interventi per ridurre le emissioni dei singoli allevamenti. Tali interventi riguardano la gestione della mandria, l'alimentazione, con particolare attenzione alla qualità dei foraggi, il rapporto foraggi/concentrati e la grassatura della razione. Inoltre, se necessario, l'allevatore si impegna ad intervenire anche mediante interventi strutturali sui

ricoveri e sul management aziendale. Infine, il disciplinare prevede, anche, un meccanismo di compensazione delle emissioni grazie all'implementazione della prima banca dei crediti di CO_2 degli allevamenti zootecnici italiani.

Workshop 4

Aggiornamenti sulla leishmaniosi

EVOLUZIONE DELLA INFEZIONE

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Introduzione: la leishmaniosi canina (CanL) è una infezione causata da protozoi (ordine Kinetoplastida e famiglia Trypanosomatidae) presenti, in quanto parassiti intracellulari obbligati, nella forma di amastigote nelle cellule del sistema reticolo endoteliale (SRE) di ospiti vertebrati quali roditori, carnivori domestici (cani, gatti) e selvatici (volpi), e uomo. La trasmissione di *Leishmania* spp. avviene quando i flebotomi vettori si alimentano su cani infetti; è dimostrato tuttavia che le trasfusioni di sangue da cani infetti e le vie transplacentare e coitale possono essere implicate nella trasmissione del parassita. Nel cane la leishmaniosi è caratterizzata da un ampio range di segni clinici, da forme asintomatiche fino a quadri clinici gravi e spesso fatali. In Europa, la leishmaniosi da *L. infantum* è endemica nei Paesi mediterranei anche se, negli ultimi anni, l'areale di diffusione dell'infezione si è espanso alle regioni settentrionali con casi di focolai autoctoni in alcune aree della Svizzera e della Germania. La prevalenza dell'infezione nei cani, in Italia, varia da 1.7 al 48.4% con un'incidenza annuale del 9.52% fino al 13.1% in animali presenti in un'area endemica della Puglia. La diffusione della LCan è influenzata da fattori ambientali ed ecologici che determinano la presenza e l'abbondanza delle diverse specie di flebotomi vettori ed il mantenimento del protozoo in una popolazione di animali recettivi. Nonostante anche altri animali quali la volpe e il ratto nero sino stati trovati infettati da *L. infantum*, la reale importanza di questi animali nel ciclo zoonotico di trasmissione non è del tutto chiara. Il cane quindi resta il principale serbatoio domestico di *L. infantum* anche se anche il gatto può essere infettante per il flebotomo. La maggior parte dei cani che vive in aree endemiche è costantemente esposto alle punture di flebotomi infetti anche se, un'alta percentale di questi animali, in aree endemiche non sviluppa segni clinici. Nonostante ciò, questa categoria di soggetti rappresenta un' importante fonte di

infezione da *L. infantum* per altri animali recettivi attraverso la puntura di insetti vettori.

Aspetti patogenetici: la deposizione del parassita nel derma dell'ospite avviene pressoché immediatamente, anche perché il pasto di sangue del flebotomo si realizza in pochi secondi. La presenza dei protozoi nel derma attiva le cellule fagocitarie del sistema immune innato (macrofagi, cellule dendritiche, neutrofili) nelle quali il parassita viene internalizzato sfruttando alcuni recettori di superficie, in particolare quelli del sistema complemento (CR3, Cr1) e quelli per le immunoglobuline (Fc-γR). L'interazione tra alcune costituenti di superficie del parassita (lipofosfoglicani-LPG) e alcuni specifici recettori delle cellule macrofagi che dell'ospite è il primo processo indispensabile per la sopravvivenza del parassita, poiché la scelta di alcune "porte di entrata" e non di altre comporta la mancata attivazione dei complessi processi biochimici che porterebbero alla distruzione del protozoo. In corso di infezione naturale i neutrofili sono considerati le cellule più attive nella captazione dei parassiti deposti nel derma. La loro breve emivita, tuttavia, fa sì che essi agiscano come "cavalli di Troia", nei quali gli stessi parassiti creano condizioni per la secrezione di chemochine che attirano i macrofagi in loco. I macrofagi sono considerati come le cellule definitive nella captazione dei parassiti, sia liberi che contenuti all'interno di neutrofili danneggiati o apoptotici. All'interno delle cellule fagocitarie la *Leishmania* assume la forma non flagellata (amastigote) all'interno del sistema fagosomiale; il risultato è la formazione di vacuoli parassitofori formati da fagosomi contenenti amastigoti maturi. Questi vacuoli, la cui composizione varia in dipendenza della specie di parassita e della cellula coinvolta possono contenere uno o più parassiti ed evolvere in maniera differente, permettendo o meno la moltiplicazione e la sopravvivenza del protozoo. E' noto, ad esempio, che i macrofagi sono le cellule più "permissive", a differenza dei neutrofili nei quali il parassita sopravvive con più difficoltà; le cellule dentritiche, gli eosinofili, e i fibroblasti sono considerate cellule a media permissività. Una volta penetrato nei macrofagi, la sopravvivenza, la crescita, la moltiplicazione e la successiva disseminazione del parassita dipendono dal tipo e dall'efficienza della risposta immunitaria del cane infetto. I macrofagi parassitati sono in grado di distruggere le leishmanie fagocitate attraverso un meccanismo di produzione di ossido nitrico (NO), una molecola particolarmente tossica per il parassita. La via metabolica che porta alla produzione di NO è attivata dalla presenza indispensabile di interferone-γ (IFN-γ), citochina chiave prodotta da cellule della linea T helper 1 (Th1) e dalle Natural Killer (NK). Altre citochine infiammatorie quali l'interleuchina-1 (IL-1), il Tumor Necrosis Factor (TNF), l'interferone-α (IFN-α) e l'interferone-β (IFN-β) sono ritenute importanti per la produzione di NO**.** In contrasto a questo tipo di attivazione macrofagica, definita "classica", è conosciuta una via "alternativa", mediate da citochine diverse dall'IFN-γ, tra cui l'interleuchina-4 (IL-4) e l'interleuchina-13 (IL-13) che favoriscono la produzione di poliamine arginasi-dipendenti, indispensabili alla crescita del parassita. La *Leishmania* è in grado di favorire l'attivazione "alternativa" di macrofagi, poiché la loro presenza all'interno degli stessi è in grado di deprimere la produzione dell'interleuchina-12 (IL-12), citochina indispensabile per l'attivazione delle cellule produttrici di IFN-γ. La

Leishmania è in grado di stimolare la produzione di altri fattori che contribuiscono alla depressione dell'attività di killing macrofagico, tra cui l'interleuchina-10 (IL-10) e il Tumor Growth Factor-β (TGF-β) o indurre l'espressione di molecole immuno-modulatorie (CD 200) in grado di deprimere ulteriormente l'attivazione macrofagica. A causa della disfunzione macrofagica indotta dal parassita, altre cellule del sistema istiocitario giocano un ruolo fondamentale per cercare di attivare correttamente la linea cellulare Th1, indispensabile per la produzione di IFN-γ. Le cellule più importanti presenti sia nell'epidermide che nel derma sono le cellule dendritiche (DCs) altamente specializzate nella captazione, processazione e presentazione degli antigeni parassitari ai linfociti presenti nei linfonodi satelliti del sito di inoculazione del protozoo. Le DCs una volta infettate dal parassita sono potenzialmente in grado di migrare verso i linfonodi satelliti e di produrre IL-12 per indurre una risposta immunitaria di tipo Th1. Va specificato, tuttavia, che esistono diversi "subset" di DCs che hanno diversi comportamenti nei confronti della captazione del parassita e della presentazione e pro cessazione degli antigeni da essi derivati. Diverse specie del parassita, inoltre, sono in grado di orientare in maniera diversa la produzione di citochine da parte delle DCs, in particolare stimolando le stesse a produrre IL-4, citochina chiave nell'induzione di una risposta negativa, di tipo Th2. Il ruolo dei diversi sottotipi di DCs nell'orientamento della risposta immunitaria, nonché la loro interazione con il parassita nell'evoluzione della patogenesi dell'infezione leishmanica resta ancora da chiarire. Sicuramente la *Leishmania,* indipendentemente dalla specie coinvolta, è in grado di manipolare o inibire numerosi segnali per l'attivazione di vie metaboliche che porterebbero alla produzione di molecole ad azione microbicida e di citochine capaci di orientare la risposta immunitaria di tipo cellulo-mediata, deleteria per il parassita. Non potendo entrare in descrizioni più dettagliate che esulano dallo scopo del presente articolo, è indubbio che alcune costituenti del parassita, in particolare i lipofosfoglicani (LPG) e alcune metallo-proteinasi (GP63) giocano un ruolo chiave nella strategia che rende non solo inefficaci i meccanismi difensivi innati dell'opsite ma innescano una cascata di eventi che porta all'attivazione di un tipo di risposta immunitaria (Th2), prevalentemente umorale, che rende suscettibile l'ospite nei confronti dell'infezione. Ovviamente sia negli animali che nell'uomo esiste una vasta gamma di individui che mostrano gradi differenti di suscettibilità o di resistenza all'infezione, probabilmente condizionati dal background genetico individuale, indipendentemente dal potere patogeno del parassita.

Risposta immunitaria nel cane: Nella specie canina gli studi sulla risposta immunitaria indotta da *Leishmania infantum* hanno avuto un particolare impulso nell'ultimo decennio. Pur non conoscendo nei singoli dettagli i fattori che determinano i diversi gradi di suscettibilità e di resistenza nel cane, sono ormai noti diversi aspetti che in linea generale contribuiscono a determinare l'evoluzione dell'infezione e il conseguente sviluppo di malattia, o al contrario conferiscono uno stato di resistenza. Ad oggi è noto che i cani resistenti allo sviluppo di forme conclamate di malattia son caratterizzati da elevati livelli di IFN-γ e TNF-α, proliferazione di linfociti helper (CD4+), linfociti citotossici (CD8+) e alcuni sottotipi di linee cellulari della linea linfocitaria B, positività al test

intradermico della leishmanina e capacità di attivazione delle cellule monocita rie del sangue periferico (PBMCs) in seguito a stimolazione con antigeni di leishmania. Al contrario, i cani che mostrano segni clinici ed alterazioni clinico-patologiche riconducibili all'infezione da *Leishmania infantum* mostrano livelli elevati di IL-10 e TGF-β, depressione dell'attività cellulo-mediata indotta dal parassita, elevati livelli di anticorpi di tutte le classi (IgG; IgM; IgE; IgA) diretti contro diversi costituenti del parassita. Questo tipo di risposta è costantemente accompagnata da elevati livelli della carica parassitaria in diversi organi e tessuti. C'è sottolineare , comunque che negli ultimi anni diversi lavori eseguiti per chiarire definitivamente il pattern citochinico caratteristico dei cani resistenti e di quelli suscettibili alla malattia non sono riusciti a definire con certezza il ruolo di tutti i mediatori di volta in volta studiati. In definitiva, è oggi noto che anche la resistenza di un cane alla malattia è mediata da un tipo di risposta immunitaria cellulo-mediata, di tipo Th1, con aumento di linfociti citotossici CD8+ nel sangue periferico. E' anche noto, inoltre, che i cani che evolvono verso fasi di malattia conclamata mostrano all'inizio dell'infezione un tipo di risposta simile a quelli resistenti, che, al contrario di quest'ultimi, subisce un netto decremento nel tempo ed è caratterizzata da diminuzione dei linfociti T CD4+ e CD8+, diminuzione dei monociti CD14+, diminuzione dei linfociti B CD21+, diminuzione dei IFN-γ, diminuzione dell'espressione di molecole di classe II del sistema maggiore di istocompatibilità (MCH II), aumento dei valori di IL-10, riduzione della capacità di killing macrofagico NO indotta e riduzione o scomparsa di positività al test intradermico della leishmanina, inizialmente positivo anche in molti animali che diventano suscettibili. Proprio per questa iniziale capacità di mostrare una risposta immunitaria potenzialmente protettiva, i cani suscettibili vengono oggi proposti come caratterizzati da una risposta di tipo misto (Th1/Th2), a prevalenza (o evoluzione) Th2. Quest'ultimo tipo di risposta, tipicamente caratterizzata da elevati livelli di anticorpi, è il principale fattore chiave per spiegare la patogenesi della malattia nel cane. Diversi studi, infatti, hanno dimostrato una correlazione diretta tra la sintomatologia, i titoli anticorpali e l'aumento della carica parassitaria nei tessuti. Tale correlazione è dimostrata per tutte le classi anticorpali. Negli ultimi anni, alcuni Autori avrebbero anche dimostrato una diversità nel ruolo che alcune sottoclassi anticorpali potrebbero avere nel cane. In particolare, elevati livelli di anticorpi di tipo IgG1 sarebbero identificativi dei cani suscettibili, al contrario di quelli di tipo IgG2, caratteristici dei cani resistenti o protetti con vaccini. Tale dicotomia, tuttavia, non è stata mai dimostrata definitivamente.

Come accennato in precedenza, la patogenesi della leishmaniosi canina è un fenomeno molto complesso e, ad oggi, non ancora definitivamente studiato. La maggior parte delle descrizioni ritrovabili in letteratura derivano da modelli sperimentali diversi dal cane e, in quest'ultimo, sono quasi esclusivamente frutto di esami anatomo-istopatologici eseguiti su tessuti danneggiati. In ultima analisi, non è nota l'esatta sequenza di eventi che conduce al danno organico, con il progredire dell'infezione. Le componenti principali alla base dei danni organici e tissutali sembrano comunque riconducibili a diversi fattori: l'azione diretta del parassita, soprattutto volta all'inattivazione e alla distruzione delle cellule del sistema monocitico-macrofagico, la formazione di infiltrati

granulomatosi in diversi tessuti, la risposta anticorpale persistente indotta dallo stesso parassita già a partire dalle primissime fasi dell'infezione, e crescente nel tempo. Quest'ultima componente si esplicita sia attraverso la formazione di autoanticorpi diretti contro bersagli *self*, sia di immuno-complessi formati da antigeni del parassita, anticorpi e frazioni del complemento che precipitano in diversi organi. In qualunque caso, la reazione infiammatoria locale amplifica costantemente il danno prodotto dalle componenti immuno-mediate e dalla presenza del parassita.

Infezione e Malattia: I soggetti giovani (soggetti di età inferiore a 3 anni) e quelli tra gli 8 e i 10 sono i più sensibili. Mentre la lunghezza del pelo non sembra influenzi la trasmissione dell'infezione, gli animali che vivono all'aperto, che provengono da aree endemiche o che vi hanno trascorso periodi di soggiorno sono maggiormente a rischio. L'anamnesi è quindi fondamentale nell'indirizzare il sospetto clinico. A causa del lungo periodo di incubazione non c'è una stagionalità a riguardo dell'incidenza della malattia. Nel cane la malattia si presenta nella forma generalizzata a decorso cronico, ed il quadro sintomatologico è pleomorfo; dopo il periodo di incubazione (variabile da 1 mese a 4 anni) possono manifestarsi inizialmente sintomi quali lieve depressione e diminuzione dell'attività fisica e, quindi, comparire lesioni cutanee non pruriginose, alopecia progressiva, desquamazione e ulcerazioni. Alcuni cani sviluppano lesioni oculari quali cheratocongiuntiviti, uveiti e accrescimento abnorme delle unghie. Si può registrare riduzione del peso, atrofia muscolare, insufficienza renale con poliuria, polidipsia, depressione del sensorio. La stadiazione clinica è decisiva per decidere se e come trattare un cane affetto da leishmaniosi, avendo sempre chiara distinzione tra lo stato d'infezione e quello di malattia. Un cane infetto, infatti, è per definizione un soggetto nel quale sia dimostrabile la presenza del parassita, con metodi diretti (microscopia, coltura, PCR) o con metodi indiretti (messa in evidenza di anticorpi specifici). La condizione di malattia è quella nella quale siano rilevabili danni organici che si esprimono con segni clinici e/o con alterazioni clinico-patologiche. Nella maggior parte dei casi, i cani infetti potrebbero non avere necessità di trattamento terapeutico, al contrario di quelli malati che devono essere sempre trattati. Un cane infetto da *L. infantum*, inoltre, prima di manifestare segni clinici di malattia, può permanere per numerosi mesi o anni in uno stato d'infezione. Tale stato può essere facilmente diagnosticabile (infezione patente) o essere al limite della rilevabilità, pur utilizzando diverse tecniche diagnostiche quali la dimostrazione di anticorpi anti-*Leishmania*, l'evidenziazione microscopica del parassita e la PCR quali/quantitativa (infezione sub patente). Per rendere più agevole l'inquadramento diagnostico e prognostico dei cani infetti e di quelli malati da *L. infantum* sono state recentemente pubblicate due diverse classificazioni cliniche (www.gruppoleishmania.org); www.Leishvet.com) che distinguono stadi differenti di infezione e malattia, con conseguenti diverse prospettive prognostiche e terapeutiche.

N.B. La bibliografia ed eventuali richieste di approfondimento e collaborazione possono essere inviate all'Autore: gaetano.oliva@unina.it

LESIONI MUSCOLARI IN CORSO DI LEISHMANIOSI, DALLA PATOGENESI ALLA LESIONE

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Le miopatie infiammatorie (MI) sono un gruppo eterogeneo di disordini muscolari diversi per eziopatogenesi e manifestazioni cliniche. Nell'uomo, le MI di maggiore riscontro sono immunomediate e sono caratterizzate da infiltrazione di cellule mononucleate citotossiche con distribuzione endomisiale, perimisiale, talvolta perivascolare ed invasione delle fibre non necrotiche.

Le miopatie infiammatorie (MI) immunomediate più comuni nei cani sono la miosite dei muscoli masticatori (MMM); la polimiosite (PM), con quadri morfologici sovrapponibili alla PM dell' uomo; e la miosite extraoculare. La Dermatomiosite (DM) si osserva in alcune razze ed è caratterizzata dal concomitante interessamento della cute e del muscolo con lesioni che si estrinsecano prevalentemente a carico dei vasi sanguigni così come avviene nell'uomo. Nell'uomo sono descritte altre forme di MI, non ancora caratterizzate nel cane, quali la miosite a corpi inclusi (IBM), la miosite focale, la miofascite macrofagica (MMF) e la miosite con prevalenza di macrofagi.

Nei cani, miopatie infiammatorie possono essere associate, come nell'uomo, a malattie infettive sostenute da Toxoplasma gondii, Neospora caninum, Ehrlichia canis o Hepatozoon canis. Inoltre, recentemente è stata caratterizzata una forma di miosite associata ad infezione da Leishmania(1).

Numerose evidenze scientifiche suggeriscono che le miopatie infiammatorie idiopatiche (MII) possono essere il risultato di alcune esposizioni ambientali in individui geneticamente predisposti; e che esse possono essere presenti in molte malattie ad eziologia parassitaria, batterica e virale come conseguenza di una risposta immunitaria abnorme.

La leshimaniosi viscerale è una zoonosi causata dal protozoo Leishmania infantum (syn: L. chagasi), essa è trasmessa da un ospite vettore, un flebotomo, è ampiamente diffusa nel bacino del Mediterraneo, in Asia e in America Latina. Nella maggior parte dei casi, il cane domestico è l'ospite principale e serbatoio del parassita. I cani possono soffrire di una grave malattia sia cutanea che viscerale caratterizzata da evoluzione cronica nel 50% degli animali infetti (2).

Recentemente abbiamo dimostrato che nei cani affetti da leishmaniosi si può osservare una vera e propria miopatia infiammatoria e la carica parassitaria può fungere da fattore scatenante la risposta infiammatoria (1).

I quadri morfologici della miopatia infiammatoria in corso di leishmaniosi del cane comprendono la necrosi fibrale, infiltrazione di cellule infiammatorie mononucleate quali

linfociti e macrofagi nell'endomisio e fibrosi endomisiale. Amastigoti di Leishmania sono stati dimostrati all'interno dei macrofagi, ma non nelle fibre muscolari (1, 2).

Lo studio fenotipico dell'infiltrato linfocitario ha mostrato linfociti CD3+ , CD4+ (T helper) e linfociti CD8 + (T citotossici) positivi nell'endomisio. Rispetto ai campioni controllo, le fibre muscolari esprimevano il complesso maggiore di istocompatibilità - MHC - di classe I e II. Inoltre linfociti CD8+ erano visibili anche in fibre muscolari non necrotiche, ma esprimenti, il complesso maggiore di istocompatibilità - MHC - di classe I (complesso CD8 /MHC-I), sia sul sarcolemma che nel sarcoplasma, aspetti caratteristici della polimiosite immunomediata del cane (1).

Diversi meccanismi possono essere proposti per spiegare il ruolo di fattori infettivi come innesco per le malattie autoimmune. La prima ipotesi comporta l'attivazione policlonale dei linfociti. Infatti diversi microorganismi sono capaci di determinare l'attivazione policlonale delle cellule B. Così come alcuni prodotti batterici possono legarsi ed attivare le cellule T CD4+ in modo indipendente dall'antigene. Queste molecole rappresentano i superantigeni che producono l'attivazione policlonale di tutte le cellule T. Alcune delle cellule T attivate saranno reattive nei confronti di auto-antigeni. L'autoimmunità sarebbe quindi causata dal "risveglio" di tali cellule (1).

Il secondo meccanismo ipotizzato è quello del "mimetismo antigenico", infatti alcuni agenti infettivi esprimono epitopi comuni ad antigeni self. Pertanto una risposta immune contro tali microorganismi può produrre reazioni dirette anche contro antigeni self (1).

La terza ipotesi è la rottura dell'anergia delle cellule T. In questo contesto, dopo che agenti infettivi hanno determinato una reazione infiammatoria in un determinato organo, alcune cellule T potenzialmente autoreattive, sfuggite alla delezione clonale, possono essere rese anergiche dall'incontro con antigeni self, espressi da cellule presentanti l'antigene nei tessuti (1). Nelle miositi da leishmania del cane si sta investigando su quale di queste ipotesi si basa l'eziopatogenesi dell'infiammazione.

Pertanto, i nostri studi hanno dimostrato che: 1) Leishmania dovrebbe essere considerata come una possibile causa di miopatia infiammatoria nel cane; 2) Leishmania non è presente all'interno di fibre muscolari; 3) amastigoti di Leishmania nel muscolo possono agire come fattore scatenante che evoca una risposta infiammatoria; ed infine che 4) il danno muscolare potrebbe essere correlato alla anomala espressione del Complesso Maggiore di Istocompatibilità sulle fibre muscolari che accende una risposta immunitaria mediata da linfociti T citotossici (CD8+).

Suggeriamo quindi che l'infezione da Leishmania spp. deve essere considerata nella diagnosi differenziale delle miopatie infiammatorie nei cani, e che simili meccanismi possano verificarsi anche nell'uomo e pertanto che Leishmania spp. dovrebbe anche essere considerata come una possibile causa di miosite nell'uomo. Studi comparativi sarebbero importanti per la definizione della patogenesi della malattia e per identificare nuove opzioni terapeutiche, tra cui farmaci anti-infettivi, anticorpi monoclonali e la vaccinazione.

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LESIONI RENALI IN CORSO DI LEISHMANIOSI, DALLA PATOGENESI ALLA LESIONE

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Nel cane le glomerulonefriti rappresentano condizioni patologiche in grado di causare sintomi di insufficienza renale cronica che possono evolvere in un processo irreversibile con perdita di nefroni e fibrosi. Alla base delle glomerulonefriti, in letteratura veterinaria, si riconoscono diverse eziopatogenesi causando così una malattia estremamente eterogenea. Tra le diagnosi differenziali maggiormente rappresentate si distinguono: 1) infiammazioni, 2) disordini immunitari, 3) neoplasie, 4) infezioni, 5) idiopatiche e 6) parassitarie.

In questo contesto, gli animali affetti da infezioni di Leishmania mostrano spesso gravi forme nefrosiche e/o nefritiche associate a lesioni glomerulari comunemente definite glomerulonefriti. Per definizione la glomerulonefrite è un processo infiammatorio causato dalla presenza di immunocomplessi (antigene-anticorpo) che interessa primariamente il glomerulo e secondariamente il comparto tubulo-interstiziale e vascolare. Gli immunocomplessi sono in grado di depositarsi a seconda delle loro caratteristiche fisiche e chimiche a livello mesangiale e di membrana basale glomerulare. Per tale ragione questo tipo di immunocomplessi vengono definiti circolanti. Esiste la possibilità che gli anticorpi siano in grado di cross-reagire contro antigeni cellulari glomerulari causando lesioni mediate da meccanismi di ipersensibilità di tipo II (citotossici) o di natura autoimmunitaria. Gli immunocomplessi mostrano un ruolo fondamentale nel meccanismo patogenetico del danno glomerulare, in quanto in grado di fissare il complemento ed innescando successivamente meccanismi infiammatori locali che determinano un'alterazione della permeabilità della barriera glomerulare e perdita di selettività del filtro. Questo meccanismo causa, nel tempo, passaggio nell'ultrafiltrato di proteine normalmente non filtrate con peso molecolare maggiore dell'albumina.

Gli immunocomplessi possono essere visualizzati attraverso esame di immunofluorescenza e tramite microscopia elettronica. Nel caso di infezioni da Leishmania, nel cane si riconoscono sulla base delle tecniche diagnostiche 3 differenti tipi di glomerulonefriti: 1) glomerulonefrite membrano-proliferativa, 2) glomerulonefrite mesangio-proliferativa e 3) glomerulonefrite membranosa.

1) **Glomerulonefrite Membrano-proliferativa**: all'esame istologico i glomeruli sono caratterizzati da alterazioni a carico del comparto glomerulare in cui si osservano globalmente ipercellularità mesangiale e incremento della matrice mesangiale. Le colorazioni PAS e PASM permettono di identificare a livello delle membrane basali glomerulari aumenti di spessore per la presenza di depositi proteici e per l'interposizione di cellule mesangiali. A carico del comparto tubulo-interstiziale si osserva con infiltrato infiammatorio e fibrosi, frequentemente materiale amorfo intra-tubulare, riferibile a proteinuria con alterazioni tubulari di vario grado fino all'atrofia. L'esame ultrastrutturale evidenzia depositi sub-endoteliali associati ad interposizione di cellule mesangiali a livello di membrana. I depositi possono essere evidenziati anche in sede mesangiale, paramesangiale e intramembrana.
2) **Glomerulonefrite Mesangio-proliferativa**: all'esame istologico, la glomerulonefrite mesangioproliferativa è caratterizzata da glomeruli aumentati di volume in seguito ad espansione dello spazio urinario e da moderato a grave incremento della matrice mesangiale. Tra le lesioni focali si riscontrano moderata ipercellularità diffusa associata a cellule infiammatorie, ispessimento della capsula di Bowman, ialinosi e sclerosi. Più raramente si può osservare, associata alla proliferazione del mesangio, la presenza di aderenze flocculo-capsulari e interposizione mesangiale. A livello tubulo-interstiziale si evidenzia fibrosi interstiziale della corticale e midollare, ispessimento della membrana basale tubulare associata ad atrofia e dilatazione tubulare. La microscopia elettronica mette in evidenza depositi mesangiali diffusi distribuiti irregolarmente. Si riscontrano depositi in sede paramesangiale, perimesangiale e sottoendoteliale.
3) **Glomerulonefrite Membranosa**: in corso di glomerulonefrite membranosa i glomeruli presentano moderato incremento della cellularità per iperplasia delle cellule mesangiali. L'alterazione patognomonica è rappresentata dal diffuso ispessimento della membrana basale glomerulare per la presenza di depositi di immunocomplessi. Attraverso le colorazioni AFOG e Tricromica di Masson è possibile evidenziare a elevato ingrandimento depositi proteici di membrana basale, come piccoli elementi di colore rosso. Le stesse caratteristiche istopatologiche sono evidenziate tramite colorazione PAMS come estensioni della membrana basale glomerulare di colore nerastro, chiamati spike, e immunodepositi di colore lucente. La microscopia elettronica mostra depositi elettrondensi localizzati nella membrana basale glomerulare: 1) sotto-epiteliale 2) intra-membrana. E' possibile evidenziare fenomeni di rimaneggiamento della membrana basale glomerulare e ulteriormente sono presenti fenomeni di riassorbimento degli stessi depositi.

LESIONI OCULARI IN CORSO DI LEISHMANIOSI, DALLA PATOGENESI ALLA LESIONE

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Leishmania spp. infection has been recognized as the causative agent of several ocular and ocular adnexal lesions. Two large comprehensive studies on canine ocular leishmaniosis reported that ocular lesions were detectable, clinically or histologically, in about 25-26% of cases of canine leishmaniosis (Peña *et al.*, 2000; Peña *et al*, 2008). Most commonly affected sites, in order of frequency, were palpebral or limbal conjunctiva, then anterior uvea (ciliary bodies and iris), cornea, sclera and choroid. Specifically, choroid was never found to be affected alone, but leishmanial choroiditis was present always and only in cases of diffuse intraocular inflammation (panuveitis, endophthalmitis, panophthalmitis). Moreover, Leishmania has been reported to cause lacrimal gland adenitis, possibly with secondary keratoconjunctivitis sicca, and orbital myositis.

In all these different intra and extra ocular sites; Leishmania has been associated to severe granulomatous or pyogranulomatous, plasmacell rich infiltration, with a variable number of Leishmania amastigotes within the cytoplasm of infiltrating macrophages. It must be outlined that some authors reported cases of intraocular leishmaniosis in which the parasitic load was so low to require immunohistochemical investigation to pose a definite diagnosis and to locate the parasite. However, cases of ocular leishmaniosis that are submitted for routine diagnostic are most commonly represented by severe diffuse granulomatous or pyogranulomatous inflammation of all eye structures (panophthalmitis) with severe effacement of normal architecture and abundant parasitic load with innumerable intracytoplasmic and free amastigotes detectable.

If single case reports and detailed large studies on ocular leishmaniosis are present in the current veterinary literature, very few studies, if any, on the pathogenesis of ocular lesions sustained by Leishmania spp. are currently available. Specifically, how Leishmania infects eye structures is not completely clear. It is obvious that conjunctival mucosa, cornea and sclera can be directly exposed to the infection, and from these sites Leishmania can spread within the eye. But how does amastigotes reach the endo-ocular compartment when surface structures (conjunctiva/cornea) are not affected? Such cases have been reported in the mentioned studies, although the pathogenesis has not been discussed.

It worth to be briefly remembered here that the intraocular compartment is protected by hemato-ocular barrier and a sophisticated system of immune deviation, known as Anterior Chamber Immune Deviation (ACAID),in order to prevent intraocular inflammation. A minimal intraocular inflammation can in fact have detrimental effects on eye function.

Therefore, how do Leishmania amastigotes elude ocular hematic barrier? Can amastigotes gain access to the uvea through the intact barrier? Or perhaps is the barrier already compromised when amastigotes reach the uvea? And, in the latter case, is the barrier damage directly related to systemic Leishmania infection?

Moreover, why the choroid, that is as rich in vessels as the anterior uvea, is spared by direct (hematogenous?) infection and involved only secondary to severe panophthalmitis, apparently by direct spread from other ocular structures?

At present we have no answers for all these questions and most likely these answer could not come from the analysis of routine diagnostic specimens but will require large comprehensive pathogenetic studies.

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EMATOBIOCHIMICO: MODIFICAZIONI E MARKERS DI RECENTE INTERESSE

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La peculiare patogenesi e immunopatogenesi della leishmaniosi determina non solo lesioni tissutali ma anche alterazioni d'organo o metaboliche responsabili di alterazioni rilevabili dai routinari esami di sangue e urine. In particolare, dopo infezione leishmania è in grado di inibire le risposte fagocitarie dei macrofagi e, nel tempo, induce uno spostamento delle risposte adattative da un quadro di prevalente attivazione dell'immunità cellulo-mediata (Th1) a una prevalenza della risposta umorale (Th2). Al calo delle difese cellulo mediate corrisponde la progressiva invasione delle leishmanie di diversi tessuti, incluso il midollo osseo che vede così alterate le proprie capacità emopoietiche per cui si sviluppa anemia, solitamente normocitica normocromica non rigenerativa. All'aumento di attività dell'immunità umorale si associa l'immissione in circolo di alte quantità di anticorpi, responsabili da un lato della gammopatia policlonale che caratterizza il protidogramma dei cani leishmaniotici, dall'altro della malattia da immunocomplessi che determina, tra le altre, le lesioni renali responsabili dei segni di nefropatia proteinurica rilevabili nel sangue sotto forma di aumenti di creatinina, urea, e fosfati e nelle urine sotto forma di aumento del rapporto proteine:creatinina urinaria.

Le alterazioni ematologiche, biochimiche e urinarie sopra citate (anemia non rigenerativa, azotemia renale e proteinuria) sono quindi considerate alterazioni fortemente compatibili con leishmaniosi e devono indurre a perfezionare la diagnosi attraverso tecniche diagnostiche dirette (es: visualizzazione del parassita, PCR) o indirette (sierologia). Recentemente sono stati proposti altri test basati su biomarker che possono fungere non tanto da indicatori di leishmaniosi visto che, come sopra accennato la diagnosi di infezione o di malattia si deve basare sui test eziologici diretti o sui marker tradizionali sopra citati, quanto da indicatori prognostici in grado di determinare la gravità delle lesioni presenti o di fornire indicazioni circa l'evoluzione della malattia, soprattutto dopo trattamento. I marker innovativi proposti a questo scopo sono finalizzati a identificare tre aspetti conseguenti all'interazione tra ospite e parassita.

1) La sede delle lesioni renali

Come in tutti i casi di ridotta funzionalità renale l'identificazione precoce della patologia è di estrema importanza per poter programmare interventi terapeutici appropriati ed allungare l'aspettativa di vita. In nefrologia veterinaria sono stati recentemente proposti

nuove marker plasmatici che possono identificare il danno renale più precocemente di quanto non lo facciano urea e creatinina. Tra questi rientrano ad esempio la valutazione diretta della filtrazione glomerulare (es: con iohexolo), il dosaggio della cistatina C o della dimetilarginina o la valutazione della concentrazione di neutrophil gelatinase associapted lipocalin urinaria. E' quindi possibile che in futuro questi marker vengano proposti anche per la gestione del paziente leishmaniotico. In corso di leishmaniosi, però, può essere anche importante stabilire la localizzazione del danno renale: la nefropatia da leishmania infatti inizia con una lesione glomerulare da immunocomplessi che induce passaggio di proteine nell'ultrafiltrato, proteine che verranno riassorbite dalle cellule tubulari. Questo può indurre a lesioni tubulari che segnano il passaggio da una forma primariamente glomerulare, che può essere tenuta sotto controllo medico o farmacologico, a una forma mista glomerulare e tubulo-interstiziale, segno di una prognosi peggiore. L'identificazione dell'una o dell'altra forma dovrebbe basarsi sull'analisi istologica di biopsie renali, che però non vengono ampiamente utilizzate nella pratica clinica. Sono stati quindi proposti marker urinari in grado di identificare il danno tubulare: tra questi l'elettroforesi con sodio dodecilsolfato (SDS) delle proteine urinarie: il trattamento con SDS linearizza e carica negativamente le proteine che quindi migrano in elettroforesi solo in funzione del loro peso molecolare: questo permette di distinguere le proteine di origine glomerulare (più grandi) da quelle di origine tubulare (più piccole). Analogamente, l'identificazione precoce del danno tubulare può essere ottenuta utilizzando enzimi o altre proteine associate alla membrana delle cellule del tubulo, che in caso di danno alla cellula tubulare vengono rilasciati nelle urine dove sono misurabili con tecniche più o meno sofisticate. Esempi di marker urinari che sono stati proposti sono la gamma-glutamil transferasi (GG) rapida ed economica da eseguire, che in cani con leishmaniosi ha già mostrato la sua potenziale utilità nell'identificazione di forme identificate come tubulari dall'SDS, la retinol binding protein (RBP) e la kidney injury molecule 1 (Kim-1), il cui utilizzo è in parte limitato dal costo delle metodiche ma che potrebbero dare informazioni precoci sull'insorgenza di complicazioni tubulari in cani con leishmaniosi

2) L'entità delle risposte infiammatorie/ossidative

Una volta che si attiva la fase sintomatica dell'infezione, può essere opportuno identificare la gravità delle alterazioni infiammatorie e monitorarne l'evoluzione nel tempo (eventualmente dopo trattamento). Le lesioni infiammatorie sono facilmente identificabili quando esterne (es: dermatopatie, oculopatie) e altrettanto facilmente si può desumere la presenza di lesioni sistemiche come la glomerulonefrite o altre lesioni da immunocomplessi. LA determinazione di marker infiammatori non ha quindi uno scopo diagnostico (identificare l'infiammazione), quanto uno scopo prognostico, teso a rilevare l'entità della risposta infiammatoria sistemica, che può correlare negativamente con la prognosi, e la scomparsa dello stimolo infiammatorio dopo terapia. Quest'ultimo aspetto è particolarmente importante in quanto alcune delle lesioni indotte da Leishmania (ad es. la stessa glomerulonefrite da immunocomplessi) sono persistenti e permangono anche

dopo trattamento efficace. E' quindi possibile che segni clinici e alterazioni di laboratorio riferibili a queste lesioni siano rilevabili anche dopo terapia, anche nel caso in cui, però, il paziente si sia liberato dallo stimolo infiammatorio. Viceversa, i trattamenti di supporto e sintomatici potrebbero mostrare transitori miglioramenti anche in cani in cui di fatto la risposta infiammatoria persiste in caso di trattamenti inefficaci.

Tra i marker più utili a questo scopo vengono utilizzate le proteine di fase acuta e in particolare la proteina C reattiva (CRP) e la siero amiloide A (SAA) che in corso di leishmaniosi aumentano modicamente nei cani sieropositivi non sintomatici e molto intensamente nei cani sintomatici. Dopo trattamento la CRP mostra sensibili diminuzioni già nella prima settimana e rientra nella norma nel giro di 3-4 settimane, più precocemente di quanto si normalizzino i titoli anticorpali (6 mesi) o i tracciati elettroforetici (45-60 giorni).

Dato che in tutti i processi infiammatori si verificano fenomeni ossidativi scatenati dai radicali ossidanti rilasciati dalle cellule infiammatorie, è stato proposto di stadiare e monitorare la leishmaniosi analizzando i livelli ematici di marker di ossidazione. Questo aspetto potrebbe essere importante in corso di leishmaniosi in quanto nelle prime fasi dell'infezione leishmania si difende dall'azione dei fagociti inibendone le risposte ossidative. I livelli plasmatici dei marker di ossidazione potrebbero così essere utili per differenziare i cani con infezione inziale/latente (marker ossidativi inferiori al normale) da quelli con malattia in atto (marker ossidativi superiori alla norma). Questo è stato dimostrato in vitro o, in vivo, nell'ambito di diversi protocolli sperimentali, nei quali è stato possibile dimostrare uno stress ossidativo solo in cani con leishmaniosi manifesta e grave, ma in condizioni di campo i metaboliti reattivi dell'ossigeno non si sono dimostrati utili nel differenziare i cani con leishmaniosi sintomatica e non sintomatica né i cani con leishmaniosi rispetto a cani con altri processi infiammatori. Questo insuccesso può dipendere dal fatto che in corso di infiammazione associata a leishmaniosi sono presenti altri radicali ossidanti, non derivati dall'ossigeno, come ad esempio l'ossido nitrico. Per ovviare a questo problema si potrebbero misurare simultaneamente diversi mediatori ossidanti oppure, più semplicemente, valutare la concentrazione plasmatica di sostanze antiossidanti. Indipendentemente dalla natura degli ossidanti infatti, la risposta alla presenza di radicali liberi consiste nella diminuzione delle difese antiossidanti. Oltre alle molecole ad azione antiossidante diretta, recentemente hanno assunto un ruolo importante, come indicatori indiretti della presenza di infiammazione alcuni metaboliti che legano il metabolismo ossidativo a quello lipidico. In corso di ossidazione, infatti, si assiste a una diminuzione della concentrazione plasmatica delle high density lipoproteins (HDL) che, quando ossidate, vengono trasformate in low density lipoproteins (LDL). LA concentrazione plasmatica delle HDL quindi diminuisce. Tra le modificazioni strutturali che portano a questa trasformazione, gioca un ruolo importante la sostituzione di un enzima antiossidante associato alle HDL, la paraoxonasi (PON1) da parte della SAA sopra citata. Di conseguenza anche l'attività della PON1 nel sangue diminuisce. In corso di leishmaniosi è stato dimostrato che sia l'attività della PON1 che la concentrazione di HDL

diminuiscono in particolare nelle forme gravi, quelle in cui i processi ossidativi sono verosimilmente più intensi, fungendo quindi da marker prognostici e indicativi di gravità dei fenomeni infiammatori. Ancora più importante è il fatto che a seguito di trattamento la normalizzazione dei livelli di PON1 e HDL avviene in 7-15 giorni e in 15-20 giorni rispettivamente, e quindi in tempi più brevi dei 20-30 giorni necessari alla normalizzazione della CRP.

3) L'equilibrio tra immunità cellulo-mediata e umorale (Th1 vs Th2)

   Ciò che determina il passaggio dalla leishmaniosi asintomatica a quella sintomatica è una diminuzione dei livelli di efficienza dell'immunità cellulo-mediata. E' stato quindi proposto di monitorare l'andamento dell'infezione valutando i livelli di linfociti CD4 (che sostengono l'immunità cellulo-mediata) e il rapporto CD4/CD8 supponendo che tali livelli sono elevati nelle fasi iniziali di infezione, diminuiscono quando il cane diventa predisposto a sviluppare la sintomatologia clinica, e si rialzano dopo trattamento. Queste misurazioni si sono dimostrate estremamente utili in lavori di ricerca in cui si sono valutati gli andamenti sequenziali delle concentrazioni cellulari durante la malattia o a seguito di diversi trattamenti. L'estrema variabilità individuale delle conte dei CD4, però, rende poco applicabile, nella pratica, l'utilizzo di questo indicatore per valutare la prognosi in condizioni di campo.

MAIN LECTURES

NEW BIOMARKERS MONITORING ANIMAL WELFARE

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Animal husbandry involves monitoring animal health, wellbeing and productivity and then responding in an appropriate way when problems are noticed. It is still largely done by humans using skills that have not changed significantly in many years, but this approach is increasingly difficult to sustain, for two related reasons: in many parts of Europe animal production units are getting larger and/or the cost of skilled labour is increasing. Both of these factors reduce the opportunity for husbandry staff to monitor animals effectively, so that husbandry becomes more difficult. Technology is used in many different ways for monitoring and improving our own, *human,* wellbeing, and the benefits that this provides to society in, for example, improved disease diagnosis, improved security, improved mobility and generally in improved quality of life are well recognized and accepted. The principle of using appropriate technologies within animal production to improve *animal* wellbeing by focusing the valuable time of animal husbandry staff onto those animals that most require attention is undeniable and compelling. Animal wellbeing is at the start of a chain that links to farmer profitability, product quality, consumer satisfaction and environmental sustainability.

In this presentation I shall focus on dairy cows. Major opportunities exist for introducing technology-based health and welfare assessment into dairy farms, but a number of barriers must be overcome:

- The data produced by existing commercial activity monitoring technologies is difficult to evaluate and does not yet provide reliable assessments of wellbeing
- Existing gold-standard biomarkers of health and welfare are plasma based, requiring manual and invasive sample collection
- The power of proteomic and metabolomic analysis has not yet progressed from the laboratory bench to the farm

We can recognize two categories of cues that can be integrated to create a technology-based assessment of an animal's state:

- Biomarkers include physiological variables such as hormone, metabolite, substrate or product concentration in relevant biological samples, ideally obtained non-invasively
- Activity measures include locomotion, feeding behavior, time budgets, social interactions and positional location

Integration of these cues identifies the individual animal's current state of fitness, which is then compared to its history and a predefined "desired fitness". A suboptimal outcome means the animal is "flagged" for the herdsman to take

action. The end stage in this process is an alert to a herdsman or manager that a particular animal requires attention. The response will depend on many circumstances, but will essentially require a modification of the animal's general management, environment or nutrition, and may require a therapeutic intervention from a veterinarian or consultant. The animal's genetic background may also be taken into account. In this scenario these factors are animal-directed, but the knowledge that builds up from identifying animals at-risk will soon start to be useful as a management-directed tool, influencing longer term nutrition, environmental design and breeding programmes.

In summary, the problem is to maintain the best possible standards of animal health and welfare together with high productivity and a minimal environmental impact in an era of increased demand for high-quality livestock products, larger livestock units and decreased contact between animals and husbandry staff. The solution has been described as "Precision Livestock Farming". I am not convinced that precision is needed, but I am convinced that the ability to understand the needs of the animal and to know whether those needs are being met is at the heart of the solution. To implement a technological solution, one must first define the animal's needs, create a set of technologies that can monitor whether those needs are being met, analyze the outputs from those technologies and identify criteria that define deviations and respond appropriately to restore the optimum. In some cases the response might be an automatic one initiated by the system, in others it might be an alert to the husbandry staff. The next step is to identify the specific biomarkers that will allow us to do all of this. In this presentation I shall review what is already known and what may well become known in the next few years. I shall also introduce our dairy animal health and welfare COST Action, DairyCare (www.dairycareaction.org). This Action will focus the talents, skills and resources of researchers, industry partners and stakeholders in the dairy-foods chain onto the topic of dairy animal health and welfare. Relevant research is happening across different disciplines (biological, ethological, technological and socioeconomic) and in many different and diverse parts of Europe. Dairycare will obtain maximum value from this research by networking, coordination and avoiding duplication. It will disseminate the many outputs widely to researchers, industry, dairy farmer end-users, consumers, policy makers and society generally. It will ensure that European dairy research remains world-leading, that European dairy animals are the best cared-for in the world, and that European consumers can take pride in European dairy industries that are competitive, responsible and sustainable.

OSTEOCHONDROSIS: A SERIOUS PROBLEM IN LIVESTOCK BREED CATTLE BULLS IN ANDALUSIA

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Origin of the fighting bull

When speaking about the bull, we cannot ignore its origin, evolution, importance and significance throughout history since ancient civilizations.

Several investigations have studied the origin of this animal, that is unique in the world, as Ortego mentions in his book "The Bull of torches". To understand the origin of the bull in ancient times, we must go back to the Pliocene (the last part of the Tertiary period) in India. The bull and all breeds of cattle arise from primitive Aurochs (*Bos primigenius*) living in North Africa, Europe and Asia 500,000 years ago, followed by *Bos taurus primigenius*, which appeared between 9000 and 4000 BC through better adaptation and subsequent domestication.

In Spain, the bulls lived in a semi-wild condition until the 17th century. The current bull is the result of genetic selections carried out since the early 18th century by farmers in different Spanish regions by empirical attempts. This breed has a heterozygous origin, as animals were selected based on their ability to fight, in different environments, giving origin to different animal morphologies. Therefore, within the breed of bulls, animals may have different morphologies, coats and shapes of horns.

In the breed of fighting bulls, sexual dimorphism is very pronounced: males have rounded shapes and increased muscle development, while females have a more environmental conformation, and more angular shapes.

Fighting bulls have no single racial prototype for all breeds, the only common denominator of all animals copies being the aggressiveness.

The bravery of bulls has been considered as a defensive instinct, triggered by rage at the moment of being bothered, or fear or cowardice of the unknown, or as a mysterious and natural violence when movement or excitement is perceived.

History of the bullfights in Spain

The first reports about bullfights are documented in Cuellar (Segovia) in 1215. The run was divided into three parts called “thirds”, marked by a clarion call.

In the first part the matador fights with the cape. A clarion call indicates that the two picadors leave the ring, standing each on one end of the square, only one runnning this "lucky".

The second part is the "luck of banderillas", where the "subaltern", "flaggers" or "bullfighters of silver" plant three pairs of banderillas on the bull.

The last third of the bullfight is the "ultimate fate", in which the fight is conducted with the crutch instead of the cape up to the end, killing the bull with a sword.

These are the most difficult moments of the bullfight, as the bull shall let run to the matador which, right in the middle of the attack, shall decide the moment to stick his sword or rapier in the heart of the animal.

If all these parts are developed with order, proficiency, elegance and aggressiveness, the transmission to the public of what is happening in the ring is called “art of bullfighting”.

However, sometimes the behavior of the bull is not corerct, because the animal shows symptoms of weakness and lack of strength, falling on the hind legs, or falling and rising quickly, or taking seconds to get up, a problem that spoils the show's run. This is what is called the “fall" of the bull in the bullring.

The fall of the bull

The term "fall" of the bull has always caused concern among bullfighting fans, stimumating studies and research since nearly a century.

According to Purroy (1992) the “fall” is described as "the fact that during the fight, the animal double limbs and/or contact with the ground with any part of his anatomy"; while the strength would be the expression of the animals, demonstrating their strength or physical form.

Regardless of rank and/or matador’s name, the “fall” represents a disturbance of the show: plasticity and beauty of bullfighting cape and crutch disappear when the bull continuously weakens on its forelegs and therefore "falls", being unable to complete passes and falling halfway.

These animals are usually more troublesome and dangerous than those who do not fall; they defeat more frequently, have torn stockings, are "uncertain", and stay in the center of the lot, that is, they defend more of those who have difficulties in movement and aggressiveness.

The defect affects both males and females of all ages: “toros” (4 years old), “utreros” (3 years old), “erales” (2 years old), “añojos” (1 year old), cows, etc. It can be detected in individuals of different farms, regardless of their weight, the category square where fighting, and distance of it from their place of origin, as reported by Castejón (1985) and Domecq (1985). Moreover in cattle breeds there are animals which have this syndrome and others which do not have, as described by Orensanz (1950).

Several hypotheses or theories in order to explain the origin of the “fall” have been advanced, attributing the problem to physical reasons, including trauma, transport, fraud, etc., up to a genetic origin. Marmol del Puerto (1967) believes that the “fall” is a problem caused by multiple factors differentiated between "predisposing", "determinant" or "trigger" causes.

Possible predisposing factors are: physical, fraudulent schemes, contribution or lack of food, parasitic diseases, excessive precocity, drugs, doping, pesticides, disease processes, rheumatism of joints, circulatory disturbances, nervous disorders, and metabolic, endocrine, genetic and behavioral alterations.

Osteochondrosis

Osteochondrosis (OC) is a disorder of the cartilage growth in humans and domestic animals. In animals, the disease has been reported in pigs, dogs, horses, Brahaman cattle, cats and rats. It is considered as the most important cause of weakness in hind limbs of pigs, and a common cause of lameness in horses and dogs.

Heritability is the most important factor in its etiology. Hemodynamic disorders are also involved in the pathogenesis of osteochondrosis: the cartilage function is altered directly by blood vessel lesions, altering the biomechanical properties of the surrounding structures. Necrosis of the vessels of the cartilage appears to be the origin of the alteration in a narrow age range during growth, causing a failure of endochondral ossification and focal necrosis.

Investigations on the “fall” of fighting bulls

The origin of the “fall” has caused several hypotheses and/or theories, from those attributed to physical causes up to a genetic origin. These studies have been the basis of our research, aimed to correlate the lesions of osteochondrosis in the “fall”, and the hypothesis that this syndrome is closely related to the heritability.

Farmers are known to have tested several solutions to eliminate the problem of the “fall” of the bulls in the bullring, but the bulls still continue to fall intermittently.

Having attended numerous bullfights in which we proved that the bulls will fall in the bullring during the fight and in the slaughterhouse, and had injuries to the extremities, we undertook a study on the “fall”.

Following my training as a pathologist, I went to the rendering plants or slaughterhouses in the bullring, to investigate which kind of injury can be found in the extremities, causing difficulty of bulls to walk and run for the fight, and sometimes fall to the ground.

At the slaughterhouses we found several lesions of the joints of the limbs. These lesions were observed in the carpal joint: intermediate radial (Cr) (Ci), ulnar (Cc), accessory (Ca), and the distal row: proximal row (C2 -C3 and C4) and pastern (M3-4).

Observing these lesions, we have set the following objectives:

1. Determine in which part of the the fight the "fall" is manifested, and what are the consequences.

2. Investigate the behavior of the bull in the bullring and the characteristic lesions of osteochondrosis (OC) found in the slaughterhouse.

3. Check whether there are differences in the presentation of the “fall” and the characteristic lesions of osteochondrosis, analyzing macroscopically, histologically and by scanning electron microscopy the distal carpal bones (ossa carpi - C2-3), and articular surface proximal metacarpal bone (ossa metarcarpalia - M3-4), depending on age and weight.

Our research was carried out on a total of 450 male cattle bulls (12 yearlings, 42 not minced steers, chopped y306 90 steers bulls). Age was expressed in months, from the date of birth to the time of fighting and death. The weight was calculated in kilograms. All the 42 farms that we investigated belonged to the same caste foundational, and 32 of them (76%) corresponded to the same strain.

Evaluation during the fight was recorded from the arrival of the animal in the arena up to its performance with “Capote” in the “Tercio de varas”, “Banderillas” and “Muleta”.

The following manifestations of the "fall" were evaluated: step shortening and/or trot, weakness of the forelimbs, support of one or both joints for less than 10 seconds, and support of one or both joints for more than 10 seconds.

The fetlock joints were collected, including the articular surfaces of the distal carpal bones (ossa carpi - C2-3), and proximal articular surface of the metacarpal bone (ossa metarcarpalia - M3-4). The diameter was measured, and the depth of the lesions was classified according to the following criteria: "normal" (when the joints showed no alterations), "moderate" (when the lesion involved less than 20% of the articular surface) and "severe" (when the injury was greater than 20% of the articular surface).

The samples were collected in individual containers, labelled with the date and place where the show was carried out, farm origin, age and weight of the animal; the samples were fixed in 10% formalin for 24 hours, subsequently included for histological decalcifying, and processed with a tissue processor (Leica® TP-1020). The samples were included with a paraffin dispenser (Shandon® Histocentre-2) and cut from 3 to 5 micrometers with a microtome (Microm HM-352®). Sections were stained with Hematoxylin and Eosin, Fraser Lendrum (for detection of fibrin), and Masson trichrome (for collagen fibers differentiation).

The histological classification included the following criteria: "normal" (no alteration of joint tissues), "erosion" (loss and thinning of the cartilage surface), and "ulcerated" (with loss of articular cartilage and subchondral bone exposure).

Additional analyzes were made by scanning electron microscopy, classifying the cartilage samples as "normal", "moderate lesions" and "severe lesions".

Results

Gross lesions: The articular surfaces of the carpal (C2-3) and metacarpal bones (M3-4) showed bilateral lesions. In the group of yearlings, 5 (41.7%) showed no lesions, and 7 (58.3%) showed moderate alterations. In the group of calves, 13 (30.9%) showed no lesions, and 29 (29.0%) showed moderate alterations with thickening of the edges of the cartilage. In the group of steers, 18 (20%) showed clear lesions, 36 (70%) moderate alterations, and 9 (10%) severe lesions with ulceration. In the group of bulls, 32 (10.5%) showed no lesions, 118 (35.6%) moderate alterations, and 165 (51%) serious injury.

Histology: The initial lesions were characterized by slight depressions, loss of continuity and thinning of the articular surface, and in yearlings by mild to moderate injuries. In calves, steers and bulls the affected cartilage showed cellular abnormalities, with chondronecrosis.

Scanning electron microscopy: Normal cartilage was observed in 4 yearlings without morphological changes; in 8 yearlings a roughened surface of the perichondrium with cellular debris was detected. Moderate lesions were observed in calves, steers and bulls, with loss of continuity, degenerated surfaces and ulceration of the articular surface. Serious injuries occurred in steers and bulls with detachment and ulcerations.

Based on the obtained results, we believe that the primary lesion of articular osteochondrosis can be defined as a "focal ischemic necrosis of the growth plate, initiated by the necrosis of the blood vessels in the cartilage canals". We consider the Lidia cattle as animals who suffer from this disease, fully manifested during the exercise in their performance.

Summary and conclusions

1.Osteochondrosis (OC) was the predominant lesion in all tested animals, appearing in all age classes. Osteochondrosis in fighting bulls seemes to have a genetic origin, linked to certain breeds and strains.

2. Based on our study, the primary lesion of articular osteochondrosis can be defined as a "focal ischemic necrosis of the growth plate, initiated by necrosis of the blood vessels in the cartilage canals" in all the animals which have been submitted.

3. The “fall” appears in all age classes, in young animals (yearlings) up to 4 years old bulls. Intense exercise carried out during the fight causes severe pain, which is responsible for the clinical manifestation of the "fall".

4. Of the 12 yearlings, 4 (33.3%) showed weakness in their forelimbs during the third part in the “Muleta”. Macroscopically, 5 of them (41.7%), did not show morphological changes in the joints, while 7 (58.3%) showed moderate lesions.

5. Of the 42 calves aging 2 years, 19 (45.2%) had weakness of their forelimbs during fighting with the “Muleta”. Macroscopically 13 (30.9%) showed no joint lesions, while 29 (29.0%) showed moderate lesions.

6. Of the 90 steers aging 3 years, in 39 (43.3%), the “fall” appeared after the "Kind of sticks", and in 26 (28.9%) with the “Muleta”. Macroscopically 18 animals (20%) had no injuries, 36 of them (70%) showed moderate aletarions, and 9 (10%) severe lesions injuries.

7. Of the 306 bulls, 117 (38.2%) showed the “fall” when leaving the “Suerte de varas", and 97 (31.7%) showed weakness of their forelimbs during fighting with the “Muleta”. Macroscopically 32 (10.5%) showed no lesions, 118 (35.6%) had moderate lesions, and 165 (51%) showed sever osteochondrosis.

8. In additional studies by scanning electron microscopy, normal cartilage was observed in 4 of the 12 yearlings. Moderate lesions were detected in

yearlings, calves, steers and bulls, with detachment and ulceration of the articular surface, irregular edges, and a fibrinous desquamative inflammatory process with hypertrophic chondrocytes. Severe lesions were detected in calves, steers and bulls, with detachment and ulceration of the articular cartilage, showing the resting zone, proliferative and germinal. The concentrations of calcium and phosphorus is not influencing the presentation of osteochondrosis in the animals in this study, and therefore also in the “fall”. Radiologically the degree of the lesion is mild to moderate or severe, with higher radiolucency in severe lesions.

9. As the fighting bull is included as a category presenting osteochondrosis, it may influence the "fall”, hindering the “art of bullfighting” exhibition.

TREATMENT OF CRANIAL CRUCIATE LIGAMENT DISEASE: WHERE ARE WE NOW?

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Cranial cruciate ligament disease (CrCLD) is a leading cause of lameness in the dog. Surgical treatment is advised for most dogs with CrCLD. A wide array of surgical techniques has been described for CrCLD dogs. They can be divided into static-constraint or dynamic-constraint (geometry-altering) methods.

Two of the most commonly performed geometry-altering techniques are tibial plateau leveling osteotomy (TPLO) and tibial tuberosity advancement (TTA). Extra-capsular techniques are another common technique used for stabilizing a CrCLD stifle.

New regenerative medicine approaches are promising, especially if combined with surgical repair. In this lecture an overview of CrCLD and meniscal injury in dogs will be given.

Biomechanical comparison (TPLO/TTA)

Although several biomechanical studies have suggested that tibial osteotomies offer a reliable dynamic stability, more recent in vivo work have demonstrated persistent subluxation in 30% of dogs. This subluxation is likely caused by a combination of factors, such as rotational stability, meniscal tears and meniscectomy and abnormal joint geometry. Recent in vitro mechanical studies conducted at the University of Florida have shown that cranial tibial subluxation and internal tibial rotation associated with CrCL transection causes a caudal shift in femoro-tibial contact, reduced femoro-tibial contact area and associated increased peak contact pressures. When 90° patellar tendon-to-tibial plateau angle was obtained (this required mean advancement of 13.5 ± 1mm), TTA restored normal femoro-tibial contact parameters. Conversely, TPLO failed to restore normal femoro-tibial contact pressure patterns(femoro-tibial contact area remained smaller and peak contact pressures were positioned more caudally on the tibial plateau, when compared with CrCL intact stifles). These findings suggest that TPLO does not reestablish normal joint biomechanics following leveling the tibial plateay to 6°. Progression of osteoarthrosis in dogs treated by TPLO may be partly caused by an abnormal cartilage pressure distribution.

Biomechanical comparison (extra-capsular techniques/tibial osteotomies)

The biomechanical goal of the extracapsular techniques is to neutralize cranial subluxation and internal tibial rotation of the tibia to allow periarticular fibrosis to develop. Although this fibrosis is often seen as a negative effect of chronicity, it should be interpreted as a normal adaptation of the joint to the instability caused by the CrCL insufficiency. The extra-capsular techniques may facilitate this adaptation by "keeping the femoro-tibial joint in close-to-normal alignment" during healing. Unfortunately, failure of the fixation may occur due to excessive activity and loosening of the implant. The major biomechanical limitation of the extra-capsular techniques is the location of their attachment points. The points of anchorage of lateral suture, for example, are not advantageous for controlling cranio-caudal tibial translation, but are better suited for neutralizing internal tibial rotation. Extra-capsular techniques are sensitive to repetitive high loads: increased level of activity will consistently cause loosening of the prosthesis. However, despite increased instability, the functional outcome may be good to excellent, suggesting that other factors such as in vivo kinematics should be considered. Because of the increased risk of failure in very active dogs, extra-capsular techniques are not recommended (personal experience) for active dogs that may stretch or break the prosthesis before adaptation is achieved. In contrast, dogs of different sizes with a sedentary life may be excellent candidates for extra-capsular techniques.

Case Selection (TPLO/TTA/extra-capsular techniques/arthroscopy)

Excessive Tibial Plateau Slope/Hypoplastic tibial tuberosity -

In cases where there is some combination of excessive tibial plateau slope and/or hypoplastic tibial tuberosity, it may not be feasible to accomplish a 90° patellar tendon-tibial plateau angle with currently available instrumentation that is limited to a 12mm wide spacer cage. Failure to adequately advance the tibial tuberosity could risk residual lameness associated with persistent postoperative stifle instability. In addition, dogs with tibial plateau angle in excess of 35° have a conformational deformity of the stifle joint that place it in a relative angle of hyperextension despite the limb itself not being in the extended position. The TTA does address this conformational deformity.

Angular and Torsional Tibial Deformity - While both procedures are somewhat focused upon reducing cranial tibial thrust instability, loss of CrCL constraint function also puts the stifle at risk of rotational instability. Angular and/or torsional tibial deformity, when present, may contribute to this rotational instability. Because TPLO involves an osteotomy that isolates the proximal articular surface (stifle) from the distal surface (hock), it permits 3-dimensional reorientation of the tibial conformation. That is to say, tibial varus/valgus and/or internal/external torsional deformity can be treated via manipulation of the TPL osteotomy whereas it would require a separate osteotomy if required at the time of TTA. In some cases additional rotational stability can be provided by an extracapsular repair technique, such as lateral suture or Tightrope CCL.

The Highly Unstable Knee - Complete CrCL tear in the absence of surrounding periarticular fibrosis often causes extreme stifle instability. This is in stark contrast to the stifle that is inherently stable by surrounding fibrosis and/or significant remaining CrCL integrity. While both TPLO and TTA reduce strain on the CrCL, the advisability of leaving remaining CrCL fibers intact is debated. Because in clinical practice, a 90° patellar tendon-tibial plateau angle is seldom achieved with TTA (despite preoperative planning), residual stifle instability can be seen following TTA-treatment of the highly unstable stifle in the author's (RP) clinical experience. A similar situation can arise following TPLO and with either technique the surgeon must be prepared to supplement with a static-constraint technique when deemed necessary.

The Stable Knee –Chronic CrCL rupture can cause progressive periarticular fibrosis and functionally stabilize the joint. In advanced stages of osteoarthritis and periarticular fibrosis, a surgical stabilization may not be needed. Instead, joint evaluation and meniscal treatment may be indicated more than a TPLO, TTA or extra-capsular technique.

COMUNICAZIONI E POSTERS

Part I

Scienze Biomediche Veterinarie

CHANGES IN SHEEP MORPHOMETRIC PARAMETERS INDICATE THE DECREASE OF THE MOUNTAIN LIVESTOCK SUSTAINABILITY FOLLOWING THE ARIDITY DUE TO CLIMATE CHANGE

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Hofmann's long-term studies demonstrated that Ruminant's digestive apparatus underwent to evolutionary steps and its evolution is still going on in addition to adaptation to environmental modifications (Hofmann, 1976, 1989, 1999), also during a short period (Catorci et al., 2014; Scocco et al., 2011, '12, '13). In Apennine grasslands, the flowering peak period yields the highest quantity of forage with the best nutritional value; in summer, a lack of green forage often characterizes the pastoral systems. So, the forage feed value decrease because it contains a greater percentage of fibers (Crofts and Jefferson, 1994). Sub-Mediterranean climate, characterized by inter-annual variability, is undergoing increase in aridity thus the worsening of semi-extensive grazing activities sustainability is expected. To test this hypothesis, we used 18 sheep nourished with dry hay and cereals during the winter season and with fresh hay during the pasture vegetative cycle; samples from rumen indicative regions (atrium, A; dorsal and ventral sac, DS and VS; dorsocaudal blind sac floor, DBF) were collected for two consecutive years (2007 and 2008). We estimate the rumen Surface Enlargement Factor (SEF and the degree of keratinization of the epithelial lining (EK) . Data on precipitation, soil water deficit, aboveground phytomass, forage chemical composition and animal body state (as BCS evaluation) were collected. SEF varied in the rumen regions in relation to the diets in both years; SEF of VS and DBF showed a trend similar to that of pasture vegetative cycle in 2007, while in 2008 the SEF trend of SD and SV were overlapping to that of pasture phytomass production. The opposite behavior was observed in the EK of both VS and A, which increased when animals were nourished with highly fibrous hay, and decreased when animals grazed on fresh fodder. Rumen VS reveals a modification trend strictly related to pasture vegetative cycle. Drought stress negatively affected forage quantity and quality. The most negatively affected plant communities are those of productive habitats. Also a decrease in the re-growth capacity after clipping was observed in dry year (2007), especially in xeric plant communities. Results indicated that a very detrimental joint effect might acts in sub-Mediterranean pastoral systems undergoing intense drought stress, in that the decrease of aboveground forage production and the increase of its lignification give rise to a combined negative effect. In fact, significant positive correlations were identified for sheep BCS with phytomass and crude protein and for EK with crude fiber and ADF. This means that, though other physiological factors undoubtedly involved in animal loss of welfare under drought event, the modification in rumen features plays a key role in the worsening of the sheep body status and thus their welfare.

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BIOLOGICAL NETWORKS ANALYSIS SHOWS THAT THE PROCESS OF LIPID REMODELING OCCURRING DURING CAPACITATION HAS A SCALE FREE HIERARCHICAL TOPOLOGY AND A SMALL WORD STRUCTURE

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Immediately after ejaculation, mammalian spermatozoa are unable to fertilize the homologous oocyte. They reach the fertilizing ability only after they reside for hours to days, depending on the species, within the female genital tract. Here, they complete the acquisition of fertilizing ability, the capacitation. This process involves virtually all the component of male gametes: the cytosol pH and ionic composition change, the proteins phosphorylation pattern modifies, the cytoskeleton reorganizes, and the plasma membrane (PM) changes its physical/chemical proprieties1. In particular, the cholesterol/phospholipids ratio changes, the microdomains reorganize, and the PM becomes more instable and fluid2. These events, recently, attracted the attention of researchers because of their possible implication in the determinism of "idiopathic infertility".

Based on the importance of lipid remodeling of PM in physiological and pathological conditions, we decided to apply a systems biology strategy to analyze this event. In particular, we realized a computational model of lipid remodeling, by using a biological networks-based computational approach: each molecule involved in that process was represented as a node, each interaction between molecules was represented as a link3.

The database representing the lipid remodeling of spermatozoa has been de novo manually compiled. That of apoptosis has been downloaded from Reactome, version v51 (http://www.reactome.org/), an open-source, curated and peer reviewed pathways database. All the networks have been realized with Cytoscape 3.1.1 (http://www.cytoscape.org/), an open source software for visualizing and integrating complex networks. All the analysis have been carried out with the plug-in Network Analyzer. As a result, we obtained a network that contains a single connected component with 237 nodes and 388 links. The node degree, i.e. the probability distribution of the number of connection per node, followed the exponential law y=118.08x-1.626 (r= 0.788, R2=0.876), while the clustering coefficient, i.e. the measure the network tendency to form clusters, the exponential law y = 0.398x-0.764 (r=0.673, R2=0.487). The node degree was correlated with the centrality of nodes within the network (r=0.855), expressed as beetweenness centrality. i.e. as the number of shortest paths from all vertices to all others that pass through a node.

This indicated that the network has a scale free hierarchical topology and a small word structure. This specific feature has several important biological consequences. As first, it is evident that the most of nodes is scarcely linked, while only a few nodes, the hubs, are highly connected. This implies that the network is robust against random failure4. In addition, the messages will spread within the networks quickly and efficiently, and, finally, it is possible to identify the nodes that show a higher level of control within the networks: [Ca2+]i, ATP, PKA, apoptosis, PKC, Protein Tyrosine Phosphorylation, cAMP, membrane symmetry, oxysterols. In our opinion, these findings could contribute to the knowledge of such important event, which lead the spermatozoa to gain their fertilizing ability.

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PROGESTERONE PREVENTS THE EPITHELIAL-TO-MESENCHYMAL TRANSITION OF AMNIOTIC EPITHELIAL CELLS

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Epithelial-to-Mesenchymal Transition (EMT) is a transdifferentiation process whereby epithelial cells acquire a mesenchymal phenotype. EMT plays a crucial role in the regulation of different events such as embryogenesis, cancer progression and stem cells biology1. This phenomenon involves also Amniotic Epithelial Cells (AEC) during the in vitro expansion thus modifying the biology of this emerging source of multipotent cells. Cultured AEC turn, indeed, spontaneously into stromal-like cells acquiring the mesenchymal markers (Vimentin and α-SMA) and losing the epithelial ones (Cytokeratin and E-cadherin)2. The EMT that occurs during the in vitro expansion is, in turn, accompanied by a dramatic reduction of AEC plasticity and immunomodulatory activities, both essential to justify their use in regenerative medicine3. But physiologically, AEC does not undergo EMT during the embryo/fetus development, when the cells are exposed to a well-defined environment characterized by low oxygen and high levels of steroids (primarily progesterone: P4), a condition that have not been yet adopted into the in vitro protocols.

The present research has been designed to verify whether P4 could prevent the spontaneous EMT in vitro by preserving AEC phenotype and genotype.

Isolated AEC and whole amniotic membrane (AM) were collected at the slaughterhouse, isolated under validated protocol4 and incubated in presence of different concentration of P4 (from 100 μM to 0.01 μM) with or without its inhibitor (RU-486). AM were analysed in terms of morphology and cell viability (calcein and propidium) over 3 days of culture. AEC were evaluated for proliferative rate (doubling-time, DT), epithelial and mesenchymal markers (Cytokeratin 8 and α-SMA) and pluripotency genes expression (Oct-3/4, Sox2 and Nanog) over 4 passages.

AM cultured in the presence of 25 μM P4 maintained unaltered their morphology and membrane integrity up to 3 days, in contrast with CTR that instead showed a significant high incidence of cell death (42.4 ± 3.7 % vs 13.6 ± 4.3 %, respectively). Similarly, the AEC incubated with 25 μM P4 maintained their epithelioid morphology and a higher positivity for Cytokeratin 8 over 4 passages. By contrast, untreated cells (CTR) acquired early a mesenchymal phenotype by increasing the expression of α-SMA. Furthermore, P4 decrease the DT of AEC (83.25 h vs 29 h of CTR). The specificity of P4 in preventing the in vitro EMT has been further confirmed through the treatment with RU-486. Indeed, AEC cultured simultaneously with P4 and RU-486 undergo EMT similar to the CTR ones. Noteworthy, P4 increases the expression of Oct-3/4, Sox2 and Nanog after the first passage. Taken together, these data demonstrate that P4-treated AEC maintain their epithelial phenotype during expansion and simultaneously increase the expression of pluripotency markers. The possibility to preserve in vitro the AEC native properties may be quite useful to improve their use in cell-based regenerative protocols after long-term expansion.

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PHARMACOKINETICS OF INTRAVENOUS TRAMADOL AT TWO DOSE RATES IN AWAKE AND ANAESTHETIZED SHEEP

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Sheep are animals widely used as experimental model for various surgical procedures. The need to find analgesic drugs which can be used in this species during the experimental procedures, sometimes also quite invasive, is thus imperative.

Aim of the study is to assess the pharmacokinetic profile of tramadol (T) and its active metabolite O-desmethyltramadol (M1) after administration of T in awake and anaesthetized sheep.

The study was approved by the animal welfare committee of the University of Padua (CEASA 80/2012). T at the dose of 4 (T4) and 6 (T6) mg/kg was administered by intravenous route in 6 healthy adult Brogna sheep according to a randomized crossover scheme (2x2), and in two groups (6 animals/group) of anaesthetised sheep undergoing spinal surgery receiving T6 and T4 respectively (parallel design). At prefixed time points, plasma samples were collected in order to determine the concentrations of T and M1 by a validated HPLC-FL method (Giorgi et al., 2009). The pharmacokinetic analyses were performed by WinNonlin 5.3.1 according to bi- and non-compartmental model for T and M1, respectively,

Plasma concentration vs time profiles of T and M1 were similar after the two doses in all treated sheep. In the anaesthetised sheep the concentrations of T were always higher than in awake animals. At the first time point after administration of T4 and T6, the concentration of T was 3.39 ± 0.21 and 4.60 ± 0.99 μg/mL in anaesthetized sheep, and 1.29 ± 0.17 μg/mL and 1.56 ± 0.10 μg/mL in the awake animals , respectively.

The Clearance value was significantly smaller in anaesthetized than in awake sheep (2.49 ± 0.28 vs 4.86 ± 1.19 L/h/kg and 3.24 ± 0.39 vs 6.31 ± 0.95 L/h/kg following administration of T4 and T6, respectively) as well as the Vd (0.77 ± 0.15 vs 1.57 ± 1.15 L/kg and 0.73 ± 0.26 vs 2.87 ± 0.12 L/kg after T4 and T6 treatment, respectively). The AUC values in anaesthetized sheep were greater than those obtained in awake subjects (1.62 ± 0.18 vs 0.87 ± 0.24 μg/mL/h for T4 and 1.87 ± 0.21 vs 0.97 ± 0.14 μg/mL/h for T6). Concerning the pharmacokinetic parameters of M1, Cmax in anaesthetized and awake sheep was 0.09 ± 0.01 vs 0.14 ± 0.02 μg/mL following T4, and 0.10 ± 0.10 vs 0.16 ± 0.04 μg/mL after T6. The Tmax of M1 was delayed in sheep undergoing surgery if compared to awake subjects (0.98 ± 0.50 vs 0.37 ± 0.33 h and 0.58 ± 0.71 vs 0.40 ± 0.27 h for T4 and T6, respectively). The AUCM1/T ratios resulted equal to 0.22 and 0.25 in anaesthetized sheep and 0.36 and 0.43 in awake animals after administration of T4 and T6, respectively

The differences in pharmacokinetics parameters of T among anesthetized and awake sheep are probably due to the reduction of the cardiac output and hepatic blood flow that incur in anaesthetized animals. Buhari et al. (2013) reported similar findings in dogs. However, an influence of the anesthetic drugs on the pharmacokinetic of T may not be excluded. The delay in the achievement of the Tmax of M1 in anaesthetized sheep might be due to the same reasons. The AUCM1/T ratios suggest a predominant metabolism of T in awake

sheep. The reason of this difference is obscure but likely due to the presence of other drugs competing for the metabolization in anaesthetized sheep.

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SUCCESSFUL COLONIZATION OF DEMINERALIZED BONE MATRIX BY HORSE MESENCHYMAL STROMAL CELLS AND THEIR DERIVED MEMBRANE VESICLES

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Demineralized bone matrix (DBM) is a natural degradable biomaterial obtained by removing the inorganic mineral component of cortical bone. It is widely documented that DBM retains an array of growth factors, including a full complex of Bone Morphogenic Proteins (BMPs), thereby displaying remarkable osteoinductive properties. For its ability to induce new bone formation accelerating the healing process, DBM is used in orthopedic regenerative medicine to fill bone defects and to treat non-union lesions.

The use of DBM as a vehicle of Mesenchymal Stromal Cells (MSC) or MSC-derived extracellular membrane vesicles (MV) could enhance the spectrum of its therapeutic applications, resulting in the combination of the regenerative properties of both elements.

In this study, a morphological analysis was performed to evaluate the compatibility of DBM and equine MSC, the degree of MSC and MV colonization and the features of their interaction with DBM.

MSC were isolated from horse subcutaneous adipose tissue. For MV recovery, cells at passage 3 were incubated for 72 h in Fetal Bovine Serum-free medium, supplemented with 0.5% bovine serum albumin. The culture supernatant was ultra-centrifuged twice at 100,000 g for 60 min and the pellet was suspended in PBS. DBM, kindly provided by Rizzoli Orthopaedic Institute, was incubated with MSC (C-DBM) and with MV (MV-DBM) for 24h at 37 °C. The experiment was conducted with DBM coated and not with collagen.

For transmission electron microscopy (TEM), samples of control DBM (Ctrl-DBM), C-DBM and MV-DBM were fixed with 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in a graded series of ethanol up to absolute, pre-infiltrated, and embedded in Epoxy resin. Ultrathin sections were mounted on 200-mesh copper grids, stained with uranyl acetate and lead citrate and observed under a Philips EM208. For scanning electron microscopy (SEM), Ctrl-DBM, C-DBM and MV-DBM were fixed with 2.5% glutaraldehyde and dehydrated in ethanol. They were then coated with gold and observed under a Philips XL30 scanning electron microscope.

DBM particles were efficiently but non homogeneously colonized by cells; in the areas of lower density, MSC showed a typical fibroblastoid morphology, appearing mainly elongated or star-shaped and flattened, with a clear roundish thickening in the nuclear region. More often, MSC formed a continuous multi-layered sheet that made impossible the observation of individual cells.

MV adhered to DBM particles individually or in clusters. However, considering the wide surface and the three dimensional environment provided by the scaffold, a very high number of MV is necessary to clearly appreciate their distribution. Collagen coating did not significantly influence cell and MV adhesion. These results suggest that DBM provides a biocompatible environment for both cells and MV that are able to adhere on particle surface. Seeding MSC and their derived vesicles on DBM, therefore, could produce a 3D living composite with significant biological potential.

IDENTIFICATION OF PLATELET-DERIVED GROWTH FACTOR-A IN THE CANINE SKIN: PRELIMINARY RESULTS

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Platelet-derived growth factor A (PDGF-A) belongs to a pleiotropic family of peptide growth factors acting through cell surface tyrosine kinase receptors (1). PDGF-A is also produced by human keratinocytes and expressed by epidermis; it acts as a mitogen involved in cutaneous wound healing. Cells of bulge hair follicle (HF) express PDGF-A during fetal development while, PDGF receptor is expressed in HF dermal papilla (2). PDGF-A is implicated in HF morphogenesis and is supposed to be a positive regulator of hair growth in vivo (3). In this regard, PDGF-A null mice show an impaired formation of dermal papilla while cutaneous injection of PDGF-A induces and maintains HF in the anagen phase.

In this work, the expression of PDGF-A was immunohistochemically investigated in the dog skin.

Normal skin samples were collected from the dorsal region and the cheek of five animals belonging to different breeds. Samples were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Skin sections were incubated with 3% peroxidase-blocking solution to block the endogenous peroxidase activity and with normal goat serum to block non-specific binding. The primary antibody, a mouse monoclonal anti PDGF-A (SCBT), was diluted 1:100 and incubation was performed overnight at room temperature. Successively, sections were incubated with a goat anti-mouse biotin-conjugated antibody. The site of the immunological reaction was detected with the ABC kit (Vector) and visualized with diaminobenzidine (Vector Laboratories).

An immunohistochemical staining was observed in some structures of the skin including epidermis, HFs, sweat glands, and blood vessels. In the epidermis, positive cells were localized in the basal layer. In the HFs, an immunohistochemical staining was observed in the basal layer of the outer root sheath in the isthmus and infundibulum, throughout hair cycle. Soprabulbar and bulbar region of anagen HFs appeared negative. Smooth muscle cells of the arrector pili muscle and blood vessels appeared intensely positive to PDGF-A as well as the myoepithelial cells of the sweat glands. No staining was evidenced in the dermal compartment of the skin.

The identification of PDGF-A suggests an involvement of this molecule in the biology of epidermis and HFs in dog. During the skin and HF development, PDGF-A expression was described in epithelial tissue, closely opposed to the receptor localized in the stromal tissue. In the dog skin, PDGF-A was always observed in the basal layer of the epidermis or the HF outer root sheath, adjacent to the connective compartment. PDGF-A was clearly expressed by smooth muscle cells and myoepithelial cells. Several studies described the expression of this growth factor by smooth muscle cells where it may stimulate cell proliferation in an autocrine or paracrine manner (1). The identification of PDGF-A in the dog skin represents an important step to understand the biological mechanism regulating skin structures, especially HFs, with implications in the clinical field.

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REGENERATIVE THERAPY FOR THE MANAGEMENT OF A LARGE SKIN WOUND IN 3 DOGS

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In companion animals, the healing of large skin defects resulted from traumatic accidents can be challenging to treat since they need a complex management. Surgical tissue reconstruction (i.e. skin graft) and medical treatments are often associated to reach the best possible anatomical and functional recovery of the damaged area avoiding debilitating effects and suffering of the animals.

The present work describes a successful therapeutic approach based on the synergic application of Platelet Rich Plasma (PRP) and autologous Mesenchymal Stem Cells (MSCs) in dogs with a very large skin wound and lack of soft tissue. Although a definitive knowledge about the molecular and cellular mechanisms through which PRP and MCSs works, several clinical and experimental reports confirms their efficacy for the treatments of patients both in veterinary and human medicine

For our 3 clinical cases we used the same therapy. Antibiogram was performed for all the patients. Systemic antibiotic and analgesic therapy was administered. PRP and MSCs was applied either by dripping or spraying the platelet concentrate over the wound surface (every 48-72 hours). The medication of the wound was performed daily, removal of old bandage, irrigation with lactated Ringer's solution, debridement of the necrotic tissue and application of PRP alone or PRP with MSCs. The hydratation of the wound was ensured. No antibiotic or disinfectant were applied locally. A variation of "tie-over dressing/bandage technique" was applied. We measured every day the wound, in the same position.

A granulation tissue was visible after 1-4 days following the first PRP application. Perception of the speed of new tissue growth, as well as of the reduction of the exposed area, was documented with photos, daily measurements and a tabular visualization. The ratio tissue growth/time reduced gradually during the period, with a fast growth during the first third of the period and then a gradual fall during the remnant 2Â° and 3Â° third. The wound never presented signs of infection or necrosis during and following the regenerative therapy.

Although a definitive knowledge about the molecular and cellular mechanisms through which PRP and MCSs exert their effects is still lacking. Large cutaneous lesions, where poor blood supply, tissue necrosis, excessive scarring, inflammation and bacterial contamination are possible complications, would take significative advantages from the application of regenerative therapy based on the synergic action of PRP and edges to the underlying tissue and a rapid tissue growth, starting from wound margin. No sign of excessive scarring, tissue contraction and fragile re-epithelialization, sometimes associated to second intention healing, were observed. These results suggest that MSCs and PRP are indeed an effective therapeutic option to manage soft tissue wounds where a large amount of tissue is destroyed. Regenerative therapy, providing trophic mediators and reprogramming resident immune and tissue progenitor cells, can be considered by surgeon to improve the quality of tissue regeneration and to speed up the wound healing process. Effective collaboration of the owner and a highly collaborative approach between clinical and biomedical staffs are essential to lead to a successful healing of complicated wounds and ensure the return of the patient to an excellent quality of life.

EFFECTS OF PLATELET RICH PLASMA ON CANINE CHONDROCYTE IN VITRO: A PRELIMINAR STUDY

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Platelet-rich plasma (PRP) is an autologous biologic treatment containing growth factors released from platelets and endogenous fibrin scaffold. Over the last decade, it has become very popular in both human and veterinary regenerative medicine(1). The basis for the use of PRP is to stimulate the natural healing cascade and tissue regeneration by a "supraphysiologica" release of platelet-derived factors directly on the site of treatment. Besides its regenerative potential, evidences show an antinflammatory and cytoprotective effects(2). Despite its wide utilization, the use of PRP remain still debated.

Several in vitro and in vivo studies have been performed on clinical and experimental use of PRP for human, bovine, equine and rabbit(3). On the other hand, PRP therapeutic use in dogs is more recent and in vitro studies are lacking.

In this study the effects of different concentrations of PRP and platelet derived growth factors (PDGFs) on canine chondrocytes were evaluated to establish their real efficacy for tissue healing and to suggest the most effective dose for intra-articular use in dog. In addition, a possible cytoprotective effect of PRP on chondrotoxicity induced by lidocaine (LD) was tested.

PRP was obtained from anticoagulated venous blood samples of healthy dogs, previous informed consent of the owner. Samples were centrifuged twice, and platelet pellet was than resuspended in PPP (Platelet poor plasma) at a final concentration of 2000000 PLT/μL. The PDGFs were obtained by incubating for 1 hour at 37°C the platelet pellet in 22 mM CaCl2 (c.f. of platelet was 2000000 PLT/μL). Samples were then centrifuged and the released PDGFs recovered. Primary cultures of canine chondrocytes and treatments. Chondrocytes were obtained from healthy articular cartilage of the femoro-tibial joints by collagenase digestion (2 mg/mL for 6-8 h at 37°C) and cultured in Dulbecco's modified Eagle's medium (DMEM) + 10% Fetal bovine serum (FBS) at 5% CO2 and 37°C in humidified atmosphere. To test the effect of PRP, cells were seeded at a density of 15x103 cells/well in 96-well plates and allowed to adhere for 24 hours at 37°C, and then treated with different concentrations of PRP or PDGFs for 12 and 24 hours. In order to test the effects of PRP on the well kwon chondrotoxicity induced by LD(4), cells, seeded as above described, were exposed to 1% and 2% of drug alone or in combination with 10% PRP for 30', after 24 hours in DMEM, DMEM supplemented with FBS or PRP. Cell viability was determined by a MTT reduction assay.

Results indicated that PRP and PDGFs are able to induce a significant increase in cell proliferation ($P<0.05$). However, PRP resulted more effective than PDGFs ($P<0.05$). In addition, PRP seem to significantly reduce chondrotoxicity LD induced, in particular when cells were pre-treated with PRP. These results offer new insights about the use of PRP in canine cartilage repair and suggest a proper cytoprotective effect against toxicity induced by local anesthetics.

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EQUINE ARTICULAR CHONDROCYTES EXPOSED TO DEXMEDETOMIDINE: AN IN VITRO STUDY

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The imidazole compound dexmedetomidine (dex) is the active D-enantiomer of the racemic mixture medetomidine. Currently, it is the most potent and selective Îś2 agonist. Recently, it has been injected via intra-articular (IA) to manage analgesia after arthroscopic knee surgery in humans(1,2,3) and dogs(4). However, studies investigating its effects on chondrocytes in vitro are lacking, even if the potential chondrotoxicity of IA medications is a cause of concern(5). Moreover, constitutive expression of $\alpha 2$ adrenergic receptors in chondrocytes has been demonstrated(6), suggesting the specific effects of these drugs on cellular pathways.

To assess the effects of dexmedetomidine on chondrocytes to improve the safety of IA administration of $\alpha 2$ agonists considered as a potentially valid analgesic drug due to the detection of adrenergic receptors in affected joints.

Primary cultures of equine chondrocytes were obtained from healthy articular cartilage of the metacarpophalangeal/metatarsophalangeal joints of horses provided by the Veterinary Teaching Hospital of our University euthanized for reasons other than orthopedic diseases and unrelated to the present study. Cells were exposed, for 15, 30 and 60 minutes, to different concentrations of dex. Cell viability was evaluated by the WST-8 assay and neutral red (NR) uptake. Cell-membrane integrity was evaluated by leakage of lactate dehydrogenase and fluorescence microscopy (double staining with Hoechst 33342) and propidium iodide (PI). Apoptosis and necrosis were assessed by flow cytometry using double staining by annexin V-fluorescein isothiocyanate/PI and by evaluation of cell morphology

Dex was chondrotoxic only at very high concentrations (0.175 and 0.25 mg/ml), while low concentrations corresponding to those administered IA in humans and dogs did not affect cell viability. The toxic effects of dex seem to be related to necrosis and late apoptosis. Doses $\leq$0.05 mg/mL of dex could be a valid alternative for IA administration of analgesics.

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EFFECT OF DIFFERENT PHYSICAL FORMS OF THE DIET FED TO GROWING PIGS ON THE EXPRESSION OF CB1 AND CB2 IN THE MANDIBULAR GLAND.

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Diet-induced morphological changes of extra-enteral organs were associated with the physical forms of the diet [1]. The endocannabinoid system consists of a complexity of endogenous molecole, namely endocannabinoids ligands and receptors. There are two types of cannabinoid receptors, named type 1 receptor (CB1) and type 2 receptor (CB2). The investigation about the localization of these two receptors in the duct epithelial cells of the major salivary glands moved on the one hand from the different expression and localization of leptin and its receptor [2] and on the other hand from the decreased effect of endocannabinoids on the salivary secretion, observed in men exposed to exogenous cannabis [3]. The aim of the present study was to test whether different physical forms of diet can induce to different extents the expression of CB1 and CB2 in the mandibular glands in response to different mechanical stimulation perceived after the intake of feed differently ground (fine vs. coarse) and compacted (mash vs. pellet vs. extruded) by growing pigs. The experiment involved a total of 32 growing pigs fed ad libitum for 4 weeks with four experimental dietary treatments. One diet was differently processed to obtain different physical forms: FP - Finely ground pelleted diet (dMEAN, 0.46 mm); CM - Coarsely ground meal diet (dMEAN, 0.88 mm); CP - Coarsely ground pelleted diet (dMEAN, 0.84 mm); CE - Coarsely ground extruded (dMEAN, 0.66 mm) diet. At the end of the experimental feeding, all animals were euthanized and both mandibular glands immediately removed, weighed and fixed in buffered formaldehyde (2,5% v/v) for 24 h at room temperature. Samples were cut and automatically embedded in paraffin. The immunohistochemical reactions were visualized on 5 μm serial sections, utilising the primary goat polyclonal anti-CB1 and rabbit polyclonal anti-CB2 antibodies, the avidin-biotin-complex and the DAB as the chromogen. Sections in which the primary antibodies were omitted, represented the control of unspecific staining. A strong positivity for CB1 and CB2 in the mandibular glands of the animals fed with CP, FP and CE diets was pointed out in comparison with the animals of the CM group. In particular, the CB1 and CB2 immuno-positivity involved duct epithelial cells with a peculiar localization in the cytoplasm of some epithelial cells near or on the apical cell membrane. In the animal fed with CM diet the immuno-positivity no longer involved duct epithelial cells but in some samples as far as the serous cells in mixed acina. The connective tissue tested negative for CB1 and CB2. The CB1 and CB2 were differently espressed in the mandibular glands of pigs fed with different physical forms of the diet. These novel findings leads us to speculate on CB1 and CB2 role, as receptors involved in the control of pig salivary secretion via endocannabinoids ligands and that these molecole likely represent an important link between the physical form of the diet and salivation. However, whether they rule on amount of saliva produced or on its composition needs to be elucidated so far.

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DEVELOPMENT OF AN ELECTROCHEMICAL SENSOR BASED ON SCREEN-PRINTED ELECTRODES FOR OCHRATOXIN A IN PORK MEAT SAMPLES

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Ochratoxin A (OTA) is a nephrotoxic, immunosuppressive and teratogenic mycotoxin produced by Aspergillus and Penicillium spp. fungi during food storage. OTA can be detected in cereal products, coffee, wine, beer, cheese and in poultry and pork meat. Many detection techniques, such as liquid chromatography coupled with immunoaffinity column or solid phase extraction cleanup, have been used for OTA determination in different samples (1). In recent years electrochemical techniques have been used for the rapid and accurate detection of OTA (2). The aim of the present study was to develop a new analytical method for OTA quantitative detection in pork meat based on electrochemical sensing, using graphite-based screen-printed electrodes and differential pulse voltammetry (DPV) as detection technique.

Experiments were performed with an electrochemical transducer Palmsens, monitored with a personal computer using PSTrace software (Palm Instrument BV, Houten, The Netherlands) for data acquisition and subsequent analysis. The electrochemical assays were performed with miniaturized disposable graphite based screen-printed electrodes (EcoBioServices & Researches s.r.l., Florence, Italy). The effect of pH (range 2-7) and of ionic strength (KCl concentration range 10-200 mM) of the supporting electrolyte solution (acetate buffer) on the DPV peak current and potentials was investigated to optimize the DPV method. The effect of the DPV parameters on OTA oxidation peak was studied. Potential pulse amplitude (Epulse) was evaluated in the range of 10-100 mV. Step height was evaluated in the range of 2-10 mV. The influence of the scan rate was examined in the range of 0.005-0.1V/s. Standard addition method was applied for quantitative analysis. The method was applied for OTA determination in spiked pork meat samples. Results were compared with those provided by a reference HPLC method.

The OTA peak current increased with increasing acetate buffer pH (from 2.0 to 7), thus pH of 7.0 for the supporting electrolyte solution was chosen. Concentrations of 75 mM KCl in the supporting electrolyte was selected. The optimization of DPV parameters indicated that best results for voltammograms were obtained from 0 to 1.1 V by using 5 mV potential step, 50 mV potential pulse, 0.01 V/sec scan rate and 0.07 sec time pulse; each scan was performed after an equilibrium time of 30 sec. Calibration graphs of peak height against concentration for OTA by DPV were plotted over the range 25-1000 μg/l in the supporting electrolyte with a LOQ of 25 μg/l. The findings obtained with voltammetric-based sensing were in good agreement with results obtained by HPLC analysis but matrix effects have been detected at lower OTA concentrations indicating the need of more selective extraction procedure. The proposed method is more rapid and inexpensive in comparison with the classical methods for OTA analysis, and can be considered a promising alternative for the evaluation of OTA in meat.

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EFFICACY OF AN IMMUNIZATION PROTOCOL BASED ON AN ATTENUATED SALMONELLA TYPHIMURIUM VACCINE BOOSTED WITH AN INACTIVATED SALMONELLA CHOLERAESUIS VACCINE IN PIGLETS EXPERIMENTALLY INFECTED WITH S.CHOLERAESUIS

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Salmonella Choleraesuis causes a systemic disease in pigs responsible of economic problems for farmers (Ku et al. 2005). Salmonella Typhimurium is the second important serovar, diagnosed in pig farms and is the principal responsible of salmonellosis in humans as a consequence of consumption of contaminated pork products (EFSA Journal, 2010). Vaccination is a decisive tool to control disease in countries with high prevalence of infection (Wales et al., 2011), unfortunately different serovars affect pigs and the cross-protection of available vaccines is not completely disclosed (Foss et al., 2013). S.Typhimurium ÎŤznuABC is an attenuated vaccine and recently we tested its safety and efficacy in mice and pigs experimentally infected with virulent S.Typhimurium (Pesciaroli et al. 2011; Gradassi, et al. 2013). The aim of this study is to assess the efficacy of this attenuated vaccine, in comparison to an inactivated S.Choleraesuis vaccine, in piglets experimentally infected with S.Choleraesuis. Eighteen piglets were randomly divided in 3 groups. Group A was orally vaccinated with S.Typhimurium ÎŤznuABC and boosted with inactivated S.Choleraesuis vaccine, group B was intramuscularly vaccinated with inactivated S.Choleraesuis vaccine and group C was unvaccinated. All groups were challenged with 5x108 CFU of virulent S.Choleraesuis at day 35 after vaccination. Animals were weighed at vaccination and before necropsy (day 47 from first vaccination). Tonsils, ileocecal lymph nodes, spleen, liver, intestinal content of ileum, cecum, colon and jejunum were collected during necropsy for microbiological analyses and gross lesions of organs were recorded. The results show that vaccination does not influence the weight gain; furthermore, the synergic action of attenuated vaccine followed by a boost with inactivated vaccine reduces fever, ileocecal lymph nodes and gut colonization caused by virulent S.Choleraesuis infection. Antibody titers of vaccinated groups (A and B) were statistically different from group C, indicating the capability of this new immunizing protocol in providing humoral response. These findings show that this new immunizing approach is more effective than the homologous inactivated vaccination protocol in controlling S.Choleraesuis infection.

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AUTOGENOUS SALMONELLA TYPHIMURIUM MONOPHASIC VARIANT BACTERIN IS EFFECTIVE IN TWO ENDEMIC FARMS OF THE NORTH OF ITALY

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Salmonella enterica serovar Typhimurium monophasic variant (S.T. 1,4,[5],12:i-) is increasingly responsible of human salmonellosis (EFSA, 2014) and pork represents the first source of infection. Salmonella can sub-clinically colonize pigs, principally during the finishing phase, and hence being introduced in the slaughterhouse, contaminating pork products destined to human consumption (Boyen et al., 2008). The biosecurity activities and management effectively decrease the prevalence in farms. However, other tools are envisaged for controlling Salmonella in endemic areas. Of these vaccination represents an efficient solution to decrease the infection in pig farms (Pesciaroli et al., 2013). Live vaccines are more effective than inactivated ones, inducing a cellular immune response that better enhances the clearance of Salmonella (Coward et al., 2014). The limitation is represented by the availability of attenuated vaccines whose protection versus different serovars, frequently diagnosed in pig farms, is not completely disclosed. In this scenario, immunization with autogenous bacterin could be more effective in endemic Salmonella farrow-to-finish or multisite pig farms (Roesler et al., 2006).The efficacy of two autogenous vaccines versus S.T. 1,4,[5],12:i- was evaluated in two multisite pig production systems of the North of Italy. Forty sows were divided in vaccinated (V) or not vaccinated and intramuscular injection of 2x109 CFU/ml of inactivated S. Typhimurium 1,4,[5],12:i- was performed at 6 and 2 weeks before the delivery. Sixty piglets from sows of group V (three from each sow) were divided into 2 groups: vaccinated group (VV) and not vaccinated group (VnV). Also, the 60 piglets born from unvaccinated sows (nV) were divided in 2 groups: vaccinated group (nVV) and not vaccinated group (nVnV). Piglets were primed and boosted at 4 and 8 weeks after birth with the same immunization protocol of sows. Microbiological and serological exams of sows were performed during pregnancy and suckling phase, conversely, piglets were monitored throughout the production cycle. Data related to weight gain, fecal shedding of bacteria, organs colonization and humoral immune response were recorded. The results indicate that the administration of inactivated vaccines in breeding and/or growing phase is differently able to improve the growth of animals and hence the productivity of farms, and to reduce the load of bacteria carried into the food processing. Vaccination of sows does not affect Salmonella shedding in sows faeces, but tends to reduce the percentage of shedder piglets. Furthermore, the antibody titers of pigs born from vaccinated sows were reduced (V/V) or remained constant (V/nV) during the observational period, while antibody titers of pigs born from unvaccinated sows had a sharp increase close the slaughtering. Particularly, a combined vaccination of sows and their piglets is the best protocol to improve the weight gain of growing pigs, to limit Salmonella colonization of organs and to reduce carrier pigs.

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SEQUENCE AND VARIANT ANALYSIS OF A PATHOGENIC FIELD STRAIN OF EQUINE INFECTIOUS ANEMIA VIRUS FROM ITALIAN ISOLATES

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Equine infectious anemia (EIA) is a persistent retroviral-based disease with a worldwide distribution significantly challenging the equine industry. In 2006 indeed, an outbreak of EIA occurred in Ireland and Italy, apparently as a result of iatrogenic transmission with a contaminated horse plasma infusion. These Irish and Italian outbreaks were characterized by cases of severe, some times fatal, disease [1,2]. Despite first clinical signs of EIA were reported as early as 1843, all the complete genomic sequences published to date are derived from just few viruses: the North American EIAV Wyoming, Chinese EIAV Liaoning, Japanese EIAV Miyazaki 2011-A and Irish EIAV IRE strains.

In the current study, we report for the first time the complete genomic sequences for Italian EIAV isolates. Unlike other authors, that previously sequenced EIAV genomes with Sanger methods, we used a strategy aimed to minimize PCR errors and chimeric clone amplification [3] and maximize low frequency mutations discovery through Next Generation Sequencing. This approach allowed for the identification of mutation accumulation in the EIAV genome that is known to correlate with the number of febrile peaks through time.

DNA and RNA of 103 samples (blood or/and organs) derived from Italian outbreaks of EIA and EIAV seropositive asymptomatic Italian horses were screened for gag and pol viral genes. Positive samples were subjected to whole pro-viral genome amplification thorough a "Long Run PCR" strategy [3] resulting in about 8000 bp single amplicons. Two selected samples derived from symptomatic animals from the same outbreak but with different clinical history (one suddenly died after infection the other euthanatized after 5 months of several febrile peaks), were sequenced using the Roche 454 Flex platform. Consensus sequences were annotated and submitted to GenBank (KM247554, KM247555) Bioinformatics analyses were carried out with an "ad hoc" pipeline performing a variant calling to evaluate mutations with respect to an Irish reference sequence (JX480631). Variants called (61 for KM247555 and 66 for KM247554) were annotated with Annovar software: 37 of those are shared between the two genomes while 24 in KM247555 and 29 for KM247554 are genome specific.

In this study we report for the first time the complete genomic sequences for two Italian EIAV isolates; performing variant calling procedures, we evaluated the samples differences with a reference genome, moreover we had different time points of observation from the same outbreak allowing the identification of polymorphisms accumulation through time. Even if we should expect most of the variability in the RNA viral genome (which we have no data) we discovered significant mutations in the proviral one. The majority of them produce non-synonymous variants, especially in KM247554 (66 SNP) highlighting that the sample derived from the animal that showed most febrile peaks, has an amount of mutations that heavily affect the protein sequence. These variants are under deeper bio-informatics characterization in order to predict the effect of the mutations on virus proteins.

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HAEMATOLOGICAL AND HEMATOCHEMICAL PARAMETERS AS ANIMAL WELFARE INDICATORS IN MINI-PIGS IN EXPERIMENTAL CONDITIONS

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Swine model plays an emerging role in biomedical research in reason of its anatomical, physiological, biochemical and genomic features, that are more closely related to human species than the rodent ones. Moreover, the prospect of obtaining genetically modified pigs further extended their biomedical potential, especially to mimic inherited human diseases such as cystic fibrosis, type 1 diabetes, vascular disorders and multiple sclerosis. Regarding neurodegenerative diseases, several transgenic pig models have already been produced. These considerations, as well as the existence of significant amounts of background data, from a regulatory perspective, provide further support for the use of this species in experimental or pharmaceutical research studies. For these reasons, it is likely that pigs and mini-pigs will become an increasingly important animal model for research and pharmaceutical development applications.

Previous studies showed that blood components are sensitive indicators of physiological and pathophysiological responses to stress. Thus in this study, the animal welfare of our Yucatan mini-pigs and Yucatan crosses with commercial breeds farmed as an experimental model of neurodegenerative disease, was investigated trough haematological and hematochemical analysis (in particular hepatic and renal indicators). On all subjects (N=6 for two years) were performed blood tests (blood count) and clinical chemistry with particular reference to indices of liver and kidney function (alkaline phosphatase, creatinine, urea, transaminase levels GPT and GOT) and electrophoresis of serum proteins. Blood tests and blood counts are performed on whole blood and serum, serum protein electrophoresis together for an assessment of the profiles organ and the reactivity of the host's immune system. For immune indices, the serum electrophoretic patterns were obtained using a semi-automated agarose gel electrophoresis system to determine serum protein. Serum lysozyme was measured with a lysoplate assay, and the haemolytic complement assay (HCA) was carried out in microtiter plates.

In all healthy subjects in fact, the values obtained in serum proteins electrophoresis show an increasing trend, indicating an involvement of the immune system. They were also highlighted an increase in white blood cells and kidney parameters fluctuations. The values, however, have always remained in the normal range in two years. In patients with neurodegenerative disease (vs healthy), significant differences related to the disease have been reported. Preliminary results indicated that more sensitive indicators of animal welfare could be some serological parameters; however more data and animals will be necessary to validate these parameters.

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PRELIMINARY COMPARISON OF MUSCLE HISTOCHEMICAL CHARACTERISTICS OF DIFFERENT HYBRID PIGS

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In recent years, in the province of Parma, beyond breeding of commercial hybrids (CH; Large White x Landrace x Duroc) for the production of fine meats, local farmers have tried to recover an autochthonous breed, the "Nero di Parma" pig, famous in the past for its high quality meat and its covering and marbling white and firm fat. With the aim to combine an ancient breed with typical territorial products, they proceeded to identify subjects with morphology similar to the "Nero di Parma" pig, the rural hybrids (RH), that were reared in conditions similar to CH or in large plots of land. As the meat of the two hybrids is different in color and taste, we compared the histo-morphometric characteristics of their femoral biceps muscle to highlight eventual differences in the myofibers composition that could justify different meat quality. The research was carried out on 8 CH and 8 RH, that were bred and fed in the same conditions. A sample of the middle portion of left femoral biceps muscle, in the point of maximum circumference of the thigh, was collected from all subjects immediately after slaughter occurred when the weight was suitable for the production of fine meats. The samples were frozen in isopentane cooled in liquid nitrogen and transversally cut in 10 μm serial cryostat sections, that were stained for myofibrillar ATPase, after preincubation at different pH values (pH 4.25, 4.6 and 10.6). Only a specific combination of pH value (4.25) and incubation time allowed the separation among type 1, type 2A and type 2B fibers. Histo-morphometric assessment was performed for each subject, within 3 regions of interest (ROI) of 6mm2 microscopically observed at 2X magnification, counting the number of whole cells, that was then expressed as number of myofibers per mm2. Moreover, the cross-sectional areas of the myofibers, in each animal, were semi-automatically established, within 3 ROI of 57,2 mm2 photographed at 10x, by means of a digital image processing software, which calculates the area enclosed by a manually traced outline of the cells. Data, expressed as means and st.err., were analyzed using statistical software SPSS 18 (SPSS inc., IBM). Statistical significance was assigned to pËĆ0.05.

The total number of myofibers per mm2 did not show statistically significant differences between the two hibrids (CH 85.6±5.9; RH 93.9±7.3), while comparing the different fiber types, only type 1 fibers were significantly more numerous in the RH (CH 12.5±2.4; RH 23.2±3.5; p0.05;). Typically, type 1 and/or type 2A fibres formed clusters, whose number was not significantly different between two hybrids (CH 4.4±0.35; RH 4.7±0.4). However, in the RH, the clusters contained a significantly higher number of type 1 (CH 2.9±0.16; RH 4.9±0.2; pËĆ0.001;) and type 2A fibers(CH 1.3±0.13; RH 2.2±0.15; pËĆ0.01;). The type 1 fibers in the RH were also larger (CH 5710±188.4 μm2; RH 8032.5±278.1 μm2; p0.001;). Differently, the size of type 2A (CH 5681±267.3 μm2; RH 6294.8±251.6 μm2) and type 2B (CH 8110.4±140.3 μm2; RH 8228.6±159.4 μm2) did not show significant differences between the two hybrids. In conclusion, our study documented the prevalence, in both hybrids, of type 2B fibers with intermediate glicolytic capacity, and the abundance of type 1 fibers, with high aerobic metabolism and fatigue resistant, in the RH. Further study are planned to compare the muscle histochemical characteristics and meat quality of CH and RH reared in different conditions.

CANINE OSTEOSARCOMA CELL LINES CONTAIN STEM-LIKE CANCER CELLS: BIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION

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Canine osteosarcoma (OSA) is a suitable model to study human tumor since they share similar biological, clinical behavior and molecular features (1-3). Accumulating evidence indicates that OSA, as several human cancers, is composed by a cell subpopulation with stem-like properties named cancer stem cells (CSCs), responsible for tumorigenesis, drug resistance and recurrence. CSCs are defined by long-term self-renewal, differentiation capacity and tumorigenicity after xenotransplantation in immunocompromised mice (4). Conventional antineoplastic agents often fail to eliminate CSCs, which are able to repopulate the tumor mass, causing relapse. Thus CSCs represent an important target for cancer therapy (5-6). It has been reported that metformin, an antidiabetic drug, reduces cancer incidence and mortality compared with other treatments in diabetic patients. In vitro findings demonstrate that metformin inhibits the proliferation of several tumor cell lines (7). Objective: CSCs were isolated from canine OSA primary cell lines cultures and preliminary bio-molecular characterization of these cells has been performed. In addition, the responsiveness of OSA stem-like cultures to metformin was assayed, aimed at evaluating the sensitivity of canine OSA stem-like cells and the reliability of the dog as a model for human OSA. Materials and methods: 2 canine primary OSA cell lines (OSA1 and OSA2) were isolated and characterized. Cells were cultured as previously described (8-9). Cell morphology changes and spheroid (sarcosphere) formation were daily monitored using an inverted contrast microscope. The expression of tumor stem cell relevant markers Oct4, CXCR4 and CD133 was analyzed. Dose-response curves were carried out by treating cells with metformin (1-50 mM) for 48h. Cell viability was evaluated by MTT assay. Conclusions: OSA1 and OSA2 primary cultures were obtained from tumor specimens (one osteoblastic productive OSA and one chondroblastic OSA). Cells were shifted to a medium devoid of FBS and supplemented with EGF and bFGF (stem-permissive culture condition); after 2 weeks, cells derived from both cell lines grew as non-adherent aggregates or sarcospheres. To investigate the self-renewal ability, OSA1 and OSA2 spheroids were dissociated to single cells and reseeded: both cultures demonstrated the ability to self-renew through the formation of secondary spheres repeatedly. OSA stem-like cells can be maintained in culture for several passages and show a sustained proliferation in vitro. Furthermore, our results indicate that both OSA1 and OSA2 stem-like cells faintly express CD133 while they are consistently positive for CXCR4 and Oct4. Then we demonstrate that metformin induce a powerful dose-dependent anti-proliferative effect. In conclusion, these data underline the feasibility of obtaining CSC-enriched cultures from canine OSA as a promising model for biological and pharmacological studies of canine and human OSAs.

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EFFECT OF DIETARY ORIGANUM VULGARE L. ON IMMUNE DEFENSE RELATED GENE EXPRESSION IN SWINE PBMCS.

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There is evidence in supporting the therapeutic usefulness of oral administration of plant extracts as antimicrobials and in inflammatory diseases. In addition, they can stimulate immune functions by restoring optimal gene expression in response to stress. Oregano (Origanum vulgare L.) is an aromatic plant of the Mediterranean flora with antimicrobial and antioxidant properties. In intensive pig farming, animals are exposed to multiple concurrent stressors (e.g., heat, cold, mixing, high stocking densities, weaning, noise, and shipping) with additive effects that can impair immune function and increase disease susceptibility. In the present study the effect of oregano on the expression of genes related to immunity were investigated. Thirty-two Suffolk hybrid pigs, with an average live weight (LW) at the beginning of the trial of 46 kg, were randomly divided (8 animals/pen) into 2 indoor and 2 outdoor pens and balanced for litter, sex and LW. After the adaptation period (35 d), both indoor and outdoor pigs were assigned to one of the following isonitrogenous and isoenergetic diets: 1) control (CTR), i.e. commercial pelleted feed (16.0% CP, 4.3% CF, 1.0% lysine); 2) CTR supplemented with 0.2% oregano essential oil (OEL). Diets were administered for 190 d until slaughter. Blood samples were drawn from the jugular vein at three time-points: before treatment (T0), after four months of treatment (T1) and before slaughtering (T2). Immediately after collection, pheripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll-Hypaque method (GE Healthcare). Total RNA was extracted from PBMCs and quantified. Total RNA was reverse transcribed using random hexamers and the Superscript III Reverse Transcriptase (Invitrogen). Successful reverse transcription was confirmed by PCR amplification of the Sus scrofa β-actin gene. Real-time PCR primers used to amplify reference genes and the genes of interest (ICAM-1, TNF-α, subunit p50 of NFkB, IL-1β, IL-8, IL-18, IL-10, STAT3, HSP90, IL1RN) were designed based on available sequences using the Primer-BLAST software. Gene expression was evaluated on all animals (8/pen) at T0, T1 and T2. Under indoor conditions, IL-1β and ICAM-1 were both decreased at T1 by the OEL diet. A tendency (P<0.06) towards an up-regulation of NFkB and STAT3 was observed in the OEL group. Gene expression analysis at T2 did not show differences between groups for all of the tested genes but STAT3 (P<0.01). In the outdoor reared pigs, a down-regulation in the OEL group for IL18, NFkB and STAT3 was observed at T1. At T2, only NFkB resulted down-regulated in the OEL group. The present experiment has demonstrated that the expression of a number of immune related genes can be affected by diet, with different effects in outdoor and indoor reared pigs. In both farming systems, the effect was shown to be greater in T1 than in T2, suggesting either animal adaptation to the environmental conditions or to the OEL supplementation. Further investigations are needed to determine possible relationship between expression of genes related to immunity and performance in indoor and outdoor reared pigs.

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USE OF OPTICAL BIOESENSOR FOR THE RAPID DIAGNOSIS OF AFRICAN SWINE FEVER VIA DSDNA:LNA TRIPLEX (PRELIMINARY RESULTS)

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Introduction African swine fever (ASF) is a highly lethal disease affecting both pigs and boars. The disease is highly infectious and is a threat to all countries where pig farming is widespread. It is due to a virus of the genus asfivirus with high survival capacity. Until a few years ago the disease was confined to the countries of sub-Saharan Africa and Sardinia, in recent years the disease has spread in the Caucasus. Given its diffusion, many efforts were focused on the development of a rapid diagnostic test for ASF to contain any contagion. Aim The purpose of this work is to evaluate the applicability of an optical biosensor for the rapid diagnosis of african swine fever. Specifically, we immobilized two different probes, namely a ssDNA probe and a chimeric ssDNA/LNA construct. The inclusion of LNA oligonucleotides in a DNA probe increases the affinity for complementary strands, as well as both sensitivity and specificity for target nucleotides (1). Furthermore the LNA oligonucleotide can bind the dsDNA in the major groove forming a dsDNA:LNA triplex (2). Materials and methods The extraction of DNA from blood samples of experimentally infected animals was perfomed using FTA mini cards. The extracted DNA was directly analyzed on a surface biosensor. In our study, two sensing surfaces (containing a probe ssDNA and one ssDNA/LNA, respectively) were developed and compared. For verification of the selectivity of the apparatus, 13 different dsDNA samples extracted from blood of experimentally infected pigs have been used. The dsDNA extract was denatured to ssDNA in order to allow the hybridization with the complementary ssDNA probe immobilized on the biosensor. In parallel, the same dsDNA samples were directly tested with ssDNA/LNA probe. After each use, the surface of the biosensor derivatized with the two types of probes was regenerated by washing with PBS (ssDNA) or with PBS-T (ssDNA/LNA). As a negative control, was used a dsDNA extract of a healthy animal and no appreciable signal was detected. Results and conclusion Our results proved that the biosensor derivatized with the probe ssDNA has poor selectivety for positive samples (4 out of 13 samples are below the background), whereas the biosensor derivatized with the ssDNA/LNA probe was able to detect appreciable signals on the same samples. Due to the higher sensitivity of the probe ssDNA/LNA and the detection capability in real time, the biosensor can provide quick responses on the presence/absence of the virus after simple treatment procedures of the sample capable of release the viral DNA. In fact, the ultimate goal of this work is to perform analysis directly on the farm by connecting the biosensor to a portable PC without the amplification step of the extracted DNA.

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SEVERE MULTIPLE INFESTATIONS OF A STRANDED ADULT MALE OF STENELLA COERULEOALBA

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Stenella coeruleoalba is an extensively studied dolphin and the most common species of our seas. Parasitism, especially of the respiratory or gastrointestinal system, is relatively common in stranded cetaceans and has been documented in animals from the North Sea, eastern Pacific Ocean, Atlantic Ocean and Mediterranean Sea. Lungworm infections associated with stranding or mortality have been documented in some species including S. coeruleoalba. This report describes the lesions found in an adult male of S. coeruleoalba stranded along Falconara Marittima coasts (Ancona, Italy) trying to clarify the role of high parasitism in stranding and mortality. One adult male of S. coeruleoalba were found moribund and died shortly after being recovered. A full necropsy examination was promptly carried out to make an accurate diagnosis of death. Representative tissue samples were removed and fixed in 10% neutral buffered formalin for routinely histological examination. 5-μm-thick sections were obtained and stained with haematoxylin and eosin, P.A.S., Wilson Disease Stain for copper and Perl's iron stain. Specimens were also aseptically collected for bacteriological examinations. Parasites were collected during the necropsy for identification. PCR-RFLP was also performed to identify the Anisakis spp. Tissues samples were also examined for morbillivirus and Orthomyxovirus nucleic acid by reverse transcription-PCR (RT-PCR). At necropsy, S. coeruleoalba showed a multiple infestations with different anatomic sites. In lung, multifocal small white-greyish nodules were found. Lungworms consistent with the family Pseudalidae were observed in multifocal granulomatous eosinophylic lesions. Macroscopic examination of forestomach showed a large chronic gastric ulcers with several adults and L4 larvae of Anisakis pegreffii. In the second chamber of the stomach, multiple nodules into the gastric wall were registered. Microscopically, gastrointestinal helminths consistent with digenea trematodes and thousands eggs were found into the gastric muscle layers surrounded by severe granulomatous reaction. Moreover, numerous cysticerci referred to as Monorygma grimaldi were found as nests of 12 retroperitoneal cyst 30 to 40 mm in diameter on the abdominal wall. In addition, areas of demyelination in the white cerebral matter, mild diffuse hepatocellular degeneration associated with copper deposits. Bacteriological exam and PCR analysis for DMV were negative. As previously suggest, certain environmental organochlorine pollutants, as well as heavy metals are responsible for a decreased immune response to foreign antigens triggering secondary viral, bacterial and/or parasitization. Since relationship among Cu, Zn and Cd, the presence of copper deposits in the liver may suggest other heavy metals storage. Chemical analysis for heavy metals will be performed. In conclusion, most of the lesions observed were caused by parasites which could be contributed or to be the primary cause of the stranding in this animal.

Part II

Sanità pubblica e sorveglianza sanitaria

LONGITUDINAL SURVEY ON EIMERIA INFECTION IN TWO GOAT BREEDS IN NORTHERN ITALY

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Intestinal parasitic infection caused by Eimeria spp. represents in dairy goats breeding up-to-now an important concern, being coccidiosis a widespread disease with an economic impact. A longitudinal survey was planned with the aim of update information on epidemiology of Eimeria infections in dairy goats, with particular regard to two different breeds: the Alpine and the Nera di Verzasca breeds. The survey was carried out in a farm located at 980 m a.s.l. in the province of Varese (Northern Italy), breeding 90 dairy goats in a semi-extensive way. Twenty adult females (10 Alpine and 10 Nera di Verzasca) were selected and monthly sampled from January to December 2012. At sampling time, individual data were collected: age, breed, number of parturitions; the distance (in days) of the sampling from parturition was calculated. Furthermore, monthly average of temperature and precipitation were obtained from the closest meteorological station. Fecal samples were examined using the FLOTAC double technique (Cringoli, 2006); for the count of Eimeria spp. oocysts, a flotation solution of MgSO4 (s.g. 1280) was used. For the morphological identification of Eimeria species (Levine an Ivens, 1970; Pellérdy, 1974), faecal samples were pooled and sporulated using 2.5% potassium dichromate. Statistical analysis was performed to analyze the risk factors associated to the infection, including as independent variables individual and meteorological data. Only two fecal samples out of 223 collected resulted negative for Eimeria oocysts, with an overall prevalence of 99.1%. E. arloingi, E. alijevi, E. aspheronica and E. christenseni were the species identified in pooled feces. A greatly variation of OPG values by sampling month (p=0.001) and by breed (p=0.007) was observed by statistical analysis: in the first part of the year both breeds showed similar OPG values, whereas since May (the month with the highest oocyst counts) Alpine goats shed more Eimeria oocysts than Nera di Verzasca goats. Alpine breed resulted therefore more at risk of infection by Eimeria (OR=1.231) when compared to Nera di Verzasca. Similarly, in the statistical model other variables resulted related to the infection: the distance from the parturition (p=0.0001; OR=0.998) and the number of parturitions (p=0.016; OR=0.934). Moreover, rainfall resulted to be a predictor of Eimeria infection (p=0.012; OR=1.042), corresponding the highest precipitation average to the month of May.

Concluding, the results indicate that Eimeria infections are remarkably widespread in goats, by analogy to previous surveys carried out on dairy goats (Abo-Shehada and Abo-Farieha, 2003; Ruiz et al., 2006), with Nera di Verzasca goats presenting a higher resistance when compared to Alpine goats. The shedding of Eimeria oocysts occurred during the whole year, increasing during the spring: environmental conditions such as rainfall are strongly related to oocysts shedding, probably favouring the sporulation.

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ENDOPARASITIC INFECTIONS IN SHEEP: PREVALENCE AND RISK FACTORS USING AGE-CLUSTERED POOLED FECAL SAMPLES

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Endoparasitism is one of the major health constraint in sheep worldwide. Gastrointestinal parasites, distomes and lungworms can lead to severe disease and relevant economic losses in ovine production (Liu et al. 2004; Rojo-Vázquez et al. 2012); aim of this work was to determine prevalence and risk factors of endoparasitic infections in sheep farms in northern Italy using pooled-fecal samples collected from different age categories. From January 2012 to August 2012, individual fecal samples were collected from 34 sheep farms located in Lombardy region. Three age categories were individuated (young, primiparous, pluriparous) and 10 animals were sampled for each age category in each herd. Each pooled fecal sample was obtained using 3 g of feces from 10 animals; all the obtained pooled fecal samples were analyzed using the FLOTAC dual technique. Fecal egg count was performed to determinate the eggs per gram (EPG) of Strongylida, Nematodirus, Strongyloides, Trichuris and Dicrocoelium dendriticum; the number of lungworms larvae per gram (LPG) was also recorded. Only qualitative analysis was implemented for Fasciola hepatica, Moniezia and Eimeria. A questionnaire survey was submitted to farmers: information on farm size, breeding management (stantial/transhumant herd), anthelmintic treatment, presence of goats and site of grazing were collected. Positions of grazing sites of studied herds were plotted using Google Earth. For transhumant herds, both positions of autumn-winter and spring-summer grazing sites were considered. From the questionnaire survey resulted that the average number of reared sheep per herd was 724 (sd 537); 82% of sampled herds reared sheep and goat and 79% were transhumant. The average altitude of grazing was 1061 masl (sd 428.54) and 146 masl (sd 112) in spring-summer and autumn-winter respectively. Anthelmintic treatment were administered to sheep in 88% of tested farm. Pooled fecal samples resulted infected by Eimeria (P=100%), Strongylida (P=99%), Strongyloides (P=83%), lungworms (P=81%), Nematodirus (P=73%), Trichuris (P=52%), Moniezia (P=38%), D. dendriticum(P=24%) and F. hepatica (P=6%). Generalized linear model and multivariate binary logistic regression analysis showed that season of sampling, farm features, management, treatment practices and spatial features can differently affect detected parasites. But Eimeria, in our survey Strongylida was the detected taxon presenting highest prevalence and abundance; egg excretion of Strongylida resulted statistically significant higher in summer, in stantial herds, in farms without goats and when herds were not treated with anthelmintc drugs. Concerning geographical features, higher egg excretion was observed at lower altitude, both in autumn-winter and in spring-summer sites of grazing; higher latitude of grazing was a significant risk factor only for autumn-winter sites of grazing. In our study, using age-clustered pooled fecal samples, we observed that prevalence of infection and egg/larval excretion of different taxa of sheep parasites were influenced by different factors. The use of representative pooled fecal sample can be useful in time consuming and expensive parasitological surveys.

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FASCIOLOSIS AND CLIMATE CHANGE IN SHEEP FARMS IN SOUTHERN ITALY: IS THERE A CORRELATION?

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The liver fluke Fasciola hepatica is a trematode parasite with an economic impact on livestock in most regions of the world (Charlier et al., 2014). F. hepatica represents also a public health problem, since the parasite is endemic in many parts of the world (Mas-Coma et al., 2014). For the development of system for infection risk prediction, knowledge of the epidemiology and spatio-temporal distribution of F. hepatica is required. Previous studies show a clustered distribution of F. hepatica in southern Italy and the main risk factors associated with the presence of fasciolosis was found to be the presence of large pastures with impermeable soil (Cringoli et al., 2002). In southern Italy, as in other regions, infection levels and incidence of disease due to liver fluke depend very much on rainfall during the period from late spring to late summer (Taylor, 2012).

The final aim of the present study was to investigate whether climate change in recent years have influenced the onset of outbreaks of F. hepatica in ovine farms of southern Italy.

In May-June 2014, a severe outbreak of F. hepatica occurred in three sheep farms in the Campania region. Clinical, coprological and necroscopic examinations were performed. Morbidity and mortality due to F. hepatica were 3-67% and 3-50%, respectively. Coprological examinations showed high values of F. hepatica eggs per gram (EPG) of faeces (860-1,240). Similarly, high adult parasitic burdens were found in animals that had sucombed (124-426 flukes). The study area was georeferenced and climatic data (temperature, humidity, days of rain and total amount of rainfall) were recorded at four georeferenced meterological stations in the study area for the period 2000-2013. The results show that there was a significant decrease ($P < 0.001$) of temperature, increased rainfall and increase in the number of rainy days compared to previous years.

It seems clear that the outbreak of fasciolosis was related to changes in climate in the study area over the study period that showed significant changes in temperature, rainfall and number of rainy days, in particular in the latest years. Effective monitoring, including active surveillance of disease outbreak, is required in order to develop suitable control strategies and to model recent and future risk of F. hepatica infection in livestock in southern Italy and beyond.

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SURVEY ON GIARDIA DUODENALIS PRESENCE IN FRUIT AND VEGETABLE BY-PRODUCTS PROPOSED AS FEED FOR LIVESTOCK

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Food waste refers to food that is still in good quality but does not get consumed because it is discarded either before or after it spoils. In industrialized countries food waste corresponds to approximately 40-50% of the total amount of biodegradable garbage produced every year (European commission, 2011). Many approaches have been proposed to reduce food waste. The use of fruits and vegetables discarded has been proposed as source of food for livestock. This approach could represent a valuable kind of recycling and may contribute to reduce the costs of animal production. However, it is important to monitor the quality and safety of these by-products, since they may be contaminated by parasites of veterinary and zoonotic concerns as Giardia duodenalis (Smith et al. 2007). The prevalence of G. duodenalis in livestock varies from 17% to 53%.

The aim of this study was to investigate the presence of G. duodenalis in fruit and vegetable by-products proposed as feed for livestock.

From January to June 2014 fruits and vegetables at the end of their shelf life were weekly collected from supermarkets (Messina, Italy). Theseby-products were individually washed in distilled water, the liquid collected into a 1 L goblets and allowed to sediment. The sediment was transferred into 50 mL tube and analyzed for G. duodenalis presence by the means of the sedimentation/flotation technique using ZnSO4 solution (1180 g/L s.g.) (MAFF). Then, the samples were homogenized and divided in two aliquots, of which one was analyzed as above and the other by a PCR targeting a specific portion of G. duodenalis DNA.

A total of 54 samples of vegetables and 24 of fruits were collected. All the samples tested negative for G. duodenalis presence at both microscopical and molecular techniques. Interesting, the absence of G. duodenalis herein reported is in contrast with the findings of other similar studies in which the parasite was found in 6% of samples collected in open-aired markets (Duedu et al. 2014). This difference could be due to the diverse type of production, storage and handing of goods between the examined markets. In fact, in open-aired markets the frequency of parasites contaminating goods is larger than in supermarkets (Duedu et al. 2014). In the present study all the samples were collected in supermarkets, in addition, fruits and vegetables were often enveloped directly at production site thus avoiding further contaminations. According to our findings, fruits and vegetables discarded from supermarkets could be safely used to produce animal feed, thus halving the general costs of waste management and livestock production. Although all the samples tested negative to G. duodenalis it is fundamental to systematically monitor and exclude the presence of parasites in order to guarantee a high level of safety for both animals and consumers.

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PREVALENCE OF TICK BORNE DISEASES IN SICILIAN CATTLE

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Tick-borne diseases (TBDs) are endemic in Sicily as well as in other regions of Italy and Europe. The development and implementation of control measures for TBDs is dependent on understanding the epidemiology of these pathogens in a particular geographical region. The distribution of Tick-borne pathogens such as Theileria spp., Babesia spp. and Anaplasma spp. in Sicilian cattle from 2012 to 2014 was evaluated in order to enhance the epidemiological knowledge of bovine TBDs. The aim of this study is to characterize the prevalence of different species of tick-born pathogens in cattle. The investigation was made using serological and molecular tests to reveal both immunological response and active infection in animals exposed to risk or with typical symptoms of TBD).

Bovine blood samples were collected in EDTA-treated and untreated tubes and stored at -20 or -80°C until analysis. DNA was extracted from unclotteded blood using PureLink Genomic Mini kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and then DNA samples were analyzed by PCR using specific primers to detect Babesia bovis, Babesia bigemina, Theileria annulata, Anaplasma marginale, Anaplasma phagocytophilum. A total of 1332 samples were analyzed to detect B.bovis, and only 3 of them were positive, with a prevalence of 0.2%. B.bigemina was found in 13 out of 1199 cattle, with a prevalence of 1.1%. The percentage of prevalence of T.annulata was approximately 76%, with a higher number of positives: 1133 from a total of 1489 samples. With regard to A.marginale, it was detected in 428 out of 661 samples, with a prevalence of 65%, and A.phagocitophylum was found in 19 out of 862 samples with a prevalence of 2,2%. Serum samples were used to carry out ELISA and IFI tests. The serological tests for B. bovis, B. bigemina and T. annulata showed a similar prevalence, 47%, 50% and 59%, respectively. Of the cattle tested for these 3 pathogens, 140 of 297 were positives for B. bovis, 422 out of 842 were positive for B. bigemina, and 558 out of 947 were positives for T. annulata.

These results suggest the existence of immunological protection for some TBDs, but about half within the populations is susceptible to infection by these microorganisms, and it is relevant not only for inside population but for animals that originate from non-endemic areas. The presence of active infection is more evident for Theileria annulata, that probably requires increased attention and deep monitoring all phase of infection from vectors to treatments. The distribution of positive animals was evaluated in order to construct a reference map showing the spread of the diseases across Sicily. The observed prevalence was generally lower when estimated by DNA rather than with serological testing. Similar differences have been found in other studies and may reflect the absence of detectable levels of bacteremia in some animals.

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EQUINE PIROPLASMOSIS IN NORTHWESTERN ITALY: NOT ONLY THEILERIA EQUI AND BABESIA CABALLI

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Equine Piroplasmosis (EP) caused by Theileria equi and Babesia caballi is a severe and problematic disease compromising the health and the international movement of horses. Both parasites are transmitted by ticks of the family Ixodidae.1 EP is present worldwide and it is endemic in Italy. In endemic areas, infection usually occurs within the first year of life, with varying degrees of severity, which can often be associated with the level of parassitemia, concurrent pathological conditions, immunologic status and treatment availability. The clinical signs of EP include fever, icterus, anemia, hemoglobinuria, bilirubinuria, and, death but in endemic areas infection is frequently subclinical and infected animals become long-term carriers.2 The aim of this study was to assess the seroprevalence of EP in Northwestern Italy and to evaluate risk factors associated with parasitic infection. Whole blood was collected from 135 horses from 12 different stables across Piedmont Region. For each horse an epidemiological questionnaire was used to collect individual and environmental factors that might influence their exposure to EP causal agents. All extracted DNA samples were tested by a seminested PCR targeting the V4 hypervariable region of 18S rRNA gene of Piroplasms belonging to the genera Babesia or Theileria.3 Positive samples were further tested by a multiplex PCR, for species-specific simultaneous detection of B. caballi and T. equi.4 All positive amplicons were sequenced to confirm species identification. A total of 17.04% (CI95%: 10.70-23.38%) of animals were found to be infected with Piroplasms. T. equi was the most prevalent species, found in 18 animals (P=13.33%; CI95%: 7.60%-19.07%). Although B. caballi was never detected, infection with parasites belonging to the genus Babesia was confirmed by sequencing in 5 horses. B. canis infection was confirmed in 3 horses (P=2.22%; CI95% 0.76%-6.33%), while B. capreoli was detected in 2 individuals (P=1.48%; CI95% 0.41%-5.24%). EP is widely present in the study area as subclinical infection. Despite T. equi being the most prevalent pathogen, different Babesia species were detected in the study animals. The natural reservoir hosts of B. canis and B.capreoli are the domestic dog and roe deer Capreolus capreolus respectively. These findings pose attention to the need of expanding classical diagnostic methods for EP in order to include different species beside T. equi and B. caballi.

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PRELIMINARY SURVEY ON TOXOPLASMOSIS IN PIGS OF SARDINIA, ITALY

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Toxoplasma gondii is an Apicomplexan protozoa considered as one of the most important responsible of food-borne parasitic zoonoses worldwide (McAllister, 2005). Although pigs usually did not show any clinical signs, they are quite susceptible to T. gondii infection, and the pork meat is considered among the principal source for human infection (Dubey et al., 1991).

The aim of this study was to carry out a seroepidemiological and biomolecular survey on Toxoplasmosis in pigs raised and slaughtered in Sardinia for human consumption. From April 2013 to May 2014, a cross-sectional investigation was carried out on animals raised in 110 pig breedings of the island. After slaughtering 418 blood samples and 107 brains were collected for the serological and biomolecular survey respectively. An ELISA commercial kit (PrioCHECK® Toxoplasma Ab SR, Prionics, Switzerland) was used for the detection of T. gondii-specific antibody on sera. A nested PCR was carried out for the detection of genomic T. gondii DNA in brain samples. Twelve PCR-positive samples were typed at 5 genetic markers including 5 nuclear loci (SAG2, SAG3, BTUB, GRA6).

Antibodies against T. gondii were found in the 52.7% (221/418) of the sera samples. The presence of T. gondii DNA was detected in the 47.7% (51/107) of the brain samples. The 53.6% (59/110) of the investigated pig breedings were found positive at least one of the diagnostic techniques. Genotyping showed T. gondii with clonal Type III.

The results herein reported showed that toxoplasmosis is a widespread infection in pigs raised in Sardinia island with seroprevalences higher than those reported in the same island by Scala et al. (2008) (52.7% vs 15.2%; $\chi 2$ = 130.12; p = 0.000). The high prevalence of T. gondii DNA herein reported suggests as the brain could be considered a good matrix for the detection of DNA of T. gondii in this specie as reported by Juránková et al. (2014). Preliminary data on genotyping, carried out for the first time in pigs in the island, showed the presence of the clonal lineage Type III. The high prevalences for T. gondii herein found should not be ignored and confirmed that pigs can be considered an important source of infection for human.

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USING STANDARDIZED HUNTING DATA TO QUANTITATIVELY ASSESS WILD UNGULATES ABUNDANCE: A TOOL FOR HEALTH RISK MANAGEMENT

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Health management of wildlife is highly sensitive to quantitative estimation of population density. Replicable and harmonized tools to model species abundance at large spatial scales are demanded to prevent or mitigate the impact of diseases on livestock and human health as well as on wildlife. A GIS-based approach was implemented to create distribution and abundance maps of wild ungulates based on hunting data. The hunting bag of seven consecutive seasons (530,000 sample points) was used to develop six abundance maps for alpine chamois Rupicapra rupicapra, red deer Cervus elaphus, roe deer Capreolus capreolus, fallow deer Dama dama, mouflon Ovis aries and wild boar Sus scrofa. Each map defines the presence area of each species with 100% positive predictive capacity. From a quantitative point of view, the GIS-based abundance map is able to precisely estimate abundance with a sensibility and specificity that can be as high as 86.4% and 87.5% respectively. The final resolution of each abundance map is 250m. The GIS-based quantitative maps are reliable tools to harmonize wildlife abundance data at large spatial scales and to identify epidemiologically sensitive areas where wildlife is present at high densities. The GIS-based process can be easily adapted and used to estimate the abundance of different species from any geographical area where georeferenced hunting data are available.

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ENVIRONMENTAL RISKS MAPPING OF PIROPLASMID INFECTION IN WILD ALPINE UNGULATES

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Piroplasms and especially protozoa of the genus Babesia, are gaining increasing attention as newly emerging zoonotic pathogens for humans. Zoonotic species like B. venatoroum and B. microti have wild animals as main reservoir hosts. Species distribution models (SDM) are usually produced by relating field observations to a set of environmental variables, presumably reflecting some key factors of the fundamental niche of a species, like temperature, precipitation, topography, and land-cover. These models are referred to as Habitat Suitability Models (HSM) as they infer a species' presence probability by describing its most suitable habitat.We propose a habitat suitability model (HSM) for Babesia/Theileria spp., as tool for institutions and policy makes for human and veterinary health to better understand the entity of Babesia presence on our territory to improve diagnosis of cryptic clinical cases in humans, and to optimize screening and preventive actions on the Regional territory. The presence probability of Babesia/Theileria spp. was estimated using MaxEnt 3.3.3 (http://www.cs.princeton.edu). This Maximum Entropy model uses presence only (PO) data obtained by molecular detection (PCR) of Babesia/Theileria infection in wild ruminants and correlates those PO data to environmental factors that can favor or limit vector and host availability. Biotic and abiotic parameters like abundance of host species, altitude, temperature, humidity, and land cover were used as covariates to train species occurrence. We developed two separate models to better discriminate the different epidemiological roles of Roe deer (reservoir of B. capreoli, and highly infected by ticks) and Alpine chamois (highly susceptible host, subject to spill-over infection from Cervids). A comprehensive model using all PO data from all ungulates species (Roe deer, Red deer and Chamois) was also developed. The overall suitable area for Babesia/Theileria spp. in this simulation is of 3723.3 km2, which correspond approximately to 15.5% of the background regional territory. Retained covariates are mean summer NDVI, solar exposure and reclassified version of LandCover. The probability of presence for Babesia sp., peaks when NDVI reaches 0.65, value that corresponds to broad-leaved forests areas, that are notoriously the most suitable environments for Ixodid ticks. LandCover confirms the high suitability of broad-leaved forests (class 9), and solar exposure shows a direct correlation with increasing Babesia habitat suitability. The increasing interaction between wildlife, humans and domestic animals has led to the emergence of several diseases. Active surveillance in wildlife has been shown to be effective in preventing disease spill-over. In the specific case of Babesiosis, vector ticks as well as different reservoir hosts are involved in the epidemiological cycle. In order to promote a more efficient and cost-effective tool for disease surveillance and management in wildlife, we developed a presence only model that can be used to better understand the role of wildlife in disease epidemiology and to focus screening efforts/preventive measures in high risk areas.

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A STUDY ON THE SANITARY ASPECTS OF WILD AND CONFISCATED INDIVIDUALS OF TESTUDO HERMANNI IN ITALY

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There is a lack of knowledge concerning the sanitary aspects of most chelonians. In particular, herpesviruses are among the most important viral pathogens in chelonians, particularly in naive population. In addition, reptiles are considered asymptomatic carriers of Salmonella spp.. In this study a microbiological and parasitological investigation on wild (W) and confiscated individuals (C) of Testudo hermanni from several Italian regions, was conducted. For this purpose, oral and cloacal sampling by sterile swabs and fresh fecal samples were obtained. In addition, the presence of endoparasites by the collection of individual fresh faecal samples was examined. Eighty-four oral swabs (OS) and 66 cloacal swabs (CS) were collected for microbiological examination. DNA was extracted from each OS using a commercial kit. A consensus nested PCR protocol was performed to amplify a conserved fragment of the DNA polymerase of the herpesviruses. The CS was inoculated in selective mediums for Salmonella spp. and the isolates were serotyped by direct slide agglutination using specific antisera. A total of 39 faecal samples, were coprologically analyzed for the presence of endoparasites, following three methods: 1) Sheather flotation technique (specific gravity 1.25); 2) formal-ether sedimentation technique; 3) microplate enzyme immunoassay for detection of Giardia and Cryptosporidium specific antigen (GSA 65 and CSA) (Prospect- Giardia/Cryptosporidium- OXOID). No active herpesvirus infection was found. However, a latent infection, not detectable by PCR on oral swabs, could not be excluded. Salmonella spp. was isolated from 15 cloacal swabs and the following serotypes were identified: Salmonella enterica Abony (W: n=1; C: n=5), Langord (W: n=5), Newport (C: n=1), Miami (R: n=1), Wedding (W: n=1) and Hermannswerder (W: n=1). The analyzed faecal samples contained a broad spectrum of parasites (total occurrence 88,74%, n=35) including different species of nematodes such as oxyurids (88,74%, n=35), ascarids (7,69%, n=3) and protozoans (Entamoeba spp. 25,64%, Balantidium spp. 12,82% and Nyctotherus spp. 30,77%). All samples resulted negative for Giardia and Cryptosporidium. In tortoises, parasites belonging to the super family Oxyuridoidea are common as well as some flagellate protozoa which are considered commensal. With regards Giardia spp. and Cryptosporidium spp., important for their potential zoonotic transmission, few data are currently available in tortoises. Tortoises act as natural hosts of many species of amoebae (E. turtle, E. testudinis, E. clevelandi etc...) which are considered commensal. However, due to the increasing number of human cases of meningoencephalitis by amoebae of animal origin, there pathogenic role should be taken into consideration. In conclusion, the zoonotic risk in managing T. hermanni should always be considered. In the case of confiscation, in which the mixing of different individuals is possible and the risk of transmission of infectious agents can be high, the management of this animals must be properly evaluated especially when a reintroduction program is planned.

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SURVEY OF ENDOPARASITES IN PET GUINEA PIGS IN ITALY

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Guinea pigs (Cavia porcellus), also known as cavies, are among the oldest domestic animals worldwide. Little information is available on the occurrence of endoparasites in pet guinea pigs (Pantchev et al., 2014) and no data are reported in Italy.

The aim of the present study was to evaluate the prevalence of intestinal parasites in cavies kept as pets in southern Italy.

Fresh fecal samples were collected from 60 pet guinea pigs in the province of Naples, Campania region, southern Italy. Twenty-three of these animals were kept in pet shops whereas 37 were privately owned. All samples collected were processed using the FLOTAC pellet technique (Cringoli et al. 2010) for detection and count of helminthic eggs/larvae and protozoan cysts/oocysts. Two different flotation solutions (FS) were used: FS2 (sodium chloride solution) (specific gravity=1200) and FS7 (zinc sulfate solution) (specific gravity=1350). For the detection of Giardia and Cryptosporidium, the specimens were also analyzed by the Xpect ® Giardia/Cryptosporidium snap test using manufacturer recommendations (Remel Thermo Scientific, Santa Fe Drive, Lenexa, KS, USA).

Intestinal parasites were detected in 19 out of 60 (31.7%; 95% confidence interval=20.6-45.1%) pet guinea pigs as described in Table1. Thirteen animals were from pet shops and six animals were privately owned. In one case, both Eimeria caviae oocysts and Paraspidodera uncinata eggs were found. None of the samples was positive for Cryptosporidium or Giardia using either the FLOTAC or the Xpect ® snap test.

Table1: Prevalences of endoparasites detected in the 60 analyzed pet guinea pig.

Parasite No. positive guinea pig Prevalence (%) 95% Confidence interval Paraspidodera uncinata 8 13.3 6.3-21.5 Nippostrongylus-like 6 10.0 4.1-21.2 Eimeria caviae 6 10.0 4.1-21.2

The results of the present study confirm that: 1)the FLOTAC techniques were effective and accurate to collect preliminary data on the parasitofauna of pet guinea pigs in Italy as already demonstrated for other pet mammals (d'Ovidio et al. 2014a, b; 2) as reported in previous reports pet guinea pigs may harbor several intestinal parasites and therefore highlight the importance of epidemiological research on the occurrence of such parasites in this species. Moreover, although the parasites retrieved in the present survey are not zoonotic, guinea pigs should still be considered potential carriers of pathogens with zoonotic potential.

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VERTICAL TRANSMISSION OF NEOSPORA CANINUM IN WILDLIFE

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Neospora caninum is an important cause of abortion in cattle and vertical transmission has been proved to occur frequently in this species. It has been reported to infect wild mammals with high seroprevalences (i.e. in Ungulates from Northwestern Italy1) but reports of vertical transmission in wildlife is limited to captive animals. Seroprevalence in dogs differs from different areas, but it is higher in dogs who live in rural or wild areas, suggesting wildlife could play a role in the epidemiology of this parasite2. Actually there is little evidence of vertical transmission in wild species even if this transmission route could be important in N.caninum maintenance in both intermediate hosts and within the sylvatic cycle of the parasite.

The aim of this study was to assess the existence of vertical transmission of N. caninum in three wild species: one wild carnivore (red fox) and two wild Ungulates: one ruminant (roe deer) and one omnivore (wild boar).

Skeletal muscle, kidney and central nervous system (CNS) were collected from 61 gravid females (n=17 fox; n=16 wild boar; n=28 roe deer) and from 190 of their fetuses (n=67 fox; n=72 wild boar; n=51 roe deer). A species-specific region of N. caninum DNA was amplified by PCR using primers Np6plus and Np21plus3. Maximum likelihood phylogenetic trees were used to characterize the sequenced isolates.

Twenty-four females (39.34%) tested positive by PCR (6/17 35.29% fox; 6/16 37.50% wild boar; 12/28 42.86% roe deer) while 53 out of the 190 fetuses tested were positive (19/67 28.36% fox; 18/72 25.00% wild boar; 16/51 31.37% roe deer). Fetuses from positive females were significantly more positive than fetuses from negative female (p=4.525-12 OR=11.98) and there was a positive correlation between age of fetuses, in all species, and N. caninum positivity ((p=0.007). Sequences from 19 positive samples have a 100% homology with that reported in rodents from the same area4.

Our results evidence that vertical transmission occurs in wild species such as fox, roe deer and wild boar and that vertical transmission could be an important source of N.caninum maintenance in the sylvatic cycle independently from the excretion of oocysts by definitive hosts that in Europe are represented only by dogs and wolf. The homology of the strains found in wild animals with the ones previously reported from both wild and domestic rodents suggest that overlapping of sylvatic and domestic cycles is likely to occur.

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GASTROINTESTINAL PARASITES IN PSITTACINE BIRDS REARED IN NORTHERN ITALY

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Introduction and aim Gastrointestinal parasites often affect captive psittacine birds (Papini et al. 2012) but lack of data exist about parasitic infection in breeding facilities. Aim of this work was to evaluate prevalence and factors predictive of gastrointestinal parasitism in psittacine bird in breeding facilities in northern Italy. Materials and Methods From October 2013 to February 2014, 12 parrots breeding farm from Milano, Varese, Como and Bergamo provinces (Lombardy region, northern Italy) were tested for gastrointestinal parasites. Tested farm reared 594 psittacine birds (mean parrots per farm: 49.5, min. 15 - max. 104) that were housed in 171 aviaries (mean aviaries per farms: 14.25, min. 5 - max. 40); in each aviary were housed 5.9 birds (min. 1 - max. 16). 35 different species of psittacine birds were reared in tested farms (mean species per farms: 5.92, min. 2 - max. 16). In our survey we sampled 147/171 aviaries, in which were reared 527/594 birds of 29 different species. Pooled fecal samples were collected from the bottom of each aviaries and were tested using centrifugation-flotation technique (flotation solution: NaNO3, s. g.= 1.2). At the moment of sampling, were collected data about: individual features of parrots housed in the sampled cage (reproducer/for selling, age < 1year old/>1 year old, species, use of a score from 1 (low) to 6 (high) to classify economic value of the reared animals; breeding management (inside/outside, antiparasitc treatment in the year before sampling, use of a score from 4 (most frequent) to16 (less frequent) to define frequency of cleaning, number of parrots per cage); and structure of aviaries (partial/complete cover, cages adjacent/separated, grid/litter/soil on the bottom, position of mangers and watering places).To determine factors that could be considered predictors of positivity to gastrointestinal parasitism, all the variables were entered in a multivariate binary logistic regression analysis which was developed by backward elimination until all remaining variables were significant (P<0.05). Reults Out of 147 cages, 76% hosted reproducers and 63% of samples were collected from aviaries with parrots that were older than 12 months. Thirty-four per cent of samples were collected from breeding facilities located outside and 33% of cages hosted parrots that were treated in the year before sampling; 15% of cages hosted one bird, 42% hosted two birds and the remaining cages hosted 3 or more parrots. Partial cover was observed in 15% of cages; 67% of aviaries were adjacent, mangers were internal in 64% and watering places were internal in 66%. On the bottom, 32% of cages presented grid, 55% litter and 13% soil. Capillarids (4%), Spirurida (2%), Strongyles (10%), Ascarids (7%), Coccidia (2%) were detected. Multivariate binary logistic regression analysis showed that factors predictors of positivity to gastrointestinal parasitism of sampled cages were: hosting reproducer, soil on the bottom, breeding facilities located outside and the lack of antiparasitic treatment in the year before sampling. Conclusion Psittacine birds in northern Italy are reared in different conditions: some of this factors affect gastrointestinal parasitism and they should be corrected. Reproducers seems to be a reservoir of gastrointestinal parasites end they should be monitored accurately.

Papini et al. 2012, Scientific World Journal, Article number 253127

AN UMBRIAN FEASIBILITY STUDY ON MONITORING ANTIBIOTIC CONSUMPTION IN DAIRY COW FARMS AND RISK ASSESSMENT REGARDING ANTIBIOTIC RESIDUES AND ANTIBIOTIC RESISTANCE SELECTION

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According to EFSA and CE, monitoring antibiotic consumption in veterinary medicine has to be supplemented by a standardized unit of measures, in order to better evaluate risks of antibiotic resistant bacteria selection, antibiotic residues in food produced by animals and antibiotic improper use. Recently, European Medicines Agency has promoted the use of units of measures already used in human medicine, such as Used Daily Doses (UDD), defined Animal Daily Doses (ADD), number of Used Daily Dose (nUDD), number of prescribed daily dose (nADD) and nADD/lactating cows (nADD/LC) (1, 2). The aim of this study was to evaluate antibiotic consumption in Umbrian dairy cow herds from 2012 till 2013 and to compare UDD and ADD in order to assess the rightness of prescriptions in Umbria and to classify each farm by risk assessment. 30 Umbrian dairy herds, representative of Umbria dairy farms situation, were chosen using a simple random sampling. UDD, ADD, nUDD, nADD and nADD/LC were calculated on veterinary drugs prescription of 2012 and 2013, received by public service veterinarians. UDD/ADD-ratio was calculated to evaluate prescription patterns per record. Ratios between 0.8 and 1.25 were regarded as appropriate, under 0.8 or above 1.25 were considered respectively below recommended dose and above recommended dose. UDD/ADD-ratios were used also to classify each farm by risk assessment in 'high risk' (n. of appropriate prescriptions < 30%), 'medium risk' (n. of appropriate prescriptions 30-50%) and 'low risk' (n. of appropriate prescriptions > 50%) (2). 360 and 277 records referred to 2012 and 2013 respectively were collected. 2566.36 nADD were prescribed in 2012, mainly beta-lactams, aminoglycosides, macrolide and quinolones. 1919.78 nADD were prescribed in 2013, with a reduction of 25.19%. The most prescribed antibiotics are in both 2012 and 2013 penicillins, cephalosporins, aminoglycosides and sulphonamides, as already shown in other European countries (2,3,4). An increase in lincosamides, polypeptides, sulphonamides and rifamycines prescriptions was seen in 2013. As to UDD/ADD-ratios, a reduction of 'below recommended dose' and 'above recommended dose' prescriptions was observed, which testifies the effectiveness of information programs on the rational use of antibiotics issued by the Ministry of Health and by the Umbria region. Analysis of prescription patterns shows a high risk of antibiotic resistance selection and presence of antibiotic residues in food for classes of antibiotic having high percentage of prescription classified as 'belove' or 'above', such as tetracyclines, macrolides, fenicols and sulphonamides. Furthermore it permits to classify farms in 'high' (3/30), 'medium' (12/30) or 'low' (15/30) risk of antibiotic resistance selection and presence of antibiotic residues in food. Despite the evaluation of drug consumption based on prescriptions received by public service veterinarians, is probably underestimated, the reduction of antibiotics prescription in 2013, the increase in appropriate prescriptions and the number of 'low' and 'medium' risk farms witness a prudent and responsible use of antibiotics in Umbrian dairy cow herds.

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γ-IFN TEST IN DETECTION OF MYCOBACTERIUM BOVIS

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Mycobacterium bovis is the causative agent of tuberculosis in cattle. It has an important impact on public health due to zoonotic implication of the disease. M. bovis has, in fact, a wide range of domestic hosts, such as swine, goats, rarely sheep, horses, cats, dogs, and also wild host, such as foxes, rodents, coyotes. Transmission of the disease occurs trough inhalation of droplets aerosol, consumption of infected milk, direct contact with faeces, urine or uterine discharge of infected animals. In livestock pathogen spreads mainly trough infected aerosol released by animals coughing or sneezing. Development of infection depends on virulence and infective dose to which host is exposed and it is most likely to occur when wild and domestic animals live in the same territory or they share the same grazing land. Bovine tuberculosis is a chronic infectious disease characterized by granulomatous lesions and exudative processes with nodules especially in lymph nodes, lungs, intestines, liver, spleen, pleura and peritoneum.

The control of the disease depends on monitoring bovine livestock by intradermal tuberculin test (IDTT), to confirm diagnosis of tuberculosis other tests are used: bacterial culture, INF-γ test, detection of lesions at slaughter (Ministerial Order 14 November 2006), isolation of mycobacteria from organs and lymph nodes. IDTT is a delayed hypersensitivity (Type IV) to intradermal injection of purified protein derivative (PPD) of tuberculin, reaction is positive if animal has a swelling 72 hours later at the site of injection. Gamma interferon assay based on the release of IFN-γ from sensitised lymphocytes during a 16-24-hour incubation period with specific antigen (PPD-tuberculin). It is a quantitative detection carried out with an ELISA using BOVIGAM ® 2G kit. This is a new version of the previous BOVIGAM ® kit, with a faster and easier procedure, that also includes Pokeweed, a mitogen essential control for cell viability.

In 2014 at Veterinary Public Health Institute of Sicily a total of 636 serum samples from 11 livestock were analyzed through BOVIGAM ® 2G. The assay was positive for 77 cattle out of 677. Only 2 cattle farms were completely unscathed from infection. Positive reactions confirmed positive results of IDTT and solved double ones. ELISA showed a higher number of positive samples and it is supposed that IDT is less sensitive. Fifty-six strains were isolated and characterized. Moreover, results were confirmed and strains were typed at the Centro di Referenza Nazionale in Brescia. Ten different spoligotypes were identified. SB0120 was the most present.

BOVIGAM ® 2G test in several cases allowed an early detection of M. bovis and thus it restricted the risk of infection of other animals in the same livestock. This type of assay was also useful as tool to recover breeding previously defined infected. Supporting the results obtained, IFN-γ test is considered by legislature an official assay to help diagnosis of tuberculosis and to predict risk of infection.

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INCREASING TOXICITY OF ENROFLOXACIN ALONG FOUR GENERATIONS OF DAPHNIA MAGNA

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Introduction. Enrofloxacin (EFX) is an antibacterial widely employed in veterinary therapy and prevention [1] as it is approved in many countries also for food producing animals. When released into the environment through the spent litter applied to agricultural fields, EFX and its metabolites steadily bind to the soil particles and can then be transferred to streams through water run-off [2]. Furthermore, EFX may directly reach the inland waters in those countries where its use is extended to aquaculture facilities. Aim. A previous study evidenced a worsening trend of EFX adverse effects on D. magna throughout two generations [3]. In the present study a four generations test was run, in order to verify if the worsening trend persists in the following generations, thus leading to a progressive damage at population level. Indeed, effects on D. magna population may reverberate on the whole aquatic ecosystem as water fleas are principal grazers of algae and primary forage for fish in lentic inland waters [4]. Furthermore, this study aimed to indicate whether EFX exposure could cause heritable damages. Materials and methods. The study was conducted following indication of the OECD Guideline 211 [5] but was extended to four generation. Due to the complexity of the experimental design only one concentration (2 mg L-1), laying in the EC20 95 Results. Mortality rate increased along generations, starting from a 50Conclusion. This study confirms the importance of multigenerational toxicity evaluations in ecotoxicology as a powerful tool for estimating pollutants effects at population level. Further studies are necessary to ascertain whether EFX can affect the cladocerian genome/methylome as already observed with other model organisms [7,8]. This investigation was funded by ex 60

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ANTIMICROBIALS RESISTANCE OF ANIMAL SALMONELLA SPP. ISOLATES

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Salmonella is an important pathogen of economic significance in both humans and animals. The majority of Salmonella infections in humans are self-limiting. In some patients the infection may be more severe and antimicrobial therapy essential, but infections by antimicrobial-resistant Salmonella strains may cause treatment failure. In the last years the emergence and spread of multi-drugs resistant Salmonella enterica strains represent a severe concern worldwide (1). The aim of this work was to evaluate the antimicrobial resistance profile of 176 Salmonella spp. isolates obtained from different animal samples, in particular from swine, poultry and reptiles. Tigecycline was included in the trial to verify the susceptibility of Salmonella to this antimicrobial. Disk diffusion method was carried out with the following antibiotics: Nalidixic Acid, Ciprofloxacin, Enrofloxacin, Ampicillin, Amoxicillin/Clavulanic Acid, Cefotaxime, Cefalotin, Ceftazidime, Gentamycin, Kanamycin, Streptomycin, Amikacin, Tobramycin, Tetracycline, Tigecycline, Sulfonamide, Trimethoprim, Trimethoprim/Sulfamethoxazole, Colistin, Nitrofurantoin, Chloramphenicol, Florfenicol. MIC was evaluated with microdilution method for antibiotics that resulted ineffective against the majority of the tested isolates and for Tigecycline. Disk diffusion method and MIC were performed as suggested by CLSI; the antimicrobial breakpoints for MIC evaluation were the following: Streptomyicin $\geq$ 64 μg/ml, Kanamycin $\geq$ 64 μg/ml, Tetracycline $\geq$ 16 μg/ml and Tigecycline $\geq$ 2 μg/ml (2). Most of the antibiotics tested resulted effective against more than 80% of isolates. In particular 88.64% and 98.30% of isolates resulted susceptible to Enrofloxacin and Amoxicillin/Clavulanic Acid, respectively, two of the most used antibiotic in veterinary medicine. For Nitrofurantoin and Chloramphenicol, the percentages of susceptible isolates were 80.68% and 93.18%, respectively. More than 90% of isolates were susceptible to cephalosporins. Low susceptibility to Kanamycin, Streptomycin, Tetracycline, Tigecycline and Sulfonamide was observed with 48.30%, 82.95%, 68.18%, 81.25% and 75.00% non-susceptible isolates, respectively. One hundred and seventy-one isolates (97.14%) resulted non-susceptible to at least one antibiotic, and a great variability in resistance patterns was observed. All but 1 of the 120 Tetracycline non-susceptible isolates were confirmed as resistant; in particular 42 isolates showed a MIC $\geq$ 1024 μg/ml. For Streptomyicin, 48 of the 146 non-susceptible isolates resulted resistant with MIC test. Fourteen of the 85 non-susceptible isolates for Kanamycin were confirmed as resistant; 11 isolates showed a MIC $\geq$ 2048 μg/ml. As concerns Tigecycline, a new antibiotic, 44 of the 143 non-susceptible isolates showed a MIC value higher than the breakpoint, in particular 24 isolates showed a MIC of $\geq$ 2 μg/ml, 17 isolates of $\geq$ 4 μg/ml and 3 isolates of $\geq$ 8 μg/ml. All isolates resistant to Tigecycline were also resistant to Tetracycline, 32/44 isolates showed a MIC for Tetracycline $\geq$ 1024 μg/ml. Our results confirm that different animal species may be reservoirs of antimicrobial-resistant Salmonella, that could represent a threat for animal and human therapy.

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GASTRO-INTESTINAL PARASITES OF WOLVES (CANIS LUPUS) FROM PIEDMONT, NORTHWESTERN ITALY

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In recent decades, wolf Canis lupus populations which have decreased in the past because of anthropogenic pressure, began to expand recolonizing parts of their natural environments as the Northwest of Italy. The analysis of parasite diversity in wild animals is an indicator of ecosystem health as it reflects conditions of interaction between parasites and hosts, observation of migratory flows and dispersion, links in trophic webs, diet changes, habits and behavior of hosts and the complexity of the structure of ecosystems.This report summarizes the results of the helminthological examination of 42 wolves, found dead in Piedmont, Northwest of Italy between 2004 and 2014.All animals were brought to the University of Turin, where they were necropsied and the stomach and intestines were examined by stereomicroscope, for helminth collection and identification. Cestodes were compressed and fixed in AFA solution (alcohol 70 °GL 92%, acetic acid 3%, formalin 5%), and subsequently stained with Langeron's Carmine. Nematodes were clarified in lacto phenol. All helminths were placed on a glass slide covered with a cover slip for morphological identification.Of the 42 individuals, 36 were parasitized (P=85.71%, CI95% 72.16 - 93.28).Eight species of helminth parasites were found, including two Cestodes (Dipylidiumcaninum and Taenia sp.) and seven Nematodes (Ancylostoma duodenale, Ancylostoma sp., Capillaria sp., Uncinaria stenocephala, Toxocara canis, Toxascaris leonina and Physaloptera sibirica). Taenia sp., and Ancylostoma sp. were the helminths more prevalent, 69.05% and 23.01%, respectively. The cestodes were found mainly with gravid proglottids. Apart for P. sibirica, that was observed for the first time in wolf from western Europe (prevalence 7.14%), all the other species of helminths had already beenreported fromwolf across the continent. Parasite diversity recorded in wolves from the study area is smaller if compared to the results reported by others studies from different geographical areas, probably due to the recent reintroduction of the wolf in the area, Which has not yet allowed the parasitic community to fully re-establish all trophic relationships among the wolf population and the others environmental components.

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EPIDEMIOLOGICAL SURVEY ON ANAPLASMA PHAGOCYTOPHILUM INFECTION IN DOGS IN CENTRAL ITALY: SEROLOGICAL AND BIOMOLECULAR EVIDENCES

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Anaplasma phagocytophilum is an intracellular obligate Gram-negative bacteria, that infects granulocytic neutrophils and eosinophils of several animal species, including humans (Woldehivet, 2010). The epidemiological circuit of the pathogen includes hard ticks (Fam. Ixodidae) as biological vectors, and domestic and wild life mammals as incidental or reservoir hosts. In Italy, the A. phagocytophilum transmission is maintained by tick species Ixodes ricinus. The canids (both free-living and domestic) act as "sentinel" in the epidemiological circuit of the pathogen, playing a very important role in the analysis of risk and spread of the infection in nature (Shaw et al., 2001).

This study aims at conducting a cross-sectional survey to set the prevalence of A. phagocytophilum in local canine population, using serological and biomolecular tools. Moreover, a biomolecular analysis of tick specimens collected in dogs involved in the survey has been performed to achieve a better understanding of the epidemiological circuit of A. phagocytophilum between host and vector.

344 dogs were randomly sampled, and a fact sheet with a detailed anamnestic history has been filled for each of them. Serological tests by IFAT for the detection of IgG antibodies against A. phagocytophilum has been performed on peripheral venous blood obtained from each dog. Furthermore, the buffy coats collected was investigated by biomolecular tools. At the same time, each dog was inspected for the presence of ticks, and specimens of I. ricinus collected were used for biomolecular investigations for the detection of A. phagocytophilum. Univariate analysis has been performed to evaluate risk factors statistically relevant to the serological positivity.

Serum samples of 35 dogs (10.1%) resulted positive for A. phagocytophilum with titers ranging between 1/80 and 1/640; the variables statistically associated ($p< 0.05$) consisted in: age, breed, type of prophylactic measures, habitat, contact with wild animals and travel in endemic areas. The nested PCR for the 16S rRNA gene target performed from buffy coats showed a positivity of 2.9%. I. ricinus was detected in 73 dogs (62.4%); biomolecular investigations conducted on ticks revealed 22 positive samples (10.4%) for A. phagocytophilum. In particular, 4 nymphs (the developmental stage most involved in the transmission of the infection) were found to be infected by A. phagocytophilum. Phylogenetic relationships (assessed using sequences obtained from buffy coats and ticks) showed that our isolates belong to taxa in which also fell strains of European ticks and wild animals.

The similarity between our sequences and isolates from different areas of Europe, suggested that the frequent practice to conduct dogs abroad, especially for hunting, in absence of adequate health controls, encourages the introduction of new bacterial strain, both in domestic and wild environments. The results obtained evidenced that A. phagocytophilum circulates in areas of central Italy, and confirmed the possibility of using the canine population as epidemiological sentinel for the pathogen. In addition, the results suggested that the dog could partly contribute to the transmission of A. phagocytophilum in nature.

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URBAN DOG FECALIZATION AND PARASITIC PREVALENCE IN THE TOWN OF CATANZARO (CALABRIA, ITALY)

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In urban areas dogs faeces are a very important but often undervaluate problem. Several recent researches in different towns have proved that the most important issues are linked to hygenic and sanitary orders. We must not undervaluate the role of dog species because are reservoir for important pathologies and parasitoses, some of which can be transmitted to humans.

Knowledge about the diffusion of parasitoses in dog population in Catanzaro is very poor. Our aim was to evaluate the canine faecal contamination in the city of Catanzaro (Calabria, Italy), and the consequent presence of canine parasitic elements (determined by coprological examinations), with particular regard to those which are potential agents of zoonosis.

The research has been done in ten of the main and popular streets of the town. In each of the ten streets when possible, 6 canine faecal samples were collected for coprological examinations. The total number of samples collected was 62. For every sample we have filled a form containing date, hour, place and macroscopic features. On each faecal sample, copromicroscopic analyses were performed using the FLOTAC dual technique having an analytic sensitivity of 2 eggs/larvae/oocysts/cysts per gram of faeces (EPG/LPG/OPG/CPG). A sodium chloride (FS2, s.g.= 1.20) and a zinc suphateâĂŘbased flotation solution (FS7, s.g.= 1.45) were used.

Our research has pointed out a large diffusion of endoparasitics in the examined samples. All streets were positive for parasitic elements and sixteen (25.8%, 95%CI=15.9%,38.7%) samples among the total (62) were positive for parasitic elements. Eggs of zoonotic parasites as Toxocara canis (8.1%,) Ancylostoma caninum (8.1%), Trichuris vulpis (3.2%) and Strongyloides stercoralis (1.6%) were found, as well as parasitic elements of non-zoonotic parasites as eggs of Toxascaris leonina (3.2%) and oocysts of Isospora canis (12.9%). Considering how things stand it is clear that the problem of canine faecal in urban areas is undervalued in Catanzaro too. In our opinion, if this situation goes on, it could cause some sanitary problems due to a lack of kicking areas suitable both for dogs and for their owners. The risk of zoonotic parasitic helminth cycle fecal-oral route is therefore more real. certainly this study should be extended other monitoring areas of the city but therefore essential that dog owners are informed about the potential risk posed by canine faeces in order to better prevent the transmission of zoonotic diseases.

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PARASITIC INFECTIONS IN COLONY CATS FROM A METROPOLITAN AREA: MARKERS FOR URBAN BIODIVERSITY

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In metropolitan areas, free-ranging domestic cats (Felis catus) living in social groups in colonies represent considerable elements in urban ecosystem. A survey on gastrointestinal and pulmonary parasitic infections in colony cats in Milan (Northern Italy) and their spatial distribution was designed with the aim of define the role of stray cats as markers for biodiversity in urban ecology. From May 2013 to May 2014, during the routine practices of health care, 156 individual fecal samples were collected from cats living in 126 colonies; colonies coordinates and individual data (sex and estimated age) were noted for spatial and statistical purposes. Macroscopic inspection (for cestode proglottids or adult nematodes) and quantitative copromicroscopic analyses by FLOTAC ® dual technique with two flotation solutions (NaNO3, s.g. 1200, and ZnSO4, s.g. 1360) (Cringoli 2006) were performed. The number of eggs/oocyst/first stage bronchopulmonary larvae per gram of feces was calculated. Fecal samples were tested for Giardia duodenalis and Cryptosporidium sp. with a commercial ELISA kit (RIDASCREEN ® Cryptosporidium/Giardia Combi, R-Biopharm). Samples resulted positive for bronchopulmonary larvae were tested by Baermann technique for morphological identification (Gerichter, 1949; Brianti et al., 2014); sedimentation technique (MAFF 1986) was performed to confirm diagnosis of trematodes or pseudophyllid cestodes infection. Statistical analysis on considered risk factors (gender and age) was carried out for each parasitic infection. Spatial analysis was performed calculating for each cat the distance to the nearest urban green areas ($\geq$1000 m2) and the distance to the city center. Parasites were detected in 59 cats (37.8%): intestinal (Toxocara cati=26.3%, Toxascaris leonina=0.6%, Ancylostomatidae=1.9%, Trichuris vulpis=1.3%) and pulmonary nematodes (Aelurostrongylus abstrusus=4.5%, Eucoleus aerophilus=0.6%), intestinal cestodes (Dipylidium caninum=0.6%, Spirometra sp.=0.6%), and intestinal protozoan (Cystoisospora sp.=8.9%, Giardia duodenalis=12.8%, Cryptosporidium spp.=1.3%) were detected. The major part of the infection was monoparasitic (27.5%), while multiparasitism was registered in 10.3% of cats. Parasites with a direct life cycle presented a higher prevalence in comparison to parasites with indirect life cycle (32.7% vs. 5.1%). Neither age nor sex resulted statistically related to any of the infections ($p>0.05$). Considering spatial analysis, any difference resulted statistically significant when compared to the distances from the city center. On the contrary, the infection by A. abstrusus, infection by parasites with indirect life cycle and multiparasitic infections resulted related with a short distance of the colonies to green urban areas. Results in the present survey are consistent to data obtained in stray cats in Europe, usually presenting higher prevalence in comparison to owned cats (Becker et al., 2012; Spada et al., 2013; Zanzani et al., 2014). With a major number of cats living near green areas presenting parasitic infections dependent on intermediate or paratenic hosts, spatial data suggest colony cats as a valuable marker of biodiversity in metropolitan areas.

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AN UPDATE ON KENNEL DOGS AND HELMINTHS INFECTIONS IN THE CAMPANIA REGION

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The endoparasites are a problem of great health importance for both owned and stray dogs, as well as for those hosted in kennels. Intestinal and lung parasites, in fact, are frequently recorded in dogs and can be responsible for severe clinical forms (Riggio et al., 2013). The life in kennel is considered a risk factor for several canine intestinal parasites, especially when there isn't an area for the quarantine for the new arrives and there are an high number of animals. Indeed, living outdoors and with other dogs may represent a great risk of acquiring parasites and may require special consideration (ESCCAP, 2006). Moreover some parasites of dogs are agent of zoonoses, e.g. Toxocara canis, Ancylostoma caninum, Echinococcus spp., Giardia duodenalis and Cryptosporidium spp. and so there is the possibility of infection both for technicians that work with animals and individuals to which the animals are subsequently entrusted (ESCCAP 2006).

The aim of the present cross-sectional copromicroscopic survey was to update the presence and distribution of helminth infections in kennels dogs in the Campania region (southern Italy).

A cross-sectional survey was conducted in 68 kennels distributed on the whole territory of the Campania region. In each kennel 20 boxes were examined; if the boxes were less, all the boxes were collected. Fresh faecal samples were collected from the ground of each box (composite sample) and preserved in formalin 5%. Each faecal sample was then examined using the FLOTAC dual technique, using two flotation solutions on the same faecal composite, namely a sodium chloride based solution (FS2; density = 1.200), and a zinc sulphate based solution (FS7; density = 1.350).

Helminth infections were found in the 100% of the 68 studied kennels as follows:

Helminths No. positive kennels Prevalence(%)95% Confidence interval Trichuris vulpis 63 92.6 82.9-97.3 Ancylostomidae 46 67.6 55.1-78.2 Toxocara canis 52 76.5 64.4-85.6 Toxascaris leonina 13 19.1 10.9-20.8 Crenosoma vulpis 9 13.2 6.6-24.1 Angiostrongylus vasorum 9 13.2 6.6-24.1 Oslerus osleri 2 2.9 0.5-11.2 Dipylidium caninum 34 50.0 37.7-62.3

The findings of the present survey show a high prevalence of helminths (including many zoonotic agents) in kennel dogs from southern Italy despite the regular use of anthelmintic treatments. This situation has important consequences on different issues concerning animal welfare, treatment and control, and public health. Because of failures in individual (use of anthelminthics) and collective (reduction of environmental contamination) preventive measures currently in place for kennel dogs, regular parasitological surveillance, appropriate treatment strategies and high quality standard of hygiene are strongly needed to guarantee the health and welfare of pets, and to enhance the safety of people.

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ENDOCARDITIS IN BLUEFIN TUNA (THUNNUS THYNNUS)

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Although in marine fishes the heart is a common target organ for bacterial, viral and parasitic diseases, little is known about cardiac pathology and, in detail, investigations on diseases involving the heart of Bluefin Tuna (Thunnus thynnus) are scarce. Moreover, in these species valvular and endocardial lesions seem to be less frequent than in other vertebrates.

Aim of this work was to evaluate gross and histopathological lesions of the heart of regularly fished Bluefin Tna earmarked for human consumption, with a special interest in thrombotic endocarditis.

Forty-two hearts of Bluefin Tuna (Thunnus thynnus), aged between 3 and 12 years, fished in the Ligurian Sea in Italy, were referred to the Department of Veterinary Sciences of the University of Turin. Gender was determined by macroscopic observation of the gonads. The age of the animals was estimated by counting the bands of skeletal growth on the first fin rays of the first dorsal fin. Serial sections 1.0 mm thick of the condyle base were obtained, dried for 24 h, observed with a stereo microscope, and the number of rings was counted to assign an estimated age. Cardiac samples were collected directly on boats and stored in 4% buffered formalin for gross and histologic investigations, stained with Haematoxylin-Eosin, Weigert-Van Gieson, Periodic acid-Schiff, Toluidine blue, and Alcian blue PAS. Macroscopical evaluation showed in two cases thickening of the leaflets of bulbo-ventricular valves. Histological examination revealed a thrombotic endocarditis in twelve out of forty-two hearts. Thrombi were detected intimately coated on the endocardial surface and on the valves.. In some cases a necrotic center surrounded by lymphocytes was observed. Valvular lesions showed a focal lymphocytic infiltration, involving the superficial layers of the valve. Only in a limited number of cases (n=8) the inflammation could be observed more deeply the tissue layers. Thrombotic lesions varied from small clumps of blood cells, adherent to injured valvular endocardium, to globular or lance-shaped organized fibrin, surrounded by endothelial cells, and elastic and connectival fibers. In the latter case endothelial cells were not present at the base of the lesions. In two cases thrombotic lesions were also associated with Ichthyophonus spp. granulomas in the valve leaflets.

The findings observed in the hearts of Bluefin Tuna are interesting from the point of view of comparative pathology. In fact, these lesions are morphologically very similar to Lambl's excrescences observed in mammals and humans. Although the lesions observed in our study involve animals phylogenetically distant from humans and other mammals, our findings are in agreement with Magarey (1949), Sinapius (1955), Pomerance (1961) and Guarda et al. (1997) which postulated that the growth of Lambl's excrescences in humans could give rise to thrombi on the surface of the valve, even after minor trauma.

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CANINE CANCER REGISTRY IN UMBRIA REGION: A WEB-BASED INTERACTIVE CANCER REGISTRATION SYSTEM

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Neoplastic disease is the one of the most important cause of morbility and mortality in dog worldwide. Similar to human, cancer registration provide information for estimation of incidence and relative risk factors for carcinogenesis giving data for epidemiological studies. In addition, based on the fact that pets are considered sentinels of environmental cancer risk, data could be utilized for comparative purposes. Canine Cancer Registry (CCR) could provide useful information for evaluation of incidence of different types of cancer in dog in a defined population. While cancer registries in human medicine evolved since the 1940s (1), the cancer veterinary registration systems are much more recent and were reviewed by Brønden (2). In Italy, a limited number of veterinary cancer registry were established and few are still working (3,4). Moreover, almost all of them relate to small geographical areas or provincial areas.

This report presents a web-based system (an integral part of the canine regional demographic registry) that represents the platform in which veterinary practitioners and pathologists communicate. Moreover, this platform serves to the pathologists for the double blind comparison and classification of neoplasm in a complete and automated classification process by the use of International Classification of Diseases for Oncology (ICD-O) including quality control and access to data. The main purpose of this paper was to describe the organization of the regional CCR (Umbria, central Italy), the procedures adopted and to estimate the size and the demographic structure of canine population, pre-requisite to ascertain and interpret prevalence data and to provide accurate estimates of cancer incidence.

In order to estimate the size of the canine regional population the official Canine Demographic Registry established in Umbria region was used as a primary source. The veterinary practitioners that collaborate in the study were contacted and invited to submit all the suspected cancer cases, removed either by surgery or necropsy, at the "Center of Veterinary Pathology for Animal Tumor Registry" established by Regione Umbria in 2013. Only the on line record procedure was adopted and a web-based information system was designed and developed. One of the strengths key of the CCR is its rigorous method of assessing the histological diagnosis in a double-blinded control procedure. Only tumors histologically confirmed by pathologists of to the Centre were coded. The tumors were classified according to the WHO's criteria for canine neoplasms (World Health Organization International Histological Classification of Tumors of Domestic Animals) and coded according to the International Classification of Disease for Oncology System (ICD-O). A double bind control procedure in which each pathologist of the Center conduct a histopathologic evaluation without knowing the previous diagnosis was planned and data were processed by statistical analysis.

The proposed process founded on the web-based system, the blinded histopathological evaluation of neoplastic samples as well as the completeness of canine demographic data, ensures the validity of this regional-based CCR.

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PILOT STUDY TO EVALUATE THE EFFICACY OF MANNAN-OLIGOSACCHARIDES (MOS) IN GOATS NATURALLY WITH CAEV

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Caprine Arthritis Encephalitis Virus (CAEV) belongs to Small Ruminant Lentiviruses (SRLVs) and it is considered one of the major source of economic loss in goat farms worldwide. At present there is no therapy or vaccination available to effectively treat and prevent the disease; for this reason the only way to control the infection is culling the infected animals and replace them with seronegative ones. In endemic areas, the possibility to reduce the morbidity can be considered an option to massive culling. The mannan-oligosaccharides (MOS) are prebiotics obtained from the wall of Saccharomyces cerevisiae. The effects of the diet integration with MOS are the result of their interaction with the gut microorganisms, which are associated to an enhancement of the immune response, stress tolerance and grown attitude of the treated animals, which in complex means a better production performance. In cattle, it has been reported an increased antibody concentration in the colostrum and a minor incidence of diarrhoea in animals fed with a MOS supplementation. The aim of the research was to test the effects of the MOS, in terms of immunity and clinical conditions, in a CAEV naturally infected goat herd. Nineteen animals were included in this study; goats were stratified by age (under and over 6 moths of age) and randomly assigned within strata to one of two dietary treatments (A and B). The two groups included either subclinical and symptomatic subjects. Dietary treatments consisted of a ration A) without and B) with the mannan oligosaccharide (Actigen® Alltech,) and the trial was performed in blind. Animals were weighed, body temperatures were taken, pH and faecal consistency were recorded once a week during the four weeks trial. The blood viral load was assessed by Real time PCR, ELISA tests were used to record oxidative stress parameters (ROMs, SH and BAPs) and cytokines (IL-2, IL-4, 1L-18, TNF-α and IFN-γ) serum concentrations. The same data, collected from subclinically infected and symptomatic animals as well as adults and kids were also recorded and compared. Statistical analyses, using ANOVA with Bonferroni post test and t test, were performed using Graphpad Prism version 6. The results do not support the hypothesis that the MOS could have any effect neither on the weight gain of goat kids, nor on the health parameters of the clinically infected animals. The faecal score and pH were not modified by the MOS supplementation in contrast to what reported in sheep. A statistically different concentration of IL-2 in adult goats treated with MOS compared to untreated ones was observed, but this result was not evidenced in treated kids. The study allowed to collect new information about viral load, cytokine profile and oxidative stress in different age classes and clinical conditions in CAEV infected goats. Statistically different concentrations of IL-18, TNF alpha were found in asymptomatic animals confirming that Th1 immunity contributes to maintain subclinical infections. Diseased animals showed higher proviral DNA copies in blood compared to sub-clinically infected subjects, this result showed to be negatively correlated with serum IL-2 concentrations. Finally, statistically higher concentrations of SH were also recorded in asymptomatic animals suggesting that CAE may result in oxidative stress.

EPIDEMIOLOGICAL SURVEY AND MOLECULAR IDENTIFICATION OF LARVAL ANISAKIS SPP. (NEMATODA: ANISAKIDAE) IN FISH OF THE TYRRHENIAN SEA

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Larval stages of nematodes of the genus Anisakis (Dujardin, 1845) are found in the viscera and musculature of many species of teleost fish that act as intermediate or paratenic hosts, whereas marine mammals are considered as definitive hosts, harbouring the adult stages. Anisakiasis is a fish-borne zoonosis that causes a clinical gastro-intestinal syndrome and a severe allergic reaction, often culminating in anaphylactic shock. Accidental human infections with Anisakis spp. larvae can occur, following the consumption of raw or undercooked seafood dishes such as sushi, sashimi, ceviche and marinated anchovies [1, 2]. Moreover the infection can affect the commercial value of fish and thus represents some economical loss for the fisheries industry.

The present work reports the occurrence of Anisakis spp. larvae in commercial fish species and their identification by molecular tools.

400 anchovies (Engraulis encrasicholus) and 33 mackerels (Scomber scombrus), caught off the Golf of Naples (Tyrrhenian Sea, Italy), were collected and kept at 4°C. All fish were subjected to external inspection, to evaluation of size, to examination of coelomic cavity and of muscular tissue by transilluminator. The larvae recovered were stored in NaCl 0.9% at -20°C. Prevalence (P), mean intensity (mI) and abundance (A) of Anisakis spp. larvae were evaluated. A molecular approach based on Multilocus Electrophoresis and PCR with Wizard Genomic DNA Purification Kit (Promega, Madison, WI) [3] was used to identify a representative pool of Anisakis spp. larvae. The sequences obtained were compared with those deposited in GenBank.

111 on 433 samples (25.64%) resulted positive and 899 larvae morphologically referable to Anisakis spp. were found, mainly localized in coelomic cavity (95%). The anchovies showed a size of 10.2-16 cm with a prevalence significantly higher in samples of size 10-12 cm (25%), with mI and A values equals to 1.863 and 0.469, respectively. The mackerels showed a size of 29.5-38.8 cm and the samples over 32.9 cm showed a heavy larval burden, with the same value of mI and A (21.52). The P referred to mackerels was 45% and the mI was 47.93, while the P referred to anchovies was 24% and the mI was 1.875. The A values were higher in mackerels population than in anchovies (21.78 and 0.45, respectively). A total of 50 Anisakis spp. larvae type I (sensu Berland, 1961) relative to 20 anchovies, and 50 Anisakis spp. larvae type I relative to 4 mackerels was identified by Allozymes Electrophoresis Multilocus. Analyzing a sequence of 629 bp obtained for 18 Anisakis spp. larvae, the result pointed out that all the larvae belong to the type Anisakis pegreffii.

A. pegreffii was confirmed as the prevalent species in fish coming from the Mediterranean Sea [4]. The prevalence obtained in the anchovies (24%) is similar to few data reported in literature [5]. Further study is important to better define the epidemiology of Anisakis spp. in mackerels from the Tyrrhenian Sea.

Acknowledgements

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THE ENZOOTIC BOVINE LEUKOSIS ERADICATION AND SURVEILLANCE PROGRAMMES IN ITALY FROM 2005 TO 2012

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Enzootic bovine leucosis (EBL) is an infectious disease of cattle and other ruminants such as buffaloes. The causative agent is the bovine leukaemia virus (BLV) that belongs to the Retroviridae family. BLV causes malignant lymphoma and lymphosarcoma; most BLV infections remain clinically silent in an aleukaemic state. The European legislation aims to eradicate the disease and prevent its spread through trade involving live animals and products. The EBL national eradication plan (Ministerial Ordinance 358/1996 and subsequent) states that all bovines, aged more than one year from breeding herds, should be serologically (AGID or Elisa) tested twice a year or annually if the herd is considered EBL free. A province is declared EBL free when all the farms in the province have been examined, and at least 99.8% of them show negative results; when all the provinces are negative even the region gains the free status.

The objective of this study was to evaluate EBL eradication and surveillance measures in Italy from 2005 to 2012.

the results of the EBL eradication or surveillance plans, recorded in the period between 2005 and 2012, were obtained from the Ministry of Health.

During the study period, we noted an overall annual decrease from 0.21% in 2005 to 0.08% in 2012 in the herd prevalence rate, from 0.06% in 2005 to 0.04% in 2012 in the herd incidence rate, and from 0.027% in 2005 to 0.015% in 2012 in the animal prevalence rate. Furthermore, the numbers of EBL-free regions were found to increase. One-hundred twenty-three outbreaks were recorded (1 January 2006 to 31 December 2012) in the National Veterinary Information System (SIMAN) on 7 November 2013. Of these, 101 had occurred in southern Italy (Sicilia, Campania), where 31 outbreaks were recorded in animals showing clinical signs, while the rest were diagnosed on the basis of serological tests. An outbreak usually lasted for a few days, but sometimes lasted for weeks. Regions officially recognized as EBL-free areas were found to have their own surveillance plans. Differences in their surveillance plans include the type of sample (serum, milk, or both), age at which the animals should be tested (12 or 24 months), and test frequency of herds (annually, or every 2, 3, 4, 5). The decreasing trend in BLV prevalence observed during the study period is largely attributed to the control measures applied. To eradicate EBL, early identification of the positive herds and early removal of the infected animals from the population is vital. However, the eradication program for EBL is difficult to implement in some Italian areas because of several factors such as incomplete herd registry, geographical location, socio-economic conditions of the region, long time period between identification of suspected cases and notification of an outbreak, and most importantly, the long time period between the notified outbreak and its termination.

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THE CANINE CANCER REGISTRY OF UMBRIA REGION (CENTRAL ITALY): PRELIMINARY DATA

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The occurrence of spontaneous tumors is one of the most important cause of morbility and mortality in dog. Next to human, cancer registration provides information for estimation of incidence and the relative risk factors for carcinogenesis giving data for epidemiological studies. Additionally, dogs are exceptional models of cancer because they have an accelerated aging process compared to humans and, moreover, they naturally develop the same cancer as humans (1). The current and prior veterinary cancer registries, as well as the inactive registries, were reviewed by Brønden (2). In Italy, the first Canine Cancer Registry was set up in the year 2000 in the Local Health Authority of Ivrea, followed by the Animal Tumor Registry (ATR) of Genoa (3) and the ATR of Venice and Vicenza provinces (Veneto Region, north-eastern Italy) (4). The others working ATR have not yet published data.

The aim of our study was to establish a Canine Cancer Registry (CCR) in Umbria Region. Incidence data and relative risk of developing spontaneous cancer in canine species were reported for year 2014.

In order to estimate the size of the canine regional population the official Canine Demographic Registry established in Umbria Region was used as a primary source. Animals data (case history, date of birth, breed, sex, etc.) and tumor data (topography, the stage of tumor, etc.) A double blind control procedure in which two pathologists conduct a histopathologic evaluation without knowing the previous diagnosis was used. Tumors were classified according to the WHO's criteria for canine neoplasms (World Health Organization International Histological Classification of Tumors of Domestic Animals) and coded according to the International Classification of Disease for Oncology System (ICD-O). The incidence rate (IR), the relative risk (RR) and relative confidence interval 95% (CI) were calculated.

A total of 949 dogs were enrolled in this study (76% with tumor diagnosis). 812 tumors were encountered: 45% of neoplasms were benign, while 55The incidence rate for all cancers was 205.43/100,000 (CI 186.77-225.46). The IR for females was 245.72/100,000 (CI 217.08-277.09) and for males 165.01/100,000 (CI 141.66-191.11); the relative risk to develop a spontaneous tumor in female dog was 1.49 (CI 1.23-1.8) higher than male dog (pâL'd'0.05). The age of dogs was grouped into classes, IR for class 0-2 years was 13.88/100,000 (CI 5.89-30.67); for class 3-5 was 66.47/100,000 (CI 46.03-92.89); for class 6-8 was 263.15/100,000 (CI 216.87-316.38); for class 9-11 was 552.37/100,000 (CI 474,83-638,96); for class 12-14 was 423/100,000 (CI 341.41-518.20); for class 15-20 was 77.99/100,000 (CI 45.15-128.28). All classes compared to class 0-2 years were statistically significant; RR of class 9-11 was the higher (RR=37.11; CI 17.44-78.94). The association between the tumor and the malignant breed dogs, was not statistically significant (p=0.2108; RR=1.13; CI 0.93-1.36). IR of purebred dogs was 225.8/100,000 and mongrel dogs was 200/100,000.

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RATING OF FARM MANAGEMENT OF MARCHIGIANA CATTLE BREED IN MARCHE REGION

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The breeding of Marchigiana cattle breed in Marche Region, has undergone major changes in recent years, rising from many small companies, to fewer farms, but with a greater number of cattle. This results in the coexistence of profoundly different management practices. The purpose of this work was to identify management strategies of 36 farms of Marche Region, assessing in particular effectiveness of these practices. A questionnaire was the instrument through which was conducted this investigation. Specifically, it takes into account three main aspects of farm management (Broom, 1991) and is divided in three sections. The first section asks about clinical and health situation of the farm, the second structural and management issues while the last deals with drug management. The collection of informations related to the first two sections was performed by the method of the interview to the farmer, and the last part was compiled by the freelance veterinarian who followed the specific farm. For each question, a score (3= high, 2= medium, 1= low) was assigned based on the importance of the question. The score of the single question is counted when the answer to the question indicates an incorrect practice by the farmer. The sum of the scores of the all questions give a maximum value of 49. Following the freelance's opinion were considered good farm (farms that apply good practices) those with scores <20, intermediate farm those with scores between 20 and 25, companies with several problems those with score>25. Of the 36 farms tested, 15 were good (42%), 15 intermediate (42%) and 6 bad farms (17%). Only 17% of farmers performs diagnostic annual screening, even in absence of symptoms. In the 24 breedings with reproductive disorders, 88% had phenomena of return to heat. 61% of farmers doesn't quarantine remounts purchased outside of their farm. Only 8% of farms ask for advice to a nutritionist. In 42% of cases, calves under 6 months are still tied with a rope. Only one company was authorized for the detention of veterinary drugs. Only in 11% of the breedings, the last three therapies were associated with complete diagnosis for a targeted treatment. The profile that emerges shows how many of the issues enteric, respiratory and reproductive, can be traced to faulty management practices, especially widespread in the smaller stables. Owners are often reticent to turn to professionals who can support them in the etiological diagnosis of the farm diseases. Furthermore breeders consider less important setting of a balanced diet, and this is inevitably reflected in deficiency states that make the animals more susceptible to infections (Amadori et al., 2002).

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DEVELOPMENTS IN TECHNIQUES FOR THE CULTIVATION OF DIENTAMOEBA FRAGILIS FROM PIG FECES

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Gastrointestinal protozoa are an important cause of morbidity and mortality in humans worldwide, and often have an animal reservoir and complex epidemiology that includes environmental transmission. Very little is known about the flagellate Dientamoeba fragilis, despite the high prevalence of infection in humans and the potential association with gastroenteric disease and Irritable Bowel Syndrome. Recent studies have demonstrated the existence of a potential animal reservoir, the pig (1). However, research on the life cycle and genetics of the parasite would benefit from the availability of purified isolates. The aim of this work was to develop a technique to support the growth of D. fragilis from pig feces.

A series of experiments were performed to define conditions that support the in vitro propagation of D. fragilis trophozoites from pig stools. Feces positive by light microscopy were used to compare the growth of trophozoites using (a) Loeffler's slope medium or modified BD medium (2) (b), microaerophilic or anaerobic conditions; and (c) different processing methods, i.e., with or without chilling and pelleting (3). All experiments were carried out at 37Â°C for 48 hrs and short-time cultures were confirmed as D. fragilis -positive using a PCR test. Short-time cultures positive were further subcultured until 5 and 7 days of incubation. Moreover, bacteriological tests were performed to identify the supporting flora in short-time cultures. Finally, D. fragilis positive cultures were tested for the presence of Blastocystis spp. using a PCR test (4).

The Loeffler's slope medium supported the short-time growth of D. fragilis, since 10 out of 15 fecal samples yielded a positive result by PCR, whereas no growth was observed using the modified BD medium. Incubation in anaerobic conditions resulted in a better growth compared to microaerophilic conditions. D. fragilis cultures were confirmed as positive after 3 in vitro passages, corresponding to 7 days of incubation. The processing method did not influence the results, as the 2 methods resulted in the same number of PCR positive samples. After 2 days of incubation in anaerobic conditions, the main bacteria identified belonged to the Streptococcus, Escherichia and Clostridium genera. Blastocystis spp.was found to be a very common contaminant of cultures.

To the best of our knowledge, this is the first report to describe conditions for the short and long-term growth of D. fragilis from pig feces. Experiments to reduce contamination by Blastocystis spp. and to define the minimal bacterial composition required for in vitro growth of D. fragilis are in progress. The availability of stable, long-term cultures will allow further studies on the biology and genetics of this parasite using a variety of techniques, from experimental animal infection to genome analysis.

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RESEARCH ON CANINE FILARIASIS CAUSED BY DIROFILARIA IMMITIS AND DIROFILARIA REPENS IN THE PROVINCE OF COSENZA (CALABRIA, ITALY)

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Dirofilaria infections are vector-borne parasitic infections mainly of dogs and cats and, in Europe, they are caused by D. immitis and D. repens. Many European countries are endemic occur mainly in the southern European countries, but the infection is gradually spreading in other areas considered immune up to now. In Calabria region the climatic conditions are in favour both of the vector and the parasite. So, considering the lacking knoledges on filariasis, we have tried to acquire updated information on the presence and dissemination of this parasites. areas considered immune up to now. area, but the infection is gradually

The aim of this work has been to value the presence in Calabria region of D. immitis and D. repens through an epidemiological research, with diagnostics exams in owned dogs distributed in different altitude above sea level. In a recent studies have indeed proved that dirofilariasis, because of climatic changes is gradually spreading in new areas where the desease had not been diagnosed.

The research has been done on a sample of two hundred dogs with an average age of 4.5 years with any prophylaxis for almost three months, lodged in doghhouses of Calabria, exactly in the province of Cosenza, placed at different altitude above sea level (Site A 250 metres and Site B 850 metres). The dogs have been examined with some taking of blood samples (2 ml of whole blood storaged in test tubed with EDTA) for the following diagnostic exams. The samples, carried in the laboratories at 4°C T, have been processed in the space of 12 hours. For the parassitological enquiry, have been utilized the exame dense drop and an enriched Knott test. Complete blood count (CBC) exams have also been done on all blood samples.

The work showed only the presence of D. immitis (3%) in site A, while in both sites were not found D. repens or other species as Acanthocheilonema reconditum. in Site B is probably referable to the altitude of the place with climate conditions less favourable to the biology of the vector. Eosinophilia and a light monocytosis have been noticed in the positive dogs. These parameters confirm those quoted in literature. Comparing the results of this works with those of others works, it is evident that in Italy the spreading of filariasis is not exclusive of the Po Valley. So, this works should be an input for researchers and veterinaries to insert the filariosis in the differential diagnosis with other dog pathologies.

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ISOLATION OF TRICHOPHYTON MENTAGROPHYTES FROM SCIURUS CAROLINENSIS, A NON-NATIVE SQUIRREL PRESENT IN UMBRIA, CENTRAL ITALY

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Wildlife species play a fundamental role in the diffusion of pathogens to other wild/domestic animals or to humans. In Umbria (Central Italy), the presence of a non-native Eastern grey squirrel (Sciurus carolinensis), an Invasive Alien Species (IAS), is considered a threat to biodiversity, in particular to the conservation of the native European red squirrel (Sciurus vulgaris).

During last three years a Management Plan was carried out, in order to trapping and suppressing S. carolinensis, in respect of animal welfare. After euthanasia, health status of grey squirrels was investigated; particularly, a mycological study was carried out in order to know the role of S. carolinensis as potentially pathogenic fungi carrier (1). It is also provided an additional contribution to the mycological data already obtained from other wild species (hare, fox, coypus) coming from Central Italy (2).

63 apparently healthy grey squirrels were subjected to necroscopy and to different laboratory analysis. For mycological investigations, hairs were collected through the use of sterile brush and inoculated on Dermasel Agar, for specific detection of dermatophytes. Plates were incubated at 26°C and daily observed for 4 weeks. Colonies were isolated and identified through standard protocol (3) and subsequently confirmed with molecular analysis (4).

Trichophyton mentagrophytes has been isolated in 4 specimens (6.3%), belonging to animals without apparent skin lesions. The traditional identification was confirmed by molecular analysis.

T. mentagrophytes is a zoophile dermatophyte, pathogenic for humans and animals. It is frequently transmitted from affected animal to human and it usually causes more severe or reactive lesions of Tinea then Microsporum canis (Tinea capitis or Tinea pedis). Very common in rabbits and other domestic rodents, it is often isolated also from dogs and cats (5). The presence of the non-native squirrel in urban areas, such as parks and gardens, and its extreme confident behavior, lead us to consider S. carolinensis an important carrier of this zoonotic agent, as demonstrated by its isolation from the specimens examined. Therefore a constant monitoring of its health status is a very important public health measure.

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OUTBREAK OF DERMATOPHYTOSIS BY MICROSPORUM CANIS IN A SANITARY KENNEL

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Dermatophytosis are superficial mycoses of keratinized tissues, involving humans and animals and their livestock. Different species, members of the genera Microsporum and Trichophyton, are responsible of dermatophytosis in domestic animals (1). Direct or indirect transmission occurs from animal to animal and from animal to human via infected hairs or contact with surfaces contaminated by spores. Dermatophytes have considerable zoonotic importance because man be infected by the same species isolated in animals. Facilities with high numbers of animals, such as kennels, can be source of contamination either animals or people working in the place.

In this paper, a recent outbreak of dermatophytosis by Microsporum (M.) canis, occurred during autumnal season in dogs housed in a sanitary kennel of Umbria region, is described.

20 dogs housed in a single building (as reserved area for particularly situations, as young and/or old subjects, pregnant bitches and convalescent animals) were submitted to sanitary control. The animals lived in single boxes (with 2-5 subjects). Except a Rhodesian ridgeback female, with her 8 puppies, the remaining animals, not purebred, were represented by 4 puppies of about 2 months old and by 7 adult (4-9 years old). All puppies showed typical skin lesions (mainly scattered on face, ears and neck), attributable to dermatophytosis. Hair and scales samples were collected from all animals by brushing and placed on Mycosel Agar and on Sabouraud Dextrose Agar, incubated at 26ÂřC for 3-4 weeks and daily observed. Colonies were identified through standard protocol (2) and then confirmed by PCR (3, 4). All hair samples were also directly processed by PCR, according to literature (4).

9 dogs were positive for M. canis (45%): of these, 5 (3 Rhodesian and 2 not purebred) showed skin lesions, while 4 were clinically negative (carrier). Colonies grew in about 5-10 days, in purity (with the macro and microscopic features typical of the species), were all confirmed by PCR as M. canis. PCR on hair samples was positive for dermatophyte only in 3 subjects (15%) of which 2 were Rhodesian puppies with lesions.

These data permitted some considerations: 1) M. canis, despite the cat represents the typical fungal reservoir, showed remarkable adaptability to dog and rapid capacity of colonization, probably due to the high environmental contamination and to the favourable climatic conditions (temperature and humidity); 2) positivity were found in immunologically predisposed dogs (puppies and animals previously affected by other diseases and/or stressful conditions); 3) a daily cleaning and the use of environmental disinfectants have been always performed but remarkable is the fungal resistance; therefore it is important in kennels an accurate animal and environmental monitoring to prevent spore transmission to animals and also to humans (volunteers, vets, citizens who show interest in the adoption); 4) further studies are needed to evaluate the sensibility of molecular analysis on hair samples (important tool to reduce response times and set an appropriate therapy).

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COMPARISON BETWEEN API SYSTEM AND PCR FOR THE IDENTIFICATION OF CLOSTRIDIUM SPECIES IN FARM ANIMALS

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Clostridia include a heterogeneous group of environmental anaerobic bacteria that are generally found in soil and in the intestinal tract of humans and other animals. Among the large number of Clostridium species (about 150 so far described), only 15 produce potent protein toxins responsible for severe diseases in man and animals (Popoffand Bouvet, 2013).These anaerobic organisms are usually identified by using the API system, but sometimes the results of these analyses are ambiguous.

The aim of our study was to compare the API system with PCR methods for the diagnosis of intestinal clostridiosis in farm animals.

In this study we focused on 46 animals (33 cattle, 11 rabbits, 1 sheepand1 goat) affected by sudden dead syndrome from Cuneo province (Italy). Necroscopies and sampling of all animals were performed within 4 hours after death. Intestinal samples were subjected to histological analyses; the identification of Clostridium spp. was performed with API system and PCR following bacterial culture.

Necroscopies revealed acute enteritis in all animals. Bacterial culture and subsequent identification with API system showed a positivity in 4 animals for Clostridium spp., 26 for C. perfringens, 12 for C. sordelli, and 1 for C. bifermentas.In three cases the cause of death was attributable to mycoplasma (2 cases) and syncytial virus (1 case).All the samples were also tested with PCR: in 65% of cases the results disagreed with API system identification.

A necroscopy associated with a prompt sampling, and bacterial cultures are necessary steps to identify clostridial disease. According to our results, API system, as diagnostic tool for Clostridiums pp. identification, doesn't appear completely reliable, as indicated by the high discrepancy between API system classification and the PCR results. Conversely, PCR seems to be more consistent, especially for Clostridium typing.However, the detection of clostridia alone is not indicative for a disease status, since they are generally found also in a healthy intestinal tract. On the contrary, the detection of the Clostridium toxins in tissues, e.g. through immunohistochemistry, is considered more useful to diagnose a clostridial disease.

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FORAGING ECOLOGY AND VEGETATION CHARACTERISTIC OF SITES INHABITED BY WILD POPULATIONS OF TESTUDO HERMANNI IN ITALY: A STUDY PREPARATORY TO AN APPROPRIATE RELOCATION IN NATURE OF CONFISCATED INDIVIDUALS

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Testudo hermanni is a tortoises distributed in Southern Europe. In Italy, wild populations are mainly found throughout the Peninsula, Sardinia and Sicily, occurring in Mediterranean coastal and hilly natural/semi-natural habitats or cultivated areas with very low agricultural pressure. T. hermanni is a protected species included in the Habitat Directive, Bern Convention and the Italian IUCN Red List. The species is threatened by habitat loss and fragmentation, as well as by translocation and illegal capture and trade. It is not uncommon that such animals are released after illegal detention. To relocate healthy animals in appropriate eco-ethological sites, food availability and dietary adaptation to the wild habitat are some of the fundamental aspects to be taken into consideration first. Studies concerning the foraging ecology of T. hermanni report that the species is primarily herbivorous; however its diet can include mushrooms, small invertebrates, even carrion, excrements, and bones. Three sites (Apulia, Tuscany and Sardinia) inhabited by wild populations of T. hermanni where sampled and plant communities were determined. The fecal micro-histological analysis was used to evaluate diet composition. The geographic characteristics of the study sites showed macroscopic differences in habitat features; however all areas own to the typical Mediterranean ecosystem characterized by a rich habitat mosaic which allows the presence of grassland and garrigue, where the tortoises can feed, as well as macchia and Quercus ilex thicket where they usually hide. About 20% of the plant species were common to the three areas. This percentage raises slightly when comparing only the edible species and doubles when the comparison is made at the genus level, which suggests phenomena of food vicariance and lowers the level of dissimilarity between sites. Preliminary results on the diet composition confirm that T. hermanni is primarily herbivore. Fabaceae species (Trifolium, Medicago and Vicia) were the most actively selected. Other selected families, in order of presence, were: Plantaginaceae (Plantago), Rubiaceae (Rubia), Smilacaceae(Smilax), Caprifoliaceae(Knautia), Poaceae (Melica, Stipa, Vulpia), Brassicaceae (Lobularia), Rosaceae (Rubus), Ericaceae (Arbutus), Oleaceae (Phillyrea), Fagaceae (Quercus), Asteraceae, Araliaceae, Lamiaceae (Teucrium) and Cistaceae (Cistus). Cistaceae, Lamiaceae and Poaceae yielded the highest negative selection index. Seeds of Knautia integrifolia and fruits of Phillyrea angustifolia, ingested before the winter hibernation, were found in some faecal samples. In addition, mushrooms, moss and gastropods' shell fragments were observed. In conclusion, like most terrestrial chelonians, foraging activity of T. hermanni is

mainly addressed to herbaceous species. The dietary dominance by Fabaceae species might be related to the high protein and calcium content, besides their digestibility when ingested at the initial phenological stage. The finding of seeds and fruits in the diet could support the hypothesis that terrestrial tortoises have a role in the distribution and germination of plant species.

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SURVEILLANCE AND CONTROL OF CANINE LEISHMANIOSIS IN THE MARCHE REGION (ITALY): FIRST DATA REPORT

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Canine leishmaniosis (CanL), a vector borne zoonotic disease transmitted by sandflies, is a public health concern in many countries of the world. In Italy CanL is endemic even if in the Marche Region the epidemiological data are scant. Recently, in this area, new foci of infection were found in kennels where a peridomestic cycle in stray dogs has been suspected. In effect, mobility of infected and asymptomatic dogs may spread new foci of infection, if a capable density of vectors is reached. Since 2013 in the Marche Region a Surveillance and Control Plan is mandatory for kennels. The main activity of the Plan is the control of the reservoir, with the aims to decrease the incidence in kennels and to provide health guarantees for the adopted dogs. All dogs, in fact, were controlled by serological test at first admission in kennel and when transferred or adopted by citizens. The dogs found infect were protected from vector bites.

- All dogs hosted in kennels are univocally identified by microchip and registered in the Regional Database;
- Clinical examination are performed at least yearly for the whole population before the vector season;
- Diagnosis, disease staging and treatment of sick animals are performed by Official Veterinarians as indicated in the International Guidelines;
- Samples are sent to the Laboratory of Istituto Zooprofilattico Sperimentale Umbria e Marche (IZS-UM) with an anamnestic form where one of alternative options of sampling can be selected: 1- surveillance, 2-transfer, 3- adoption, 4- disease suspect.
- The reference test in the Plan is the Indirect Fluorescent Antibody test (IFAT) produced by National Reference Center for Leishmaniosis (CRENAL, IZS of SICILY) and performed at IZS-UM as recommended by OIE;
- Cut-off for positive sera was set at 1:160 in agreement with the epidemiological status of the Region. Dogs with a title of 1:40/1:80 are considered doubtful (and re-tested 2-4 months later) as recommended by National Guidelines (Istituto Superiore di Sanità, Roma).
- Positive dogs (IFAT >1:160) are considered infective. Positive cases without clinical symptoms are further analyzed with Real Time PCR, protidogram, or for kidney and liver functionality.

YEAR: 2013 / 2014
DOGS IN KENNELS: 4259 / 3976
ANALYZED (%): 2037 (47.8%) / 1599 (40.2%)
IFAT $\geq$ 1:160 : 78 / 81
PREVALENCE : 3.83 % (IC: 3.29-4.46) / 5.07% (IC: 4.30-5.94)

During two years of surveillance, a total of 2037 and 1599 dogs in 37 kennels were tested in 2013 and 2014 respectively. Prevalence data as reported in the table, are obtained by randomly sampling of different beings (options of sampling: 1-2-3). The values reported confirm the ipo-endemic status of the Marche

Region, in contrast with the iper- endemic status of the Southern Regions and the sporadic cases documented in the north of Italy. Early diagnosis and protection of infect dogs from vector bites, are core activities for the Plan as well as treatment of sick dogs. In fact, in kennels where a wider amount of vector is found, the risk of disease spreading is higher. Preventive actions in order to cut down the host-parasite cycle are provided by the Plan. This is the expected result for the veterinary public service in the coming years.

MONITORING ANTIBIOTIC RESISTANCE IN SALMONELLA SPP. ESCHERICHIA COLI AND ENTEROCOCCUS SPP. THROUGH THE UMBRIAN ECOSYSTEM ANIMAL-FOOD-MAN IN 2014

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Use of antibiotics is linked to the emergence of antimicrobial resistance (AR), one of the most serious cause of concern worldwide. Programmes to monitor the occurrence of AR among animals, food and humans are strongly recommended and should be implemented both worldwide and locally(1-2).Our aim was to define the occurrence of AR in 3 species of bacteria isolated in Umbria from humans, food and animals: Salmonella spp., Escherichia coli and Enterococcus spp. Salmonella spp. (258 strains), E.coli (125 strains) and Enterococcus spp. (76 strains) were isolated. Salmonella spp. were isolated from human (151-Umbrian hospitalized patients), animal (29-bovine(B):3, swine(SW):1, ovine(O):5, avian(A):9, others(Ot):11) and food (78-meat/meat products(M/MP):72, Ot:6) and serotyped according to Kaufmann-White-Le Minor. E.coli strains were isolated from animals (43-B:14, SW:13, O:2, A:8, lagomorphs:6) and food (82-M/MP:44, carcass sponge:14, dairy products(DP):19, Ot:5) and Enterococcus spp. only from food (M/MP:46, DP:26, Ot:4). E.coli and Enterococcus spp. were isolated from matrices sampled for official controls and/or self-control plans. Antimicrobial susceptibility was tested in vitro using Kirby-Bauer method and standard CLSI VET01-A4/S2. Ampicillin (10μg-AMP), cefotaxime (30μg-CTX), chloramphenicol (30μg), ciprofloxacin (5μg-CIP), gentamicin (10μg-CN), kanamycin (30μg-K), nalidixic acid (30μg-NA), streptomycin (10μg-S), sulfonamide (300μg-S3), tetracycline (30μg-T), trimethoprim+sulfamethoxazole (1.25-23.75μg) and cephalothin (30μg-KF) were assayed for Salmonella (human); colistin (10μg-CT), amoxicillin+clavulanic acid (20/10μg-AMC), enrofloxacin (5μg) and ceftazidime (30μg-CAZ) were added for Salmonella (animals+food). The latter panel + florfenicol (30μg) and + erythromycin (15μg), nitrofurantoin (300μg), penicillin G (10UI), rifampicin (30μg) and vancomycin (30μg) were assayed respectively for E. coli and Enterococcus spp. Strains showing resistances against more than 3 antimicrobials were considered multi-resistant. Software R vs 3.1.0 was used for statistical analysis. S.Typhimurium, and its monophasic variant (4,5,12:i-) was the most isolated serovar from human and food and it showed the high number of resistances. Swine and pork products were the most frequently contaminated. Resistant strains are 64% (Salmonella spp.), 62% (E.coli) and 100% (Enterococcus spp.) Multiresistant strains are Salmonella spp.:47%, E.coli:38% and Enterococcus spp.:100%.Some resistance patterns (S.Infantis i.e.) are maintained between strains isolated from humans and food, showing the transition within the food chain. The 3 bacteria species show the high number of resistances against S3,T and S, similar to which is observed in Europe (1) and this result could testify Enterococcus spp. role transmitting resistance genes through germs(3). Salmonella spp. and E.coli showed high percentage of AR to AMP and AMC. Enterococcus spp. resulted to be susceptible to these molecules and extremely resistant to CAZ, CTX, KF, CT, CN, K and NA(2). Salmonella spp. showed low levels of resistance to CIP, CN and NA; this is very important, considering that these antimicrobials are widely used in human medicine(1). Our results reflect the situation of AR pointed out in Europe and underline the transmission of AR through bacteria in the food chain.

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AFRICAN SWINE FEVER VIRUS IN SARDINIA: AN UPDATE ON MOLECULAR CHARACTERIZATION

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African swine fever (ASF) is a highly contagious hemorrhagic fever of domestic and wild suids. ASF has been present in Sardinia since 1978 and is still endemic although control and eradication plans. African Swine Fever Virus (ASFV) is a large icosahedral DNA arbovirus, the only member of Asfarviridae family. The ASFV genome consists of a linear dsDNA of about 170 to 193 Kbp in length. Many of the length variations are in the multigene families (MGF), while smaller length variation are associated with the number of tandem repeat sequences (TRS). Sequence analysis of p72 (B646L), p54 (E183L) genes and Central Variable Region (CVR) has been used to perform molecular epidemiological investigations. Genotyping of ASFV isolates by partial sequencing of the B646L gene identified 22 genotypes (1). A previous study based on the combined p72, p54 and CVR approach, showed that the Sardinian strains have high similarity between them and clustered into ASFV p72 and p54 genotype Ia (2).

A further differentiation of so closely related strains requires the analysis of other molecular markers. For this purpose we selected three ASFV regions, CP204L, I73R/I329L and EP402R to better discriminate between old and recent Sardinian isolates collected in the years 1978-2014.

ASFV isolation was performed on primary swine leukocyte cultures using homogenized tissues from PCR positive naturally infected domestic pigs and wild boars. Viral DNA was extracted from infected cell culture. The PCR amplicons of the CP204L (p30), 173/I29L and EP402R (CD2-like protein) regions were purified and used in cycle sequencing reactions. Purified products were run on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The nucleotide sequences of 45 Sardinian isolates were edited and analysed by BioEdit Sequence Alignment Editor.

Comparative analysis shows that CP204L and I73R/I329L regions are 100% identical in all viruses, however we found a variation in the number of the aminoacid hexamer repeats in the EP402R gene. Particularly, in the oldest viruses (1978-1990) the analysis revealed the presence of 9 repeats (PPPKCP), with the insertion of the motif SPPKCP; an additional hexamer presents one aminoacidic substitution (PPSKPC) like the international strains Benin 97 and E70. The viruses collected in the years 1991-2014 have all the same sequence, but show 8 PPPKCP repeats instead of 9. Just one isolate shows one insertion of two repeats. These results, even though confirming a remarkable genetic stability of ASFV, show that a genetic variability exists among ASFVs circulating in Sardinia and such data are very important for studying the ASFV evolution.

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HEPATITIS E VIRUS: FIRST DESCRIPTION IN A PET HOUSE RABBIT. A NEW TRANSMISSION ROUTE FOR HUMAN?

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Hepatitis E virus (HEV), the aetiological agent of hepatitis E, belongs to the family Hepeviridae which is divided into the genera Orthohepevirus (all mammalian and avian HEV isolates) and Piscihepevirus (cutthroat trout virus). Strains of HEV were recently identified from farmed and wild rabbits from USA, Mongolia and France (1), although HEV prevalence and epidemiological knowledge in these countries remain unknown. We identified for the first time HEV in a pet house rabbit

An adult 7 years old female of domestic rabbit (Oryctolagus cuniculus) was referred for the necropsy to the Department of Veterinary Sciences of the University of Turin, due to sudden death. The dead animal and another domestic rabbit were regularly vaccinated for RHDV and MV. They had no contact with pigs or wild boars; rabbit diet consisted of hay (a self production in organic farm), vegetables, fruit and pineapple juice. Rotavirus, RHDV and MV were considered and investigated by indirect immunofluorescence (MV) and by PCR (RHDV and Rotavirus). RNA from liver and whole blood samples was tested by a one-step real-time RT PCR assay, targeting a highly conserved region of ORF3 according to Jothikumar et al. (2). The 5' region of the ORF2 gene, coding for the putative capsid polypeptide, was selected for phylogenetic analysis.

Although necropsy and laboratory investigation revealed that HEV was not the cause of death, as a Pasteurella spp. was isolated from lungs, we report the first detection of HEV in a pet house rabbit. Most notably, the resulting phylogenetic tree showed that the HEV strain identified was closely related to the human TLS-18516 strain and group together into an isolated monophyletic branch. This finding reawakens concerns regarding the zoonotic risk represented by HEV in animals and expands to house rabbit the spectrum of potential source of infection for humans. In Italy, no data were available about circulation of HEV both in farmed and pet house rabbits. In north-western Italy, HEV has been demonstrated to circulate in swine and wild boar populations in previous investigations by our group. On the basis of collected anamnestic data, the rabbit may have become infected with HEV in several ways; consumption of contaminated domestic food (vegetables and fruits) as well as contaminated water may have represented a transmission route. Nevertheless, hay provided to pet owner by an organic farm could also be take into account as primary source of infection. Another hypothesis is that, as the rabbit lived in an external environment (garden) only in the summer season, it might have come in direct contact with infected wild rabbits, which are known to be a potential reservoir of HEV circulation. Awareness by veterinary practitioners and, above all, the knowledge of good hygiene practice for the pet owners are crucial in the prevention of zoonotic diseases since our data proved that also domestic rabbit should be considered in the epidemiological scenario of HEV infection.

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DETECTION OF BOVINE VIRAL DIARRHEA VIRUS GENOTYPE 1 (BVDV-1) FROM ALPACA FETUSES (VICUGNA PACOS) IN SOUTHERN ITALY

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Bovine viral diarrhea virus (BVDV) is a member of the Pestivirus genus that includes Border Diseases virus of sheep and Classical Swine Fever in swine. BVDV infection in camelids was reported in llamas (Llama glama) and alpacas (Vicugna pacos) in association with respiratory diseases, abortion, illthrift, diarrhea and neurological diseases (1-5).

In this study we describe the detection and genetic characterization of BVDV in fetuses from alpaca herd with a history of abortion.

The herd was situated in the Sicily Region. The animals were not in contact with other ruminants. From 2009 until today, 15 episodes of abortion occurred in pregnant animals between 1 and 6 months of gestation. The fetuses were submitted to a full necropsy, bacteriological and virological examinations were also conducted. BVDV was isolated on MDBK cell monolayers from placenta, spleen and liver homogenates. Reverse transcription, sequencing, and polymerase chain reaction (PCR) assay targeting a 288-base pair (bp) region of the 5'-UTR were performed on positive BVDV samples.

At necropsy no gross abnormalities were observed in fetuses. In 2 cases, acute enteritis, pulmonary edema and pericardial effusion were observed. A non-specific bacterial flora was detected by standard bacterial examinations. Four fetuses resulted BVDV positive at virological investigations. PCR products of the expected size were obtained from spleen and placenta from 2 fetuses and used for sequencing. The phylogenetic analysis indicated that they belonged to the genetic subtypes 1d and 1e. BVDV-1d had a nucleotide similarity ranging from 92,0% to 96,7% with the other sequences of the same group while BVDV-1e showed an intragroup identity of 88,0-92,0%. This is the first evidence of BVDV circulation in an alpaca herd from Italy. In other countries, BVDV-1a, 1b, 1i and sporadically BVDV-2 have been evidenced in camelids (6). Because this farm is not epidemiologically related by common trade practices with other farms, we cannot exclude that BVDV circulated in the area prior to our study. It will be necessary carry out further, more extensive surveys to obtain a better picture regarding the distribution of these BVDV genotypes to clarify the epidemiological pattern in this geographical area. Currently there are no BVDV vaccines licensed for use in camelids thus, as for other infectious diseases, good farm biosecurity measures are essential, associated with quarantine of the animals after the purchase from other alpaca herds.

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THE PREVALENCE OF INTESTINAL HELMINTHIC INFECTIONS IN WILD BOAR KILLED DURING THE HUNTING SEASON IN 2013-2014 IN THE CATANZARO PROVINCE (CALABRIA, ITALY)

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Wild boar (Sus scrofa) in Calabria has lately had a strong increase in population, also due to the presence of S. scrofa imported from abroad on the Italian territory such as S. scrofa raiseri and in some cases mix with pig characterized by both a greater reproduction and greater sizes. These factors have caused the disappearance of the autochthonous species S. scrofa majori linked to an increasing population definitely superior to the necessary to assure a natural biological balance. From several researches of other countries, it emerges that wild boar has an important epidemiological role, because it is like a reservoir for many disease and parasites also transmissible to other species of zootechnical interest.

Knowledge about diffusion of helminth in wildness wild boar population in Calabria is very poor or absent. The aim of our research is therefore the acquisition of data, in the province of Catanzaro, about the prevalence of helminth in wild boar, taken through copromicroscopic exams.

Our research has been done on a group of 60 wild boar killed during the 2013-2014 hunting season and homogeneous for place and hunting period. The animals came from a geographical area of about 215 Km2 situated in catanzarese Sila and Presila. The research has foreseen an individual faeces collection, carried out during the evisceration. To point out parasitic elements we have used FLOTAC dual technique with 2 EPG/OPG/LPG sensitivity. For the research and the count of parasitic elements, we have used one solution flotation on the basis of NaCl (s.g.1.200) and for the heavy elements on the basis of ZnSO4 (s.g.1.350).

Our research, has pointed out a large diffusion of helminth in Sus scrofa living in the province of Catanzaro. The most widespread helminth are the Ascaris suum 90%, they are followed by gastrointestinal strongyles 83%; Tichuris suis 38%; Strongyloides ransomi 15%; Dicrocoelium dendriticum 3%. Our results, except for A. suum and T. suis line up with the ones of other researches made in Italy. In our opinion, the clear prevalence of A. suum and T. suis could depend on the breeding method in our area where there is still both a wild and half wild practice. Among the inspected animals exactly in 10% there is the presence of mix with pig. The parasitological context emerged from our research, could have sanitary effects on wild and half-wild cattle breeding farms of the autochthone races of our area such as the black pig of Calabria. We think it is right to carry out some strategies both for a sanitary monitoring and a demography control of wild boar, in order to protect both wild species and domestic ones in Calabria.

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Part III

Scienze cliniche - Clinica medica

FROM FELINE BLOOD DONORS TO WHOLE BLOOD UNITS: CHANGES IN SELECTED HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

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As demand for feline transfusion services increases the characterization and pre-donation examination of feline blood donors to ensure the quality and safety of blood units becomes evermore important.

Aim of the study is to investigate changes in selected hematological and biochemical parameters during the transfer of blood from the feline blood donor to a whole blood unit. It was hypothesized that hematological parameters would not change significantly from donor to unit but that significant changes in biochemical parameters would occur, due to the effects of the anticoagulant.

With owner consent 27 feline blood donors (15 male and 12 female) were enrolled in this study. Procedures for blood donation were as previously described [1]. Blood (10 ml/kg body weight to a maximum of 60 ml/cat) was collected from the jugular vein in three 20 ml syringes containing citrate-phospate-dextrose-adenine1 (CPDA1) anticoagulant with an anticoagulant:blood ratio of 1:7. Selected hematological and biochemical parameters (complete blood cell count, % hemolysis, glucose, sodium, potassium) were evaluated in the blood donor before blood donation and in the corresponding donated blood unit within 24 hours of preparation. Distribution of the data was assessed with Kolmogorov-Smirnov test, differences between parameters in the blood donor and blood unit were tested with a t-test or Wilcoxon test according to whether or not data was normally distributed. Significance was set at $P<0.05$.

Significant decreases occurred between blood donor and blood unit in RBC count (mean donor RBC count 7512 x 103/μL, mean unit RBC count 6448 x 103/μL, $P<0.0001$, mean difference -1064 x 103/μL), HB concentration (mean donor Hb concentration 10.6 g/dl, mean unit Hb concentration 8.9 g/dl, $P<0.0001$, mean difference -1.6 g/dl), PCV (mean donor PCV 30.7%, mean unit PCV 26.1%, $P<0.0001$, mean difference -4.6%), RDW (mean donor RDW 17.6%, mean unit RDW 16.7%, $P=0.0003$, mean difference -0.9%), WBC count (mean donor WBC count 7136/μL, mean unit WBC count 4886/μL, $P<0.0001$, mean difference -2168/μL) and potassium concentration (mean donor concentration 4.5 mmol/l , mean unit potassium concentration 3.1 mmol/l, $P<0.0001$, mean difference -1.4 mmol/l). Significant increases occurred between blood donor and blood unit in glucose concentrations (glucose donor concentration 92 mg/dl, unit glucose concentration 549 mg/dl, $P<0.0001$, mean difference +458 mg/dl) and in sodium concentrations (donor sodium concentration 158 mmol/l, unit sodium concentration 178 mmol/l, $P<0.0001$, mean difference +19.6 mmol/l). Mean values of RBC, HB, PCV, glucose, sodium and potassium in the blood units were outside the normal feline reference range for these parameters. Contrary to our hypothesis, when using a donation protocol with intravenous fluid administration mid-way through the donation and a blood collection protocol with a CPDA:blood ratio of 1:7, , there were significant changes in both the hematological and biochemical characteristics between the blood donors and blood units. Despite these changes the feline whole blood units obtained were still deemed suitable for transfusion purpose in anemic patients.

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DETECTION OF BACTERIAL CONTAMINATION AND DNA QUANTIFICATION IN CANINE AND FELINE STORED BLOOD UNITS.

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Blood transfusions in veterinary medicine have become increasingly more common and are now an integral part of lifesaving and advanced treatment in small and large animals (1).Several guidelines suggest what infectious agents to screen for in canine and feline transfusion medicine (2,3).While the risk of bacterial contamination of blood products during collection, processing, storage and administration is not considered in veterinary medicine, it has emerged as a cause of morbidity and mortality in human transfusion medicine.

The purpose of this report is to describe the detection and quantification procedures applied in four cases of bacterial contamination of canine and feline blood units, which suggest the need of further investigative studies and monitoring to optimize patients'safety in veterinary transfusion medicine.

Four red blood cell units which showed a color change were included in the present report. The first one (case A) was collected from a 3-year-old female mongrel dog from a Blood Bank; the second one (case B) from a 7-year-old male mongrel dog, the third one (case C) from a 3-year-old male mongrel dog, and the last one (case D) from a 7-year-old male European cat, from another Blood Bank. The blood was collected from healthy animals according to the guidelines and immediately refrigerated in a blood-storage refrigerator at 4 °C, where it can be stored up to 40 days.The massive visible color changes were noted at day 31 of storage in case A, at day 20 in cases B and C and day 32 in case D. These units were removed from the Blood Bank for further investigations. Microscopic evaluation of a smear from each of the blood bags revealed heavy bacterial contamination.DNA was isolated from each of the blood bags and bacterial DNA load per sample was assessed by qPCR modifying Nadkarni et al procedure(4). The bacterial Genome Equivalent number (GE/mL of template) was 1.18 x107 GE/mL in case A, 3.64 x107 GE/mL in case B, 8.38 x107 GE/mL in case C and 5.22 x108 GE/mL in D. PCR products were purified and after alignments in the EMBL GenBank database, the sequences matched perfectly with Serratia liquefaciens in A, Pseudomonas putida in B and C, and Pseudomonas fluorescens in D. CONCLUSIONS: The current study showed that bacterial contamination was present and with high bacterial DNA load. These findings confirm data of human transfusion medicine. Instead, when reviewing the veterinary literature, actual reports of bacterial contamination of blood bags are remarkably rare and many post-transfusion reactions could probably be misdiagnosed or overlooked. Since thousands of blood transfusions are performed each year on dogs and cats and the demand for blood products continues to grow (5), the present report emphasizes the importance of carefully designed protocols to prevent bacterial contamination of blood collected for transfusion and to optimize patients' safety in veterinary transfusion medicine.

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CANINE STORED WHOLE BLOOD UNITS: WHICH IS THE REAL EXTENT OF BACTERIAL CONTAMINATION RISK?

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Actual reports of bacterial contamination of blood units has emerged as a cause of morbidity and mortality in human transfusion medicine, but they are remarkably rare in veterinary literature (1-3).The results would be that many post-transfusion reactions could probably be misdiagnosed or overlooked.Furthermore, an underestimation of the extent of the risk of the bacterial contamination of blood units during collection, processing, storage and administration could be expected (1,2). This study aims to detect and quantify bacterial microorganisms in 49 canine whole blood (WB) units during their shelf-life and to estimate the bacterial contamination rate during collection, processing and storage of blood units. The blood was collected from healthy animals according to the guidelines and immediately refrigerated at 4 °C, where it can be stored up to 35 days. Eight sterile samples from tubing segments containing 500ul of WB from each units were tested for bacterial culture (Blood agar, MacConkey agar and Mannitol Salt agar) on days 0 (T0), 1(T*), 7 (T1), 14 (T2), 21(T3), 28 (T4), 35 (T5), 42 (T6). Moreover, after DNA extraction, a real-time qPCR assay was performed on T0, T3, T5 according to Nakardini et al. procedure3. PCR products were purified and subjected to direct sequencing. Obtained sequences were submitted to a BLAST analysis with the GenBank reference database to reveal the amplification source genera. On bacterial culture, 47/49 (96%) samples were negative for all the time points. One sample was positive for Enterococcus spp at T0 and at T* and 1 for E.coli at T5. After the PCR analysis, 26/49 (53%) samples were positive in at least one time point. Sequences were assigned to Propionobacterium spp. (n=6), Corynebacterium spp. (n=2), Caulobacter spp. (n=4), Hypomicorbium spp. (n=1), Pseudomonas spp. (n=6), Enterococcus spp. (n=2), Serratia spp. (n=2) and Leucobacter spp. (n=2). The unique E.coli growth as a contaminant by laboratory techniques since the same sample resulted negative to PCR assay. In one case both blood culture and PCR assay identified Enterococcus spp. and the species of bacteria was the same for both the assays. The process of bacterial culture is slow, as there is need for the microorganism to grow and reach an appreciable number of cells, and a low quantity of bacteria can also not be detected with bacterial culture. That could explain the significant difference between culture methods and molecular detection. Most of the organisms detected by qPCR assay tend to be skin-associated organisms or widespread bacteria (soil and water) not usually implicated in transfusion reactions1. With qPCR assay, bacterial load varied from 4 to 80 Equivalent Genome (GE)/mmc, and we can state that bacterial contamination of blood units resulted very low. Moreover, these results could include the detection of dead or degraded bacterial DNA. Possible mechanisms of bacterial contamination included: donor bacteriemia, contamination during the WB collection procedure, contamination of the collection pack and contamination during the blood processing procedure. We emphasize the importance of carefully designed protocols to prevent bacterial contamination of blood units to optimize patients' safety in veterinary transfusion medicine.

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HAEMOSTATIC PROFILE IN CANINE MULTIPLE MYELOMA: A COHORT STUDY IN 210 DOGS

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Human patients with MM have short survival times associated with frequent complications such as thrombosis.1 Considering their life span, dogs with MM in comparison to humans, have a longer survival times. Hypercoagulable complications in canine MM are not known and prognostic factors linked to haemostasis have been not thoroughly investigated.2

Aim of the study: a) to describe the haemostatic profile in dogs with MM at presentation, b) to assess whether coagulation parameters have a prognostic value, and c) to detect a possible hypercoagulable state.

Haemostatic abnormalities in dogs with MM (Group 1, #70) were evaluated via search of the electronic data-base (P.O.A System Plus 9.0 ®) of the San Marco Veterinary Clinic, between 2002-2015. Dogs included in Group 1 met the criteria: bone marrow plasmacytosis (plasmacells $\geq$15%), osteolytic lesions, serum mono-biclonal gammopathy. All groups had a haemostatic panel taken at presentation. Two groups of dogs matched for age, breed, and sex were enrolled as case-control: healthy dogs (Group 2,#70) and dogs affected by various diseases (Group 3,#70). The analytes investigated were: Platelet count (PLT), activated Partial Thromboplastin Time (aPTT), Prothrombin Time (PT), Fibrinogen, Thrombin Time (TT), Fibrin-Fibrinogen Degradation Products (FPDs), D-Dimer and Antithrombin (AT). In addition, within the MM-dogs the haemostatic profile between bleeding (B-MM, #42) and non-bleeding (NB-MM, #28) dogs was evaluated. Statistical differences between groups was evaluated by Kruskal-Wallis test and post-test analysis were performed by Wilcoxon-Mann-Whitney. Risk to death within B-MM and NB-MM dogs was evaluated by Pearson's X2 test. ROC curves were used to identify the best analyte to predict death. The significance level for all statistical test was set at $p<0.05$.

aPTT, PT and TT were significantly increased in Group 1 compared to Groups 2 and 3. PLT count and AT concentrations were significantly decreased in Group 1 compared to Group 2 and 3. Fibrinogen concentration was significantly decreased in Group 1 compare to Group 3, while no difference was present between Group 1 and 2. No difference were present between Groups 1 versus Group 2 and 3 for FDPs and D-dimer. PLT count and AT concentration were significantly decreased in B-MM compared to NB-MM; aPTT and PT were significantly increased in B-MM compared to NB-MM; finally, no differences between B-MM and NB-MM were present for TT, FDPs, D-Dimer. B-MM dogs showed lower mortality rate in respect to NB-MM patient ($p<0.028$). AT resulted the best haemostatic analyte in predicting death in dogs affected with MM ($p<0.04$; AUC 64%; 95% CI 0.50-0.78).

Primary and secondary haemostasis are highly compromised in dogs affected by MM while tertiary haemostasis appears to be not altered, suggesting that a hypercoagulable state, opposite to humans, is unlikely in dogs with MM. Surprisingly, in dogs with MM bleeding seems to have a protective effect against death. The best haemostatic assay to predict the mortality in canine MM at 90 days after the diagnosis is the AT.

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EFFECT OF REPEATED ARTHROCENTESIS ON CYTOLOGIC ANALYSIS OF SYNOVIAL FLUID IN HEALTHY HORSES

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Arthrocentesis is a common practice used for the diagnosis and therapy of many articular pathologies. The aim of the present work was to evaluate the influence of repeated arthrocentesis on synovial fluid composition in healthy horses.

Approval to conduct this study was obtained from the Ethics Committee on Animal Experimentation of the University of Pisa (n. 14875 - 20/11/12). Four horses not affected by muscoloskeletal diseases were submitted to repeated arthrocentesis of both the intercarpal (IC) joints. In particular, the right IC joint was sampled at Time 0 (T0), at 2 (T2), 7 (T7) days and then every week for 3 times (T14, T21, T28, respectively), while the left IC joint was sampled at T0 and then every 10 days for two times (T10 and T20, respectively). An arthrocentesis was also performed on both IC joints 60 days (T60) after T0. Arthrocentesis were always performed by the same operator. The synovial fluid samples were collected in EDTA and processed within 1 hours to evaluate: 1) total protein (TP) concentration by a refractometer (1); 2) total WBC count by an automatic hematology analyzer (Lasercyte® Idexx, USA) with hyaluronidase pretreatment (2,3) to reduce the viscosity; 3) differential leukocyte count after cytospin preparation (1500 gpm, 5') (Cytofuge 2, StatSpin, USA) to improve smear's quality (3), with a modified Romanowsky staining (Diff Quik® Dade Spa, Milano, Italia) and microscope evaluation at 100X. Data distribution was performed with KS test; Anova for repeated measures and Bonferroni test as post hoc were applied to verify differences related to sampling times. Significative level was set at $p<0.05$.

Data were expressed as mean$\pm$standard deviation. The total WBC count did not change over time as well as the percentage of lymphocytes, eosinophils and neutrophils, while a variation over time was observed for clasmatocytes and total protein concentrations. Both decreased from T0 to T14 (right IC joint) and T10 (left IC joint), then rose again over time till T60 with values similar to T0.

Our results are in line to what reported in dogs and cows (4-8) and suggest that repeated arthrocentesis has no effects on cytological analysis of synovial fluid.

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LONG-TERM PRESERVATION OF URINE SEDIMENT WITH FORMALDEHYDE: DOES IT WORK?

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Microscopic examination of the urine is an invaluable technique in the diagnosis of the urinary tract diseases. A practical difficulty is the need to observe a freshly collected urine specimen, since with delay the urine sediment deteriorates, rendering diagnosis impossible (1). This causes problems in teaching diagnostic urine microscopy, since representative samples cannot be stored without preservative and fixatives, that prevent degenerative changes in urine cells and structures (1,2,3,4). Aim of this study was to evaluate the preservation of structures and cellular elements present in urine sediment by the addition of formaldehyde. Thirty-six samples of pathological urine sediments from dogs (n=22), cats (n=13) and horse (=1) were included. Urine sediment was examined within one hour to collection (T0), added with 4% formaldehyde in water and stored at 4°C. Microscopic analysis on stored urine was performed at 24 hours (T1), 48 hours (T2), one week (T3), two weeks (T4), one month (T5) from collection; at T5 samples were centrifuged twice and reconstituted with saline solution. The urine sediment parameters included white blood cells (WBCs, normal value: <3/hpf), red blood cells (RBCs, normal value <3/hpf), epithelial cells and casts (normal value: absent or few), crystals, sperms and bacteria (described as absent or present). After storage WBCs (dog n=12, cat n=3) were well preserved, while RBCs (dog n=7, cat n=9) were reduced in 18% of samples (1 dog ad 2 cats) at T4, except for one cat (T2); occasionally they appeared shrunk and pale as described in literature (2). Crystals (dog n=7, cat n=3) disappeared at T1 in 80% of samples: they were all struvite; the addition of formaldehyde preserved the bilirubin (n=1) and the calcium carbonate crystals (n=1), and the amorphous calcium phosphates (n=1). Urine casts (dog n=6, cat n=1) were not preserved in 2 canine samples (28%), both at T1. Squamous and transitional epithelial cells (dog n=5, cat n=3) were reduced in 38% of cases, at different time (T1 and T2 in dog, T5 in cat). No differences were observed neither for sperms (dog n=5, cat n=1) nor for bacteria (dog n=7, cat n=3). In conclusion, in urine diagnostic microscopy we obtained good preservation results with the addition of formaldehyde to urine sediment, providing samples whose characteristics are similar to those observed in fresh urine specimens. Urine particles and cells could be adequately preserved for teaching diagnostic microscopy for at least one month. Furthermore, for routine urinalysis the storage of samples with formaldehyde at 4°C could offers the possibility of postponing the timing of sediment analysis.

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PLASMA FREE METANEPHRINE AND FREE NORMETANEPHRINE IN DOGS (PRELIMINARY RESULTS)

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In humans, detection of increased plasmatic concentrations of metanephrines is the gold standard test for pheochromocytoma. Pheochromocytoma is a catecholamine-producing tumor derived from chromaffin cells in the adrenal medulla that yields vague and nonspecific clinical signs. Because of this, over 50% of pheochromocytomas in dog are an accidental finding during ultrasonographic evaluation or necropsy. Adrenal gland ultrasonography has been indicated as a useful clinical screening modality to evaluate pheochromocytomas in dogs (Besso et al.1997), but definitive diagnosis of pheochromocytoma in the dog currently relies on adrenal tissue histopathology. Only one study, with a small sample size, described the diagnostic accuracy of plasma free-metanephrines measurements in the dog (Gostelow et al. 2013). The aim of this study was to determine the diagnostic accuracy of plasma free-metanephrines concentration for canine pheochromocytoma.

Sixteen dogs were selected for inclusion and underwent blood collection and abdominal ultrasonographic evaluation followed by histopathological evaluation, either post-mortem or after adrenalectomy. Plasma free-metanephrines concentration, metanephrine (fMN) and normetanephrine (fNMN), were evaluated by LCMS/MC (liquid chromatography-tandem mass spectrometry). Abdominal ultrasound evaluated dimensions, shape, echogenicity, distinction between adrenal cortex and medulla together with number, dimension and location of focal lesions. Samples were divided into three groups on the basis of histological results: dogs with non-adrenal diseases, dogs with adrenocortical tumors and dogs with pheochromocytomas. Dogs were classified as healthy (control group) based on normal physical examination, serum biochemical results, abdominal ultrasonographic evaluation and the absence of owner-reported clinical signs.

Of sixteen dogs included in the study, seven presented adrenocortical tumors, 3 non-adrenal diseases, 5 were healthy and 1 was diagnosed with pheochromocytoma. Seven adrenocortical tumors were diagnosed histologically and identified as carcinomas. The dog with pheochromocytoma was bilaterally affected and presented local invasion of the caudal vena cava with tumor thrombus phenomena. Dogs with non-adrenal diseases presented respiratory disease or splenic and testicular malignant neoplasms. Ultrasonographic median diameter was 3.2 cm (range 1.6-4.8 cm) for adrenocortical tumors and 7.0 cm for pheochromocytoma. Plasma fNMN concentration was consistently higher in the dog with pheochromocytoma than in all other considered groups. Free NMN concentration in pheochromocytoma showed no overlap with values measured in other groups than was shown for fMN concentration. Plasma fMN and fNMN measurement by LCMS/MS appears to be an accurate method of identifying affected dog and is minimally invasive. Pheochromocytoma is a rare occurrence in dogs. A larger sample size would be needed for accurate and statistically significant comparisons.

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EVALUATION OF HEMOSTASIS IN PIEMONTESE NEW BORN CALVES BY MEANS OF THROMBOELASTOMETER

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Developement of hemostatic system is different among mammals. In some species, hemostasis is relatively immature at birth and remains so for the first few weeks of life.(1) Studies conducted in newborn calves reveal the efficiency of coagulation system with small differences compared to adult cattle (1). Thromboelastometric analysis provide in vitro assessment of global coagulation and detects the viscoelastic properties of whole blood from beginning of clot formation to fibrinolysis.(2)

The aim of this study was to evaluate the hemostasis system in Piemontese newborn calves during the first weeks of life by means of thromboelastometer (ROTEM).

Fifteen Piemontese newborn calves clinically healthy according to physical examination and complete blood count (CBC) were enrolled after colostrum intake. Calves that were born premature, by caesarean section or dystocial, were excluded. Samples of whole blood were obtained from each calves within 24 hours of life (T0) and subsequently, after 8 and 15 days of birth (T8, T15) by jugular venepuncture. Coagulation was analysed by means of standard coagulation profile [prothrombine time (PT), activated partial thromboplastin time (aPTT) and fibrinogen] and ROTEM analysis by in-TEM, ex-TEM and fib-TEM profile. For each profile was assessed: clotting time ([CT],s); clot formation time ([CFT],s); maximum clot firmness ([MCF],mm); α angle (α,°). Analysis of variance for repeated measures, whether variables were normally distributed otherwise Friedman's nonparametric two-way analysis of variance, was used to assess the differences among T0, T8, T24. Statistical significance was set P $\leq$0.05 and data were expressed as median (minimum-maximum).

Thromboelastometric analysis has shown statistical significant differences at T0 compared to T8 and T15 only in the ex-TEM and fib-TEM profiles. At T0, in the ex-TEM profile, the CFT [T0:73(55-83); T8:79(68-85); T15:79(58-85)] was prolonged (T0vsT8, P=0.001; T0vsT15 P=0.004) and the α angle [T0:71(66-74); T8:76(67-152); T15:74(67-83)] less wide (T0vsT8 P=0.001; T0vsT15 P=0.003), and in the fib-TEM profile the MCF [T0:24(19-39); T8:43(24-57); T15:32(23-38)] was reduced (T0vsT8 P=0.001; T0vsT15 P=0.020) compared to other times evaluated. Standard coagulation profile has shown a prolonged aPTT at T0 [42(31-52)] compared with T8 [33(28-45)] (P=0.0001) and T15 [32(25-36)] (P=0.0001); fibrinogen concentration was significantly low only when T0 [291(174-455)] was compared with T8 [413(233-627] (P=0.009). Results obtained in this study, related to standard coagulation profile, agree with literature, which reported the efficiency of hemostatic system in newborn calves (1). In this study fibrinogen level, according to Gentry et al (1994), has shown at birth an initial lower concentration, followed by an increase at T8 and a decrease at T15 (not statistically significant). Thromboelastometric differences coud be related on low fibrinogen concentration at birth. Indeed, in the ex-TEM profile CFT and α angle are affected by platelet number and fibrinogen.(2) In the fib-TEM profile platelet function was inhibited, then only fibrinogen level influence the MCF (2).

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A POSSIBLE TREMORGENIC MYCOTOXICOSIS BY ROQUEFORTINE C IN A BOVINE HERD

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A total of 15 beef cows and calves were referred for history of neurological signs. The animals (12/15 Chianina breed, 3/15 Limousine) were grazing in 300 ha area, fed with grass and hay. Inspection of the hay reveled macroscopic alterations, consisting of diffuse and heavy mold contamination of many hay bales. Due to the not cooperative attitude, the animals were only visually examined in the field; the neurological signs observed were ataxia, intentional head tremors and muscle twitching. Only 3 calves with severe neurological signs were housed in a medication area and underwent a complete clinical exam. All 3 calves showed intentional head tremors and muscle twitching; 1/3 presented severe ataxia and stiffness gait, while 2/3 calves were recumbent and unable to rise. The most important clinical data were: hyperthermia, tachypnea, tachycardia and long capillary refill time. The neurological examination showed deficits of V and VII cranial nerves. Calves could swallow, but they were unable to grab the food. Based on history and clinical examination the following differential diagnoses were considered: tremorgenic mycotoxicosis, nervous ketosis, nervous BVD form, BHV1-5, Listeriosis and WMD. Blood samples were collected for CBC count and biochemistry panel (TP, urea, creatinine, total and direct bilirubin, GGT, AST, CPK, Mg, Se and vit E), urinalysis was performed for ketone bodies. Calves were also tested for infectious diseases (Listeriosis, BVD, BHV 1-5). Multiple samples of altered hay were analyzed for mycotoxins and hay balls were removed in all animals' stock. The grazing animals recovered spontaneously within 1 week along with 2/3 hospitalized calves, while 1/3 calf was euthanized due to poor general conditions. CBC, biochemistry panel, vit E and oligo-minerals resulted within normal ranges and no positivity for infectious agents were detected. Food analysis showed high concentrations of roquefortine C (RC): 345 μg/kg DM. Presence of RC in livestock food is highly reported, in particular in visibly moldy areas (1). RC intoxication causes anorexia, paralysis and several reports attribute it neurotoxic properties (2). In mice experimental intoxications induced muscle contractions, ataxia, prostration and intermittent seizures. RC intoxication, resembling penitrem A (PA) intoxication, has been reported in dogs (3). Moreover, RC is considered a sensitive biomarker for PA exposure (3). PA is a tremorgenic fungal toxin which intoxication causes ataxia, tachypnea, and sustained tremors (4). The pathophysiological mechanism by which mycotoxins affect the CNS is unknown but the biochemical lesions are reversible. Diagnosis is based on the clinical signs, demonstration of the mycotoxins in the feed and identification of the fungal elements in blood and feces. Affected animals recover completely when they are removed from infected pastures (5). Based on neurological signs, recovery after altered food removing and results of food analysis, the diagnosis of tremorgenic intoxication was hypothesized. Limits of this report are: lack of PA dosage in the food and lack of RC and PA evaluation in blood and feces of affected animals.

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PANCREATITIS AND ACUTE KIDNEY INJURY (AKI): RETROSPECTIVE OBSERVATION OF 41 DOGS WITH AKI MANAGED BY HEMODIALYSIS

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Acute Kidney Injury (AKI) is a severe disease associated with a sudden onset of renal parenchymal injury most typically characterized by generalized failure. AKI may to be severe and culminated with the requirement for renal replacement therapy (RRT) or death. Acute pancreatitis is a potentially reversible condition, but severe disease that can cause systemic and local complications and recognized more commonly as an etiology as well as a complication of AKI. Aim With this retrospective study we investigate the role of pancreatitis in dogs with AKI managed by hemodialysis (HD), and we evaluate how this morbidity influences patients' outcome. Material and methods This study includes 41 dogs, managed by intermittent hemodialysis (IHD), with anamnestic, clinical, imaging and laboratory findings of AKI or AKI/CKD. All Dogs were divided into two groups: 1) patients affected by AKI and Pancreatitis (n=13) and 2) dogs affected by only AKI (n=28). Diagnosis of pancreatitis was established by physical examination, diagnostic imaging findings and measurement of pancreatic lipase concentration in serum dog (cPLI ®). We excluded patients with positivity of cPLI ® but negative abdominal ultrasound. We consider laboratory findings of all dogs at moment of presentation. Data were statistically analyzed using GraphPad Prism ® for Mac. Result Dogs with pancreatitis were 31,7% (13/41) of all subject; patients with pancreatitis that died were 84,6% (11/13) while only 25% (7/28) of the dogs without pancreatitis and managed with HD had worse outcome. T-Test unpaired showed not significant difference in the concentrations of creatinine (p=0.668), phosphorus (p=0.511), albumin (p=0.496), cholesterol (p=0.197), and ionic calcium (p=0.751) between two groups at presentation. The quantitive proteinuria (UP/UC) was evaluated with t-test unpaired between two groups and wasn't statistically significant (p=0.293). Chi Square test instead showed a significant difference (p=0.016) between number of dogs who survived or died in relation to the presence of pancreatitis. The test was also evaluated in relation with the presence of disseminated intravascular coagulation (DIC) and wasn't statistically significant. Conclusion Dogs with AKI and affected by pancreatitis had a worse outcome than patients without pancreatitis, but at presentation there weren't any significant difference between two groups in hematologic parameters. Pancreatitis is reported in veterinary medicine as common complication in renal failure and in human medicine is documented that risk of acute pancreatitis in patients on long-term hemodialysis is significantly high. We haven't also showed any correlation with presence of pancreatitis and CID.

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TRANSITIONAL CELL CARCINOMA IN A HORSE: CLINICAL PRESENTATION, DIAGNOSTIC PROTOCOL AND OUTCOME

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Bladder tumors are uncommon in the equine clinical settings. Presentation can be variable, but affected horses usually show various degrees of hematuria, and sometimes stranguria. Prognosis is guarded, and non steroidal anti-inflammatory (NSAID) drugs could be used to treat or at least control clinical signs.

Aim of the study is to describe the clinical presentation, diagnostic protocol and outcome of a case of transitional cell carcinoma in a horse.

A 13-year-old warmblood gelding was admitted with hematuria lasting for six months; antibiotic treatment was initiated, without improvement. At admission the clinical examination was unremarkable, but on rectal palpation it was possible to detect a mass cranial to the pelvic bones, that on ultrasound and endoscopy appeared to involve the bladder: it measured more than 8cm in diameter and was located on the ventral wall of the organ, its surface presented areas of necrosis and fibrin deposits; biopsy samples were collected during endoscopy and a diagnosis of transitional cell carcinoma was made according to histopathology; COX-2 immunohistochemistry on neoplastic cells was negative. Blood work was within normal ranges but urinalysis detected blood. Abdominal and thoracic ultrasound, abdominal fluid and laparoscopy showed no signs of metastatic spreading. Therapy was initiated with antibiotics (Marbofloxacin, 2mg/kg IV SID) and NSAIDs (firocoxib, 0,1mg/kg IV SID). After two months, the horse was clinically worsened, and rectal findings and cystoscopy confirmed that the mass was enlarged. The owner decided for euthanasia. At necropsy, almost 90% of the urinary bladder mucosa was replaced by a poorly demarcated mass, with an irregular and often ulcerated surface, that was spreading dorsally and cranially, massively infiltrating the pelvic muscle and circumferentially surrounding but not compressing ureters. Two whitish nodules with central necrosis, about 10 cm in diameter, were noted in the left caudal lobe of the lung. At histopathologic examination, the urinary mass as well as the mass that invaded the pelvic muscles and the lung nodules were all characterized by a densely cellular, non encapsulated, poorly demarcated and markedly infiltrative growing mass. Neoplastic cells were arranged in cords, lobules and trabeculae, showed a moderate anisocytosis and anisokaryosis and a high mitotic rate. Squamous differentiation and intravascular embolization of neoplastic cells were multifocally observed.

Transitional cell carcinoma is a rare tumor in the horse, but it should be considered in differential diagnoses for hematuria and stranguria. A complete clinical and laboratory examination is warranted, to have a definitive prognosis. Use of COX-2 selective NSAIDs has been advocated in some cases of carcinoma in both human and veterinary medicine, with good results, alone or together with the removal of the primary tumor. To confirm the effectiveness of the NSAIDs therapy, immunohistochemistry to detect the expression of COX-2 receptors by neoplastic cells should be performed: a negative result warrants a poor prognosis, and, especially with large tumors, should call for euthanasia.

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CLINICAL AND HEMATOLOGICAL ADVERSE EFFECTS AFTER ADMINISTRATION OF TWO DIFFERENT CHEMOTHERAPY PROTOCOLS IN DOGS AFFECTED BY LYMPHOMA

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Lymphoma is the most common canine hemopoietic neoplastic disorder. Chemotherapy can prolong the survival time of patients, even if Adverse Effects (AEs) may occur. The rate and severity of common AEs of two chemotherapy protocols were investigated.

Medical records (2007-2014) of 24 dogs with multicentric lymphoma were reviewed. Thirteen dogs were treated with COP protocol (cyclophosphamide, vincristine, and prednisone) at the Veterinary Teaching Hospital, while 11 were treated with Wisconsin-Madison (UW) protocol (cyclophosphamide, vincristine, prednisone and l-asparaginase) at a Private Veterinary Clinic. The appearance of fever, weight loss, vomiting, diarrhea, anorexia, anemia, neutropenia, and thrombocytopenia, during the first nine weeks of therapy were evaluated. The occurrence of dogs showing different severity of AEs weekly (AEsW) or during the entire period of treatment (AEsT), were collected. Each AE was classified into 6 grades using VCOG criteria and 101 and 99 observations (dogs treated with COP and UW, respectively) were carried out. Results underwent statistical analysis (Fisher's exact test for AEsW and Chi Square tests for AEsT).

In UW-treated dogs anemia of grade 2 at 4th week was statistically significant in comparison to COP-treated dogs ($p= 0.01$). Other AEs during the several weeks of treatment were not statistically significant. The comparison between the severity of AEs during the entire period showed the following results. Fever was an uncommon sign (23% COP-treated vs. 36% UW-treated) while weight loss was detected in the early weeks of treatment especially in COP patients (62% COP vs. 55% UW; $p=0.0019$). Gastrointestinal AEs were common but rarely affected the patient quality of life (vomiting 46% COP vs. 27% UW; diarrhea 23% COP vs. 36% UW; anorexia 46% COP vs. 55% UW) ($p> 0.05$). The common hematological AEs were anemia (85% COP vs. 82% UW; $p> 0.05$) and neutropenia (85% COP vs. 55% UW; $p=0.0018$) compared to thrombocytopenia (38% COP vs 45% UW; $p> 0.05$).

This retrospective survey pointed out some interesting findings. Fever was not associated to neutropenia, probably because UW-treated patients have never showed severe neutropenia and in COP-treated dogs antibiotics were preventively administered. Both protocols caused severe weight loss during the first weeks of treatment (grade 2 or higher); in COP-treated patients there was a significant weight loss. The gastrointestinal AEs were mild and cases of severe vomiting or diarrhea (grade 3 or higher) were not observed. Vincristine causes more gastrointestinal and hematological AEs; vincristine/cyclophosphamide combination could be the trigger of neutropenia that has been reported more frequently and severe in COP-treated cases. Regarding anemia and thrombocytopenia, the possible myelo-suppressive effect of drugs used could worsen the clinical condition of some patients (dogs with stage V lymphoma and bone marrow involvement); for these two AEs a correct etiology is difficult to trace. Chemotherapy drugs used in the present study for the canine lymphoma treatment are well tolerated, they prolong the survival time and assure a good quality of life with low frequency and severity of AEs.

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PREVALENCE, LESION DISTRIBUTION AND RISK FACTORS FOR EQUINE GASTRIC ULCER SYNDROME IN PLEASURE HORSES IN ITALY: PRELIMINARY RESULTS

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Equine Gastric Ulcer Syndrome (EGUS) is a multifactorial disease of the horse most common in animals subjected to a supposed high level of stress, especially exercise-related. Between 90 and 100% of race horses develop gastric lesions during their career. In the last few years the prevalence of the disease in categories of horses considered less susceptible has been studied. It appears that 40% of Polish pleasure horses without clinical signs and 70% of thoroughbred mares at pasture can be affected by the disease.

To evaluate the presence of gastric ulcers in pleasure horses in Italy and identify possible risk factors.

134 adult horses, aged between 2 and 27 years underwent gastric endoscopy. The exam was performed as described in the literature, using a portable processor (Gastropack, Karl Storz, Germany) and a 3m scope (Karl Storz, Germany). Videos were recorded and stored. Lesions were graded according to Equine Gastric Ulcer Council grading system and the owners were questioned about the management of the animal (stable vs paddock, number, type and quality of feeding, exercise level, colic episodes, etc): the answers and the grading of each part of the stomach were recorded in a database for statistical analysis. Routine descriptive statistics were used. Individual risk factors were analyzed by Odds Ratio calculated in 2 x 2 contingency tables.

Our population was characterized by a high percentage of saddlebred horses (57%) and Thoroughbreds and related breeds (40%); almost equal number of animals aged between 1 and 10 years (49%) and between 11 and 20 years (43%); females outnumbered males and geldings (59.7%, 14.18% and 26.12%, respectively). Lesions were present on the pars esophagea in almost 30% of the horses (17.9% grade 1, 9% grade 2, 1.5% grade 3, 0.7% grade 4) and on the margo plicatus in almost the 27% (8.2% grade 1, 14.9% grade 2, 3.7% grade 3); the pars glandularis was not affected in all animals. Gasterophilus larvae were found in 46.3% of the horses. Possibile risk factors identified were horse breed (56.3% of Thoroughbred type horses had gastric ulcers) and temperament (25% of hot-blooded horses had lesions), not having access to a paddock (45.8% with EGUS), having mild/recurrent colic sings (10.4%), and having lived with the owner for less than 6 months (39.6%). The presence of Gasterophilus larvae seems to be an added risk factor.

Our study is the first one in Italy that takes into account the prevalence and risk factors for gastric ulcers in pleasure horses. Few data are available regarding this category of animals in literature. Our prevalence is lower than that reported in Poland and no informations were provided for risk factors in that country. Our data corroborate that EGUS is a disease widespread in the equine population; further studies are needed to understand the complex pathophysiology of this disease in a population considered in principle as not at-risk for ulcers. These information should be kept in mind when dealing with recurrent colic in pleasure horses, especially ones were other risk factors are present.

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MONITORING OF CANINE LEISHMANIASIS IN THE PROVINCE OF VIBO VALENTIA (ITALY, CALABRIA): DIFFERENT DISTRIBUTION BETWEEN DOGS WITH OWNER AND POPULATION FROM DOG POUND

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In the last decade there was an increased incidence of leishmaniasis associated with a geographic spread from areas traditionally endemic to areas previously considered not endemic. It is obviously linked to the spread and survival of Phlebotomus perniciosus, main vector of leishmaniasis in Italy. Concomitant factors including phenomena such as stray dogs and the changed environmental climate were involved on spread of leishmaniasis. The increase of human cases and the incidence in the territory has meant that in control thereof have been realized in Italy several plans for monitoring and surveillance, last of these has been implemented in the Calabria region.

A control plan of canine leishmaniasis was performed by Calabria Region (Decree 181 of 28 November 2012) in the years ranging from 2010 to 2014. Four shelter located in the Province of Vibo Valentia were monitored. Five hundred-sixty- nine subjects of three public shelter (Municipal Doghouse of Vibo Valentia, Oasi Canina and Mondo di Pluto) and five hundred-fifty-four dogs were tested by IFAT (Immunofluorescence AntibodyTest).

The presence of anti-L. infantum antibodies was detected by indirect immunofluorescence antibody test (IFAT) in according to the recommendation of OIE using MHON/TN/80/IPT1 as a whole-parasite antigen fixed on multispot slides (Bio Merieux Spa, Florence, Italy) and fluorescently labelled anticanine gamma globulin (Sigma Aldrich, Milan; Italy) as conjugate. Positive and negative controls were included on each work session. Positive sera were serially diluted and tested to establish the maximum reaction titer. A dilution 1:160 was considered positive.

The results showed an higher prevalence to IFAT of subjects owned than the dogs housed in kennel. The 46% (n. 181/388 cases) of dogs owned found positive to IFAT. Only 10.1 % (n. 51/503 cases)of dogs living in the shelter was positive. The breed belonged or a different immune response th1 Vs th2 may be relate to the different positivity detected on subjects examined in our study.

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DONKEY GASTROINTESTINAL HELMINTHIASIS IN CENTRAL ITALY: PREVALENCE AND RISK FACTORS

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Interest in the welfare and diseases of donkey is in constantly increasing. Despite this, no information are available about prevalence of gastrointestinal helminthiasis in Italian donkeys.

Aim of the study is to To establish the prevalence and risk factors relating to gastrointestinal helminthiasis in donkeys reared in central Italy.

Between January 2014 and March 2015,fecal samples for detection of gastrointestinal parasites were collected by rectal sampling from 137 animals (20 male,117 female),with age ranging from 1 month to 16 years (mean 6.7 y, s.d. 4.0 y) belonging to 4 farms of different nature and size. Three farms were located in Marche Region and included one herd for milk production (n=32) and two centers for onotherapy (n=15 and n= 41,respectively).The last farm was located in Umbria Region and was a herd for milk production (n= 49).The history referred all animals were annually treated with alternatively fenbendazole or ivermectine.

Quali-quantitative coprological analysis were performed. Faecal samples were processed by flotation and modified McMaster techniques (MAFF, 1984). All samples with FEC (fecal egg counts) $\geq$50 eggs per gram of faeces (EPG) were subjected to coproculture to generate third-stage larvae (LIII) for species differentiation (Bevilacqua et al., 1993). Animals were categorized as young (0-2 years), adults (3-10 years) and aged (=>11 years). The effect of age, gender, location, and kind of farm were statistically analyzed by Analysis of Variance (ANOVA). Significance was set at $P < 0.05$. Results and conclusion. Fecal examination revealed the following parasite eggs (prevalence, abundance [mean number of EPG]): Strongyle eggs were recorded in 75.2% (103/137-105.1 EPG) of the donkey and Parascaris equorum 1.46% (2/137- 1218.6 EPG). The technique of coproculture (performed on 103 samples) has allowed the identification of the following kinds of gastro-intestinal strongyles together with the prevalence values: Strongylus vulgaris 51.5% (53/103), Triodontophorus spp. 14.6% (15/103), Strongylus edentatus 8.7% (9/103) and small strongyles (Cyatostomine) 100.0% (103/103). There was no statistically significant difference in level of helminthic infection with respect to the gender and between age categories. The farms resulted to be statistically different with respect of the amount of isolated parasites ($P< 0.05$). However, the two farms for onotherapy represented an exception, since resulted to have a similar amount of isolated parasites (P=0.6). The findings of the present study indicated a high prevalence of helminthic parasites, especially Cyatostomine, which may compromise the health, welfare and production of donkeys. Age or gender seems to not affect the kind or number of the parasite. This is in agreement with some studies (Mezgebu et al., 2013) and in contrast with other (Wosu et al., 2014). Nonetheless, geographical location and the attitude of animals seem to affect the entity of parasitosis. Treatment could not be affective with the schedule currently used in most farms. Further studies are needed to better understand the proper preventive strategy to be used in donkeys and the influence of age and gender on prevalence.

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PREVALENCE OF ENTERIC PROTOZOAN PARASITES IN KITTENS AFFECTED BY INTESTINAL DISORDERS IN UMBRIA REGION

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Kittens are frequently infected by several protozoan parasites that may cause a wide range of intestinal disorders. Giardia duodenalis and Cystoisospora felis are frequently associated to diarrhoea in kittens living in overcrowded conditions (e.g. catteries or multiple-cat households etc), where the cysts/oocysts shed can persistently contaminate the environment. Cryptosporidium felis was described in kittens affected by severe diarrhoea (Scorza et Tangtrongsup, 2010) and Tritrichomonas foetus has been recently recognized as an emergent causative agent of large-bowel disease in young cats (Tolbert et Gookin, 2009).

In order to help veterinarians to better plan diagnostic and preventative strategies regarding intestinal disorders in young cats, a cross sectional survey on the prevalence of enteric protozoans associated to diarrhoea was conducted.

From August 2014 to January 2015 individual faecal samples were collected from 92 kittens having an history of intestinal disorders (i.e. abdominal discomfort, acute or chronic diarrhoea etc). The kittens, from 2 to 8 months old belonged to catteries (37) and private owners and breedings (55) of Umbria region. Each fecal sample was divided into 3 aliquots (5 g/each). The 1st aliquot was examined by routine flotation technique (Zinc Sulfate 33% solution sg 1.300); the 2nd aliquot was processed by a commercial DFA (MeriFluor ® Cryptosporidium/Giardia, Meridian) for the detection of G. duodenalis and Cryptosporidium coproantigens and the 3rd one was used for the detection of T. foetus by molecular tools (Gookin et al., 2002). All cats scored positive for protozoan infections were submitted to specific treatments and then re-examined clinically and coprologically.

Overall 21.74% (20/92) of the kittens scored positive for at least one protozoan species. G. duodenalis was the most predominant agent diagnosed (13/92, 14.13%), followed by C. felis (10/92, 10.87%) and T. foetus, detected in 2 animals (2.17%). Co-infections were observed in 3 kittens (3.26%), one harbored G. duodenalis and C. felis and 2 animals showed a mix-infection by G. duodenalis and T. foetus. The 2 single cases positive for T. foetus infection belonged to a owned breeding. The intestinal disorders observed in private owned cats were less associated to the presence of protozoan parasites (13/55, 23.63%) in comparison with that observed in kittens from the catteries (12/37, 32.43%) even if not significantly. The full-recovery and the negative results of parasitological examinations, following the specific treatments, support the primary role of the protozoan parasites in the investigated intestinal disorders.

This survey showed that protozoan parasites are quite frequently associated to enteric disorders of kittens. Since no significant difference was observed on the occurrence of Giardia/Cystoisospora in owned kittens in comparison with animals from catteries, they should be included in the differential diagnosis for young cats with intestinal disorders regardless to the housing contest. On the other hand tritrichomoniasis seems to be more commonly associated to breeding conditions; however due to the scant sample size, the results might be confirmed by a large-scale sampling.

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ON THE PRESENCE OF LINGUATULA SERRATA NYMPHS IN MESENTERIC LYMPH NODES OF SMALL RUMINANTS IN SOUTHERN ITALY

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Linguatula serrata, (Fröhlich, 1789) commonly called tongue worm, is a cosmopolitan species of the phylum Pentastomida (1). The adult form of this parasite inhabits the nasal airway, frontal sinus, and tympanic cavity of canids and felids (2). The intermediate hosts of these parasites are usually sheep, cattle, or rodents (3). In humans linguatulosis can be caused by either the egg (visceral form) or nymph stage of the parasite (nasopharyngeal form) (4). Nasopharyngeal linguatulosis, often occurs after consumption of the raw or undercooked viscera of infected animals (5). Data on L. serrata infection in Italy are scant. Rare case reports are available on linguatulosis in humans (6) and dogs (7). The presence of L. serrata nymphs in intermediate hosts has not been investigated in Italy, except for an old study conducted on cattle (8). The clinical aspects associated with the infection in the intermediate hosts have not yet been described carefully (9). The aim of this study is to investigate the presence of L. serrata nymphs in small ruminants at autopsy and to describe the clinical, laboratory and post-mortem changes registered in natural infected animals.

A total of 201 small ruminants going to slaughter (160 sheep and 41 goats) were included in the study. Before slaughtering animals underwent clinical examination and blood sampling to perform complete blood count, serum protein electrophoresis and biochemical tests. Post-mortem examination was performed and all organs were visually examined for the presence of L. serrata nymphs and gross changes. Parasites and parasitic stages were collected for subsequent identification.

The presence of mobile nymphs were revealed in four out of 201 investigated animals (1.99%) (2/41 goats, 4.88%; 2/160 sheep 1.25%). They were morphologically identified as L. serrata nymphs (10). Parasitic stages were localized only at the mesenteric lympho nodes. Positive animals were all females, aged from 6 to 9 years and came from Apulia region. All of them had a body condition score of 1/5 and had pale mucous membranae. The most common laboratory alterations were anaemia, hypocalcemia, hypomagnesemia and changes in phosphorus concentration shared by all positive animals.

Results from this study indicate the presence of L. serrata infection in small ruminants, with a higher infection rate in goats, suggesting a potential zoonotic risk for humans in the investigated area. The clinicopathological changes shared by all infected animals (anemia, weight loss, low milk production and laboratory alterations) might be associated with the chronic presence of the parasites or at least it could be argued that linguatulosis could have contributed to the severity of the clinical picture in these animals.

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FIRST REPORT IN ITALY OF CAPARINIA TRIPILIS (ACARINA: PSOROPTIDAE) IN AN AFRICAN HEDGEHOG (ATELERIX ALBIVENTRIS)

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Mange by Caparinia spp. is an ectoparasitic skin disease reported in Europe (England, Germany, Poland), America (USA, Brazil, Chile, Costa Rica), Kenia, Korea and New Zealand [1-3]. Among 5 known species of the genus Caparinia, only C. tripilis and C. erinacei infest hedgehogs; among them C. tripilis shows higher pathogenicity, especially in conjunction with other infections.

The African hedgehog (Atelerix albiventris) is one of the newly exotic pets which have been observed with increasing regularity in veterinary clinics, but information about its diseases is scarce. The aim of this case report is to describe for the first time the infestation by C. tripilis mite in a native Italian African hedgehog.

In October 2014, an adult male of African hedgehog was brought to the Dept. of Veterinary Medicine (Perugia, Italy) with a respiratory symptomatology (dyspnoea and open-mouthed breathing), periorbital oedema and diurnal restlessness. It was treated with i.m. injection of enrofloxacin (20 mg/Kg SID) and subsequent with norfloxacin 5% p.o. for 10 days. After an initial improvement, the hedgehog began to present new symptoms: pruritic dermatitis with scales and crusts on the muzzle and on the dorsal surface, loss of spines, poor appetite and lethargy. Skin scraping samples were collected and examined by microscope. The slides showed numerous mites, at all developmental stages, identified, based on the morphological features [3-4], as Caparinia tripilis. Because of the seriousness of mange a therapy was made with ivermectin (0.4 mg/kg) administered subcutaneously and topically (mixed 1:9 with water for topical use). The next day a further local treatment was made with Moxidectin and Imidacolprid solution spot on, but there were no improvements. After about 24 hours the hedgehog died and it was subjected to skin biopsy. Histological examination showed mild dermal lymphomonocitic infiltration, diffuse epidermal hyperplasia and severe hyperkeratosis with intracorneal neutrophilic pustoles and serocellular crusts; embedded within keratin scales were present myriads of yeasts and sections of a mite with spiny cuticle and striated muscle.

This represent the first case of a hedgehog mange by C. tripilis in Italy. C. tripilis (Michael, 1889) is an astigmatal non burrowing mite of the family Psoroptidae, characterized by the presence of three long setae on the tarsi of legs III and, in males, of two typical trilobated opisthosomal excrescences behind a wide dorsal opisthosomal shield. Both sexes have a long dorsal propodosomal shield, which is wide in its median portion. Two long setae are present at its sides, reaching the opisthosomal margin of idiosoma. The male is 310 μm long; the female (400 μm) lays big elongated eggs from which larvae (160 μm) hatch and become quickly protonymphs (210 μm) and then deutonymphs (290 μm).

The introduction of exotic pets has forced veterinarians to acquire knowledge about these species; therefore, we consider it very important to point out new pathogens present in Italy, in order to allow the practitioners to have more conscious approach towards these new pets.

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NASAL EUCOLEOSIS IN DOGS FROM CENTRAL ITALY: CLINICAL, DIAGNOSTIC AND THERAPEUTIC ASPECTS

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Eucoleus boehmi (syn. Capillaria boehmi) is a trichuroid nematode that infests the mucosa of the nasal cavities and sinuses of both domestic and wild canids, causing a parasitosis known as nasal eucoleosis (Campbell et al., 1991). In the past few years, nasal eucoleosis has been increasingly reported in dogs from different areas, including Italy. Nonetheless, nasal eucoleosis is a neglected parasitosis which is rarely included in the differential diagnoses of upper respiratory tract disorders of the dog, mainly because the knowledge of clinical importance of E. boehmi is still poor and fragmentary. The most common respiratory signs described for nasal eucoleosis are catarrhal nasal discharge, repeated sneezing, epistaxis and impairment of scenting ability (Baan et al., 2011); moreover, the nematode has been recently incriminated as a cause of intracranial disease and meningoencephalitis due to aberrant migration (Clark et al., 2013).

The present study describes clinical cases of nasal eucoleosis diagnosed in dogs from Italy, in order to provide new insights into clinical features of the infection and to promote awareness among veterinarians.

This study was performed between November 2012 and December 2013 and involved dogs with clinical pictures compatible with nasal eucoleosis for which the diagnostic work-up or treatment had not led to a diagnosis. The dogs were recruited from veterinary clinics and kennels in central Italy. For each dog history details and clinical data were collected. Faecal positivity for E. boehmi was determined by double sampling of faeces subjected to flotation in a sugar-based solution (specific gravity 1,200) and to McMaster's technique. A polymerase chain reaction based on amplification of cox1 gene of the Subfamily Capillarinae (Di Cesare et al., 2012) was used to confirmed the identity of the parasite in copropositive samples. All dogs resulted positive were treated with a single dose of a spot-on product containing imidacloprid 10%-moxidectin 2.5% (Veronesi et al., 2014). Four weeks after treatment a copromicroscopic control was performed and the animals resulted positives were treated again.

Twenty out of 120 dogs with upper airways disorder resulted positive for E. boehmi eggs both to copromicroscopic examination with a mean of 581.1 EPG both to molecular identification. Most of the infected animals (70%) were hunting dogs which lived in rural or suburban areas of central Italy, often in crowded conditions. The most frequently observed clinical sign was nasal discharge (16/20), followed by repeated sneezing (11/20) and cough (7/20). Other respiratory signs, e.g. epistaxis, and impaired sense of smell were also found, although less frequently. No general clinical signs were present. Eighteen out of 20 dogs were successfully treated with a single dose of imidacloprid 10%moxidectin 2.5%, while 2 dogs needed of a second treatment for achieved faecal negativity.

These results suggest that E. boehmi should be included in the differential diagnoses of upper respiratory tract disorders of the dog and show the promising activity of topical moxidectin in the treatment of the infection.

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CANID HERPESVIRUS-1 IN ITALY: BIOMOLECULAR AND SEROLOGICAL SURVEY IN A BREEDING KENNEL

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Canid herpesvirus (CaHV-1) is a worldwide spread pathogen causing reproductive, respiratory and neurological disorders in adult dogs as well as neonatal death in puppies. According to studies, a higher seroprevalence exists in kenneled dogs than in household pets(1,2), however, there is a sparsity of prevalence studies reporting viral shedding and antibody titres. Additionally, the real prevalence of CaHV-1 in Italy is still unclear. The aims of this survey were to analyze immunological changes and detect CaHV-1 DNA in an at-risk dog population - a large breeding kennel - in order to find any evidence of circulation of the virus.

For this survey we chose a breeding kennel in central Italy that hosted 243 dogs (160 breeders), which have never been vaccinated against CaHV-1. The experiment group consisted of 23 dogs (14 females and 9 males). All of the females were in heat contemporary. Blood samples and vaginal swabs were taken from bitches at three critical points: during estrus, before mating, and immediately after parturition. Blood samples were also taken from male dogs before and 3 weeks after mating. Aborted fetuses, stillborn pups, umbilical cords and placentas were collected as well. Serum neutralization (SN) assay was performed on serum while vaginal swabs and other samples were tested using nested PCR assay. The external genitals were examined for CaHV-1 associated lesions.

None of the dogs showed any external genital signs and none tested positive during the SN test. All of the other submitted samples tested PCR-negative.

CaHV-1 is maintained in nature by persistence in its canine host and by direct spread from infected animals. Viral shedding occurs sporadically and usually when animals are under stress, such as those in high population densities, those being transported or those that are pregnant. Serologic prevalence in domestic dogs has ranged from 30% to 100%(3,4,5,7), however, our survey showed no association between antibody titres and risk factors for CaHV-1 transmission despite the association of predisposing risk factors present in the kennel. Indeed, this dog population was large and animals were allowed to travel outside of the kennel and do various normal activities - such as contests or breeding - which may have exposed them to other infected dogs. An Italian study in 2014 showed a low seroprevalence (14.6%) in southern Italy(6), bringing attention to the problem of worldwide distribution of CaHV-1. In conclusion, similar values of seroprevalence obtained in canine populations of Italy imply the existence of a lesser circulation of CaHV-1 in our country when compared to the world mean, which attested between 40% and 93% with an incidence of 60-80%(7). Despite the low number of available bitches in heat contemporary in the kennel, it has been interesting to observe that there was no evidence of circulation or reactivation of viral infection in the population studied, neither before nor after breeding, rising new questions concerning the epidemiology of this pathogen.

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CONTRAST-ENHANCED ULTRASONOGRAPHY IN NORMAL AND NEOPLASTIC UVEA IN CATS

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CEUS is a valid diagnostic tool for study several districts. There are numerous pathological conditions of the eye in which CEUS can be very helpful or detrimental.

Objective: To determine the feasibility of using quantitative contrast-enhanced ultrasonography (CEUS) to detect uveal perfusional changes in uveal tumors in cats.

In this prospective study, 9 healthy cats (18 eyes) and 6 pathologic cats (8 eyes) affected by primary and secondary uveal tumors (melanoma and lymphoma) were examined. In all animals an ophthalmic and ultrasonographic examination of the eye was performed. A standard grey-scale scan was performed, followed by the CEUS examination. After subjective evaluation of the videos, 3 region of interest (ROI) were drawn for each eye at the iris (I), ciliary bodies (CB) and choroid (C). Time-intensity curves were generated using commercial software (Qcontrast, Bracco) and perfusion parameters were automatically calculated. Peak intensity (PI) and time to peak (TTP) were statistically compared.

In the normal group, PI of the iris was significantly lower when compared to ciliary bodies and choroid. (31.18% versus 39.10 e 58.50%; $P<0.05$), with TTP values between 20 (I, CB) and 15 seconds (C). In the pathologic group, the PI values of the anterior neoplastic uvea were not significantly different when compared to choroid, not affected by the disease process (42.94% versus 46.1 e 64.49%, $P>0.05$). PI of the neoplastic group was increased when compared to controls, but not significantly.

Contrast-enhanced ultrasonography can detect uveal perfusion changes in primary or secondary tumor of the anterior uvea, but it was not possible to identify cutt of values that differentiate the normal uvea from neoplastic.

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BIOCHEMICAL PROFILE IN SERUM OF LOGGERHEAD SEA TURTLES (CARETTA CARETTA) RESCUED ON THE COAST OF SICILY DURING 2014

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The loggerhead Sea Turtle (Caretta caretta) is the most frequently reported marine turtle species in the Mediterranean basin. The threat of extinction of these reptiles is related to habitat loss, fishery interactions, predation, human activities and marine pollution. Loggerhead Sea Turtles are now well studied as a protected species, but limited data is available regarding their biochemical parameters. The assessment of biochemical profiles provides useful information to treat and monitor the status of a rescued animal. The aim of the study is to compare the biochemical profiles of Loggerhead Sea Turtles rescued on the coast of Sicily with values obtained from the same species in apparently healthy animals.

Between March 2014 and February 2015, the Centro Regionale di Recupero for sea turtles, located at the Veterinary Public Health Institute of Sicily (Palermo, Italy) monitored Sicilian coastal areas to detect stranded sea turtles. Each rescued turtle was registered, identified by its morphological traits, sexed, measured and weighed. A physical examination was carried out to evaluate their health conditions and to identify the presence of ectoparasites and/or external lesions. Venous blood samples were collected from the dorsal cervical sinus and centrifuged at 3000 rpm for 5 minutes to obtain serum specimens. Biochemical analysis was performed within 4 hours of collection by DPC Konelab T60i at the animal welfare laboratory of the Veterinary Public Health Institute of Sicily. Biochemical profile included 18 parameters such as creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total bilirubin, triglyceride, creatine kinase (CK), total protein, calcium, glucose, chlorine, sodium, potassium, phosphorus, and magnesium.

A total of 44 Loggerhead Sea Turtles identified as Caretta caretta was studied and 18 of these were sampled more than once during the study. Fishing hooks, lines and plastic ingestion were the most frequent causes of stranding. Compared with data previously reported from other studies on healthy sea turtles, low creatinine levels suggested that no rescued sea turtles had kidney distress, even though urea was higher probably due to dehydration. However, AST and GGT were often high, perhaps because of the mobilization of stored lipids to meet energy demands as they generally became aphagic and emaciated. Moreover, CK was always found close to the maximum value, suggesting immobilization and debilitation of the sea turtle, with decrease in muscle tone visible at the first physical examination. This parameter, as well as AST, had previously been used to evaluate the health status of sea turtles and to improve veterinary care at rehabilitation facilities through the calculation of a summarized health index (SHI). The maximum value of glucose was 291 mg/dl, more compatible with stress than with a state of pathological hyperglycemia. All the other values of the parameters examined were consistent with results found previously.

The present study shows that sea turtles retrieved on the coast of Sicily were in poor health because of the ingestion of plastic or/and fishing equipment, regardless of the age of the animal. According to their biochemical profile, Loggerhead Sea Turtles were frequently stressed, dehydrated and showed muscle weakness.

THE VETERINARY PROFESSION AND THE NON-PUNISHMENT OF OFFENCES AGAINST ITS EXERCISE

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On March 16, 2015, the legislative decree n. 28/2015, that establishes the non-punishment of offences considered particularly mild and occasional, was adopted. The principle behind the new rule provides that a crime offence, that is both mild and the result of a non-habitual criminal behavior, can be punished by the milder provisions of the civil law and not by the criminal law. The rule applies to offences punishable by a term of imprisonment not exceeding a maximum of five years or by fine, or by both. The unlawful practice of a profession is included.

The paper aims to analyze the rationale underlying the new decriminalization bill, with reference to the unlawful practice of a profession as a crime and to its potential impacts on the veterinary profession.

The Authors will analyze all the provisions governing the unlawful practice of a profession as a crime and modifying the related criminality framework. They include the art. 348 of the Criminal Code of Italy, concerning the "Unlawful practice of a professionâĂİ; the Legislative Decree 16/03/2015 No. 28 on decriminalization of mild crimes; the new art. 131bis of the Criminal Code of Italy, concerning the "Exclusion of criminal liability for the tenuous nature of the fact"; the art. 41 of the Veterinary Code of Professional Conduct. The juridical analysis will be carried out in particular in light of the fact that, just over a year ago, the Italian Senate of the Republic approved the bill n. 471 aiming to amend both the Criminal Code and the Italian T.U.LL.SS., regarding the unlawful practice of health professions. The bill included tightened sanctions against âĂIJan offence that is of particular social alarm". The main well-known cases where the unlawful practice of the veterinary profession is involved will be presented. In order to check the logic underlying the decriminalization of the unlawful practice of the veterinary profession, the Authors will also examine: - the criteria for the application of the new sanction decision (scale of the offence, circumstances of the crime, offender's personality and habitual behavior, nature of the property that is protected, instances of the victim and the accused) sifting them in perspective, with respect to the practice of the veterinary profession and to the veterinary code of conduct; - cases of interest in the veterinary field to which the new art. 131bis of the Criminal Code of Italy shall not apply, despite the new decree.

The decriminalization of the crimes identified as 'mild' and 'not habitual' should only be carried out following a defined, rigorous assessment of these conditions by the court. In these cases, the State will give up applying a penalty and will implement a claim for redress and / or restitution as in typical civil proceedings. If it will be applied to the crime of unlawful exercise of the veterinary profession, that provision could have serious repercussions on the sectors in which the profession itself is exercised, putting on the line the health and welfare of animals, the public health and food safety in products of animal origin. The absence of deterrent penalties could undermine the legitimate exercise of the veterinary profession and stands in contrast to the previous normative orientation aiming to introduce a more rigid, rigorous, effective penalty system.

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Criminal Code of Italy

INFORMED CONSENT IN VETERINARY MEDICINE

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The veterinary profession is constantly changing, always according to its own duties and proper principles, so that all the connected items require to be adapted to the socio-cultural context in which it performs. The Code of Ethics is a set of ethical standards and behavior issued to help the professionals to conduct their actions in accordance with the professional duties and ethical values encoded in it. The actual, renewed conception of relationship between man and animal directS more and more the category to take care of animal welfare. The professional conduct should be updated in relation to these changes. This involves also the question of which is the correct ethical procedure for a veterinary clinical decision. A procedure for a responsible and informed clinical decision must be considered a component of a trusted relationship with clients that takes on a special meaning, because of the additional "critical" valence coming from the affective nature of the connection owner / animal.

The Authors want to explore the concept of informed consent (I.C.) and its role in the nowadays world of veterinary medicine. The research is focused on the informed consent as a tool to help the communication between the vet and the client/owner/carer and hence to further a due process of responsibility when operating with sentient beings such as animals. The aim is to gather some explorative preliminary information regarding the use of I.C. by veterinarians active in Italy.

For the experimental part of the research, the Italian situation regarding the use of the I.C. in veterinary medicine was analyzed and deepened. A questionnaire was proposed to a sample of 213 veterinarians all over Italy, by telephone survey carried out in the months from September to December 2014. The data were analyzed to evaluate the effective use of I.C. in the veterinary practice, by interviewing a sample of professionals working in private ambulatories (138 units) and clinics for small animals (75 units). In particular, the knowledge of art. 32 and art. 33 of the New Code of Veterinary Ethics, providing an obligation to inform the customer and to obtain I.C., was investigated.

The main results showed that 74% of the respondents claim to do a routine use of I.C., especially for sedation, surgery and euthanasia (90,4%). They mainly use a template provided by their professional association (57%) or self-made (31%), only few respondents (12%) choose format from other origins. The 72% of the veterinarians that claimed they do not use the informed consent confirmed not to be willing to use it. Finally, 53% of the respondents do not know art. 32 and art. 33 of the New Code of Veterinary Ethics. The informed consent paradigm in veterinary practice deals with animals as properties, which therefore are not considered capable of deciding for themselves. The complete and comprehensive information has to be provided by the vet to the animals' owner and it is a duty of "good practice". In perspective, the use of the informed consent appears quite common in veterinary medicine, but not sufficiently so. It should be implemented, not only as a good practice, but also in force of its legal value against any possible charges of negligence, imprudence, unskillfulness, or any other infringement of laws or regulations, orders or disciplines.

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CARDIOVASCULAR EFFECTS OF CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP) IN DOGS UNDER ANESTHESIA ASSESSED BY DOPPLER ECHOCARDIOGRAPHY

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General anesthesia impairs respiratory function by the development of atelectasis in association with altered ventilation at pulmonary bases1. Applying Continuous Positive Airways Pressure (CPAP) in spontaneously breathing patients reduce the work of breathing, increases functional residual capacity and is often recommended to prevent or reduce alveolar collapse1. In human medicine it has been widely recognized that an increase in intra-thoracic pressure is associated with a decrease in cardiac output because of the reduction of venous return2. Nevertheless, conflicting results (increases, decreases, or no change) in CO have been reported and it was studied that the effects of CPAP on cardiac function were influenced by increasing CPAP levels. Doppler echocardiography represents a way to investigate how cardiac parameters can be affected and can change during CPAP application. What about CPAP application and its cardiovascular consequences in dogs?

The aim of this study is to investigate by Doppler echocardiographythe cardiovascular effects of a low level of CPAP(5 cm H2O) in dogs under anesthesia.

20 dogs have been enrolled in the study and divided into two groups. Both groups underwent anesthesia with a standard protocol and in Group A (10 dogs) CPAP was administered (5 cmH2O). Group B (10 dogs) served as control group and did not receive CPAP. Cardiovascular parameters (heart rate, mean arterial pressure and echocardiographic indices) were registered before (T0) and 15 minutes after anesthesia induction (T1), during anesthesia with or without CPAP (T2) and during recovery (T3). All patients were anesthetized with acepromazine (20 μg/kg), morphine (0.3 mg/kg), propofol (4 mg/kg) and isoflurane (end-tidal concentration 1.3 %) in spontaneous ventilation. Standard echocardiography (Esaote ultrasound system MyLab30) was performed using a 2.5 MHz transducer. The following parameters were calculated as indicators of cardiovascular function: ejection fraction (EF%), left and right cardiac index (CI), left ventricle end diastolic volume index (LV-EDVI ml/m2), ratio of isovolumetric contraction time to ejection time (IVCT/ET), ratio of early rapid filling peak to atrial peak filling of trans atrioventricular inflow (E/A), time velocity integral of atrioventricular inflow (TVI), aortic max velocity (AoVmax) and pulmonary max velocity (P Vmax).

On the whole no statistical differences have been revealed in cardiovascular parameters at T2 compared to T1 in both groups. Interestingly a significant difference was revealed in EF between CPAP group and control group at T2. Myocardial diastolic properties remained unchanged. The right cardiac index do not change. Results of this study suggest not only that application of low levels of CPAP during anesthesia of healthy dogs does not negatively affect cardiac function and hemodynamic parameters, but also that it could positively influence the global left ventricular systolic function. In conclusion the application of this level of CPAP during anesthesia in healthy dogs could be considered safe.

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CONTRAST-ENHANCED ULTRASONOGRAPHIC FINDINGS IN DOGS WITH LUNG LOBE TORSION

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Lung lobe torsion (LLT) is a pulmonary condition characterized by twisting of a lobe around its bronchovascular pedicle. This causes bronchial obstruction and pulmonary flow compromise, resulting in congestion of the lung lobe. Progressive lobar congestion leads to parenchymal and alveolar edema, hemorrhage and necrosis. While LLT is considered rare in dogs, it is life-threatening and require surgical approach. Therefore, this condition has to be accurately distinguished from others with similar signs that not require surgery. Thoracic radiographs and ultrasonography may be nonspecific, computed tomography is useful but requires a specialized equipment and general anesthesia. Lack of contrast enhancement after contrast medium administration during CT study can be seen when a lung lobe is completely rotated. Contrast-enhanced ultrasonography (CEUS) has been described as a valuable imaging method for the study of the vascularity of the peripheral lung consolidation in humans. Compared to contrast-enhanced CT, CEUS is less expensive, does not require anesthesia and ionizing radiation, and eliminates the contraindications of iodinated contrast media. The purpose of this study was to describe CEUS findings in a group of dogs with confirmed LLT. We hypothesized that CEUS may provide useful findings in the diagnosis of twisted lung lobe. Three dogs with a final diagnosis of LLT that underwent CEUS were included. All dogs were placed in lateral or sternal recumbency with only manual restraint and both conventional ultrasonography (CUS) and CEUS were performed. A bolus of sulfur hexafluoride microbubbles stabilized by a phospholipid shell (SonoVue ®) was infused by hand through a peripheral intravenous catheter placed in the cephalic vein. Suspected twisted lung lobes were assessed for the presence or absence of enhancement after the injection of the contrast agent. Enhancement patterns were evaluated for 2 minutes and compared with the surrounding tissues (other lung lobe, liver or intrathoracic vessels). CT of the thorax before and after contrast-enhancement was performed prior to surgery to further characterize the lesions in cases 2 and 3. CEUS showed the absence (case 2) or reduction (cases 1 and 3) of the pulmonary vascularization secondary to twisting of the lung lobe around its bronchovascular pedicle. Marked vascular enhancement was visualized within a second partially consolidated lung lobe (case 1), at the periphery of the lung lobe (case 2) and within the liver (case 3). Reduction of the enhancement was due to partially twisted lung lobe (180°) in case 1 and recent torsion of the lung lobe in case 3. The reduction of the pulmonary vascularization was confirmed by CT in cases 2 and 3. The results demonstrated that CEUS may provide useful information and should be considered in the diagnostic algorithm in dogs with LLT.

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PRELIMINARY "IN VIVO" STUDY ON PERIPHERAL VENOUS PRESSURE (PVP) IN THE EQUINE FINGER IN STATIC AND QUASI-STATIC MOVEMENTS AND "FOOT PUMP"' MECHANISM

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There is a marked interest in the mechanism and function of the finger and the hoof of the horse, but there is a lack in the literature of "in vivo" investigation of the behavior of the Peripheral Venous Pressure (PVP) in the finger and the local effect of the mechanism of the "foot pump". Two theories explain this mechanism:1)the blood is withdrawn thanks to the dilation or "elaterio" of the posterior foot region (Strasser 2001); 2)the hoof squeezes the blood during the load phase (Pollit 1996). Aim of the Study The aim of this "in vivo" preliminary work is to study the PVP in the horse foot in static and quasi-static conditions in order to investigate the effect of the mechanism of "foot pump" on the local peripheral circulation. The mechanism of the "foot pump" has been evaluated by the PVP changes in relation to the different loads on the front legs during different moviments (static and quasistatic). Materials and methods The surveys were conducted on the forelimb in "clinically healthy" horses: 7 competitive (4 shod and 3 unshod) and 3 non-competitive never shod horses. The lateral digital vein was canulated under local anesthesia and PVP measuremed after 120 min in order to exclude possible influences on the vascular tone. Local PVP was electronically evaluated with the horse's forelimbs in static and quasi-static conditions: a)four Feet Standing, on the ground (4FS-g), examined foot lifted and loaded; b)four Feet Standing, both forelimbs on the podoblocks (4FS-p) examined foot lifted and loaded; c)standing, controlateral forelimb lifted and loaded (3FS-g); d)standing, both forelimbs on the podoblocks, controlateral forelimb lifted and loaded (3FS-p); e)standing, forelimb on the podoblocks, controlateral forelimb lifted and palmar angle variation on the examined foot (-15°/0°/+15°) (3FS-a); f)four Feet Standing, head and neck turned laterally (4FS-l). The statistical analysis is performed by analysis of variance with ANOVA and t student. Result No differences in baseline values of PVP were observed between right and left limb of the same horse. Shod and unshod horses in standing position showed a significant difference ($p<0,05$) in PVP, independently from their competitive activity. Significant local PVP variations were recorded in the examined leg when loaded after lifting ($p<0.05$), both when evaluated on the ground or on the podoblock. When the examined foot was lifted (4FS-g), a significant reduction in PVP was recorded (-18,31$\pm$3.02 mmHg) after loaded. Lifting the contralateral leg in 3FS-g condition, the PVP value showed a rapid slight increase followed by a significant reduction (-11$\pm$3.4 mmHg) and a slow return to baseline. Comparing the two results, the difference is statistically significant ($p<0.05$). A similar variation was observed in conditions 4FS-p and 3FS-p.The lifting of one forelimb causes a concomitant involvement of the contralateral leg: the influence of this active quasi-static condition, as in 3FS-g and 3FS-p, in the horse foot may be attributed to the isometric muscular contraction rather then to the different distribution of the weight. This evidence supports the theory that attributes the "blood pumping" action to the phalangeal flexion-extension since there is a concurrent muscular action on the local vascular system and on the corium microcirculation (Pollit 1996).

The study was communicated to Italian Ministry of Health 24 January 2014.

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INNOVATION OF CANINE WOUND MANAGEMENT: AN ALL-IN-ONE DEVICE "ONE VET". A PRELIMINARY CLINICAL STUDY

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The need to find new substances characterized by low toxicity and reduced risk of inducing antibiotic resistance is one of the drivers currently leading towards a constant innovation in medical and scientific research. This necessity has opened the door to herbal medicaments such as Neem (Azadirachta indica) and St John's Wort (Hypericum perforatum).

To verify the effectiveness of an herbal based medical device 'one VET' in the treatment of chronic wounds in dogs kept under poor sanitary conditions.

The study was carried out with the approval of the Bioethical Committee of the University of Perugia (no. 2014/027). Seven dogs of ENPA's shelter, presenting overall 17 cutaneous wounds (bedsores and traumatic injuries) lasting at least 4 months (which according to Werdi et al., 2009, are defined as 'non-healing' or 'chronic' wounds) were admitted to the study. All wounds were treated using the device 'one VET' as sole primary dressing, according to the 'all-in-one' protocol. Wounds were washed daily using saline solution, dried with sterile gauzes and sprayed with 'one VET'. Microbiological swabs, relative antimicrobial susceptibility tests and digital images of each wound were made weekly, starting at time 0. The images were used for calculating initial and intermediate wound area, until epithelialization, using Autocad 2005 software, in order to obtain the Stashak Epithelialization Rate (SER: ($\sqrt{\text{Area cm2}}/\pi$)/Time to Heal) (Stashak, 1991).

Thirteen out of 17 wounds reached complete healing (Time To Heal: 27.18 $\pm$ 10.47 days); SER was 0.05$\pm$0.04 cm/day. Microbiological swabs demonstrated the presence of several microorganism (Staphylococcus pseudintermedius, Staphylococcus aureus, Escherichia coli, Klebsiella, Pseudomonas aeruginosa), all presenting multi-resistance against antibacterial drugs.

The majority of the lesions healed in a time laps that was always three times shorter than the wound duration time. Since start of treatment, a continuous turnover of bacterial populations was detected, nonetheless the 'oneVet' device showed to be able to contrast the well known anti-healing effect due to the presence of pathogen microorganisms on the wound's surface (DeLeon et al. 2014). This is probably due to the presence in the 'oneVET' formulation of free fatty acids with antimicrobial activity (Desbois and Smith, 2010). Three out of the four not healed wounds were related to dog affected by Leishmania, which is known to hinder wound healing (Mc-Mahon-Pratt and Alexander, 2004). One wound haven't healed for the persistence of the traumatic cause. The 'all-in-one' 'oneVET' protocol seems able to heal wounds, without the use of antimicrobial or cytotoxic disinfectants, also in presence of bacterial contamination that usually prevent healing. Further studies aimed to include control groups in the study design are warranted.

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MEASUREMENT OF ALDOSTERONURIA IN HEALTHY AND CARDIOPATHIC DOGS: EARLY EVALUATION OF TWO ELISA METHODS

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Degenerative mitral valve disease (DMVD) is the most common acquired cardiovascular disease in dogs (75%) and it is characterized by a long pre-clinical period [1]. There are conflicting data concerning neuro -hormonal (RAAS) activation and about its role in early DMVD pharmacological treatment. Preliminary data showed that plasma aldosterone levels are significantly higher in asymptomatic affected dogs than in healthy dogs [2]. This observation suggest that aldosterone can be involved in the early course of DMVD and plays a key role in disease progression [3]. To date, ELISA kits to determinate aldosteronuria in dogs are available, but they are very expensive, and for this reason, they are not currently used in veterinary practice.

The aim of the present study was to compare two commercial ELISA kits, one specific for canine species (Aldosterone EIA Kit-Monoclonal - Cayman Chemical Company, USA), and the other specific for human beings (Aldosterone ELISA - DRG Instruments GmbH, Germany). The ELISA kit for humans, has a double advantage: it is cheaper than the canine kit and the execution time is short (4 hours vs 21 hours of the canine kit).

5 healthy dogs (named A-E) and 5 DMVD dogs (named F-L) were recruited in the Veterinary Teaching Hospital of the Department of Veterinary Sciences in Turin. Dogs were assigned to the groups after a physical examination performed by a veterinarian, specialist in cardiology. Urine samples were collected by cystocentesis and they were analyzed using the two kits, twice and in duplicate. Urine samples of healthy dogs were stripped using dextran charcoal (0.5 g/ml) and fortified with different concentrations (0, 20, 200, 500, 1000 pg/ml) of aldosterone (Sigma Aldrich, Milan, Italy) to evaluate the sensibility and the accuracy of the two kits. A single concentration (500 pg/ml) of cortisone was added to all stripped samples and they were analyzed with both kits to evaluate specificity. Data were analyzed with GraphPad Prism 5.0 software using One-way Anova and Bonferroni's post test ($p<0.05$).

No statically significant differences were highlighted among all the samples analyzed with both kits. Also the fortified samples didn't shown statically differences with the two different kits. Cortisone was added to stripped samples to verify cross-reactions and both kits didn't measure cortisone. Surprisingly, the dog C, belonging to the group of healthy dogs, was statistically different from the dogs of its group, but similar to the group of pathological dogs, showing no statistically differences with them. This case was reported to the veterinarian that examined first this dog for further investigations. The results of this study seemed to highlight that ELISA kit to measure aldosteronuria in humans might be use also for dogs. This means a saving of time and money. Moreover, the data of dog C support the hypothesis that aldosteronuria could rapidly increase in early DMVD phase [4]. Further studies should be encouraged to improve specificity and sensibility of this test, comparing this trial with a gold standard method (i.e. LS-MS) and using a huge number of dogs to prove if this method might be a useful diagnostic and prognostic tool.

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INFLUENCE OF OMEGA-3 FATTY ACIDS ON THE LEARNING ABILITY OF THE GUIDE DOG DURING THE TRAINING

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Some scientific evidence shows omega-3 fatty acids, particularly docosahexaenoic acid (DHA), to affect the neurological development of children and puppies by improving visual skills, memory and cognitive learning. The brain development occurs in the last stage of pregnancy and continues until a few months after birth, both in human and in dog (Clandinin et al, 1980; Hinemann et al, 2005; Bauer et al, 2006). In this phase a selective accumulation of arachidonic acid (AA) and DHA happens both in brain and in retina. The demands of DHA in dog can be satisfied by conversion of alpha linoleic acid (ALA), by DHA synthesis in other tissues and by DHA exogenous administration. The DHA supplementation seems more effective than the which one with high amount of ALA for improving retinal function, measured by electroretinogram (Heinemann et al, 2005). Some studies show the usefulness of supplementation with long chain poliunsatured fatty acids (LCPUFA) in puppies during training through the evaluation of the ability of learning and memory (Kelley et al, 2004; Reynolds et al, 2005; Zicker et al, 2012).

The purpose of this preliminary study was to evaluate the effect of DHA supplementation on learning abilities in a group of future Guide Dogs for the Blind during the phases of education and training.

Two groups of six Labrador dogs (A, study group and B, control group), belonging to School of Guide Dogs of Tuscany (Italy), random selected, were included in the study. All subjects were included in the preliminary training program for guide dog and were fed the same diet (Purina Pro Plan Puppy LB ®); to each puppy was ensured a 0.06% of DHA. For one year, to the group A a further dose of 35 mg/kg of DHA once a day has been administered while the group B received a placebo. The administration was double-blind. To evaluate the effect of supplementation, we used the standardized tests of the School as they are internationally recognized and can establish both character and learning skills of puppies. The tests were repeated three times during the first year: at 7-9 weeks, at 6-8 months and after one year. The differences of tests were evaluated using the Mann-Whitney test (Wilcoxon), W test, that compares the medians of the two populations.

Some differences were observed between the two groups in some tests results; in particular in those that require a greater visual and sensory capacity and motor coordination as the grid (or abnormal surface) test and the tilting table test. Furthermore the response to fearful stimuli showed a worse outcome in not supplemented subjects in all three tests. All the 6 puppies belonging to group A have passed the training phase and were all eligible for the next guide training (100 %); only three puppies of group B were admitted to the guide training (50%), 1 was selected for Assisted Activities with Animals (16.6%) and 2 were discarded (33.3%). The results obtained in this study could confirm the usefulness of a 35 mg/kg DHA supplementation during the first year of life for improving cognitive skills in dogs.

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DETECTION OF BACTERIAL CONTAMINATION AND DNA QUANTIFICATION IN CANINE AND FELINE STORED BLOOD UNITS.

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Blood transfusions in veterinary medicine have become increasingly more common and are now an integral part of lifesaving and advanced treatment in small and large animals (1).Several guidelines suggest what infectious agents to screen for in canine and feline transfusion medicine (2,3).While the risk of bacterial contamination of blood products during collection, processing, storage and administration is not considered in veterinary medicine, it has emerged as a cause of morbidity and mortality in human transfusion medicine.

The purpose of this report is to describe the detection and quantification procedures applied in four cases of bacterial contamination of canine and feline blood units, which suggest the need of further investigative studies and monitoring to optimize patients'safety in veterinary transfusion medicine.

Four red blood cell units which showed a color change were included in the present report. The first one (case A) was collected from a 3-year-old female mongrel dog from a Blood Bank; the second one (case B) from a 7-year-old male mongrel dog, the third one (case C) from a 3-year-old male mongrel dog, and the last one (case D) from a 7-year-old male European cat, from another Blood Bank. The blood was collected from healthy animals according to the guidelines and immediately refrigerated in a blood-storage refrigerator at 4 °C, where it can be stored up to 40 days.The massive visible color changes were noted at day 31 of storage in case A, at day 20 in cases B and C and day 32 in case D. These units were removed from the Blood Bank for further investigations. Microscopic evaluation of a smear from each of the blood bags revealed heavy bacterial contamination.DNA was isolated from each of the blood bags and bacterial DNA load per sample was assessed by qPCR modifying Nadkarni et al procedure(4). The bacterial Genome Equivalent number (GE/mL of template) was 1.18 x107 GE/mL in case A, 3.64 x107 GE/mL in case B, 8.38 x107 GE/mL in case C and 5.22 x108 GE/mL in D. PCR products were purified and after alignments in the EMBL GenBank database, the sequences matched perfectly with Serratia liquefaciens in A, Pseudomonas putida in B and C, and Pseudomonas fluorescens in D. CONCLUSIONS: The current study showed that bacterial contamination was present and with high bacterial DNA load. These findings confirm data of human transfusion medicine. Instead, when reviewing the veterinary literature, actual reports of bacterial contamination of blood bags are remarkably rare and many post-transfusion reactions could probably be misdiagnosed or overlooked. Since thousands of blood transfusions are performed each year on dogs and cats and the demand for blood products continues to grow (5), the present report emphasizes the importance of carefully designed protocols to prevent bacterial contamination of blood collected for transfusion and to optimize patients' safety in veterinary transfusion medicine.

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ULTRASONOGRAPHIC FINDINGS OF RIGHT-SIDED CARDIAC DISEASE ASSOCIATED WITH AN INTRAVENTRICULAR THROMBUS IN A CANINE FOETUS

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Congenital heart defects represent one of the most common causes of mortality in still born dog and puppies1. In adult dog congenital heart defects represent 23.5% of all cardiac disease and tricuspid dysplasia and pulmonic stenosis was reported in 7.5% and 23.3% of cases respectively2.To our knowledge, ultrasonographic findings of right-sided cardiac disease, associated with intraventricular thrombus, in canine foetus were not reported.

The aim of this study was to describe for the first time, ultrasonographic findings of right-sided cardiac disease, associated with intraventricular trombosis in a canine foetus.

A 5 years-old newfoundland pregnant bitch (Day 55 post ovulation) was presented at the day-hospital service provided by the Obstetric and Gynaecology unit of the Veterinary Teaching Hospital of Department of Veterinary Medicine to monitoring foetal viability by ultrasound (My Lab 30 Gold Esaote, Genova, Italy). The pregnant uterus was visualized by transversal and longitudinal sections. The foetus viability was assessed by visualization of movements and heart frequency rate.

The ultrasonographic examination showed, in one foetus, a large homogeneous, hyperechoic mass in the right ventricular lumen. Right ventricular and atrial enlargement, abnormal tricuspid valve (thickened and fused valve leaflets) and, patent foramen ovale were also observed. Pulmonic valve was not clearly visualized. A presumptive diagnosis of right-sided cardiac valves malformation (tricuspid stenosis and pulmonic stenosis/hypoplasia) associated with an intraventricular thrombus was made. The great number of foetuses, during the late gestation, not allowed the visualization of all foetuses. The bitch delivered 11 puppies, four of which dead few hour after parturition and were subjected to gross and histopathological examinations. At necropsy, there was diffuse subcutaneous edema, mild serous thoracic and abdominal effusion and enlarged liver with mottled surface. The right atrium was moderately enlarged with large interatrial communication; the right atrioventricular ostium was restricted and delimited by a whitish fibrous band in which no valvular leaflets were present. The right ventricle was enlarged and contained a large mural thrombus attached to ventricular free wall and finally the pulmonary valve was stenotic with post-stenotic dilation of pulmonary common trunk. The other three puppies showed tricuspidal dysplasia with stenosis: two had very enlarged right atria, one of which contained a thrombus. Histologically the right atrioventricular ostium was composed by loose connective tissue with myxoid extracellular substance and focal cartilaginous metaplasia. The pathological diagnosis confirmed ultrasonographic findings of tricuspidal and pulmonic stenosis associated with an intraventricular thrombus. The diagnosis of puppies' heart disease may be underestimated, because in most cases necropsy is not performed. Therefore, prenatal diagnosis of congenital heart diseases could be important to estimate their incidence and useful in the choice of potential candidate for an optimal breeding.

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REPORT OF MYASIS BY LARVAE OF RHINOESTRUS PURPUREUS (DIPTERA, OESTRIDAE) IN A HORSE IN UMBRIA (CENTRAL ITALY)

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Rhinoestrus purpureus (Brauer, 1858) is a fly belonging to the suborder Brachicera, order Diptera, family Oestridae. It is found mainly in Asia, but is also widespread in Africa and Europe. In Italy this species should be considered rare and mainly affects horses [1]. In other countries hosts of this insect are also zebras, giraffes, antelopes, warthogs and hippos.

The aim of this paper is to report the finding, in Central Italy, of R. purpureus in a horse, and the exceptional complete development in the laboratory of the larvae taken by an endoscope.

- Clinical case: in May 2014 a seven-year-old Sardinian Anglo-Arab mare was admitted at the Veterinary Teaching Hospital of Perugia (Central Italy) because of bilateral nasal discharge and cough. The referring vet treated the animal with Cefquinome (1 mg/kg IM for 5 days) with partial remission. At admission, the clinical examination was unremarkable, a radiography of the head showed sinusitis of maxillary sinus, and an endoscopy of upper respiratory tract allowed to detect the presence of larvae among the ethmoidal turbinates and on the pharyngeal recess. The horse was treated with Cefquinome for 7 days and ivermectin twice every 20 days. During a second endoscopy, two days after admission, larvae were collected using a biopsy forceps. A follow up examination, performed after 3 weeks, showed no abnormal findings in both radiographic and endoscopic examination, except for a slight discharge coming from the right sinus, seen on the drainage angle in the middle nasal meatus. After the discharge from the hospital, the horse showed no signs of disease until September 2014, when it was returned to the vendor because of orthopedic problems.

- Parasitological examination: at the Lab. of Entomology (Dept. of Veterinary Medicine, Perugia), the larvae III removed by an endoscope (May 19, 2014), were placed in jars containing cotton wool and absorbent paper, at an ambient temperature of 24° C and a Relative Humidity of 75%. Among four mature larvae III, only 2 impupated (May 20, 2014) and only one hatched (June 18, 2014). The fly was kept under observation at 24° C and 75% RH, until its natural death occurred (July 2, 2014).

The adult insect (about 1 cm long) was similar to Oestrus ovis, fly parasite of sheep which is very common in Italy. However, it differed for the distribution of cuticular tubercles, which extended even to the pro-thorax, scutellum and dorsal region of the abdomen. It was identified as R. purpureus. It is a viviparous species and its larvae are launched in flight on the eyes and, more often, on the nostrils of the host. The larvae I (1 mm) have solid mandibular hooks and a body covered with spines: those on the sides are particularly prominent and can easily push it forward making it quickly get down to the maxillary sinuses and pharynx. Here the larva reaches the second instar and then the third instar (2.5-3 cm). This localization causes especially phenomena of rhinitis, wheezing and coughing.

The report of R. purpureus in Italy is rare, but the development of the fly in the laboratory should be considered an exceptional event [2] because this insect develops only if the larva III has reached full maturity and it is in the biological phase before its expulsion from the animal.

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ABDOMINAL FOREIGN BODY MIMICKING AN ASYMPTOMATIC ABDOMINAL MASS IN A DOG

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Clinical case discussion: A female, neutered, 8 years old Whippet dog was referred to the Department of Small Animals, Leipzig University, cause presenting acute para-paresis of the hind limbs. Radiographs of the lumbar spine showed a possibly narrowed intervertebral disc space (Th13/L1); magnetic resonance investigation resulted normal. Abdominal ultrasonography revealed the presence of a not vascularized structure with two rounded, inhomogeneous, main portions. It was occupying the middle abdomen cranially to the bladder, and measured 2,6 x 2,6 and 3 x 3 cm; the distal abdominal aorta was significantly compressed. Computed tomography (CT), suggested that this dumbbell-shaped structure could be possibly related to a neoplasm or an abscess. An organ allocation was not possible. It was finally decided for a laparotomy that allowed the resection of the structure that was firmly adherent to the large omentum. A diagnosis of a surgical gauze left in previous surgery, incorporated in a very reactive omentum, was made. The structure removed underwent bacterial culture, resulted negative, and histopathology that confirmed the absence of neoplastic tissue, and confirmed the foreign body with surrounding hemorrhage and necrosis, inflammation and fibrosis. Even if the diagnostic plan included a second level diagnostic imaging (CT), the diagnosis of foreign body was not achieved until surgery was performed, showing that the retrieval of a surgical swab could be a difficult diagnosis. It is also peculiar how the foreign body behaved, because in literature similar cases are frequently (not always), reported as symptomatic (nonspecific symptoms, bacterial infections, granulomas, neoplastic transformation, transmural migration, enterocutaneous fistula, etc.) [1-13], while in the present case it was an accidental finding.

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Part IV

Scienze Cliniche - S.I.C.V.

EYE ENUCLEATION AND APPLICATION OF A SILICONE PROSTHESIS IN 20 STANDING HORSES

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Enucleation consists in removing all the ocular structures from the orbit1. Although it is pain-relieving, cosmetic results are poor and it is generally associated with surgical procedures aiming to improve the final outcome.

The purpose of the study is to describe surgical technique, cosmetic results and outcome of silicone prosthesis (PROST) application in standing horses.

20 horses were admitted between 2008 and 2014 for unilateral enucleation. Collection of data included signalment, history, clinical findings, anesthetic protocol, post-operative treatment, complications and outcome. Horse were prepared for standing surgery.Sedation was maintained through CRI of an alpha-2 agonist2. Regional nerve blocks and retrobulbar block were performed to achieve good quality analgesia and akinesia before transconjunctival enucleation procedure.A large curved hemostat was used to clamp the optic pedicle before complete closure of vessels was achieved with a surgical knot.A sterile PROST was inserted in the resulting gap without any supporting suture.Such device presents 2 surfaces: the spherical one fits the orbital cavity, the convex one remains in contact with the eyelids.It is manufactured in three different sizes*.As the implant is often bigger than ocular cavity it was necessary to perform a lateral canthotomy in most cases.Eyelids were closed in a simple interrupted U pattern and a stent bandage was applied for 3 days. Post-operative management included NSAIDs, antibiotics and a protective mask.A period of visual rehabilitation was advised to owners. Follow-up was obtained through phone call and ranged from 10mo to 6yrs (mean:4 yrs). Reasons for enucleation were: 8 severe ocular trauma end 6 stage uveitis 4 perforated corneal ulcers 1 panophthalmitis and 1 neoplasia. Surgical time ranged from 29' to 125' (mean:58'). Intraoperative complications: head jerking associated with depth of sedation; optic pedicle hemorrhage managed successfully with a new ligature in one horse. Postoperative complications: moderate swelling at the surgical site; mild serous drainage for 20 days in one horse. Outcome was good in 18 cases: the PROST remained in situ and no signs of pain or discomfort were reported. Infection and dehiscence in 2 cases.The implant was removed and the infection resolved. Long-term follow-up included 16 horses, and 15 had the PROST still in situ.

Although invasive, removal of the eye is advocated for untreatable ocular diseases. Enucleation can be performed in anesthetized or standing horses3 : minor bleeding, reduced anesthetic risks4, fewer facilities requirements and reduced costs are the main advantages of standing technique.The application of a SP is technically easy and safe to perform and time required is short. There are several techniques for ocular PROST application5 some of which giving perfect cosmetic results, but they require multiple surgical interventions, daily care of the implant and expensive materials.Cosmetic results of traditional enucleation are poor and horses present a sunken orbit.PROST application represents a good compromise between cosmetic outcome and horse welfare.

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STANDING COMPUTED TOMOGRAPHY (CT) IN THE EQUINE PATIENT

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Computed tomography (CT) allows a better evaluation of the equine skull than conventional radiographs, providing excellent contrast and spatial resolution. The availibility of CT examination in the equine practice is often limited by the need of general anesthesia and procedure high costs. To avoid the anesthetic complications and reduce the overall cost of the procedures, custumized CT scanners for standing equine patients examination have been developed. The aim of this study is to describe the CT standing system with particular relevance to acquisition protocols and positioning through the analysis of four clinical cases. In addition, advantages and limitations of the procedure will be investigated. In the study were included 4 horses (age 10-21 years) referred for dental or sinonasal disease that underwent a standing CT examination. A multi-detectors 16 slice CT system was used for the study. The horses were sedated with alpha2-agonist and opioids and positioned standing squarely on an air pallet platform connected to the CT table. Thanks to the air-cushion the friction on the floor was almost nulled allowing to the CT table to pilot the horse into the gantry. The equine head was placed in extended position on the CT table and a weight was put on the neck to minimize its movements during acquisition. Images were acquired in contiguous helical mode with 1,25 mm slice thickness. Scanning parameters were 140 kVp and 300 mA. Duration of each scan was in a range of 30-35 seconds. In one horse a remaining dental fragments of the teeth 209 and the presence of an abscess and necrotic tissues in the caudal maxillary sinus were observed. One month after surgical removal of the fragmented teeth, the CT was repeated because of the presence of monolateral nasal discarge. The follow up CT allowed to recognize the presence of a communication between the oral cavity and the cranial maxillary sinus even if the plug was in situ. In two horses a sinonasal cyst was diagnosed. In one horse, referred for headshacking CT exam revealed: cyst like lesion close to the apex of 106 root, hickening of the right nasal bone, fluid accumulation in the right cranial maxillary sinus and thickening of the mucosa of right dorsal concha. In all horses CT examination was performed easily and without complications and an accurate and definite diagnosis was reached in 75% of cases. Minimal motion artifacts were noted without affecting the diagnostic value. The limited availability of CT scanner for standing horse and the possibility to have motions artifacts are the major problems of this technique. A good sedation protocol and well trained staff is mandatory to obtain good quality images safely both for the patient and the personal. The standing CT examination eliminates the risks of general anesthesia and allows to submit the patients to follow-up CT scans without increasing the risk of morbidity. In addition, performing a CT examination on a standing horse affected by neurological disroders is safer and has the same capability of a CT scan performed on a horse under general anesthesia. Last, but not least, performing CT in standing patients can rather reduce the costs of the examination.

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ULTRASONOGRAPHY OF SUSPENSORY LIGAMENT IN THE HORSE AFTER PERINEURAL ANESTHESIA

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Proximal suspensory ligament (PSL) disease frequent causes lameness. The diagnosis is made using clinical and lameness exam, diagnostic analgesia and imaging. Ultrasonographic (US) exam is particularly challenging in this region, however PSL scanning after diagnostic analgesia could potentially lead artifacts or false positive findings. Zekas and Forrest in 2003 described changes in US findings immediately and 24 hours after a subcarpal high palmar and palmar metacarpal nerve block and a low palmar and palmar metacarpal nerve block were performed. In clinical settings many veterinarians perform the block by injecting the anesthetic solution more diffusely in and around the origin of the suspensory ligament.

Aim is to describe US changes in PSL after injection of the palmar metacarpal nerves and in and around the origin of the ligament at 24 hours post-injection.

6 Standardbred mares (10 to 16 years old) and 12 forelimbs were used. The study was approved by the Institutional Animal Care and Use Committee of the presenting Institution. All horses were healthy. After sedation (detomidine, 20mcg/Kg) the palmar aspect of both metacarpal regions was clipped, washed and aseptically prepared; 6 ml of 2% mepivacaine hydrochloride were used. Limbs were divided in 3 groups, each group was composed by 4 limbs: A) medial and lateral approach to the palmar metacarpal nerves with empty needle hub; B) lateral approach only to both palmar metacarpal nerves with empty needle; C) lateral approach only to both palmar metacarpal nerves with needle filled with anesthetic. In all limbs the anesthetic solution was also injected in to the ligament and dorsal to it. US scan of the proximal third of the suspensory ligament was performed prior, immediately after and 24 hours after injection. Longitudinal and transvers scans were acquired with a 7,5 MHz linear probe (Esaote Mylab30gold). Dorsopalmar thickness of the PSL, presence of gas, changes in echogenicity, and changes in surrounding tissues were recorded. Results: there were not significant differences among groups. There were no significant differences in dorsopalmar thickness of the PSL before and after injection. Subjectively, there were no changes in echogenicity or fiber pattern in all groups. The dorsal hypoechoic space was significantly increased in size in 8/12 limbs at the first post injection scan. This was still detected at 24 hours post injection. In 5/12 limbs there was loss of definition between the dorsal margin of the PSL and the dorsal hypo echoic space, due to increased echogenicity of the latter. This persisted 24 hours post injection in 3/5 limbs. No gas was detected in any of the limbs at any time.

Due to local anesthetic infiltration, soft tissue changes may be present thus the interpretation of ultrasound examination may be confusing. In this study there were no significant differences in the PSL between baseline measurement and pattern and post-injection measurements. Nonetheless correct identification of the PSL limits was not easy due to increased echogenicity of the dorsal hypoechoic region. This suggest that diagnostic ultrasonography of the origin of the PSL should be interpreted with caution if performed within 24 hours after diagnostic analgesia.

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ULTRASONOGRAPHIC FINDINGS IN HORSES WITH TENOSYNOVITIES OF THE DIGITAL FLEXOR TENDON SHEATH: SENSITIVITY OF THIS DIAGNOSTIC TECNIQUE IN IDENTIFICATION OF SEPSIS AND TENDON INJURIES

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The digital flexor tendon sheath (DFTS) is a complex synovial cavity containing the superficial digital flexor tendon (SDFT), deep digital flexor tendon (DDFT) mesotenons, manicae flexoria and vinculae. Diagnostic ultrasonography is the most commonly used technique for evaluating the digital flexor sheat (DFTS) in horses.

The aim of this retrospective study was to investigate the diagnostic sensitivity of ultrasonography (US) to detect tendon lesions during tenosynovitis of the DFTS and to assess the most sensitive US findings to identify septic DFTS.

Medical records of horses with a diagnosis of tenosynovitis of the DFTS were included. The horses were divided into two groups: septic (S) and non-septic (NS) tenosynovities. All horses underwent an US examination of the DFTS and diagnostic or therapeutic tenoscopy when required. The following US findings were considered: degree of effusion (absent,mild,marked), synovial thickening (mild,marked), echogenicity of the sinovial fluid (anechoic, echogenic),presence of hyperechoic spots (no,yes) and presence of fibrinous loculations (no,yes). For each parameter sensitivity (Se), specificity (Sp), accuracy (Acc), positive (PPV) and negative (NPV) predictive value were calculated for the diagnosis of sepsis; the NS group was used as control. The presence of tendon lesions were extrapolated from medical records and Se, Sp, Acc, PPV and NPV of the US examination for the detection of tendon lesions were calculated; tenoscopy was used as diagnostic gold standard.

36 horses of different breeds were included (20 group S and 16 NS) aged between 1 month-19 years; 18 fore and 18 hindlimbs. 30/36 horses underwent tenoscopy. US of the DFTS represents a usefull diagnostic technique for detect synovial sepsis. Several sonographic findings may be correlated with sinovial sepsis: marked synovial effusion was detected ultrasonographically in 85% of horses in group S and in 68% in NS (Se 90%, Sp 69%, Acc 81% PPV 78% and NPV 85%). Marked synovial thickening was detected in 60% in group S and 45% NS (Se 63, Sp 56, Acc 60, PPV 63, NPV 56). Hyperechoic spots and echogenicity of the fluid showed a high specificity: in group NS hyperechoic spots were not detected and the fluid was always anechoic. US showed also high sensitivity detecting tendon injuries: tendon lesions were detected in 86% of the horses involving: 35% SDFT, 58% DDFT and 6% other structures. Lesions were confirmed by tenoscopy in 64% of cases: 62% in group NS and 50% in group S (63% lesions of SDFT and 61% lesions of DDFT). Se, Sp, Acc, PPV and NPV in the NS group for the detection of lesions of the SDFT were: 89%, 95%, 93%, 89% and 95%, for lesion involving DDFT: 87%, 93%, 90%, 93%, 88%, especially in detection of longitudinal tears of the DDFT (88%, 50%, 75%, 78% and 67%). Diagnostic care of US in the detection of the DDFT lesion in group NS is in accordance with literature but there are no data on traumatic injury. Regarding SDFT lesions data are comparable to what observed for DDFT lesions in the present study: no data are present to author's knowledge on SDFT lesion.

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CELIAC PLEXUS BLOCK IN EQUINE ILEUS: A PILOT STUDY

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Celiac plexus block (CPB) is considered useful for the treatment of abdominal pain and for postoperative ileus. The aim of this study was to determinate feasibility and efficacy of the CPB in horses with ileus or postoperative ileus. Nine horses with decreased/absent small intestinal motility, confirmed by ultrasound abdominal examination, were enrolled. A numerical descriptive "motility scale" was used to identify motility degree: 0= absence of intestinal motility, 1= slight intestinal contraction, some less dilated loops, no ingesta movement. 2= contractions of at least 50% intestinal loops, 3= normal intestinal motility, 4= hypermotility. Under ultrasound guidance, a spinal needle (20 SWG x 120 mm) was inserted for all its length, between transverse processes of L1-L2 in dorso-ventral direction, parasaggital to the spinous processes, approximately 8-10 cm abaxially. To verify the exact retroperitoneal position two drops of saline were positioned in the needle cone. If these were aspirated by the intra-abdominal negative pressure, the needle was slightly retracted and lidocaine 1% (40 ml) and ropivacaine 1% (10 ml) were injected on both sides. After 30-45 minutes, ultrasound examination was performed to evaluate the intestinal motility. For each horse, CPB was performed every 6 hours for 48h. HR, RR, gastric reflux, haematocrit, intestinal motility, appetite, defecation and bilateral flank sweating, were recorded before and after every CPB.

Eight patients underwent surgery, 1 received only medical treatment. In 7/9 patients, ultrasound examination demonstrated increases in intestinal motility, 2 patients reached a score of 3 within 48 hours. Mean "motility scale" score was respectively 0.5 ± 0.4 and 1.3 ± 0.6 before and after the block. In all horses after the CPB sweating of the flank was present bilaterally. The effectiveness of CPB is supposed to be due to the inhibition of vasoconstriction occurred in splanchnic organs as a consequence of sympathetic activation. To completely understand the mechanism trigged by the sympathetic block, an evaluation of intestinal myoelectrical activity should be made. The correct positioning of the needle was always associated to flank sweating, increased intestinal motility, evaluated by ultrasound examination, progressive decrease of gastric reflux, increasing appetite and presence of feaces. This procedure can be used by veterinarians without great experience and also in situations that do not involve a hospital or ambulatory structure. Local anaesthetic combination takes advantage of the rapid onset (lidocaine), and prolonged duration (ropivacaine). Preliminary results are promising, further studies will be performed.

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SURGICAL TREATMENT OF AN ABDOMINAL ABSCESS CAUSED BY A FOREIGN BODY INVOLVING THE SPLEEN AND THE LARGE COLON: A CASE REPORT

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Foreign bodies on the equine intestine have been diagnosed to be the cause of colic syndromes in the equine patients; several cases are reported in literature about occlusive lesions of different part of the small intestine especially the large or small colon or due to fecaliths that occlude the lumen of the involved organ. Migrating intestinal sharp foreign bodies causing chronic capsulated suppurative lesions involving different organs within the abdominal cavities are not well represented in literature for the equine patient.

The aim of the study is to describe the diagnostic trial, the surgical treatment and the long term follow up of a horse treated for an abscess involving the spleen and the large colon caused by a metal foreign body as diagnosed after an exploratory laparotomy.

A 13 years old mixed bred filly was admitted to a veterinary teaching hospital for chronic episodes of colic. The horse underwent an in depth clinical and ultrasonographic examination: at presentation the horse was bright with vital parameters within normal limits and no signs of abdominal pain. The ultrasound examination revealed the presence of a mass involving the spleen with an iperechoic line producing an acoustic shadow on the inside: it was suspected to be an abscess involving at least the ventro-cranial part of the spleen with a possible air/foreign body contained into it. Bloodworks revealed leukocytosis and high values for SAA and fibrinogen. It was decided to perform an exploratory celiotomy for a definitive diagnosis and possible treatment of the lesion. Upon abdominal exploration a capsulated mass involving the spleen and the lateral wall of the most cranial part of the left ventral colon was evident; there were adhesions between the involved segment of the spleen and the abdominal wall. A surgical approach via a colotomy was tempted to manually open the capsule within the colon and avoid possible abdominal contamination from its contents: during the procedure the manual exploration of the cavity revealed the presence of a piece of iron-wire of an approximate length of 4 cm. The foreign body was removed, the colotomy closed and the adhesions between the spleen and the abdominal wall removed manually. The horse recovered uneventfully from anesthesia and did not have any post operative complication. Follow up informations were obtained by the referring vet: after 6 months the ultrasonographic examination revealed no evidence of the mass anymore, the fibrinogen level underwent a slow but constant decrease in the two months after surgery until normal limits and the horse did not suffer of colic episodes anymore.

Sharp foreign bodies causing lesions of the large intestine are a rare condition in horses; the surgical treatment is possible but attention has to be paid to avoid abdominal contamination by the abscess content. Opening of the capsule via a colotomy is a reasonable technique that allows the surgeon to keep the purulent material contained in the abscess walled off from the peritoneal cavity.

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EFFECTS OF XYLAXINE, ROMIFIDINE, DETOMIDINE AND DETOMIDINE-BUTORPHANOL ON HORSE'S TEAR PRODUCTION

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The veterinarian must improve animal's comfort during diagnostic and therapeutic procedures, and even the simple attention to corneal dryness and, therefore, the prevention of a possible discomfort is important. Several drugs are known to decrease tear production, in particular in dogs and cats, and for these species the use of tear substitutes is frequent in order to avoid the onset of corneal dryness. No data are available for the horse.

Aim of the study is to analyze the effects of four sedation protocols on tear production in horses.

The study consists of measuring the tear production using Schirmer Tear Test I in 45 horses sedated with different protocols. The patients were divided in four groups: the first group of horses was sedated with detomidine 15 μg/kg iv, those of the second with detomidine 10 μg/kg plus butorphanol 9 μg/kg mixed in the same syringe and administered iv, those of the third with xylazine 0,72 mg/kg iv and the horses of the fourth group were sedated with romifidine 0,04 mg/kg iv. Schirmer Tear Test I was performed at different times during sedative procedures: before sedation and 5, 15, 30, 60, 120 and 180 minutes after drugs administration. Data were analyzed by SAS 9.4.

A significant decrease in horse's tear production was recorded after 15 minutes in detomidine group and after 60 minutes in detomidine plus butorphanol group. No significative differences were noted in xilazine and romifidine groups. These results suggest that xilazine and romifidine don't affect tear production while when medetomidine alone or in combination with butorphanol are used for sedation, it's necessary to employ tear substitutes in order to avoid corneal dryness also in horses.

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EFFICACY OF TWO TOURNIQUET TYPES FOR INTRAVENOUS REGIONAL LIMB PERFUSION (IVRLP) WITH MARBOFLOXACIN IN STANDING DAIRY CATTLE

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Antimicrobial IVRLP has become a complementary therapy for treatment of distal limb infections in large animals (1, 2). There are no previous published studies investigating the use of marbofloxacin in IVRLP in cattle. The goals of the present study were to evaluate the safety and the efficacy of 2 tourniquet types detecting potential violative residues in milkings after a single IVRLP with marbofloxacin in clinically healthy, standing non-sedated dairy cows. Ten adult animals were included in the study, which was approved by the local ethical committee (Prot. 41/2012/CEISA). One pelvic limb of each cow was randomly selected and assigned to 1 of 2 groups (5 limb/group). Group 1 had a wide rubber elastic tourniquet (10 x 500 cm, 6-8 full circumferential turns; Esmarch Bandage) and group 2 had a manual pneumatic tourniquet (11 x 76 cm cuff at 300 mmHg; VBM ® Germany). The dorsal common digital III vein was used to perfuse the pelvic limb. After the tourniquet was applied by the same clinician above the tarsus around the distal portion of the tibia, a 19 g butterfly needle was introduced into the vein and 0,67 mg/Kg of marbofloxacin (Marbocyl 10%® Vàtoquinol) diluited to 60 ml with sterile water for injections was infused manually by a slow bolus injection over 60-90 seconds. Blood samples were collected from the jugular vein on times: 0 (before injection), 0.08, 0.25, 0.5 (immediately after the tourniquet was released), 1, 2, 4, 8, 12, 24 and 48 hours after injection. Synovial samples were aseptically collected from the tibiotarsal joint on times: 0, 0.5, 1, 2, 4, 8, 12, 24 and 48 hours after injection. Composite milk samples were manually collected from each gland of every cow (pool of all quarters) at the following time: 0, 12, 24, 36 and 48 hours after injection. All samples were analyzed for marbofloxacin concentration using liquid chromatography tandem mass spectrometry. The pneumatic tourniquet resulted in significantly higher synovial fluid mean peak marbofloxacin concentrations in all cows (77.33μg/ml $\pm$ 6.85 at 1 hour in group 2 versus 6.17μg/ml $\pm$ 1.86 at 0.5 hour in group 1) in combination with lower mean plasma concentration before tourniquet was removed (1.19 μg/ml $\pm$0.36 at 0.08 hour and 1.24 μg/ml $\pm$0.36 at 0.25 hour in group 2 versus 2.18 μg/ml $\pm$1.15 at 0.08 hour and 1.48 μg/ml $\pm$0.68 at 0.25 in group 1) and then higher just after the tourniquet was released (2.87 μg/ml $\pm$1.46 in group 2 at 0.5 hour versus 0.66 μg/ml $\pm$0.28 at 0.5 hour in group 1). All milk residual values were below the maximum permitted levels by current European Legislation. Based on the obtained results in this study it is assumed that the high concentrations of marbofloxacin achieved in synovial fluid with the application of the pneumatic tourniquet may have an effective therapeutic effect against the major bacterial pathogens involved in bovine distal limb infections. This study represents an initial step in evaluating the potential application of marbofloxacin for treatment of deep digital septic conditions in dairy cattle when administered by IVRLP.

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THE USE OF COMPUTED TOMOGRAPHY IN THE DIAGNOSIS OF TRAUMATIC ACETABULAR FRACTURES IN A COLT

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Pelvic fractures are uncommon in horses even if a predisposition in female horses less than 2 years old is reported in literature. Fractures involving the acetabulum are related to a trauma, often a fall. A definitive diagnosis can be reached by standing radiography, ultrasonography, computed tomography (CT), scintigraphy and diagnostic arthroscopy. This case report describes the radiographic and CT findings in a colt with an acetabular fracture. A five month old Barockpinto colt was referred for an acute severe lameness of the left hindlimb of 3 weeks of duration. The colt was treated by the referring veterinary surgeon with phenylbutazone and box rest for one week with a mild improvement of the lameness. At presentation , the colt showed good body condition and clinical parameters were in normal range. A moderate swelling on the left coxofemoral region was noted. The colt was lame at walk and the abduction of the limb induced a moderate pain. The colt was sedated with alpha2-agonist and latero-lateral and ventro-dorsal radiographic obique views of the hip were taken with the horse in standing position The colt underwent CT of the pelvis in dorsal recumbency, under general anesthesia. Radiographic examination revealed a left coxo-femoral diastasis, irregular margins of the acetabulum associated with radiolucent areas and subchondral sclerosis of the fovea capitis. Computed tomography findings consisted in multiple fractures involving the dorsal acetabular margin in association with dorso-cranio-lateral dislocation of the femoral head. In proximity of the caudo-ventral aspect of the acetabular fossa were present several small mineralized fragments. Moderate sclerosis was seen in the fovea capitis and a mild osteopenia of the left femoral head. Despite the radiographic views allowed to recognize the presence of a femoral head luxation and irregularities of the acetabular margings, only CT images revealed the presence of multiple fractures along the acetabular rim improving the prognostic value for the patient. Compared with radiography in dorsal recumbency with the limbs in a frog-legged posture, extended positioning of hindlimbs used fot the CT acquisition, induced less stress on the fracture3 In conclusion, on the basis of our experinece and in accordance with other studies, CT examination provides more valuable information than radiographs in the diagnosis of the coxo-femoral joint disease.

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PRELIMINARY RESULTS ON A NEW CT PROCEDURE FOR EVALUATING THE ELBOW IN THE DOG

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Computerized Tomography (CT) is increasingly used for musculoskeletal imaging and a number of studies had demonstrated its importance in canine elbow dysplasia. Anyway, in spite of different protocol studies proposed, it still not exists a standardized CT procedure for canine elbow study. All the proposed CT procedures require a stressful positioning of the neck with the head pulled laterally or caudally in order to insert only the elbow joints in the gantry.1 Unfortunately, the breeds predisposed to the elbow dysplasia often are at the same time frequently affected by Wobbler syndrome. Consequently, the suggested positioning could be particularly dangerous to the patient. Therefore, we proposed a new CT procedure in which the positioning used is not stressful for the cervical column.2

Aim is to evaluate a new CT procedure for canine elbow study.

50 elbows, from 25 dogs, were included. All the studies was performed with the dog in lateral recumbence, with forelimbs cranially pulled and closely placed against the neck. Care was put in removing air between the forelimbs and the neck in order to reduce streak artefacts and to obtain the smallest field of view (FOV) including both elbows. CT studies were performed with a helical single slice scan (Prospeed plus, General Electric) using axial contiguous 1 mm slices and a bone convolution filter; the slices were acquired at 120 kV and 200-260 mAs. Every study was evaluated on transverse slices, sagittal and dorsal reconstructed images and using a bone window (WW: 3500; WL: 500).

In all dogs, but two, the images were clean and without artefacts. The FOV ranged from 15 to 10 cm, mean FOV was 12.7 cm ($\pm$2.00). All the studies were diagnostic: 36 fragmented medial coronoid process (FMCP); 26 elbow incongruity (EI); 3 osteocondritis dissecans (OCD) of the humeral condyle; 1 ununited anconeal process (UAP).

The two studies with strike artefacts in the proximal portion of elbow belonged to the first performed with the new CT procedure and artefacts were referred to the presence of residual air between the forelimbs and the neck. However, the most of the studies had satisfactory quality (good spatial resolution and good noise to signal ratio) as we already assessed in a previous study.2 Therefore, the new CT procedure had a series of advantage: simple and non-stressful positioning; reduced dose radiation to the patient; simpler image evaluation, due to the simultaneous visualization of both elbows. The next step of our work will be to develop a customized positioning device in order to reduce artefacts, to standardize and simplify the positioning procedure and to obtain the symmetrical alignment of the elbows.

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FEASIBILITY OF A REAL-TIME, ULTRASOUND-GUIDED, LUMBO-SACRAL EPIDURAL CATHETER PLACEMENT IN DOGS: CADAVERIC STUDY

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Epidural catheterization is performed in veterinary medicine to prolong postoperative analgesia. In small animals the failure rate of the blind technique for epidural injection has been reported to be 25-30% (1). Several factors may influence the success of the blind technique, including patient obesity, vertebral abnormalities and operator's experience (2). To decrease the failure rate, different ultrasound-guided regional techniques have been described with a widespread interest in using them in clinical practice.

Aim of the study is to establish the feasibility of a lumbosacral epidural catheterization in dogs by using an in-plane ultrasound (US) needle guidance system.

Ten dogs, euthanized for reasons unrelated to the study, were used. Three cadavers were used to describe the US anatomy and to establish the US-guided injection technique. The remainig 7 cadavers were positioned in sternal recumbency with the hindlimbs pulled forward. Three variations of the technique were performed: a blind technique (BT), a US-guided two-operators (US2) and single-operator techniques (US1). BT and US2 were performed by 3 different operators: an anesthetist trained in blinded locoregional techniques but without ultrasound experience (A), a student without experience in locoregional technique and ultrasound (B), an experienced sonographer without experience in locoregional techniques (C). US1 was performed by operator A and C. An epidural catheterization kit with a Tuohy needle (18 gauge x 90mm) was used. Anatomical landmarks location, procedural execution time and the numbers of attempts were recorded. Procedure was considered failed after 10 attempts or 10 minutes. Epidural catheterization was considered successful when the catheter was directly identified in the epidural space. Data were analysed by ANOVA ($p< 0.05$).

US anatomical landmarks were identified as previously described (2). A sagittal longitudinal approach, performed with a microconvex 5-8 MHz probe was chosen. The probe was caudally armed with an in-plane, 18 gauge, US needle guidance, with a fixed angle needle path of 15°, that enabled to visualize the needle insertion according to on-screen software guidelines. A total number of 56 procedures were performed. BT failed 3 times by operator C, US2 1 time by operator B, and US1 2 times by operator A and 1 time by operator C. Anatomical landmarks location time was statistically lower for operator A with BT compared with US1 and US2, and with US2 compared with US1. Operator A had a statistically higher time of location with US1 than operator C with BT. Procedural time was statistically higher for operator A with US1 than US2 and BT, and higher with US2 than C with BT. Procedural time was higher for operator C with BT than US2. Number of attemps were higher for A with US1 than US2 and BT, and lower with BT than C with US2. Operator C had higher number of attempts with BT than US2. Operator B had a statistically higher number of attempts with US2 than BT.

This technique is suitable for lumbo-sacral epidural catheterization in dog. US needle guidance can help in needle insertion specially for inexpert operator by using US2. Both US1 and BT require a training period.

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PITUITARY CHROMOPHOBE CARCINOMA IN A DOG: CLINICAL, TOMOGRAPHIC AND HISTOPATHOLOGICAL FINDINGS

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A 9 year old, male mixed-breed dog was presented for evaluation of oral dysphagia and progressive aggressiveness towards the owner and the operators. The aim of this work is to describe clinical, tomographic and histopathologic features of pituitary chromophobe carcinoma in a dog. At the clinical examination the patient was normothermic, polypnoic (>50 apm) and tachycardic (>140 bpm). The neurological evaluation revealed normal postural reaction and normal cranial/spinal reflexes, mental depression, aggressiveness and crotaphyte muscles atrophy. Due to the impossibility to establish a specific neuronal localization, the diagnostic procedure included blood analysis with leukocyte formula, chest x-rays and abdominal ultrasound, with no relevant findings detected. Due to the aggressiveness and the mental depression after five days the patient was referred for brain Magnetic Resonance Imaging (MRI). MRI revealed an intense ventricular asymmetry, discrete left deviation of the falx cerebri, enlargement of the third ventricle and the presence of a large (18x20x15mm) spheroidal mass in the sellar/parasellar region charcterized by isointense on T1 weighted images and discretely hyperintense on T2 weighted and FLAIR. In the dorso-lateral portion of the mass, a circular lesion (6 mm diameter) characterized by intense signal hyperintensity on T2 weighted images was detected After intravenously paramagnetic contrast medium administration, the mass showed dishomogeneous intense enhancement. A pituitary macroadenoma (invasive adenoma/adenocarcinoma) characterized by the presence of a necrotic/cystic lesion was suspected. Because of the invasive natur of the lesion the owner decided to euthanize the patient. Brain histopathology was performed, confirming the presence of a pituitary chromophobe carcinoma. Pituitary carcinomas have been rarely observed in old dogs, moreover, cases of pituitary neoplasm with intense cellular pleomorphism and elevate mitotic index in absence of metastatic lesions are extremely rare. These neoplasms can cause serious functional disorders due to the destruction of the pars distalis of the neurohypophysis. In humans the distinction between invasive adenoma and pituitary adenocarcinoma is based on the finding of intracranial or systemic metastasis .It is believed that adenocarcinoma originate from malignant transformation of pre-existing adenoma, after a variable latency period. In the presented case, despite the absence of systemic and intracranial metastasis, the infiltrating growth pattern and the presence of neoplastic cells that arrive and surround the third ventricle, together with the intense cellular pleomorphism, guided the diagnosis to a malignant transformation of the neoplasm.

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"COUNTS" REALLY "COUNT"?

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Bone scintigraphy is widely applied in Veterinary Medicine, especially in equine patients. Its usefulness is strictly correlated to the knowledge of its added value but also the recognition of its limits. A complete clinical investigation based on orthopaedic evaluation and diagnostic analgesia still remains paramount for a correct localization of the lameness.

The aim of this retrospective study was to evaluate in which clinical cases the scintigraphic examination is mandatory for a certain diagnosis and, in the other hand, in which horses the scintigraphic findings could have a poor diagnostic value or mislead the diagnosis. Images obtained form Radiographic and Magnetic Resonance (MRI) examination were correlated with clinical and scintigraphic findings in order to evaluate the diagnostic capability of scintigraphic examination in different body region and define guidelines for the exam requests for the reverring veterinarian surgeons.

All horses included in the study underwent bone scinthigraphy, MRI and/or radiographic examination. Scintigraphic findings were cross-referenced with other imaging modalities and patient clinical data were evaluated.

Thirty-one horses (20 lame horses and 11 affected by poor performance) had been included in this study. Twenty horses underwent scintigraphy and radiography, four horses underwent scintigraphy and MRI and seven horses underwent scintigraphy, radiography and MRI. Eighteen horses underwent total body bone scan while in five horses the study focused on one anatomical region (i.e. forelimbs) and in eight horses images from two regions (i.e. forelimbs and neck) were obtained.

Only in 6 horses scintigraphy was able to show pathological findings ascribable to a correct and definitive diagnosis. In these horses, the lesions involved the origin of the suspensory ligament, the sacro-iliac joint, the coxo-femoral joint and the navicular bone. In 14 horses bone scintigraphy identify the localization of lameness but not the cause and, in 10/14 cases the combined use of different imaging modalities permitted to reach the final diagnosis. In 11 horses,scintigraphic examination revealed findings of ambiguous clinical interest but, because of the referring veterinarian surgeon did not perform the dignostic blocks, nor the localization neither the cause of the lameness were identifiyed.

As in literature also in this study scintigraphic examination alone was able to lead to a definitive diagnosis in which sacro-iliac and coxo-femoral joint were involved. In horses with suspensory ligament and navicular bone pathology scintigraphy shows high sensibility and good accuracy but MRI provides relevant informations both for treatment and prognosis. In most cases scintigraphy should be considered as a part of a diagnostic process due to the poor specificity. Often ambiguous findings were detected during scintigraphyc examination to wich it was not possible to attribute a clinical relevance due to a lack of anamnestic data. Nevertheless, sensibility and specificity can be increased by a complete and detailed orthopedic evaluation.

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MINIMALLY INVASIVE PERCUTANEOUS FLUROSCOPIC TECHNIQUE TO INJECT THE LUMBO-SACRAL DISK IN THE DOG: PRELIMINARY STUDY IN TWO DIFFERENT POSITIONINGS

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Lumbo-sacral pathology is one of the most diffused degenerative spinal disease in small animal geriatric medicine. Aim of the work is to evaluate two different approaches (lateral or sternal recumbency) for lumbo-sacral disk injection in the dog with a percutaneous minimally invasive technique under fluoroscopic guidance.

Ten dogs (2,8-25,6 Kg.) died for causes unrelated to this work were enrolled in the perspective study. Eight intervertebral lumbo-sacral disks have been injected with a gelatinous radiopaque compound. Two disks have been injected with an alcoholic radiopaque solution. The two different solutions were associated with methylene blue for the subsequent gross anatomic examinations. Five dogs have been positioned in lateral recumbency with the lumbo-sacral joint in neutral position, five dogs in sternal recumbency with the hind limbs extended cranially along the body and the lumbo-sacral joint flexed. The correct injection of the compunds within the disk was assessed by Computed Tomography (CT) and subsequent gross anatomic examination.

Lateral recumbency approach required less time of execution and minor attempts to reach the correct position of the spinal needle. In two cases leakage outside the disk was observed with one of them involving structures within the vertebral canal. The injected lumbo-sacral disks were successfully visible by CT, while necroscopy resulted satisfactory only in five patients.

Fluoroscopy could successfully applied as a feasible modality to guide the percutaneous injection of the lumbo-sacral disk. Lateral recumbency approach resulted in an easily time-saving procedure that will probably be helpful in the future for delivering safely injectable therapeutic solutions, as in case of chemonucleolysis.

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RADIOGRAPHIC FINDINGS AFTER ORO-GASTRIC DECOMPRESSION IN 24 DOGS WITH GASTRIC DILATION VOLVULUS

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Gastric dilation and volvulus (GDV) is an acute, life-threatening condition that primarily affects large and giant breed dogs. GDV is most often diagnosed by means of a right-lateral radiograph of the abdomen. Immediate goals in treatment of GDV include restoring circulating volume and gastric decompression with trocarization or oro-gastric tubing. Radiographic appearance and position of the stomach after oro-gastric tubing is poorly documented.

The aim of this retrospective study is to evaluate the radiographic appearance of the stomach after GDV decompression by means of orogastric tubing and to understand if any radiographic pattern can predict persistent volvulus.

Post oro-gastric tubing radiographic reports of 24 dogs with a diagnosis of gastric dilation and volvulus were collected; initial classification as "resolved" and "unresolved" volvulus was recorded, as well as time elapsed up to surgery. All radiographs were reevaluated to find and describe any common signs that can help in distinguish cases. Surgery reports, when available, were used to confirm the position of the stomach.

14 dogs had a "resolved" volvulus radiographic report and other 11 had a "unresolved" volvulus radiographic report. All the unresolved cases had the same radiographic pattern: the stomach showed a horseshoe shape with ventral concavity associated to variable amount of endoluminal gas. All of them underwent surgery; only four surgical reports described the gastric position and all of them confirmed the volvulus. The resolved cases were less homogeneous: 6 dogs showed a normal gastric axis, and in 8 cases we were not able to evaluate stomach position from radiography, due to a lack of endoluminal gas or to an "unusual" gastric appearance not belong to horseshoe shape or normal gastric axis. 11/14 dogs underwent surgery and 1/14 dog died before surgery. One dog with normal gastric axis had a clinical relapse after 9 hours and repeat a radiography showing an "unusual" gastric appearance; one case with lack of endoluminal gas was refilled with a mild amount of gas, showing a horseshoe shape.

The "horseshoe shape" pattern indicates a partially decompressed stomach with persistent volvulus. A presumably normal gastric axis does not rule out persistent volvulus. In several cases the gastric position cannot be properly assessed radiographically due to a lack of endoluminal gas; in such cases no conclusion can be drawn about resolution of the volvulus.

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MODIFIED MAQUET PROCEDURE (MMP ORTHOMED ®) FOR TREATMENT OF CRANIAL CRUCIATE RUPTURE IN LARGE BREED DOGS: FIRST EXPERIENCES AND OUTCOME

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The modified Maquet Procedure (MMP) uses the same principle as the tibial tuberosity advancement (TTA) for stabilization of the cranial cruciate ligament deficient stifle in the dogs. The original technique was first described by Maquet in 1976 in humans. The procedure obtain the tibial tuberosity advancement leaving intact a distal bony attachment to the tibial shaft. In the MMP, the plate and fork, originally described by Montavon et al., are replaced by a titanium foam wedge and an eight figure wire circlage act as tension band.

The objective is to describe the MMP technique, radiographic bone healing, degree of degenerative joint disease (DJD), clinical outcome in 11 dogs.

Eleven dogs with CrCl failure, underwent to MMP TTa. For every patient in the preoperative (T0) were taken radiograph of hip and knee and the degree of DJD was evaluated. The follow-up consisted in clinical and radiological evaluations, once a month for 6 months (T2 to T6). All X-Ray study were evaluated for degree of DJD, as described by Morgan in 2010 (1). Healing of the osteotomy site, cutaneous healing, and minor or major complications were also evaluated clinically.

Mean age and weight are respectively 36±8,7 months, 39,7±11,4 kg. Median lameness score was 3. DJD degree scores did not differ at the different time points No major complications occurred. Two patients had a fracture of distal bone attachment to the tibial shaft, after an accidental trauma. One dog had a lateral patellar luxation. Clinical bone healing occurred by a mean of 6.8 weeks (range 4 to 12 weeks). At the 12-week follow-up, all dogs had radiographic signs of complete bone healing.

The MMP deserves consideration as a primary treatment option for cranial cruciate ligament rupture in dogs. The results of this study suggest that it is technically possible to achieve an advancement of the tibial tuberosity using a titanium foam wedge. The advantages of this technique consist in reducing the soft tissue trauma due to the minimal surgical approach. Furthermore compared to the classic TTa this technique allows to reduce surgical and recovery time by using a well tolerated biomaterial according to the concept of "biological repair". However cage restraint is advisable for young and brisk dogs in the first week post surgery. Long- term follow-up and a larger sample would be necessary to a better evaluation of the techniques.

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POROUS TTA (CANARIA) IN THE TREATMENT OF CRANIAL CRUCIATE LIGAMENT RUPTURE IN SMALL ANIMAL

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The presented study describes an innovative way of performing the Tibial Tuberosity Advancement (TTA) procedure in the surgical treatment of the rupture of the Cranial Cruciate Ligament in companion animals. Such new technique avails itself of a custom-made porous scaffold made of titanium Ti6Al4V ELI, specifically engineered to be highly biocompatible for bone tissue and to exert osteoinductive and osteoconductive properties when sustaining cyclical charge,as precedently demonstrated in studies on an ovine experimental model.

The aim of the study was to evaluate and validate the orthopedic employment of the described porous scaffold. Specifically for its employment in the TTA technique, the scaffold was tested both in its osteoinductive and osteoconductive aptitude,and as a mean of further stabilization of the osteotomy site. This with the final aim of proposing a simpler, more efficient, and less expensive technique in the treatment of CrCL rupture.

Starting on January 2014,eligible patients were chosen to be included in the study among the spontaneously referred cases of CrCL rupture to our department: up to the present day, the technique has been performed on 14 animals (13 dogs and one cat).Before undergoing surgery, every patient was subjected to a throughout radiographic study designed to evaluate the degree of tibial advancement to be obtained by the surgery, and thus, the dimension of the scaffold to be employed. The surgical procedure was executed as a standard TTA technique, with the insertion of the porous scaffold in the osteotomy site and the aid of a titanium plaque to guarantee the maximum stabilization of the Maquet-hole. Thirteen different sizes of scaffolds and seven sizes of plaques were available to tailor the procedure on very different kinds of patients.

Immediately upon surgery, on every patient was performed another RX study to verify the obtainment of the desired 90 degrees knee angulation, necessary to attain a stable joint. Further clinical and RX investigations were repeated at scheduled times to estimate the degree of functional recovery and the quantity of newly-formed bone tissue around and within the scaffold in the osteotomy site.

Today, upon the results obtained in our study we can sustain that the Porous TTA represents a valid alternative to the surgical techniques traditionally employed in the CrCL rupture. Such technique proved to be objectively advantageous for multiple reasons, such: the feasibility on a very wide selection of patients, ranging from giant to medium-small sized; the short time required to complete the procedure ;the very low employment of extraneous material and the mini-invasive approach which both guarantee minimum post-surgical pain;the particularly rapid recovery of a completely functional gait in the majority of patients; the efficacy as a revision surgery after faulting interventions on the knee; and finally, the low expense required.

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FORCE PLATE ANALYSIS FOR COMPARISON OF LATERAL FABELLAR SUTURE AND MAQUET MODIFIED PROCEDURE TIBIAL TUBEROSITY ADVANCEMENT TECHNIQUES FOR TREATMENT OF DOGS WITH CRANIAL CRUCIATE LIGAMENT DISEASE.

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Subjective evaluation of a clinician is only able to perceive a few kinematic variables at a time, but a kinetic analysis system can capture, analyze, and store hundreds of observations per second1. Force plate gait analysis is a valuable method to obtain objective data on limb loading in dogs, and is increasingly being used to evaluate the outcome of surgical or medical treatments for orthopaedic conditions2. Previous studies3 of the gait analysis for the comparison of surgical techniques in dogs with cranial cruciate ligament rupture compared extra articular stabilization with tibial osteotomies. In literature, there are no reports about gait analysis of dogs treated with Maquet Modified Procedure (MMP) Tibial Tuberosity Advancement (TTA).

The purpose of this study is to compare 6 months outcomes after lateral fabellar suture stabilization (LFS) and MMP tibial tuberosity advancement for the treatment of dogs with cranial cruciate ligament disease and to determine through pressure platform gait analysis whether one of these techniques has better patient outcomes.

Twenty-two dogs with naturally occurring unilateral cranial cruciate ligament disease were randomly assigned to undergo LFS (n = 11) or MMP (n=11). Outcome data were obtained using a pressure platform for gait analysis and were collected prior to surgery (T0) and at 4 (T1), 12 (T2) and 24 (T3) weeks after surgery. Peak vertical force (PVF), vertical impulse (VI), both converted in percentage of body weight (BW), and loading time (LT) were recorded. The same investigator collected five correct attempts for each study. The aspect of the gait curve was observed at each control.

PVF of affected hind limbs at a walk was 5% higher for dogs in the MMP group (33.3$\pm$3.5) versus those in the LFS (31.64$\pm$3.17) group, during the 6 months after surgery. No difference in the analysis of VI between two groups. A better response was observed in the improvement of LT, appearing 8% higher for dogs in MMP group (0.6$\pm$0.1) versus those in the LFS group (0.55$\pm$0.04). The gait curve improved during the follow up in both groups, with tendency to a progressive normal shape at the first evaluation.

Dogs in both groups had good to excellent outcome at 6 month follow up, but kinematic analysis results indicated dogs that underwent MMP tibial tuberosity advancement had better outcomes than those that underwent LFS.

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CLINICAL AND RADIOGRAPHICAL ASSESSMENT OF A SINGLE INTRA-ARTICULAR INJECTION OF PRP ON OSTEOARTHRITIC JOINT IN DOGS: PRELIMINARY STUDY

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Osteoarthritis and articular degeneration are the most important causes leading to a poor life quality both for patients and the owners. Many approaches had been developed in veterinary medicine and in the last decades clinicians are paying attention to infiltrative substances such as non steroid anti-inflammatory or steroid drugs, hyaluronic acid and blood derivates. One of the latter is platelet rich plasma (PRP), whose use is increasing but lacking of evidences and standardized procedures of preparation and administration. Its regenerative potential had already been established in human medicine, both in vitro and in vivo, and numerous experimental animal in vitro studies had been performed suggesting a promising outcome (1). Therefore in vivo studies are required in order to assess a real efficacy of PRP, a safety and relatively easy clinical application (2). AIM-The aim of this study is to point out the effect of a single PRP injection in osteoarthritic joints of dogs in a period of three months examining clinical and radiographical scores, other than owners satisfactory grade.

Autologous PRP preparation - Autologous anticoagulated venous blood samples were centrifuged twice, and platelet pellet was than resuspended in platelet poor plasma (PPP) at a final concentration ranging from 7 to 10 folds above whole blood platelet count under aseptic conditions .Patients - Six dogs (3.6 ś 2.56 years old, body weight 35.05ś16 Kg) with osteoarthritis involving a single joint were enrolled. All patients underwent a general visit and serum blood analysis. After sedation and intravenous anaesthesia, the autologous PRP was injected intra-articularly, after a clinical evaluation of synovial fluid aspect. Clinical and radiographical evaluations - Patients underwent a clinical evaluation (lameness, clinical objective assessement, response to manipulation) using standard tables with three grades at 0, 15, 30, 60 and 90 days from the infiltration. Radiographs of both the affected joint and the normal one were made at day 0, 30, 60 and 90 from injection. Projections for elbows were lateral standard at 130°,at 90°, at 45°, at maximum extension, and standard antero-posterior and antero-posterior with 15° of pronation; for other joints, standard lateral antero-posterior and postero-anterior were made. Same radiographical parameters were maintained during the study. Radiographs were evaluated following the International Elbow Working Group graduation by two radiologist blinded to the study. Satisfactory grade of the owners were assessed using the Liverpool Osteoarthritis in dogs questionnaire at the first visit and at day 90 of the follow up. CONCLUSIONS -An overall positive effect of the autologous treatment was observed in all dogs. Radiographical scores showed no statistical difference between all time points of the study, suggesting a stability of both affected and normal joints. Clinical lameness scores at 90 days were significantly different from those observed at 0 and 15 days ($p<0,036$); while clinical objective assessements and manipulation response were significant between day 0 and 90 days ($p<0,05$). All owners' questionnaires indicate a reduction in lameness and pain.

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STRAY DOGS AND ROAD ACCIDENTS IN THE URBAN AREA OF NAPOLI: GIS BASED ASSESSMENT AND ANALYSIS

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Stray dogs are the highly exposed to traffic accidents, especially in urban areas. In the literature there are many studies that use geographical information systems (GIS) for pedestrian safety,1 while there are no studies on the use of such technologies to assess the risk of road accidents for dogs.

The aim of study was to assess and analyze the road accidents involving stray dogs in the urban area of Naples (southern Italy), using GIS technology.

A retrospective study was performed on clinical records of 251 stray dogs hospitalized in the years 2012 and 2013 (data from the Regional Centre for Urban Veterinary Hygiene (CRIUV), the Veterinary Hospital of the local public health NA1 and the Interdepartmental Center of Veterinary Radiology). Traumatic lesions were diagnosed using clinical and imaging findings. Street name, date of the accident, type of the lesion, breed, body size, age, and gender were recorded for each stray dog. These dogs were classified into three age classes: 0-2 years, 2-7 years and >7 years; in addition, according to their body size, they were divided in large (>25 kg), medium ($\leq$25 >8 kg) and small ($\leq$8 kg). The chi-square test was used to evaluate the influence of age, sex and season of the year on the accidents. All road accidents were geocoded using Google Earth. In addition, a GIS software (ArcGIS 10.2.2, ESRI, Redlands, CA, USA) was used to develop a map of the accidents (density surface) using the Kernel density analysis.

Kernel density estimation method identified six areas distributed in four neighborhoods of Naples, in which stray dogs had a higher risk to undergo to road accidents. Three of the above mentioned neighborhoods are located in the outskirts of the city while one is near the city center. However, all the areas with higher risk of road accidents are characterized by the presence of many high-speed motorways. Our data showed a significant higher prevalence of road accidents in young dogs during the spring ($p<0.05$). This result may depend on less experienced dogs moving in the breeding season and therefore more prone to undergo to road accidents. The geolocalization of the car accidents involving stray dogs may help to elaborate road-risk maps useful also for the safety of driving people. In fact, on the basis of the risk maps, it is possible to develop strategies to prevent accidents, such as the positioning of specific road warning signs, speed bumps, etc. Our results may be considered as a first step to obtain a database that, if conveniently integrated with available database used for human road accident risks, 2 could lead to develop a platform for local public health and public administration within the concept of One Health.

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TREATMENT OF LONG BONE FRACTURE IN A WILD RAPTOR POPULATION: A LONG TERM RETROSPECTIVE STUDY

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Hunting accidents, vehicle collisions, impact with anthropogenic structures, competition for food, poisonings, gunshots and poaching, represent just some of the most frequent causes of trauma and injuries among wildlife fauna including birds of prey1- 3. Being a predator and hence a member at the top of the ecological food chain, raptors more than other predators, suffer the huge anthropogenic impact on the environment observed in recent times1-3. The apparent increased risk of fractures and skeletal trauma highlights the need for more in depth study on both causes of morbidity in raptor traumatology2 and the best treatment for orthopaedic problems that allow for a more rapid return back into their habitat. Evaluation of the results of conservative and surgical treatments carried out on raptors, with orthopaedic fractures, which were admitted alive to the Wildlife Recovery Centre "Corpo Forestale dello Stato, Nucleo Territoriale per la Biodiversità" CRAS Pescara Italy, between 1 January 2005 and 31 December 2014. A sample of traumatised raptors including orthopaedic and non-orthopaedic patients was obtained by the review of medical records of mixed avifauna admitted to the centre over a 10 year period. From this sample, a sub-sample of raptors became the object of this study including only the traumatised raptors that were admitted alive to the recovery centre presenting with fractures of the skeletal system and requiring conservative or surgical therapy. The species belonging to the orders Falconiformes and Strigiformes, were classified according to the type of injury sustained, the bone segments involved, the types of treatments received, the length of hospitalisation and outcome provided. The most common type of fracture was primarily concerned with the wing bone segments such as: ulna, humerus, radius and carpometacarpus; while the lower limb was marginally affected: femur, tibiotarsus, tarsometatarsus and ossa digitorum pedis4. The treatments were performed according to conservative or surgical stabilisation methods with a similar percentage of subjects receiving each treatment. Conservative treatments provided were: figure-of-eight wing bandage, wing-body wrap and splints, associated to a variable period of cage rest. Surgical treatments provided were: intramedullary Kirschner pin, cerclage and bendage, External Fixation with intramedullary tie-in configuration pin and imping. Of the total raptors admitted and treated, only a small number were classified as unrecoverable and became permanent residents, instead the majority were released with success back into their habitat. The first objective evidence indicates the bone segment most affected by fractures was the ulna, highlighting wing pathology as having greatest incidence in all orthopaedic pathology in raptors.

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FRACTURE REPAIR IN WILD RAPTORS USING LOCKING COMPRESSION PLATES: A BIOMECHANICAL STUDY

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Treatment of wing fractures in raptors is a challenging problem for orthopaedic surgeons and is one of the most debated topics in wild animal surgery. Currently External Skeletal Fixation (ESF) is the treatment of choice for fracture fixation in birds1. Although bone plates actually have clear advantages in many species compared to other fixation methods, this technique is less frequently used in avian surgery2. A new generation of bone plates called Locking Compression Plate3-5 have recently been developed in veterinary orthopaedic surgery. These plates could be ideal for fractures repairs in birds of prey due to their innovative characteristics. The clinical relevance the biomechanical advantages, the occurrence of complications and the surgical technique options, are unknown in these species. The purpose of this study was to evaluate the use of LCP in experimental fractures of humeral and ulnar diaphysis in wild birds and to determine if LCP provides adequate stability in a cadaveric humeral fracture model.

Specimens preparation: 11 humeri and 11 ulnae were obtained from different species of raptors (Buteo Buteo, Strix Aluco, Tyto alba, Circus Approximans) that were admitted at the Wildlife Recovery Centre in Pescara (Corpo Forestale dello Stato, Nucleo Territoriale per la Biodiversità). A radiological examination of the specimens was taken to assess skeletal maturity, the absence of pre-existing pathology and to make morphometric measurements. A four or six hole plate (Fixin 1,9-2,5 series with 4 screws ø 1,9) was dorsally applied to each isolated bone segment, and then a mid diaphyseal ostectomy was created in all constructs. The tests were performed using a specific mechanical device; some components of this machine were designed and realized specifically for the correct positioning of the specimens. Static bending test: static test was performed on 4 humeri, one of each species. The test consisted of the application of a flexion stress force until the failure of constructs occurred. This force was registered as Maximum Load of Breaking (MLB). Dynamic cyclic bending test: this test was performed on 16 specimens; for each test a maximum load (75% of MLB), preload (5% of maximum load), frequency (600 rpm) and number of cycles (21.000) was set to allow for elastic deformation. The data obtained from the mechanical tests were collected and processed with NI Signal Express Project software. At the end of the tests, all specimens were evaluated macroscopically and x-rays were performed to determine the presence of any bone lesions, their localisation, mode of propagation and the location of the maximum stress.

The four-point bending flexural test has allowed a detailed characterisation of the bone-LCP construct in bending elasticity, flexural stress-strain response and breaking point. Using the high-cycle fatigue test, the weakening of the specimens caused by repeatedly applied loads was measured. This biomechanical cadaveric study, validates the application of this surgical technique on birds of prey and confirmed that the LCP can be a solid fixation method to treat wing fractures.

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SURGICAL EXPERIENCE IN THE FIRST YEAR OF THE SICILIAN REHABILITATION CENTER FOR SEA TURTLES

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The loggerhead sea turtle is considered in danger of extinction and is protected by C.I.T.E.S. (Convention on International Trade in Endangered Species of Wild Fauna and Flora). The Mediterranean basin remains a nesting area for this animal, but they are frequently victims of human activities that result in injury and death.

Between March 2014 and February 2015, the Centro Regionale di Recupero for sea turtles, located at the Veterinary Public Health Institute of Sicily (Palermo, Italy) monitored Sicilian coastal areas to detect stranded sea turtles. Each rescued turtle was registered, identified by its morphological traits, sexed, measured and weighed. Physical and X-rays examinations were conducted to evaluate the health condition of the animal and to identify the presence of ectoparasites, external lesions and/or ingested fishing gears. Following the techniques previously described by Di Bello (Di Bello et al., 2013), surgical procedures to remove accidentally ingested hooks and monofilament lines were performed after few days of rehydration therapy. Esophagotomies through the ventral portion of the neck, and enterotomies reaching the coelomatic cavity by the prefemoral fossa, were performed under inhalational general anesthesia. Anesthetic protocols provided for a premedication phase (Medetomidine, 50 μL/Kg, IM) followed by an induction phase (Propofol, 5 mg/kg, IV), and, when the tipping reflex disappeared, the patient was intubated and anesthesia was maintained with isofluorane (1.5-2%) and oxygen.

A total of 100 loggerhead sea turtles were rescued alive during one year of activity at the Centro di Recupero Regionale for sea turtles, 60 of which were in critical condition and died in the first hours of hospitalization. All turtles were identified as belonging to the Caretta caretta species. Clinical examination findings suggested surgical approach in twenty-six cases. Briefly, 9 esophagotomies, 11 enterotomies and 4 instances of both procedures were performed to remove fishing gear. In two turtles, fishing hooks were removed from the oral cavity under anesthesia without any incisions. Ten turtles had compromised intestinal and coelomic conditions, such as intestinal intussusception, volvulus, severe intestinal congestion and celomitis that did not allow them to recover. Serious injuries highlighted during surgery were confirmed by post-mortem examinations, and the most frequent pathology was coelomitis followed by other pathological signs such as serum hemorrhagic effusion, pericardic effusion, intestinal volvulus, intestinal perforation and pulmonary emphysema.

Anesthetic and surgical techniques for sea turtles are now very successful. However therapeutic success depends on the health condition of the animals. The present study shows that the poor conditions of the patients at recovery are associated with poor prognosis. Therefore, a more rapid recovery of the turtles in difficulty would improve this prognosis, safeguarding this protected species.

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VALIDATION OF THE ITALIAN VERSION OF THE UNESP-BOTUCATU MULTIDIMENSIONAL COMPOSITE PAIN SCALE FOR THE ASSESSMENT OF POSTOPERATIVE PAIN IN CATS

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Recognizing pain and assessing its intensity are mandatory for its correct management. The use of pain scales represents a valuable diagnostic aid as such systems provide the veterinarian with an objective, ready-to-use tool. Specific pain scoring systems have been developed for the evaluation of pain in dogs and/or cats (Wiese et al., 2015). However, an instrument validated in one language is not automatically valid when translated in another language and culture. The validation of a pain assessment scale in a language or culture different from the original one requires a rigorous and thorough process of translation, cultural adaptation and evaluation of the psychometric properties in that language or culture (Guillemin et al. 1993).

The purpose of this study was to validate the Italian version of the UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS) to assess postoperative pain in cats, by mean of video analysis and psychometric tests.

The already validated English version of the scale was translated into Italian by two independent translators, and synthesized in one version by a third individual. The synthesized version was then back-translated and minor adjustments were made. The scale was reviewed by two anaesthesiologists with expertise in feline pain management, and the final Italian version was set up. Thirty cats undergoing ovariohysterectomy were previously video recorded at specific time points during the perioperative period (Brondani et al., 2009). Five observers with different clinical experience with Italian as the first language watched the videos and determined the pain scores as well as the cats that should receive analgesics using the Italian version of the scale. Videos were reanalyzed in a different order about one month after the first assessment. Obtained scores were then submitted to psychometric validity, responsiveness and reliability tests.

Significant changes in pain scores in response to surgery and analgesics confirmed content and construct validity as well as responsiveness (Wilcoxon test, $p < 0.001$). The agreement between the evaluations of the 'gold standard' (researcher that developed the scale) and the observers supported the criterion validity. Of the three domains identified by factor analysis, the internal consistency was excellent for 'psychomotor changes' and 'protection of the painful area and vocal expressions of pain' (Cronbach's alpha coefficient: 0.949 and 0.836, respectively), while 'physiological variables' showed moderate internal consistency (0.563). Inter- and intra-observer reliability, evaluated by intra class correlation coefficient, ranged from good to very good for all scale items. The cut-off point for rescue analgesia identified by the Receiver Operating Characteristic curve was ≥ 7 with a sensitivity of 94.4% and specificity of 97.0%.

The Italian version of the UNESP-Botucatu MCPS is a valid, responsive and reliable instrument for assessing acute pain in cats undergoing ovariohysterectomy. The cut-off point for rescue analgesia provides an additional tool for guiding analgesic therapy.

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ANALGESIC, SEDATIVE AND CARDIOVASCULAR EFFECTS OF CLONIDINE AS AN ADJUVANT FOR SPINAL ANESTHESIA IN SHEEP UNDERGOING ORTHOPEDIC SURGERY

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Clonidine is an $\alpha 2$ agonist extensively used in human medicine to provide spinal analgesia for acute and chronic pain.1 Clonidine mediates analgesia by activating spinal $\alpha 2$ adrenoreceptors resulting in a presynaptic inhibition of the release of substance P, a postsynaptic inhibition of the dorsal horn wide dynamic range neurons and an increase of concentration of acetylcholine in the cerebrospinal fluid.2

The aim of this study was to evaluate the analgesic, sedatives and cardiovascular effects of clonidine administered in combination with lidocaine and buprenorphine in sheep undergoing an experimental surgical procedure of the stifle joint.

After the approval of the ethical committee 20 healthy female sheep were involved in the study. All sheep underwent to the same surgical procedure, which contemplated the implantation of a scaffold on the medial condyle of the right femur. Sheep were randomly assigned to two groups, characterized by a different spinal anesthesia's protocol. In all animals sedation was achieved by the administration of 0.4 mg/kg IV of midazolam and spinal anesthesia was performed at the level of the lumbosacral space with a combination of 2% lidocaine (2 mg/kg - 0.1 mL/kg) and buprenorphine (300 μg - 1ml), moreover in the CLON group (n = 10) clonidine (2 μg/kg, 0.013 ml/kg) was added to the spinal anesthesia solution while in the control (CTR) group (n = 10) an equal amount of saline solution was added. The times between the midazolam administration and the execution of the spinal anesthesia and between the latter and the loss of the anal reflex were recorded. Persistence of the anal reflex was considered as a sign of failure of the spinal block. The time between the spinal anesthesia and the beginning of surgery was recorded. Five minutes before the administration of midazolam (PRE), 5 minutes after midazolam administration and at 10 (T10), 20 (T20), 30 (T30) and 40 (T40) minutes after spinal anesthesia the following physiological parameters were recorded: HR, RR, mean arterial pressure (MAP), SpO2 and T . At the same time intervals the level of sedation was scored. During the recovery an operator unaware of the anesthetic protocol recorded the following parameters: the time of return of skin sensation on the hind limbs, anal sphincter reflex, spontaneous movements of the hind limbs, first attempts of standing and time of standing. After standing, every 10 minutes the degree of ataxia was evaluated by means a numeric score. Data were compared between the two groups with the two way ANOVA for repeated measurements followed by the Student-Newman-Kleus test. Non parametrical data were analyzed by means the Kruskal-Wallis test.

The results of this study suggest that the addition of clonidine to a solution of lidocaine and buprenorphine for spinal anesthesia in sheep produces a faster onset and a long lasting motor and sensitive block as compared to the sole lidocaine and buprenorphine combination. Moreover clonidine increases intraoperative sedation and postoperative ataxia.

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COMPARISON OF INTRANASAL AND INTRAMUSCULAR ADMINISTRATION OF DEXMEDETOMIDINE IN HEALTHY DOGS

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Among different routes of administration of anaesthetic agents, the intranasal (IN) route is widely employed in human medicine thanks to several advantages such as a large drug absorbing surface by the nasal mucosa, avoidance of hepatic first pass and direct transport to the Central Nervous System; the latter occurring either by diffusion to the subarachnoid area or after internalization of the active molecules by olfactory neurons and then axonal transport up to the olfactory bulb (1). The IN route has also been used to induce sedation in different animal species (2, 3, 4) including dogs (5). The aim of this study was to compare the sedative and cardiorespiratory effects after IN or IM administration of dexmedetomidine in dogs.

This study was approved by the Ethical Animal Care and Use Committee of the University Federico II of Naples under project license number 2015/0016561. Twenty ASA I or ASA II dogs undergoing sedation for diagnostic imaging or minor surgical procedures were enrolled in the study. Dogs were randomly assigned to one of two groups both receiving 0.02 mg kg-1 dexmedetomidine (DEX- Dexdomitor ® 0.05%, Pfizer Italia srl) either intramuscularly (IM) or intranasally (IN). In the IM group dogs DEX was injected into quadriceps femoris muscle, while in the IN group dogs the solution was divided equally spraying half of the volume in each of the two nostrils using a Mucosal Atomization Device. Sedation score (SS) was assessed using a modified numeric rating scale (0-13) (6). Heart rate (HR); respiratory frequency (fR); haemoglobin oxygen saturation (SpO2), temperature (T°C) and indirect arterial pressure (SAP, DAP, MAP) were recorded using a multiparametric monitor (Mindray Express PM-9000, Mindray Medical Srl) for 45 minutes following drug administration. Physiological variables' trends over time were explored using a two-way analysis of variance (2-way ANOVA); post hoc all significant values ($P < 0.05$) were explored by a Sidak's multiple comparison test using a program for statistical analysis (GraphPadPrism 6.01, 2012; La Jolla, USA). Conclusion: Our results substantiate the use of the IN route to produce effective sedation by dexmedetomidine in dogs; in fact SS was significantly higher in the IN group compared to the IM group between 10 and 45 minutes ($p < 0.0001$). Further to that DEX onset time after IN administration was shorter compared to IM route and closer to that reported in literature after intravenous administration (7). A 55% HR decrease from baseline values in the IM group coincided with peak sedation time (PST), while only a 18% HR decrease was recorded at PST in the IN group. No significant differences in SPO2 and fR were found between the two groups at all times points. No undesirable effects were observed in any of the dogs. The rapid and effective sedation produced in dogs by IN Dexmedetomidine relies on the high degree of vascularisation and drug permeability of the nasal mucosa (1), as well on direct passage of DEX to the brain.

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ALTERATION OF PULSE WAVES FORM DURING SURGERY FOR PATENT DUCTUS ARTERIOSUS IN A DOG

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Patent ductus arteriosus (PDA) is the pathologic persistence after birth of ductus arteriosus. The resulting left-to-right shunt causes volume overload of left atrium (LA) and left ventricle (LV) which predispose to heart failure and death (1). Closure of PDA is recommended if right-to-left shunt is not present (2). At closure, reduction in preload and increase in afterload causes left ventricular pressure and diastolic blood pressure (DBP) to increase while temporary reflex bradycardia can happen in case of fast closure.

To report a case of an alternating modification of pulse waves form during and after ligation of PDA in a dog.

A 1 year old intact mongrel male dog, weighing 20 kg was presented for surgical ligation of PDA. Transthoracic ultrasound confirmed clinical diagnosis of PDA. Echocardiogram revealed dilation of LA and pulmonary artery (PA), eccentric LV hypertrophy and dilation and a minimal PDA diameter of 6 mm. Doppler interrogation of the PA demonstrated high-velocity (peak systolic velocity of 5.5 m/sec) continuous ductal flow directed towards the pulmonic valve and mild mitral valve insufficiency. Electrocardiography (ECG) showed sinus rhythm, normal mean electrical axis and signs of LV enlargement. The dog underwent standard ligation of the PDA through left thoracotomy. After premedication with fentanyl e.v. 5 μg/kg and midazolam e.v. 0.3 mg/kg, anesthesia was induced with propofol e.v. 3 mg/kg and maintained with sevoflurane EtSevo 2.1% in O2 Fi 60%, with volume controlled ventilation, tidal volume 320 ml, PEEP 5 mmHg, EtCO2 42 mmHg and fentanyl CRI 7 μg/kg/h. Invasive blood pressure (IBP) trace, heart rate (HR), ECG and SpO2 traces were recorded during surgery until 30 minutes post-operatively. Before final closure of PDA, a 90" temporary closure by compression of PDA was performed to anticipate eventual adverse effects. After 3 minutes, final closure of PDA was performed by 2 ligatures with 1 USP silk suture.

Either in the simulated as in the final closure of PDA, diastolic blood pressure (DBP) increased from 60 to 102 mmHg and systolic blood pressure (SBP) remained at 125 mmHg while HR decreased from 100 to 50 bpm and soon returned to more normal values. Starting from the simulation of the closure and until the period of awakening, pulse waves of reduced amplitude (weak pulses) unexpectedly arose at periods in the IBP trace, in regular fashion alternated with normal pulse waves, without no relate alteration in the ECG nor with ventilation. The difference in SBP between normal and weak pulses ranged from 15 and 18 mmHg while mean blood pressure was around 110 mmHg with no vasopressor administered. The dog had good awakening and improving clinical and echocardiographic follow up, which showed the complete closure of PDA and a decrease in LA and LV size.

Pulsus alternans was our interpretation for the pulse wave trace, a relative uncommon detection, found in patients with impaired LV systolic function due to various pathologic conditions (3), not yet documented in PDA. It is likely that pulsus alternans can be induced also by substantial afterload and pressure increases as it occurs in the ligature of PDA.

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PERIOPERATIVE ANALGESIC EFFICACY OF A PREEMPTIVE INTRA-ARTICULAR LIDOCAINE OR LIDOCAINE WITH EPINEPHRINE BOLUS IN DOGS DURING ELBOW OR SHOULDER ARTHROSCOPY

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Lidocaine is a local anesthetic, widely used on veterinary practice, that produces complete blockade of sodium channels of sensory nerve fibers; the blockade prevents the inflow of Na+ into the channel, preventing the conducting axons of peripheral nerves and it is administered through several methods. Several studies have demonstrated the perioperative analgesic efficacy of intrarticular (IA) lidocaine during arthroscopic surgery in humans. However, some studies have reported that local anesthesia was not adequate in controlling pain during arthroscopic surgery. Therefore, data on effectiveness are not conclusive. To the author's knowledge there are no studies regarding the preemptive use of IA lidocaine with or without epinephrine for arthroscopic surgery in dogs. The purpose of this study was to evaluate the intraoperative and postoperative analgesic efficacy of a preemptive bolus of IA lidocaine (or lidocaine with epinephrine, L+A) during arthroscopy in dogs under general anesthesia. Forty-three dogs with joint diseases referred to the Veterinary Teaching Hospital of the University of Perugia and scheduled for arthroscopic surgery of the shoulder or elbow, were included in the study.

The dogs were assigned to two groups: shoulder group (SG, n=20) and elbow group (EG, n=23). In the SG, 7 subjects received the IA lidocaine (lidocaine group, SGL), 7 received IA saline (control group, SGC) and 6 received IA lidocaine with epinephrine 1:100,000 (SGL+A). In the EG, 7 dogs received IA lidocaine (lidocaine group, EGL), 9 received IA saline (control group, EGC) and 7 received IA lidocaine with epinephrine 1:100,000 (EGL+A). All dogs were premedicated with intramuscular acepromazine (10 μg/kg). General anesthesia was induced with propofol (4-6 mg/kg), and after intubation, it was maintained with a mixture of isoflurane and oxygen (50-100 ml/kg/min) via a circle breathing circuit with spontaneous ventilation. Physiological parameters were monitored continuously and recorded every 5 min. Particularly, were recorded: time Baseline (B), IA injection of lidocaine (or L+A or saline) (Joint Access, JA), introduction of the arthroscopic trocar (T), and 30', 45', 60' from the joint access. During the procedure if an increase in HR or blood pressure or RR (more than 20% compared to the baseline values) was observed, an intravenous bolus of sufentanil (0,1 μg/kg) was administered as rescue analgesia. Numbers of boluses and the total dose of sufentanil were recorded as well. The postoperative pain was evaluated by the Hellyer and Gaynor pain scale, at 5', 30', 1 and 2 hours after extubation. The results of this study demonstrated that no significant differences were evident between the groups that received IA lidocaine (or L+A) and control groups, as regards the intraoperative analgesia. The pain score in the postoperative period until 60' from extubation, showed a lower value in groups EGL, EGL+A and SGL, SGL+A than control groups. The use of preemptive intrarticular lidocaine (with or without epinephrine) bolus for arthroscopy of the elbow and shoulder, in acepromazine/isoflurane anesthetized dogs, does not provide adequate intraoperative analgesia, and offers only a weak and short postoperative analgesia.

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BRONCHOPLEURAL FISTULA HEALING BY AUTOLOGOUS STEM CELL ENDOSCOPIC TRANSPLANTATION IN A GOAT MODEL

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Lung cancer is the leading cause of cancer death worldwide. Despite the efforts to identify new therapeutic strategies, surgery still represents the only opportunity to cure patients with NSCLC.. Unfortunately, sometimes the excellent long-term results of surgery can be nullifyied by post-surgical complications such as bronchopleural fistula (BPF) that leads the patient to death in an extremely high percentage of cases (up to 71.2%). It appears therefore of paramount interest the development of effective BPF treatment procedures.

Our study is aimed to assess the feasibility, safety and efficacy of autologous mesenchimal stem cells (MSCs) endobronchial transplantation to solve an induced bronchopleural fistula after lung resection in an animal model.

DSA total of 12 goats underwent to the same surgical procedure to create the artificial BPF and were randomized in two groups: a) experimental group (n=6); b) control group (n=6). Autologous MSCs isolated from the bone marrow were inoculated in the broncho pleural fistula together with a fibrin-glue (Quixil ®) after infection with a lentiviral vector codifing for a report gene, the galactosidase gene (LacZ). In the experimental group, 5 millions of infected MSCs/ml in 2.5 ml of Quixil ® were injected into the BPF either in the submucosal layer around the fistula and into the bronchial lumen. The control group followed the same procedure using Quixil ® alone. A mid term (15 days) evaluation was performed using bronchoscopy. After one month the animals were sacrificed and specimens of the bronchus and the surrounding tissues were collected and immediately frozen. The samples were submitted to histological, immunohistochemical and imaging analyses (CT/MR). All animals were treated in accordance with the European Communities Council directive (86/609/ EEC), to the Italian Health Department law and regulations on animal welfare (D.L.G.S. 116/92).

The disease model of this study proved to be feasible and the surgical technique showed niether major discomfort nor complication for all animals enrolled. The MCS endoscopical transplantation was always effective even if the reproducibility of cells injection has to be improved. Differently from the control group, microscopical and imaging analyses highlighted the full closure of the fistula one month after MCS transplantation.

The proven efficacy of MSCs transplantation in the airway may represent a first step for cell therapy as a treatment for any type of post-surgical fistula (digestive, genito-urinary etc.) thus expanding clinical indication of MSCs transplantation. Given the extremely high mortality rate of BPF (up to 71.2%), the

proposed technique could drastically improve post BPF survival rate, providing the patients an effective therapeutic chance in an otherwise highly fatal situation.

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OSTEOCHONDRAL LESION TREATMENT WITH PDLLA IN AN OVINE EXPERIMENTAL MODEL: TOMOGRAPHIC AND MICROTOMOGRAPHIC ASPECTS

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Treatment of chondral defects is a yet to be resolved problem for orthopedic surgery because in this kind of disease both the cartilage and the subchondral bone are affected. The current teraphy is mosaicoplastics and the use of osteochondral scaffolds. An innovative research field for the recovery of such defects is tissue engeneering with the creation of "biomaterials" whose goal is the reconstruction of biologic tissues: this can be achieved by growing cells on artificial scaffolds.1 Those scaffolds are made up of biomaterials designed to permit cellular proliferation both in vitro and in vivo; they can be reabsorbed by metabolism or they can stay in place and give mechanical aid.2

The aim of the study was to evaluate in a macroscopical and microscopical fashion the regenerative effects of a new polymerich scaffold made up of polilactic D-acid (PDLLA) with two different gradients (homogeneus porosity and varying porosity), when implanted on osteochondral lesions in an ovine experimental model.

After approval of the ethical committee 5 female sheep (bodyweight ranging between 35 and 45 Kg) were included in the study. During a surgical procedure an osteochondral lesion was practiced on the femoral condyles and then in the lesion was inserted the described Scaffold. The homogeneous scaffold was placed on the medial condyle (OM-Med Group) while the scaffold with varying porosity was placed on the lateral condyle (DG-Lat Group).Immediately after surgery (T0) all the sheep were subjected to RX and CT investigation to evaluate the degree of osteochondral lesion and the initial Hunsfield Unit densities. These first results have been compared to subsequent investigations at 3 and 8 months after surgery. At the 8th month the sheep were euthanasized and the condyles removed to be subjected to a Morphologic Characterization by means of Micro-CT. The obtained results have been compared with the ones previously given by Macro-CT: the values of Hunsfield Unit densities for the macro-CT showed a major and faster osteoinductive capacity of the homogeneus biomaterial compared to the biomaterial with varying porosity, but in contrast the Micro-CT exam clearly showed a lacking of substantial differences between the two biomaterials. Both scaffolds appeared equally succesfull in promoting osteoconduction, osteoinduction and osteointegration on the subcondral bone.

This study demonstrated both the utility of the PDLLA scaffold as a mechanical support and its ability in promoting osteoinductive and osteogenetic activity, necessary for the reparation of osteochondral lesions. These biomaterials could be utilized clinically as bone substitutes and as well as frameworks for cellular growth, thus favouring regeneration of bone and osteochondral defects.

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BIOMIMETIC POROUS TITANIUM SCAFFOLDS FOR LARGE BONE CRITICAL DEFECT RECONSTRUCTION AN EXPERIMENTAL STUDY ON LARGE ANIMAL MODEL

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Large bone defects reconstructions still represent a hard challenge in many critical situations such as tumor resections,non-unions,infections,some traumatisms and in prosthesis revisions.The main goal of the treatment is directed to guarantee a precocious loading of the affected limb even if most of the scaffolds proposed in the last decade did not achieve such an objective.In the present paper the authors propose a new technique to reconstruct large bone defects by use of Biomimetic porous titanium scaffold custom made with EBM (Electron Beam melting) technology that evidenced to be suitable to reach the purpose.

The study was conducted in compliance with the Italian Animal Welfare after the approval of the Ethical Committee.A complete resection was practiced in the diaphysis of the right tibia of six sheep and replaced with a five centimeters framework of EBM-sintered titanium,which was stabilized with custom-made EBM-titanium plates and screws.The scaffolds were fixed to the custom made plate by two small screws inserted in the pre-shaped holes of the scaffold.The outcome was followed-up by periodical X-ray and clinical investigations with a follow-up of 12 months.At 9 months the plates were removed,at 12 months the sheep were euthanized and the tibia were subjected to histological and immunoistochemical investigations.

The post operative X-ray showed a good position of the plate and the proximal and distal border of the scaffolds were perfectly adherent to the bone to permit the process of osteointegration.After surgery the sheep were allowed to move freely in the stables.The clinical evaluations at two months evidenced no problems related to the gait and the absence of pain to the passive movements of the operated hind-limb. The radiological aspects at two months after surgery demonstrated that the bone re-modelling was taking place and a periostal callus was already visible.Nine months after the X-ray showed a remodelling of periostal callus with a well-defined cortical bone, the scaffolds were completely integrated in the dyaphysis of the tibia and consequently the removal of plates was performed.The histological investigations were executed on bone-metal interface (axial and horizontal sections) to evaluate the osteointegration of the EBM-titanium implants. The non-stained thick section (1.5 mm) showed bone growth among the titanium bars.The sections stained with von Kossa's,suitable to observe calcified bone, showed how bone trabeculae have bridged the titanium trabeculae,forming a metal-bone interconnected network with a histological pattern suggestive of bone remodelling.Bone trabeculae have been formed around the EBM-sintered titanium trabeculae creating an interconnected system and the tight joining among them suggested a very good tissue-metal interaction The geometry of the porous implants seems to better promote osteointegration of the implant itself. In conclusion this kind of implant,used to repair large bone severe defects in a large animal model, can guarantee the desirable immediate body-bearing, a precocious functional recovery and a good osteointegration.

This study demonstrated both the utility of the PDLLA scaffold as a mechanical support and its ability in promoting osteoinductive and osteogenetic activity, necessary for the reparation of osteochondral lesions.

These biomaterials could be utilized clinically as bone substitutes and as well as frameworks for cellular growth, thus favouring regeneration of bone and osteochondral defects.

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RELATIONSHIP BETWEEN INTRAOCULAR PRESSURE AND CENTRAL CORNEAL PACHYMETRY IN HEALTHY DOGS; PRELIMINARY STUDY

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An accurate measurement of dog's intraocular pressure plays a fundamental role in the management of varios ophtalmic diseases; yet IOP (intraocular pressure) is still rather difficult to standardize. Although the real IOP is mesured by inserting a catheter into the anterior chamber (direct pressure), in practice we have varios instruments capable of measuring the endocular pressure from the outside (indirect method), by evaluating the corneal resistance. In medicine the evaluted intraocular pressure is always related to the central corneal thickness.

The aim of this study is to verify if there is a relationship between the IOP and the corneal pachymetry in awake dogs. Materials and methods. Both eyes of 49 German Shepherd dogs (belonging to the Guardia di Finanza Corps) without ocular diseases at the moment of the detections, were enrolled in this study. All dogs were placed in sitting position on a veterinary exam table for the measurements. After instillation of a local ocular anesthetic (two drops per eye; benoxinato 0,4%), IOP was measured by a tonometer (Tonopen Avia, Reichert Thecnologies) always by the same operator (first operator). For the assessment of corneal thickness, pachymetry was measured by an Optical Coherence Tomography (OCT iVue, Optovue) always by the same operator (second operator). The acquisition of all data was obtained in two sessions in the same time slot in the morning at the "centro allevamento e addestramento cani" of Castigion del lago PG.

A statistically significant correlation between the he corneal thickness and intraocular pressure detected by tonomenter was found. In the order of 0.36 mmHg per 10 μm of thickness of the cornea that departs from the average of pachymetry of the group that turned out to be 559 $\pm$ 44,318 μm. The value of IOP was 17,20 $\pm$ 2,646 mmHg. In conclusion, the measurement of IOP in dogs is influenced by corneal thickness as a result of the different resistance provided by the cornea in relation to the stresses made by the tip of the tonometer.

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PLASMA-COATED PCL SCAFFOLD IN OVINE MODEL OF OSTECHONDRAL DEFECT: PRELIMINARY MICRO-CT RESULTS

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Cold plasma processes both at low and atmospheric pressure have been shown to be very useful to functionalize material surfaces, with no change of the bulk, to tailor the surface composition of scaffolds and improve their cytocompatibility, as well as to synthesize functional surfaces for direct cell growth and biomolecules immobilization, for depositing non-fouling coatings, nano-composite bacterial resistant coatings or micro/nano-structured surfaces [1-3]. Aim of the study has been to test in vivo a plasma -Coated Polycaprolactone (PCL) scaffold in an ovine model of osteochondral defect.

Production and plasma modification of materials 3D PCL scaffolds (7mm dia, 10mm thick) were produced with solvent casting/particulate leaching The samples were plasma coated with radiofrequency ,glow discharges, in a low pressure plasma reactor. C2H4/N2 deposition at 47Pa, 50W, 30min, followed by H2 plasma post- treatment (53Pa, 20sccm, 20W, 3min). 20 sheep have been randomly assigned to the following experimental groups: Groups of sacrifice at 3 months: a. Group with implantation of scaffolds in PCL untreated; b. Group with implantation of scaffolds in PCL treated with PDE process: N / H2; c. Group with implantation of scaffolds in PCL treated with PDE process: N / H2 kept in culture for 15 days with mesenchymal cells from bone marrow; Groups to be sacrificed at 6 months: a. Group with implantation of scaffolds in PCL untreated; b. Group with implantation of scaffolds in PCL treated with PDE process: N / H2; c. Group with implantation of scaffolds in PCL treated with PDE process: N / H2 kept in culture for 15 days with mesenchymal cells from bone marrow An osteochondral defect 7mm in diameter was produced in the medial condyle of the right femur; the defect was then filled with the scaffold according to the group of belonging of the subject. After surgery each sheep was subjected to X-ray examination of the knee joint in the left orthogonal projections. The micro-morphology of 3D PCL scaffolds was studied by means of Micro Computed Tomography (Skyscan 1172, Bruker MicroCT), producing reconstructed 3D images of scaffolds.

The scaffolds treated with Plasma (group PCL + Plasma) showed already 3 months of implantation, a better osteontegration, especially for the deposition of new bone formation into the the scaffold , both on the boundary, where the presence of bone tissue was organized according to a similar architecture to the healthy bone. In cartilaginous side we also appreciates the presence of dense material that moves from the periphery to the centre position in scaffold, supported by a subchondral bone in training. This finding is related to the formation of new tissue attributable to joint cartilage, the quality of which require further investigation by biochemical and himmunohistochemical analysis. In Group PCL + Plasma sacrificed at 6 months these findings are confirmed in a more intense showing more clustering of biomaterial and thickening of trabeculae, particularly on the interface scaffold-bone. This phenomenon may be related to bone remodeling in elapsed times more, expression of a greater maturity of bone neoformed. Both groups treated with PCL + Plasma + BMSC cells, showed excellent osseointegration than the other groups. This reveals therefore a good ability of the biomaterial coated with plasma in providing support to cells MSCs and ameliorate healing process.

USE OF THE CADAVER IN SURGICAL EDUCATION

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Since the beginning of its development surgery has been based on the use of animal or human bodies for research or educational purposes. Several surgical theaters, so called Teatrum Anatomicum, grew up in Europe during the 16th century. Resident surgeons showed anatomy and surgical techniques to the public, students and colleagues, on dissected, fresh or embalmed, bodies. In the modern era of surgery the use of cadavers is still an important part of the education of a surgeon, even though different new tools has been proposed and used for ethical, economical reasons or simply by choice of the teachers.

Evaluation if the use of animal cadavers is still a satisfactory educational tool to teach surgical anatomy and techniques to veterinary undergraduates.

Two groups (n=8/each) of fifth year undergraduates were enrolled in a Surgical Anatomy and Techniques Course. During the course 8 dogs and 4 cats, plus 4 kits were used. Teaching was divided in 3 (Group A) or 4 (Group B) steps: 1) tutorial with slides and videos (Group A/B), 2) surgical anatomy teaching on cadavers (Group A/B), including some easy surgical procedures (Group A), 3) easy surgical procedures performed on anatomical kits (Group B), 4) surgical procedures on entire animal cadavers (Group A/B). During the all steps students were allowed to consult tutorials concerning the topics. The embalming techniques were done on donated animals, dead or euthanized for different medical reasons, using a carotid arterial access for dye injection, while drainage were achieved through a femoral vein cannulation. Almost 24 hours resting of the animals at 4°C ended the procedure.

The use of cadavers for the surgical anatomy lab resulted helpful to fix concepts of topographic and surgical anatomy in both groups. The evaluation of the surgical skill improvement in the two groups displayed a positive trend in the Group B compared to the Group A.

The use of the cadavers and tutorials demonstrating efficacy is probably related to the long time passed from the first anatomy courses. The minor improvement of the Group A could be related to the difficulties to approach standard surgical techniques on the bodies simulating a real surgical setting, on the other side an intermediate passage on isolated organ showed that the Group B reached the supposed surgical skills proposed in the course.

This teaching program, providing early exposure to cadaver training using only for anatomy background, ensures that basic anatomical skills are mastered before students are exposed to cadaver surgical practice. Otherwise the use of single bench organs, as a bridge to the cadavers, improves the student's ability and the handleability of the surgical techniques. In fine the ultimate step for the students is to merge all the anatomical and surgical skills on the entire body as a real surgical simulator.

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CLINICAL AND COMPUTED TOMOGRAPHY TUMOUR DIMENSION ASSESSMENTS FOR PLANNING WIDE EXCISION OF INJECTION SITE SARCOMAS IN CATS: HOW STRONG IS THE AGREEMENT?

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The tumour dimensions of injection site sarcoma (ISS) in cats are among the first aspects evaluated for calibrating surgical doses.1 Despite this role, a standardized approach to measuring the size of ISS in cats is currently lacking.2-5 The discrepancy between clinical and computed tomography (CT) measurements of the same tumour may led to possible bias that affects the surgical dose and prognosis analysis. The aim of this study was to investigate prospectively the agreement between clinical and CT measurements of tumour size in newly diagnosed ISS in cats.

Fifty-three client-owned cats that underwent both clinical and CT measurements of the length and width of an ISS6 were included. The agreement between two measurements was evaluated with the Bland and Altman approach.7 A measurement of overall concordance using the concordance correlation coefficient (CCC) was obtained.

Computed tomography measurements showed a tendency towards being larger than clinical dimensions, both for length and width, and this difference increased with increasing tumour size. The CCC also suggested unsatisfactory concordance between the two measurement methods.

This result suggested wider lateral margins of excision in cases of clinical measurements relative to CT evaluation. The excision with 5-cm margins recently proposed for clinical dimensions might not be sufficient in cases of large tumours, leading to the hypothesis that for large palpable tumour a CT evaluation could be more useful for determining the extension of tumour tentacles. The usefulness of contrast-enhanced whole-body CT for ISS in cats is not only linked to the role of planning lateral excision margins, but it also has the ability to estimate deep margins and to detect distant metastasis. These aspects are crucial points to discuss before surgery with surgical staff and the owner.

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TUMORS VOLUME CORRELATION BETWEEN MANUAL CALIPER AND HIGH FREQUENCY ULTRASOUND IN A MICE MODELS OF COLORECTAL CANCER

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The volume of tumors is an important metric of disease progression and response to therapy in preclinical drug development and in clinical small animal practice. Noninvasive methods, i.e. manual calipers or Ultrasonography (US), might be routinely used. US evaluation of such pathologies is becoming more widespread and it is cost effective. Aim of our study was to assess if any difference occurred between three techniques of superficial tumor volume measurement to evaluate the effect of Peptide R, a new CXCR4 antagonist peptide, in the development of mice models of colorectal cancer(CRC). Twelve balbC/nude mice were subcutaneously injected with human CRC cell lines HCT116 (2*106). Tumor growth was measured using a transcutaneous Vernier caliper (caliMax, Wiha, UK - 0.1 mm reading), the electronic caliper integrated on 2D US images and with the integrated 3D US mode (Vevo ® 2100 Imaging System, VisualSonic Inc., Canada). The tumors' volumes (TV) were calculated applying the ellipsoid volume formula (V-caliper = [length x width2] x [Π/6]; V-2D-US = [length x width x height] x [Π/6]) or directly by the 3D integrated Vevo 2100 software (V-3D-US). HCT116 human subcutaneous xenograft (>=50mm3) were treated with 1) Peptide R alone 2) 5FU +OX 3) 5FU +OX + Pep R i.p. for 2 weeks.Statistical analysis: Mean ± SEM within the three measurement techniques was calculated. A linear regression was applied to explore if any correlation existed between the three measurement modalities and a Spearman's ρ correlation coefficient was calculated, as well. Mean TV was 180.2 ±48.2 for V-caliper, 64.9 ±13.0 for V-2D-US and 66.3 ±16.0 for V-3D-US mm3. Linear regression showed a strong relation between the three techniques (V-3D-US - V-caliper r2=0.99, P<0.0001; V-3D-US - V-2D-US r2=0.71, P<0.0004; V-2D-US - V-caliper r2=0.78, P<0.0001), confirmed by the Spearman's ρ correlation coefficient (ρ=1, P=0.0006, for all). Concomitant chemotherapy plus CXCR4 new antagonist Peptide R reduced tumor volume in mice model of human colon cancer. In clinical small animal practice, monitoring TV is essential to assess the response to therapy, either when surgery is not applicable or before planning a surgical intervention. US is a cost effective imaging modality and it has been demonstrated to be more accurate in measuring TV, when assuming as the reference standard the tumor mass, whereas caliper volume variance resulted 1.3-fold higher than for ultrasound. Our data set, are in according with Ayers et al. V-caliper was on average 3-fold and 2.5-fold higher than V-2D-US and V-3D-US, respectively. The two US-based techniques were quite similar (V-2D-US 1.2-fold higher than V-3D-US). We did not compare the three volume methods measurements because the manual caliper is biased by the contextual measurement of cutis and subcutaneous tissues. The strong correlation

between the two techniques leaves a free choice to the clinician, remembering that both modalities are quite subjective (operator-dependent) and susceptible to bias.

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TREATMENT OF EYELIDS AND THIRD EYELID TUMORS IN DOGS WITH RADIOSURGERY: A PRELIMINARY STUDY

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In canine eyelids surgery, intraoperative bleeding may obscure the surgical field and lead to increased swelling, bruising and pain. Therapies for the canine lids and nictitating membrane tumors include surgical excision, cryosurgery, or carbon dioxide laser ablation (Stades and Van der Woerdt, 2013). Potential disadvantages of cryosurgery are severe postoperative swelling, depigmentation and the unwanted loss of normal tissue. Laser requires safety precautions, including wavelength-specific eye wear and the hazard of beam scatter and or reflection.

Objective is to evaluate the efficacy of high-frequency radiowave surgery in treating tumors of eyelids and third eyelid in dogs.

Seven dogs (7 eyes) referred with eyelids tumors and 3 dogs with third eyelid tumors were enrolled in the study over a 12-month period. Ablation was performed using high-frequency radiowave surgical unit (Ellman Surgitron FFPF) at a power setting on 2.5 (4 cases) or 5.0 (6 cases) with "needle electrode" (TA2B, TA3B, TA8B) or âĂIJempire microincision electrode (TEE305) in the Cut/Coag mode (5 cases: 2 eyelid papilloma and 3 third eyelid tumors) or cut mode (5 eyelid tumors).

In all cases surgery was performed in a very short time with the advantage of an excellent haemostasis and minimum onset of oedema using both modes (cut or cut/coag). Surgical incisions were extremely precise and accurate. Two dogs with papilloma on the upper eyelid, treated with a needle electrode and a power setting on 3.0 showed dehiscence of the surgical wound one week postoperatively. One dog with melanocitoma of the third eyelid showed transient depigmentation of the inferior eyelid which resolved after two months. In all other cases (2 meibomian gland carcinomas, 2 adenomas and 3 epitheliomas) no notable complications were observed and the results of the scar was judged as good by surgeons and dog's owners. Follow-up (up to 1 year) showed no recurrence in any case.

To the authors' knowledge, treatment of eyelid and third eyelid tumors with high-frequency radiowave surgery is not wide described in dogs and there are not published clinical studies. Results of this study demonstrated that it may be a simple, safe and effective surgical treatment for eyelids and third eyelids tumors in dogs. Dehiscence of surgical wound after the excision of an eyelid papilloma in two cases could be due to the use of low power setting in cut/coag mode. At this setting, a less precise incision and a slower passage through the tissue were achieved, increasing tissue damage and delaying healing. In the same way, needle electrodes with cut/coag mode, used for third eyelid excision in a dog, could have caused a minimal amounts of lateral heat, responsible of depigmentation of the inferior eyelid margin. The "empire microincision electrodes" produce a high concentration of energy with the least amount of thermal damage to adjacent tissue. Therefore, they are the optimal electrodes for eyelid surgery. This technique offers potential advantages over some others in use, like CO2 laser, including minimal safety precautions, self cleansing and relatively low cost. Choosing optimal power settings and the correct electrode will prevent increased tissue damage.

Frans C Stades et al in: Veterinary Ophthalmology, Fifth Edition. Edited by Kirk N. Gelatt, Brian C. Gilger, and Thomas J. Kern. John Wiley & Sons, 2013; pp 832-893

HOW TO GET PROFITABLE BIOPSIES FROM NASOPHARYNGEAL SPACE-OCCUPYING LESIONS IN CATS MAKING PATHOLOGISTS SMILING: 16 CASES (2012-2015)

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Feline nasopharyngeal space-occupying lesions include nasopharyngeal/middle ear polyps, lymphoplasmacytic rhinopharyngitis (LR) neoplasia and cryptococcical granuloma. While diagnosis of nasopharyngeal polyps is straightforward, the definitive diagnosis of other proliferative disorders requires exhaustive case history, thorough clinical exam, imaging, anterograde and retrograde rhinoscopy along with multiple biopsies (1).

To describe a novel endoscopic bioptic technique to obtain adeguately sized and possibly multiple diagnostic biopsy samples from nasopharyngeal lesions in cats.

Inclusion criteria were cats presenting with clinical signs of nasopharyngeal space-occuping lesions like stertor, dyspnoea, mono/bilateral nasal discharge, frank epistaxis or sneezing (2). General anesthesia was induced in all cases, and both anterograde and retrograde endoscopic examinations were performed with a 2.7 mm 0° arthroscope without sleeve and a 4.9 mm video-bronchoscope, respectively. Nasopharyngeal specimens of 0.2-0.6 cm were collected under retrograde continuous rhinoscopic guidance, through anterograde insertion of bioptic opposing-cups forceps or other dedicated devices such as sharp curettes, ear loops and otological knives into the nostrils. In some cases larger bioptic samples were obtained through aspiration of dislodged fragments. Cytology was performed in selected cases, and histology in all. Biopsy sampling, in selected cases, was repeated to check for lesion progression or to confirm LR vs lymphoma diagnosis.

Pinch biopsies from 16 cases were collected. Most cats were domestic shorthair (9), male (75%) with a mean age of 11.8 years (range 6-18). Number of biopsies varied from 1 to 24 (mean of 7.9). Cytology was available in 8/16 cases and was obtained by impression (2), squash preparation (2), impression and squash preparation (3) and fine-needle aspiration (1). An agreement between cytological and histological diagnoses was obtained in all cases. Diagnostic distribution of lesions was LR (5), lymphoma (8), malignant epithelial neoplasia (2), and hemangiosarcoma (1). For each case, a mean of 4.4 biopsies was often necessary to achieve a correct diagnosis. A lymphoplasmacytic inflammation was concurrently present in 6 out of 11 neoplastic lesions. Bleeding was the most common complication but never life-threatening. CONCLUSIONS: The recognition of primary proliferative nasopharyngeal diseases is not considered straightforward, and the diagnosis of neoplasia is often delayed for the simultaneous presence and prevalence of inflammation. This was more frequent in cases of lymphomas where inflammation obscured often the neoplasia. In our caseload, the availability of "profitable" multiple biopsies allowed to detect malignant endothelial cells arising in a case of a previously diagnosed cavernous hemangioma. In humans, an average of 6 biopsies from diseased and apparently healthy tissues are suggested as the minimum number required for an undoubtful diagnosis of nasal tumors. A consensus conference to reach multidisciplinary agreement on standard definitions, imaging techniques and adequate sampling protocols with multiple large size biopsies (minimum 4-5), should be required for a definitive diagnosis of some challenging and nasopharyngeal diseases in cats.

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TRANSSPHENOIDAL HYPOPHYSECTOMY AS TREATMENT FOR MACRO-ADENOMAS IN DOGS: CASE SERIES

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Pituitary tumors can be classified according to the ratio between the pituitary height and the brain area (P/B value) with a cut-off of 0.31 mm-1 between micro and macro-adenomas1. The aim of the study was to retrospectively review the surgical therapy and the related complications of dogs with macro-adenoma treated with transsphenoidal hypophysectomy at the DIMEVET of the University of Bologna.

Dogs with a pituitary mass diagnosed by CT or MRI and treated surgically between 2011 and January 2015, were included. In all dogs a complete diagnostic work-up, comprehensive of endocrinological evaluations, were performed. For transsphenoidal hypophysectomy all dogs were sedated with midazolam and fentanyl; induction was performed with propofol, anaesthesia was maintained using propofol in costant rate infusion (CRI) and isoflurane in oxygen. Analgesia was provided by fentanyl CRI. All the surgical procedures were performed according to the microsurgical technique described by Meij in 1997 2. In the post-operative period dogs were monitored for neurological deterioration, electrolytes and fluid balance. Antibiotics, gastroprotection and opioids were administered. Hormonal supplementation consisted of desmopressin, corticosteroids and thyroxine administration. The histological evaluation of the removed masses was carried out.

Seven dogs were included and 8 surgeries were performed. One dog was operated twice for recurrence of the tumour. The main represented breed was the Labrador Retriever (2/7 dogs), the median body weight was 34,5 kg (range 12-41 kg), the median age was 7,5 years (range 4-9 years) and 6/7 dogs were male. Of the 7 dogs included, 4 had pituitary-dependent hypercortisolism, one dog had hypopituitarism and 2 dogs had a hormonally inactive tumor; 4 dogs showed severe neurological signs before surgery. All the 7 cases had an enlarged pituitary gland, the median P/B value was 0,8 mm-1 (range 0,48-1,28 mm-1). In one dog there was a moderated bleeding during surgery and one dog developed septic shock when recovered from anaesthesia. In the post-operative period, complications were hypernatremia and hypertension (5/8 cases), neurological deterioration (4/8), exophthalmos (2/8), stupor (2/8) and venous thrombosis (2/8). Four dogs were discharged, while 1 dog died due to sepsis and 3 dogs were euthanized for deterioration of clinical and neurological conditions. The histological diagnosis was adenoma in all cases. Survival time for the 4 dogs was two years, for the dog operated twice, 5 months and one dog is still alive at the moment of writing this study (3 months post-operatively).

Transsphenoidal hypophysectomy represents a solution to decrease the mass effect in enlarged pituitary adenomas. A surgical learning curve is necessary for the surgeon. Nevertheless the complete removal of the mass is not always possible. In surgery the main difficulties were the exact localization of the fossa hypophysalys and to recognize and completely remove the neoplastic tissue. All the cases presented had macro-adenomas, some of them with very enlarged pituitary gland. In these dogs various complications were encountered in the post-operative period. Surgery represents the curative therapy, in cases of complete removal of the mass, both for the neurological signs and the eventual endocrinological disease.

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2. Meij et al, Vet Surg, 1997

SUCCESSFUL MANAGEMENT OF ACUTE BABESIOSIS IN A DOG

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Canine babesiosis is a tick-borne disease caused by Babesia spp. Dogs with uncomplicated babesiosis typically show pale mucous membranes, fever, anorexia, depression, water-hammer pulse, and splenomegaly. The complicated form can include acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, immune-mediated hemolytic anemia (IMHA), acute respiratory distress syndrome (ARDS), hemoconcentration. This case report describes the presentation, diagnosis, and management of acute systemic inflammatory response syndrome (SIRS) in a dog affected by Babesia canis. A Border Collie, intact male, 8-years-old, was presented in emergency setting showing weakness, anorexia and 'pigmenturia' started 2 days before. The dog was used as cattle dog in Piedmont region and recently moved to Tuscany. Dog showed fever (38.2°C), tachycardia with weak pulse (110 bmp), dyspnea (30 rr), pale mucosae. The thorax ascultation revealed mild attenuation of lungs sounds. Bilateral miosis, positional nistagmus and absence of pupillary light reflex were observed. Mean arterial pressure (MAP) was 67 mmHg. An hypovolemic shock was suspected. Oxygen via face mask at 5 Lt/min and isotonic cristalloyds IV at 30 ml/kg as bolus were administered. Synthetic colloids (Infuplas ®) were then also administered at 5 ml/kg IV. After 45 minutes dog rise, MAP slightly increase to 75 mmHg. Blood collection was carried out. Laboratory findings revealed a mixed acid-base disturbance with hyperlactatemia, hyperbilirubinemia (1.33 mg/dl); mild (Hct 27.8%) normocytic-chromic non-regenerative anemia, severe leukopenia (1.17 K/μl) with neutrophil left shift, thrombocytopenia (9 K/μl); hyperfibrinogenemia (812 mg/dl); increase of ALKP (936 U/L), AST (239 U/L), ALT (144 U/L), CK (789 U/L), mild hypoalbuminemia (2.3 g/dl) and elevated C-reactive protein (2.60 mg/dL) and urea (131 mg/dL); severe hematuria. SIRS was suspected. Pleural effusion and alveolar pulmonary pattern was detected at chest X-ray. Abdominal ultrasound revealed acute inflammation of liver with venous stasis and abdominal fluid.The fluid cytological exam was subtyped as aseptic transudate. A blood smear exam revealed the presence of pyriform-shaped organisms singly or paired within RBCs referable to Babesia canis. Antibabesial (imidocarb) and antimicrobial (doxicicline) drugs with supportive care (fluid therapy, vitamins and liver support) are the mainstays of babesiosis treatment. Dog was hospitalized for a week and discharged in good condition. This case of canine babesiosis was successfully treated. Until the protozoan was evidenced in the blood smear the diagnosis remained presumptive.The development of neurologic signs is associated with a high mortality rate. Neurological symptoms quickly improved after fluidstherapy and oxygen support; we hypothesize that the neurological symptoms could be related to the SIRS. Babesiosis includes manifestations that can not be explained by haemolysis alone but appear to be the result of the systemic inflammatory response to the parasite, rather than the actions of the parasite itself.

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A CASE SERIES OF SWIMMING PUPPY SYNDROME IN PUPPIES: CONSERVATIVE THERAPEUTIC OPTIONS

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Swimming puppy syndrome is an infrequent developmental abnormality seen in neonatal puppies, in which the hind limbs and sometimes the front legs, are splayed laterally. 1, 2 This condition is mostly observed in chondrodystrophoid and brachicephalic dogs breeds that have short legs and wide thoracic cavities, and an association with pectus excavatum has been previously reported.3 The cause of the syndrome is unknown, although various undocumented theories have been formulated. These include altered neuromuscolar synapse function, improper or delayed myelinization of peripheral motor neurons, slow muscular development and ventral horn neuropathy.4 The differential diagnosis of this disease includes encephalomeningitis, canine distemper, toxoplasmosis, neosporosis, myopathies, and spina bifida.2, 5 A specific treatment protocol has not been documented in any literature.

The purpose of this study was to report clinical signs and successful conservative treatment with limb realignment, bandages, intensive physiotherapy, and hydrotherapy, on five cases of Swimming Puppy Syndrome

Four-week-old, two Pointer sibling puppies, weighing 2.832 g (female) and 2.874 g (male); a five-week-old, Labrador retriever, weighing 2.335 g (male); four-week-old, two English Bulldog sibling puppies, weighing 2.034 g (male) and 2.567 g (female) were presented in our Hospital with the inability to stand or walk and to adduct both hind legs. Physical and neurological examination was considered normal and treatment was started by applying soft elastic gauze bandages, supported by self-adherent tape. Puppies were hospitalized on a soft but rough surface and put in a standing position with all legs in adduction for 10 minutes, seven to eight times daily. Passive range of motion (PROM) exercises to promote muscle tone were done for 20 minutes, three times daily. Every limb was pushed up by placing a hand under the paw to create a form of active-assisted range of motion. After 15 days, hydrotherapy was started for 20 minutes, two times in a week to stimulate muscle activity through swimming.

Many treatment options such as hobble bandaging, thoracic splinting, physiotherapy, and oral Vit. E and Selenium supplementation are reported. In this study, hobble bandaging, physiotherapy and hydrotherapy were used and at the end of the 5th week of the therapy process all puppies were able to walk as good as their littermates.

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CHYLOTHORAX IN CAT: MACROSCOPIC AND MICROSCOPIC ANATOMICAL EVALUATION OF THE COMPLEX CISTERNA CHYLI - THORACIC DUCT BASING ON A NEW SURGICAL APPROACH

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Chylothorax is a collection of lymph in the pleural space caused by congenital or acquired underlying diseases. The cisterna chyli is composed by a ventral sacculated portion connected to a plexiform dorsal one. The dorsal part, after receiving the lymph from the ventral one, passes through the aortic iatus and continues into the thoracic duct. During it's course and before connecting into the jugular vein, the thoracic duct could be single or double.

Aim of the study is to identify a fixed topographic framework of the cisterna chyli and the thoracic duct, in order to detail the lymph transport pathway from the abdomen to the chest.

26 cat cadavers were perfused with an embalming solution (Metaflow and Introflant - The Dodge Company Ltd, UK). In 1 cat the diaphragm, the thoracic and abdominal aorta, the thoracic duct, the cisterna chyli, the esophagus and the greater splanchnic nerve were removed en-block and suspended using surgical sutures to a customized cubic metal cage . The suspended specimen was fixed in 10 % buffered formalin for three days, embedded in paraffin and 23 cross sections, 2 mm spaced, were obtained for haematoxylin and eosin (H&E) staining, All the remaining 25 cadavers underwent two different surgical approaches to the thoracic duct and cisterna chyli. In 5 cats a ventral midline celiotomy was made in dorsal recumbency with the dissection of the left dorsal muscular portion of the diaphragm. In 20 cats a transdiaphragmatic approach was performed through a left paracostal celiotomy in right recumbency, using the left kidney and the left adrenal gland as anatomical landmarks for the dissection of the left crus of the diaphragm. In all subjects the portion of the thoracic aorta and the thoracic duct from the last intercostal artery to the emergence of the celiac trunk was harvested for the histological analysis.

The two surgical approaches were feasible even if the transdiaphragmatic approach through the left paracostal celiotomy seemed to be more effective and easier. The histological analysis of the en-block specimen showed, in the caudal mediastinum, the dorsal portion of the cisterna chyli that leaving the thoracic duct near the emergence of the celiac trunk. In the medium mediastinum the main duct is accompained by two little ducts placed laterally to the left intercostal arteries. The same ducts conformation is present in the cranial mediastinum. By this evidence of the first case the thoracic duct starts to run alone two millimeters before the emergence of the celiac trunk. In 23 cats (88,4 %) of the surgical group the histological analysis showed one single duct dorsally to the aorta 2 to 4 mm cranial to the emergence of the celiac trunk, while in the other two cats (11,6%) an additional little duct laterally to the aorta was present long the right part of the mediastinum.

Left paracostal laparotomy in right recumbency represents the best and easiest surgical approach. A single thoracic duct has been the most represented in our series.The emergence of the celiac trunk has been proven to be a useful surgical landmark. Our data showed a new abdominal approach to the thoracic duct while the anatomic analysis could help to better understand the area around the left emi-diaphragm.

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A 15-YEAR STUDY OF THORACOLUMBAR DISC HERNIATION IN DOG

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Intervertebral disc (IVD) degeneration is a common condition in dogs and it is usually associated to many different diseases. The most common localization of the IVD extrusion is the thoracolumbar spine and it can be observed in dogs of different age, sex and breed.

The purpose of this study are: to describe prognostic factors, outcome and recovery time among ambulatory, paraparetic and paraplegic dogs, that underwent decompressive surgery, through a hemilaminectomy, for Hansen Type I and II IVD disease; to evaluate the clinical outcome of dog that had conservative treatment.

Medical records (January 2000-December 2014) on all dogs that had thoracolumbar IVD disease were reviewed. Signalment, severity of signs, neurological deficits and postoperative outcome were registered for each dog. A panel of 580 dogs has been categorized based on the scheme proposed by Scott and McKee, 1999 and a score from 1 to 5 was used to describe symptoms. The choice of the therapy was through the severity of the clinical signs. Medical therapy has been used in all dogs with minor neurological deficits on hindlimbs, usually characterized only by back pain; dogs with severe, and acute, clinical signs were treated surgically with lateral spinal decompression, after CT scan or myelography for the correct identification of the IVD interested.

Male dogs were more affected than female (p=0.0012) and the most common sites were T12-T13 (25.9%) and T13-L1 (25.4%). In the sample analyzed, the 44% of dogs had surgical treatment and the 83% of them showed complete recovery. The best response to surgery has been observed in dogs with acute lesions treated within 48 hours from the beginning of the signs. The most dogs had clinical signs for a longer time and their prognosis has been strongly related to the severity of initial signs and the persistence of deep pain perception.

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Part V

Produzioni animali e sicurezza alimentare

EFFECT OF DIETARY LACTOSE SUPPLEMENTATION ON THE INTESTINAL ECOSYSTEM OF ADULT DOGS

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Whey, a by-product derived from cheese making, is commonly used in swine nutrition and consists of lactose (70-75%), protein (10-13%), and minerals (8%)., Due to the low ability of adult mammals to digest lactose, lactose may act in adult dogs as a prebiotic and, therefore, be of interest to the pet food industry (Mäkivuokko et al., 2006). Thus, the aim of the present study was to evaluate the effect of feeding adult dogs with increasing levels of lactose on their intestinal ecosystem. Fourteen adult household dogs, mixed breed, were fed an extruded commercial diet that did not contain any prebiotic ingredients, but instead included silica (0.5% wt) as a digestion marker. After a 20-d adaptation period, during which all dogs received the same dry diet, increasing doses of lactose were added to the dogs' diet (0.5, 1 and 2 g/kg BW0.75/d). Each feeding period lasted 20 days. From each dog, a fecal sample was collected at the end of each feeding period for microbial (by qPCR) and chemical analyses. Feces excreted by each dog during the last five days of each feeding period were collected and pooled in order to evaluate nutrient digestibility. Data were analyzed using linear and quadratic contrasts to determine the nature of the lactose feeding response. Data were subjected to statistical analysis using ANOVA with differences considered significant at $P<0.05$. Four dogs refused the diet with added lactose at 0.5 g/kg BW0.75/d and were excluded from the trial. When lactose was fed at 1 g/kg BW0.75/d, two dogs developed diarrhea and were also excluded from the trial. Conversely, 8 dogs remained healthy throughout the study. Fecal ammonia concentrations tended to decrease linearly when dogs were fed increasing doses of lactose (40.8, 37.7, 35.5 and 33.9 mmoles/g of feces for diets containing lactose at 0, 0.5, 1 and 2 g/kg BW0.75/d, respectively; $P=0.094$), while fecal pH and moisture were not affected by dietary treatment (average fecal pH was 6.64 while average moisture was 65.7%). Lactose resulted in linearly lower proportions of fecal isovalerate (2.72, 2.95, 2.01 and 1.46 mmoles/g of feces for diets containing lactose at 0, 0.5, 1 and 2 g/kg BW0.75/d, respectively; $P<0.05$), but did not affect concentrations of total volatile fatty acids. No changes in fecal microbial populations were observed as a result of lactose supplementation. Average concentrations of Lactobacillus spp, Enterococcus spp and C. perfringens were 5.66, 5.57 and 6.38 log copies dsDNA/g of feces, respectively. Apparent total tract digestibility of macro nutrients (crude protein, ether extract, crude ashes and starch), macro minerals (calcium, phosphorus, magnesium, sodium and potassium), and trace minerals (zinc, manganese, iron and copper) did not differ among treatments. In conclusion, eight dogs out of 14 tolerated the intake of lactose at 2 g/kg BW0.75/d without exhibiting any gastrointestinal signs. Moreover, despite the fact that some evidence of reduced proteolysis was observed when lactose was added to the dogs' diet, a strong prebiotic effect of lactose in adult dogs was not observed.

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EQUINE HINDGUT METHANOGENS DIFFERENTIATION BY RDNA PCR-RFLP

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Methanogenic archaea are part of the anaerobic microbial community of the animal gut. Recently these microbes received a great attention, due to their ability to synthetize methane. The production of methane represents a loss of energy for the animal and also the predicament of global greenhouse gas emissions. Equidae represent hindgut fermenters and produce less methane than ruminants. However, methane production by large, herbivorous monogastric animals, such as horses, donkeys and mules, is substantially up to 80 l per head per day. The number of studies of methanogenic bacteria present in monogastric animals is however quite limited. This work aims to assess the diversity of methanogenic population along the horse digestive tract by restriction fragment length polymorphism (RFLP) analysis. The total DNA was extracted from frozen and lyophilised samples of cecum, colon and rectum content of an adult and healthy horse fed with meadow hay and concentrate (3:1 ratio) twice daily. Archaeal DNA was amplified by specific primers targeting 16S rDNA gene (Wright and Pimm, 2003) and cloned into plasmid vectors pCR4. 16S rDNA was re-amplified by M13 primers and subjected to restriction enzyme analysis using DraIII, MluI, and MboI. Representatives of different restriction patterns underwent sequence analysis. 240 16SrDNA archaeal clones from horse hindgut were categorized by RFLP analysis. The enzyme DraIII digestion resulted in only 2 patterns, MluI distinguished 5 different patterns, and MboI discriminated even 12 dissimilar patterns. 17 unique archaeal RFLP samples were characterized by sequencing and subjected to phylogenetic analysis. Neighbor-joining method resulted in phylogeny showing four different clusters. Cluster I closely related to Methanobrevibacter, cluster II distantly related to Methanomassiliicoccus, cluster III closely related to Methanocorpusculum and cluster IV not related to any known methanogen. Cluster IV, representing the unknown uncultured archeons, was moreover divided into three subclusters. The MluI and MboI restriction enzymes are suitable for categorization of Operational Taxonomic Unit (OTUs) of equine methanogenic archaea. The Methanomassiliicoccus cluster II contained only samples distinguished by MboI, while the Methanocorpusculum cluster III contained only samples distinguished by MluI, therefore the application of both the enzymes is recommended. The unknown cluster IV covered samples with different MluI as well as MboI RFLP profiles. The samples of cluster IV are not related to any known or even uncultured archeons, which indicate that the horse digestive tract is populated by new genera of methanogenic microorganisms.

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Wright and Pimm - "Improved strategy for presumptive identification of methanogens using 16S riboprinting" - 2003

THE USE OF INFRARED THERMOGRAPHY FOR ASSESSING THERMAL CONDITION OF PIGS DURING UNLOADING AT SLAUGHTERHOUSE

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Infrared Thermography is a completely non-invasive technique that allows recording measurements of animal skin temperature on subjects difficult to reach or to approach, or moving subjects. The use of this technique is particularly interesting at the slaughterhouse, where there is a need to detect the temperature increase in response to the pre-slaughter handling. In order to evaluate the use of thermography at the slaughterhouse for assessing pig's surface temperature, a survey was conducted during 10 deliveries occurred from February to June 2014 involving 1400 subjects supplied by one farm. Distance from farm to slaughterhouse was 15 km, corresponding to a journey time of about 30 min. The deliveries were carried out using double trailer lorries with three hydraulic deck. The lorries had natural and mechanical ventilation system, with fans placed on the left side of the trucks. Unloading at the slaughterhouse was carried out by a platform (m 2.7x9.3x0.9) adjustable at the level of the lower deck. The vehicle was always offloaded firstly and then the rear trailer. Maximum surface temperatures of dorsal region and ears were recorded by Avio Nec G120 EX thermocamera on pig's groups of each unloaded deck drove along the platform. Images involving at minimum of 20 subject on the total of 23-24 pigs held in each deck were examined by software NEC InfRec Analyzer and Grayess IRT Analyzer. A total of 7000 thermal images involving 1222 pigs were analysed to determine the maximum temperatures in repere areas. Environmental temperature and relative humidity were recorded during the unloading of each deck. Data of surface skin temperature recorded by thermocamera was analysed using PROC MIXED of SAS using a model including the fixed effect of the truck deck, the random effect of day of slaughter and their interaction. This latter affected significatively ($P<0.05$) both temperatures of dorsal surface and ears. Irrespectively of the environmental temperatures, pigs loaded into the middle deck of both vehicle and lorry showed the highest surface temperatures on the dorsal area at unloading for the majority of slaughter days (80

INNOVATIVE APPROACH TO DETECT RESIDUES OF ANTIBIOTICS IN FOOD

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For the first time in Italy an instrumental multiclass method was developed and validated for the simultaneous determination of 62 veterinary drugs belonging to ten different families in meat and milk. Samples were extracted and analysed by liquid chromatography coupled to a hybrid high resolution mass spectrometry. The results of a preliminary survey carried out on meat samples collected from local markets demonstrated the fundamental role of multiclass methods in the residue control of veterinary drugs.

Several classes of antibiotics are normally used in farm to treat or prevent diseases, but they can also be illegally used posing a risk of residues occurrence in products. Therefore, control laboratories have to manage a considerable number of samples and analyse a large number of analytes. In this context, multiclass approaches are of great interest and in the last decade they have become reality thanks to the widespread diffusion of instruments based on mass-spectrometry technology. Aim is to develop and validate a multiclass method for antibiotic determination in food covering screening, confirmatory and quantification functions. Aim is to develop and validate a multiclass method for antibiotic determination in food covering screening, confirmatory and quantification functions.

Sample preparation (muscle and milk) consisted in the extraction with a mixture of acetonitrile and water. The redissolved extracts were injected in a Thermo Ultimate 3000 Ultra High Performance Liquid Chromatography system coupled to a Thermo high resolution Q-Exactive mass analyzer (Thermo Scientific, Bremen, Germany). The acquisition (positive ionization) was achieved in full scan mode (screening) and in data depending scan for confirmatory purposes. The chromatographic separation was performed in gradient mode within 30 minutes.

Sixty-two antibiotics belonging to ten different drug families (amphenicols, beta-lactams, diamino- pyrimidine, lincosamides, macrolides, pleuromutilins, quinolones, rifamycins, sulphonamides and tetracyclines) have been successfully included in the scope of the method. The list of analytes has been preliminary selected considering both veterinary practices and EU Regulation 37/2010 [1]. Its performance characteristics were compliant with the European criteria [2]. Seventy-one bovine meat samples have been collected at retail and analysed. The bad news is that, regardless their origin, the calf meats ($\leq$ 8 months of age) were largely contaminated (30%). The good news is that all positive samples were compliant, i.e. containing concentrations lower than the fixed Maximum Residue Limits, MRLs [1]. Tetracycline was the most found antibiotic class.

The proposed method largely improves the control of antibiotic residues in food and it could replace the combination of microbiological tests and instrumental single-class procedures currently used. This approach can finally provide an analytical support based on risk assessment principles improving the cost-effectiveness of food safety policies. Studies are in progress to evaluate the method applicability to other food of animal origin.

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DETERMINATION OF CADMIUM IN WHITE AND BROWN MEAT OF WARTY CRAB (ERIPHIA VERRUCOSA)

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European Union regulations establish the cadmium maximum levels for crab taking into account only concentrations found in muscle of claws and appendages (white meat), therefore excluding other organs and tissues (brown meat). Since in certain populations of Mediterranean region, such as Italy, the consumption of whole crabs including brown meat is not infrequent. The aim of the present study was to evaluate Cd levels in white and brown meat of warty crab (Eriphia verrucosa) collected along northern coast of Campania region (Italy) and to assess the health risk related to human consumption of warty crab for Mediterranean population.

Forty samples of warty crab were caught along the coast of Campania region between May and July 2014. The crabs were weighed, then sealed in decontaminated polyethylene bags, frozen at -20 °C and kept at the same temperature until delivery to the laboratory. The white and brown meat from each animal were individually separated and weighted and their percentage were calculated. Each tissue was subsequently homogenized and stored at -20 °C until further analyses. Aliquots of each sample were digested in ultrapure 65% HNO3 and H2O2 in a microwave digestion system. Cd concentrations were determined by atomic absorption spectrometer (GF-AAS).

Cadmium concentrations in white meat were below the limit of quantification in all tested samples. In contrast, Cd in brown meat was quantified in all samples and 47,5% exceeded the EU ML referred to the muscle meat, confirming the data reported by the EU Commission note of 2011. The Estimate Weekly Intakes (EWI) values were calculated assuming the only consumption of white meat and the consumption of whole crab (both white and brown meat) so as to take into consideration even food habits of specific population groups. The EWI value considering a weekly consumption of 100 g of warty crab meat was found 55.78 μg/week for the consumption of whole crab. This values accounted for 32% of the Tolerable Weekly Intake (TWI) set by the EFSA. Considering the negligible level of Cd in white meat, the contribution of the metal exposure derived by this crab tissue did not increase the EWI. In contrast, the consumption of whole crab could increase the Cd intake reaching high EWI value even nearly comparable to that set by the EFSA, if other main contributors to dietary Cd intake, i.e., fish and seafood products, cereals and cereal products were included in the risk assessment.

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FRUIT AND VEGETABLE BY-PRODUCTS SILAGE: PHYSICO-CHEMICAL, NUTRITIONAL AND PARASITOLOGICAL CHARACTERIZATION

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Food waste corresponds to approximately 40-50% of the total amount of biodegradable garbage produced every year. Recently, in order to reduce the amount of food waste dumped in landfill sites, its use has been proposed for livestock feed. This could represent an alternative of high interest since it may allow to halve the cost of animal production. Recently a trial has investigated the possibility to use fruit and vegetable by-products (as fed) in the unifeed diet for cattle, replacing a portion of protein and fibrous content (Chiofalo et al. 2014).This study has also underlined the impairments in conferring these perishable goods daily to the farm, and silage has been proposed as an alternative way to solve this problem allowing to keep fruit and vegetable by-products for long time. However, in this process of recycling of by-products it is important to ensure a high level of safety for animals and humans. In fact, fruit and vegetables can be contaminated by numerous pathogens including zoonotic protozoa such as Giardia duodenalis that is recognized as the most common food-born parasite (Smith et al. 2007).

The aim of the present study was to investigate the physico-chemical, nutritional and parasitologicalcharacteristics ofa fruit and vegetable by-products silage. This is a part of the innovative project PON "Save" - Smart Cities and Communities and Social Innovation.

Fruit and vegetables, at the end of their shelf life, were collected in Despar food store supermarkets (Messina, Italy) and conferred to a cattle farm. In the farm, at D0,these by-products were homogenized with straw (20%) and stored in trench for 40 days. Three samples were collected every 10 day sand divided into two aliquots up to the trench opening (D40). One aliquot was used for the following analysis: pH, moisture, ash, crude protein, crude fat, crude fiber; the other was analyzed using microscopical and bio-molecular techniques for the detection of G. duodenalis.

The silage test was repeated twice, in September and in November. A total of 24 samples were collected, i.e., 12 for each silage. The pH at the beginning of the test was 4.8 and ten days after it dropped to 3.8 without showing any further variations. At D0 moisture was 78.34% and decreased to 75.26% at D40. Crude protein and crude fat increased from 4.75 % and 1.52% at D0 to 6.27% and 2.65% at D40, respectively. The crude fiber was increased from 33.15% (D0) to 36.55% (D40). The nutritional characteristics of this product were comparable to those of other silages commonly used for cattle feeding, but with reduced costs of production.All the samples tested negative for G. duodenalis at both parasitological and bio-molecular techniques. According to the results of this study fruit and vegetable by-products can be successfully ensiled and used in animal feeding. This recycling process could reduce the discharge of a large amount of these by-products and thus halving the environmental impact and costs of livestock production.

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DEVELOPMENT OF LEGISLATION ON MOBILE SLAUGHTERHOUSE FOR RURAL POULTRY MEAT PRODUCTION

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The Regulation EC/853/2004 shall not be applied in relation to the direct supply, by the producer, of small quantities of meat from poultry and lagomorphs slaughtered on the farm, to the final consumer. The exemption is mainly on the limited requirements of the slaughterhouse that are not defined as "approved establishments"(1). In Umbria Region, there is an increasing interest in free range local poultry production (2) that is performed in small farms that produce less than 10,000 birds per year (1). The lack of slaughterhouses strongly discourage the producers and forced them to limit the production to less than 500 birds per year. A possible solution to bring access to inspected slaughtering to such a community and improve local production could be a mobile slaughterhouse, equipped on a small truck that directly reaches the different poultry farms. The development of new structures forced Local Authorities to point out rules to allow the activity of these slaughtering facilities not approved, and setting up specific procedures.

The aim of this work is to provide the first attempt in Italy in the development of a specific legislation on mobile slaughterhouse for small quantities of poultry meat production.

With reference to mobile slaughterhouse for poultry meat production the Regional Authority of Umbria Region allow the use of one mobile slaughterhouse that work for different farms (3). The food business operator and each farmers that intent to apply the slaughterhouse have to provide a notification to the Local Authorities (4). Furthermore the slaughterhouse structurally has to fulfill several requisites, set by Reg EC/853/2004 (Annex II, Capter I, III and IV point 1) and by Local Authority (5). In particular, stunning, bleeding and plucking have to be performed separately from evisceration. Stunning have to be performed according to Reg EC/1099/2009. Meat chilling and storage have to be performed in specific structures at slaughterhouse or at farm level. Animal by-products have to be managed according to Reg EC/1774/2002. Cleaning and disinfection of the structure and equipments have to be performed at the end of each slaughtering session in the farm level or in a specific staging area of the truck. Meat have to be labeled with the day of slaughtering, farm code and farm address. A proper traceability system have to be set up.

Not all the poultry farms have similar characteristics and an evaluation of the better farm site for slaughtering, as well as the water supply and wastewater management, are needed. A rotation of the slaughtering days among the farms have to be suggested. The microbiological criteria set by Reg EC/2073/2005 for such slaughtering system need further evaluation.

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GLUCOCORTICOIDS MODULATION OF FKBP51 EXPRESSION IN BOVINE THYMUS

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FK506 binding protein 51 (FKBP51) belongs to immunophilins, a family of proteins that binds immunosuppressive drugs. In the signal transduction of androgens, progestins, glucocorticoids (GCs) and mineralocorticoids, immunophilins act as co-chaperones for HSP90 chaperone complexes, hormone binding and intracellular trafficking of their cognate receptors [1]. In turn, these receptors regulate FKBP51 expression [1]. This study investigated FKBP51 expression in the thymus of veal calves and beef cattle experimentally treated with GCs to establish whether the FKBP51 gene can be considered as a biomarker for the detection of GCs abuse in bovine husbandry.

In trial 1, 22 Friesian veal calves, 6 months old, were divided as follows: gr. A (n=6) treated with 5 mg/week of estradiol benzoate (E2) for 6 weeks and 0.4 mg/die of dexamethasone (DEX) for 31 days; gr. B (n=8) treated with 15 mg/die of prednisolone (PRD) for 31 days; gr. K1 (n=8) was the control. The calves were euthanized 3 days after the last treatment. In trial 2, 18 Charolaise beef cattle, 17-22 months old, were divided as follows: group C (n=6) treated with 0.7 mg/die of dexamethasone 21-phosphate disodium salt (DEX) for 40 days; group D (n=6) treated with 15 mg/die of PRD for 35 days; group K2 (n=6) was the control. The animals were euthanized 6 days after the last treatment.

Samples of the cervical and thoracic thymus were collected from each animal and subjected to quantitative PCR (qPCR) for FKBP51 and GRα. Furthermore, qPCR for ERα was performed in the thymus of trial 1. Statistical differences were determined by ANOVA, followed by Dunnett's post test.

In trial 1, E2 and DEX (gr. A) significantly down-regulated FKBP51 expression in both cervical ($P<0.01$) and thoracic thymus ($P<0.05$), whereas GRα expression was down-regulated only in the thoracic thymus of group A ($P<0.05$). No effect on ERα expression has been detected.

In trial 2, DEX (gr. C) down-regulated FKBP51 expression in both cervical ($P<0.01$) and thoracic thymus ($P<0.01$). The treatment with PRD (gr. D) reduced FKBP51 expression in both cervical ($P<0.01$) and thoracic thymus ($P<0.01$). No effect on GRα expression has been detected in beef cattle thymus.

It has been reported that GCs administration induces the up-regulation of FKBP51 expression [1]. In turn, the FKBP51 generally attenuates the GR transcriptional activity, sequestering the receptor to cytoplasm and reducing the hormone binding affinity [1] Most of these studies regards in vitro experiments with short-term treatments (hours). Conversely, in our experiments, animals have been treated for a long period (> 30 days) that may have led to a prolonged reduction of GRα activity and so to the down-regulation of the target genes, like FKBP51. Moreover, it should be pointed out that the subcellular localization and consequently the activity of steroid receptors, like GRα and Erα, is also affected by the phosphorylation status and the redox milieu [3]. Our results suggest a thymus differential response to PRD compared to DEX, probably due to the different age of animals or their different metabolism [4].

Although further studies are needed, FKBP51 expression in the thymus appears to be a promising biomarker for the detection of GCs abuse, particularly in beef cattle.

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EFFECTS OF NANDROLONE PHENYLPROPIONATE AND RACTOPAMINE ASSOCIATION ON VEAL CALVES TARGET ORGANS

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Growth promoters use in beef industry is banned in the European Union according to EU 96/22 and 96/23 directives. Beef industry system is based on a strong industrial rationalization that, in many cases, results in the illicit use of pharmacologically active substances, in order to improve animal performances and to increase profits [1]. Natural or synthetic growth promoters are widespread in this field, particularly beta-agonists, glucocorticoids and sex hormones. Androgens exert their biological action through the androgen receptor that induces either transcriptional activation or repression of target genes in responsive tissues [2]. The most employed anabolic steroids for growth promoting purposes are testosterone and its derivatives that are usually administered with an ester side-chain attached. Chronic administration of beta-agonists influences muscle growth inducing the increase of the myofibrillar protein gene expression [3]. The present study examines the effect of nandrolone phenylpropionate (nandrosol) and ractopamine on testis, sex accessory glands and skeletal muscle of veal calves. Fifteen male veal calves were divided in two groups: group A (n=7) untreated controls and group B (n=8) treated with a combination of nandrosol (150 mg/animal, im) administered four times every 15 days and ractopamine (80 mg/day/animal, os) for last 31 days. Testis, prostate, and bulbourethral glands were sampled for histological investigation; samples of Longissimus dorsi, Vastus lateralis, and Biceps brachii muscles were collected for qPCR analysis. The study was approved by the Italian Ministry of Health and the Ethics Committee of the University of Turin (D.L. 27/01/1992 no. 116). Group B testis showed weight reduction ($p<0.001$), and the histological evaluation did not show differences in the germinal line between experimental groups. Moreover, in treated animals, seminiferous tubules diameter and mean tubular area were significantly reduced ($p <0.001$). The prostate and bulbourethral glands of group B revealed mild epithelial hyperplasia associated with moderate hypersecretion and cystic dilatation of ducts. Gene expression analysis on group B skeletal muscle showed a different gene regulation in three muscle types. In particular, Myogenic Regulatory Factor (MRFs) genes, involved in differentiation process, were up-regulated by the treatment in Vastus lateralis, whereas Myosin Heavy Chain (MyhC) gene expression was increased in Longissimus dorsi. In conclusion, our results show that besides the morphologic changes, already reported following sex steroid androgens administration [4], nandrosol and ractopamine seem to be able to influence skeletal muscle genes expression involved in the differentiation process. Additional in vivo and in vitro studies are necessary to better understand the mechanisms related to this complex process.

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ISOLATION OF FOODBORNE PATHOGENS IN LIVER OF WILD BOARS HUNTED IN LIGURIA

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During last decades, the wild boar population (Sus scrofa) growing in Liguria has induced a consequent consume increase of meats derived from these animals. The animal slaughter is frequently performed by hunters; in certain cases, the application of inappropriate hygiene procedures could determine a poor sanitation of meats (i.e. contamination by pathogens, such as Yersinia and Campylobacter), that may therefore affect human health (1-2). However, only few data on the presence of these pathogens isolated from liver and meat in wild boars are to date available (3).

The aim of the present study is to evaluate the safety of meats derived from wild boars hunted in Liguria.

In the last hunt season (1 October 2013-31 January 2014) 1,027 liver samples were collected in Liguria (Imperia 225, Savona 289, La Spezia 56, Genoa 457) and checked for the presence of Yersinia enterocolitica and Campylobacter spp. The specimens were analyzed in according to ISO 10273-2003 and ISO 10272-2006, respectively; colonies suspected for the Yersinia enterocolitica presence were confirmed by biochemical tests, biotyping and serotyping using specific antibodies: anti-O8, O9, O3, O5 and O1,2.

Regarding the analytical research of Yersinia enterocolitica in Savona, Imperia and La Spezia provinces, no positive results were obtained; in Genoa province 26 (2.5%) samples were positive. Only 2 strains of isolates belonged to the 1B biotype, considered as pathogenic for humans; the remaining part was classified as non-pathogenic 1A biotype. In province of Genoa the prevalence was 5,7%; so the territory was divided in different areas of hunt. Seven of them showed a prevalence of Yersinia enterocolitica lower than 10% (Polcevera, Paradiso, Petronio, Fontanabuona, Aveto, Scoffera and Sturla); 4 areas showed values included between 10% and 20% (Golfo, Genoa, Stura and Scrivia). The difference of prevalence between male and female animals was considered not significant (Fisher's Exact Test, P >0.05). As reported by EFSA (1), it's possible assume that 1A biotype strains are widely spread in the environment and are often isolated from animal and human stools and foods; our data indicate, despite of circulation of Yersinia enterocolitica strains, a low risk of yersioniosis in association to the food consumption. Regarding Campylobacter spp, 21 samples were positive (2%). This bacteria showed an homogeneous distribution. The highest percentage of positive cases was in Imperia (2.6%), and Genoa (2.6%) followed by La Spezia (1.8%) and Savona (0.7%); concerning the isolation of Campylobacter in Province of Genoa, 7 areas showed a prevalence lower than 10% (Genoa, Sturla, Scrivia, Scoffera, Fontanabuona, Graveglia and Stura); two areas were characterized by values included between 10% and 20% (Entella, Trebbia). Our results evidence the presence of Campylobacter spp. and 1B biotype Yersinia enterocolitica in wild boars of the Liguria, outlining a possible risk for public health. As previously suggested, this data may be related to the carcass' contamination that could occur when animals are eviscerated and skinned under insufficiently hygienic conditions.

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EVOLUTION UNDER DIFFERENT GROWTH CONDITIONS OF ROPY SLIME-PRODUCING BACTERIA ISOLATED FROM COOKED MEAT PRODUCTS

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In this study, our aim was focusing on spoilage of cooked cured meat product, with emphasis on the formation of ropy slime on the surface of vacuum-packaged products, which determines their deterioration and subsequent elimination from the trade. After the isolation and identification of strains involved in the formation of ropiness, the purpose of the research was identifying best growth conditions and the survival at heat treatment.

Samples of stretchy filaments from the surface of cooked meat were collected and two strains were isolated and cultured on de Man Rogosa Sharpe broth (MRS, CM0359, Oxoid, Basingstoke, UK) at 30°C for 24 hours, reaching the concentration of approximately 10^8 cfu/ml. The growth curves' study started from an initial concentration of 10^3 cfu/ml on MRS broth; then they were incubated at different temperatures: 44°C, 37°C, 30°C, 20°C, 12°C, 4°C. Samples from different storage conditions collected every 12 hours the first three days, then every day for a week, then, for lower temperatures one day a week. Thermal resistance was evaluated at 60°C, 70°C, 80°C.

Growth curves demonstrated that these strains were not able to grow at 44°C while they reached 10^8 cfu/ml after 48 hours of incubation at 37°C and after 24 hours at 30°C. At 20°C a complete inhibition was observed after 7 weeks (<30 cfu/ml) and at 12°C after 11 weeks. At 4°C the concentration was still 10^6 cfu/ml after 16 weeks. The strains was sensible at the core temperature of 70°C and 80°C while at 60°C measured D values are much higher.

Ropy slime-producers belong to Lactobacillus spp. and Leuconostoc spp. and are able to produce a large amount of exopolysaccharides, which forms stretchy filaments on the surface of vacuum-packaged cooked meat. Previous studies demonstrated that ropy slime-producing bacteria do not survive heat treatment so it was concluded that contaminations occurs mainly after heat treatment. This is an important aspect not only for the necessity of improving hygiene management after heat treatment but also because these strains are able to growth and produce filaments at refrigeration temperatures.

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OCCURRENCE OF OCHRATOXIN A (OTA) IN SICILIAN SALAMI: PRELIMINARY RESULTS

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In Sicily, in the Peloritani, Nebrodi and Madonie mountains, the traditional family-run farms where the autochthonous pigs are reared in the open air are still present. In these farms, the animals are of the native race 'Nebrodi Black Pig', traditionally called by farmers 'U Porcu Nivuru'. The breeding method of wild and semi-wild state implies that animals are nourished of the natural vegetation of the undergrowth (acorns, tubers, chestnuts, hazelnuts, etc.) that can be integrated with barley and field beans, and ground or pelleted feed. The meat of black pig can be used for the production of salami of superior quality and able to satisfy the most discerning palate. Ochratoxin A (OTA) is a mycotoxin produced by the secondary metabolism of moulds of the genus Aspergillus and Penicillium. OTA is a possible human carcinogen (group 2B, IARC) and probably responsible of Endemic Balkan Nephropathy (BEN). The main sources of human exposure to ochratoxin A are cereals and cereal products, wine, beer, grape juice, coffee, cocoa and its derivatives, spices, and pig meat. In Sicilian salami, many ingredients can be added: black pepper, cloves, nutmeg, cinnamon, garlic, chili, pistachio nuts, fennel, coriander, and red wine.

The aim of this study was to assess the occurrence of OTA in salami sampled in Sicily and made with meat from black pig, or even mixed with pork from the conventionally produced pigs. We analyzed handmade salami produced in farmhouses, and in small and medium size salami factories to assess if content of OTA in these products is a real risk to human health, also in consideration of the characteristic breeding and feeding methods of the black pig.

In this study, we sampled 55 Sicilian salami that differ for the mode of production and the content of ingredients added to the pork meat. They were purchased in the provinces of Messina, Siracusa and Enna. For analysis of OTA, we used the extraction procedure described by Bozzo et al. (2012) slightly modified, followed by purification on immunoaffinity columns (IAC). The analyses were performed by high performance liquid chromatography with fluorescent detection (HPLC-FD).

The sample preparation procedure with IAC clean-up has proved a suitable and effective method for OTA evaluation in salami samples. The rate of recovery was about 97%, LOD and LOQ were 0.05 μg kg-1 and 0.20 μg kg-1 respectively, far below the guideline value of 1 μg kg-1 established by the Italian Ministry of Health. Ochratoxin A was found in 5 samples, and only one (1.03 μg kg-1) exceeded the recommended residue limit.

The clean-up procedure with IAC and analysis via HPLC-FD has proven to be an effective method for OTA determination in heterogeneous meat products like salami. Despite the limited number of samples, our results indicate that Sicilian salami produced totally or partially with meat from black pig, and added with various spices, are a safe food as regards the presence of ochratoxin A. This research will continue also involving salami factories from other Sicilian areas.

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ACTIVITIES OF A FORMULATION OF LACTIC BACTERIA DAIRY OF ORIGIN AGAINST SELECTED PATHOGENS IN DRY-CURED SALAMI

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The aim of this study was to evaluate the effect of selected lactic acid bacteria starter culture (LAB) against selected pathogens in a laboratory scale production of dry-cured salami.

Before sausage production, starters and pathogens were prepared. The strains of starter cultures were incubated aerobically in Nutrient Broth (NB, CM0001 Oxoid, Basingstoke, UK) at 37Â°C for 24 hours. The LAB were inoculated in purity in sterile milk for an initial ratio of cocci: bacilli: enterococci of 2: 1: 1 and a concentration of approximately 107 cfu/ml. Selected pathogens from the collection of the Laboratorio di Ispezione degli Alimenti di O.A.(Escherichia coli K12 CSH26, Staphylococcus aureus 27R, Salmonella Derby 27, Pseudomonas fluorescens ATCC 12983, Listeria innocua ATCC 33090 and Clostridium sporogenes ATCC 19404), were individually inoculated in sterile milk to obtain an initial concentration of 104 cfu/ml. Once the ingredients were added to the ground meat, the mixture was divided into three batches: i) LAB, ii) pathogens, iii) LAB + pathogens. The sausages were prepared, suspended and stored in maturation cell in the pilot plant for 30 days. Samples were collected from each batch at day 0, 3, 7, 13, 21,30.

Analyzing the growth trend of the LAB inoculated with pathogens, it was possible to highlight that the LAB growth was constant and not influenced by the presence of pathogens. On the contrary, the growth of the pathogens was gradually decreased due to the presence of LAB, the growth level was at least 1 log lower than in the pathogens batches.

This study clearly demonstrated that, under experimental conditions, the LAB formulation was able to decrease the exponential phase of growth of the major foodborne pathogens of interest. This research demonstrated that alternatives to the use of additives are possible.

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MULTIPLE DNA BARCODING FOR FISH SPECIES IDENTIFICATION IN SUSHI PRODUCTS

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The aim of this work was to perform a molecular survey based on DNA barcoding to identify the seafood species used in the preparation of ethnic products (sushi). Twenty-one raw products (each composed of 3 to 8 pieces, for a total of 88 samples) were purchased in ethnic restaurants in the provinces of Pisa (11), Lucca (2), Livorno (3) and Florence (5). The total DNA extracted (1) was evaluated by gel electrophoresis and amplified using universal primers for mitochondrial (COI, 16SrRNA) or nuclear genes (PEPCK) depending on the species (fish, mollusk or crustacean) and the level of DNA degradation. Different primers (2,3,4,5,6,7) for the amplification of a long (~700 bp) or a short (~139-200 bp) fragment were used. Ninety-five PCR products were obtained (for some products two genes were analyzed). Of these, 30 have already been sequenced (Experimental Zooprophylactic Institute of Latium and Tuscany (Rome)). The sequences were elaborated with Clustal W in Bioedit 7.0.9.0, and analyzed by a BLAST analysis on GenBank and by using the Identification System on BOLD. A top match with a sequence similarity of at least 98% was used to designate potential species identification (8). DNA was degraded in almost one third of the samples. This was probably due to rice acidification, to repeated cycles of freezing/thawing or to prolonged storage. The degradation was confirmed by PCR amplification. In fact, we obtained long amplicons in 72.6% of the cases (n=69) and short amplicons for 27.3% of the samples (n=26). The average length of the long sequences was 595 bp for the COI FDB and 490 bp for the PEPCK gene, while the length of the short sequences was ~210bp for the 16S rRNA and 139bp for the COI MDB. All the samples were identified at least at the genus level, with identity values ranging from 99 to 100%. Although for some samples it was impossible to achieve a specific identification, the results were informative enough to verify the information given by the producers. No samples were found mislabeled. Even though the COI gene represents the most exploited target for seafood species identification, issues were found during amplification and comparison with the databases. Thus, in order to increase the PCR output, new universal primers, able to amplify a wide range of taxa, would be desirable. Finally, in case of degraded DNA samples, where the number of diagnostic mutation is limited, a multiple gene analysis is advisable.

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EVALUATION OF THE CONCENTRATION OF STEAROYL-CO-A DESATURASE IN COW MILK AND CORRELATIONS WITH DESATURASE INDICES

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Mammary gland is able to desaturate several fatty acids, leading to the synthesis of unsaturated fatty acids (UFA). This process is carried out mainly by fatty acid desaturase 1 and 2 (FADS1, FADS2), and delta9 stearoyl-CoA-desaturase (SCD). SCD preferably introduces a double bond in delta9 position of myristoyl-, palmitoyl-, and stearoyl-Co-A. The activity of SCD can be indirectly measured by the calculation of desaturase indices: the aim of this work was to evaluate the content of SCD in bovine milk and to relate the concentrations to the common desaturase indices.

Milk samples were taken from 13 healthy multiparous Valdostana cows: milk samples were collected at 40±5 days, 70±5 days, and 130±5 days of lactation, and analyzed by gas chromatography for UFA, namely C14:0, C14:1-cis, C16:0, 16:1-cis, C18:0, C18:1n-11-cis, C18:1n-9-cis, as percentages of a total of 37 fatty acids. The desaturase indices for C14 (delta14), C16 (delta16), C18 (delta18) were calculated as reported by Mele et al. (2007). A total desaturase index (Tdelta) was also calculated. The concentration of SCD was performed through an ELISA kit specific for bovine substrates. Lecture was made at 450 nm wavelength, corrected at 540 nm wavelength with a microplate reader. The optical density of samples was converted into SCD concentration (pg/ml). A two-way repeated measures ANOVA mixed model was performed in order to evaluate differences in UFA/SCD between groups (fixed factor) and subject (random factor) on log-transformed values: significance was set at pâL'd'0.05.

Data analysis evidences a strong individual component, mainly in C14 and C18 acids, in delta14, in delta14 and in Tdelta. The lactation period significantly influenced the C14:1-cis percentage with an increase (0.93±0.30 1.09±0.24,and 1.07±0.23 % at 40, 70 and 130 days, respectively, $p<0.05$), and its desaturase index delta14 that decreases during lactation (5.81±1.23, 6.84±1.14, and 6.91±1.20 at 40, 70 and 130 days, respectively, $p<0.01$); SCD concentration decreased significantly during the follow-up (215.6±140.3, 122.5±49.5, and 135.1±126.4 pg/ml at 40, 70 and 130 days, respectively, $p<0.05$), while no statistical significance was found after calculating the Spearman correlation coefficients between all desaturase indices and SCD milk concentration.

The results suggest that during the follow-up the considered UFAs change only in a limited fashion, with important individual features. The only fatty acid changing in percentage (increase) is C14:1-cis, while the the Stearoyl-Co-A desaturase concentration shows a decrease during lactation. No correlations were found between SCD concentration and desaturase indices. This feature could be contrasting, but agrees with other studies in which SCD activity is not correlated with desaturase indices (Archibeque et al., 2005). This behavior could be due to the higher cellular turnover occurring in the mammary gland at the onset of lactation when apoptosis is marked (Dessauge et al. 2011). Therefore, SCD concentration seems not to be a valid index of desaturation activity of mammary gland during lactation in cattle.

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NICASTRESE GOAT MILK ASSESSMENT THROUGH FT-IR METHOD: A PRELIMINARY STUDY

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Goat dairy products have an important impact on the local economy of South of Italy, e.g. Calabria Region. In particular, the products made using milk from autochthonous breeds, such as the Rustica di Calabria, the Aspromontana and the Nicastrese, represent an important economic issue for local market.1 Only 4.975 head of Nicastrese goats were catalogued in 2013 from Calabria Region.4 This number seems to suggest that the Nicastrese is an endangered breed (FAO, 1992).1,2,4 The Nicastrese goat is breeding using extensive and semi-extensive grazing management, mainly in the local area of Catanzaro.2 The main dairy products are the canestrato and caprino cheeses, appreciated by customers for their peculiar texture and aroma. The physical-chemical characterization of goat milk allows providing suitable information of manufactures and supporting the local dairy industries. Currently, the Fourier transform infrared spectroscopy (FT-IR) is widely used in dairy industry to physical-chemical characterize milk and provide information about its composition. No studies are actually available about the use of FT-IR analysis to physical-chemical characterization of Nicastrese goat milk.

The aim of the work was the physical-chemical characterization of Nicastrese goat milk composition using the FT-IR analysis.

In May 2014, in a family dairy farm, located in the province of Catanzaro, a total number of 42 multiparous Nicastrese goats, between the 7th and the 8th week of lactation, were randomly selected for the analysis. At this time, the pasture type was extensive. Goat milk was manual hand milking collected into graduated measuring bucket, mixed and finally sampled into 50 ml sterile tube (Falcon). Samples were stored at +4°/8°C and analysed the same day at University of Catanzaro, Laboratory of Food Analysis. A MilkoScanTM FT+ (FOSS) was used to carry out the following parameters: fat (%), total proteins (%), lactose (%), casein (%), true protein (%), acetone (mM), BHB (mM), milk freezing point (mC), and urea (mg/dL).

The resultant data demonstrated that the Nicastrese goat milk contains: fat (4.59±0.6%), total proteins (3.47±0.31%), and lactose (4.2±0.18%). All these values agreed data reported in bibliography.3,4,5 The Nicastrese goat milk was stable over time and the milk composition did not change for fat, total proteins, and lactose. Furthermore, the true protein, casein, milk freezing point, and metabolites of Nicastrese goat milk were physical-chemical characterized, for the first time, using FT-IR analysis. The following results are below reported: true protein (3.13±0.31%), casein (2.55±0.27%), milk freezing-point (542±6.2 mC), urea (57.8±8.5 mg/dL), acetone (0.08±0.08 mM), and BHB (0.01±0.01 mM). The resultant data also demonstrated that Milkoscan FT+ equipment allows characterizing the milk quality and compositions derived from Nicastrese breed. The association among grazing diet, milk quality and autochthonous breeds can characterize the composition of local milk manufactures and improve the economy of marginal areas of Calabria Region.

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SCREENING OF BIOGENIC AMINES PRODUCTION BY BACTERIA ISOLATED FROM "PECORINO" CHEESE

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Biogenic amines (BA) are naturally present in many foods and beverages, especially fermented ones. Due to their toxicity high levels of BAs in food can be a health risk. In cheese the most abundant amine is tyramine (TYR) which is the main cause of the so-called "cheese reaction". BAs presence in food is mainly caused by aminoacids decarboxylating bacteria.

Aim of the study is to evaluate BAs in vitro production by bacteria isolated from a semi-hard "pecorino" cheese. Materials and Methods: 72 strains of potential BAs producers -enterococci (Ec), mesophilic lactobacilli (Lb), Enterobacteriaceae (Eb)- were isolated during the cheesemaking and the ripening phases of a "pecorino" cheese, manufactured in a dairy factory in Tuscany. Ec (34) were identified by PCR [1], Lb (28) were identified by API 50 CH (Biomàl'rieux) and confirmed by PCR [2,3,4], Eb (10) were identified by API 20E kit (Biomàl'rieux). After 72h incubation in a decarboxylase broth added with 1% w/v of the precursor aminoacids, the production of 7 BAs (triptamine, 2-phenylethylamine (2PHE), putrescine (PUT), cadaverine (CAD), histamine, TYR, spermidine, spermine) was quantified on the acid extract of the cultural medium by HPLC analysis as described in a previous study [5].

All the strains tested produced BAs although in varying degrees. Overall our data on BAs production by the different microbial groups agree with previous studies [6,7,8,9]. Eb were confirmed as good PUT and CAD producers, both for number of decarboxylating strains (100% and 90%, respectively) and for BAs concentrations (on average 341 and 785 μg/ml, respectively). All Ec strains produced TYR, with very high mean amounts (1608 μg/ml), and many of them gave not negligible 2PHE, PUT and CAD production (on average 184, 121 and 146 μg/ml, respectively). These data agree with Ladero et al. [10] hypothesis that TYR production is a genomic trait of E. faecium and E. faecalis species. On the other hand the same Authors speculated that PUT is a genomic trait of E. faecalis, while in our study 50% of the tested strains of this species showed no detectable PUT production. Indeed, other Authors reported PUT-negative strains of E. faecalis [6,9,11]. Strains that produce high amounts of BAs, although with a low prevalence, when growing to high cell counts can substantially contribute to BAs formation [9]. In view of this, it is noteworthy that, although our data confirm that Lb are overall not good BAs producers, few Lb strains produced very high amounts of TYR (810, 1766 and 1959 μg/ml).

BAs presence in cheeses is not solely associated with undesired bacterial groups (Eb, Ec), but technologically useful microorganisms, like Lb, could play a role in TYR accumulation in ripened cheeses, especially considering the high Lb loads reached and maintained throughout the ripening period.

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CHARACTERIZATION AND INVESTIGATION OF SEASONAL VARIATIONS OF TYPICAL ITALIAN CHEESE PECORINO OF MONTE PORO THROUGH THE FATTY ACID COMPOSITION AND VOCS PROFILE

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"Pecorino di Monte Poro" is a semi-hard Calabrian cheese produced in the area of the Monte Poro in the province of Vibo Valentia. This cheese is traditionally made with raw ewes' milk, without the addition of any starter cultures, and using rennet paste to coagulate the milk. Pecorino di Monte Poro is today manufactured in small dairies mainly near the sheep shelter; it is a small round cheese weighing about 700-1000 g. It obtained the nomination as regional traditional dairy product of Calabria and nowadays, thanks to the growing interest towards traditional foods, it is marketed and appreciated also outside of the region. 1 Typical characteristic of the milk, the special geo-climatic conditions of this region, the natural pastures, and the traditional technology used for the production may explain its unique sensorial characteristics. At present, no studies on the volatile fraction and fatty acid composition have been reported so far. 2

This study intends to verify if the volatile constituents and the fatty acid profile differ among seasonal production considering a great variability of pasture essences most characterised in spring by the presence of Medicago sativa L. and Hedysarum coronarium L., and during winter, by the presence of Avena sativa L. cultivation. The study was performed in a family dairy farm located on the plateau of Poro. 10 wheels of pecorino of 1 kg, at about 70-80 days of aging, were used of which 5 aging in January 2014 (arising from pasture from April to October with Medicago and Hedysarum as representative essences) and 5 aging in March 2014 (pasture from November to April with Avena as major grass). Cheesemaking process was performed with lamb rennet no older than 20 days. The pecorino's samples were analysed, to assess: moisture content, ashes, fat, protein, fatty acids composition, aromatic profile, and to check for seasonal differences between the dairy batches. The use of a gas chromatograph equipped with a flame ionization detector (FID) has allowed the determination of the fatty acids composition. The study of the volatile fraction was performed using headspace solid phase micro-extraction (HS-SPME) and gas-chromatography mass-spectrometry (GC-MS). All data were reported as mean $\pm$ sd.

The results highlighted important differences in the cheese composition, arising from the different seasonal pasture essences. In fact, from an high-Avena sativa diet (cheese produced in March) there is an higher concentration of protein (29.09$\pm$2.11%), conjugated linoleic acid (0.15$\pm$0.02%), omega-3 (0.66$\pm$0.23%), omega-6 (2.2$\pm$0.03%), mono- (31.84$\pm$0.5%) and poly-unsaturated (2.89$\pm$0.07%) fatty acids, with a lower percentage of saturated fatty acids (65.12$\pm$0.48%), if compared with the cheese made in January. Indeed, from an organoleptic viewpoint, it is important underline the high level of sulfur compounds detected in cheese produced from an high-Medicago sativa diet. In conclusion, the organoleptic and nutritional characteristics of Pecorino of Monte Poro are clearly influenced from the feeding of the flock in term of pasture essences.3 This can impart an uniqueness at the manufacturing process, that must to be protected and preserved over time, as a precious patrimony of the territory to ensure the continuation of the survival of an ancient tradition and the authenticity of the products.

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THE RISK PERCEPTION OF ITALIAN CONSUMERS ABOUT HAZARDS ASSOCIATED WITH RAW FISH FOODS

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The traditional japanese food prepared from raw fish has become very popular (1-2). The consumption of raw fish preparations has been increased in Italy. This should be attributed to the eastern culinary fashionable trend. Anyway, in Italy raw food consumption such as marinated fresh anchovies has deep cultural roots.

The objective of the study was to determine consumer perception and awareness to food safety hazards associated with raw fish.

Questionnaires were designed to obtain information on demographics (age, gender, educational level etc.) of respondents, trends and type of raw fish consumed, consumer's food safety perceptions (awareness of foodborne pathogens etc.) and safety practices. These respondents were interviewed face-to-face or indirectly by using social networks.

A total of 324 randomly selected consumers of raw fish foods were interviewed. The total sample was composed of 62.1% women and 37.9% men. As for demographic attributes of respondents, 48% of the respondents were under the age of 30, whereas the 33% part of them were among the ages 30 and 50, and 19% of the respondents were over 50. There were more participants with higher education (94.7%). The majority of participants (45.8%) consumed raw fish dishes once per month. The results of this study showed a low level of knowledge about raw fish related hazards among Italian consumers. Three clusters of consumers were identified. These three sub-samples differed in terms of age. The consumer's behaviors of these sub-samples differed in terms of food consumption habits. The young people (under 30) are the strong consumers of sushi and sashimi. In particular, these products are consumed at sushi bars or japanese restaurants. The people from 30 to 50 years old eat both of the traditional raw fish foods (marinated fresh anchovies) and sushi/sashimi at home. The older people (over 50 years old) are oriented to eat just traditional raw fish foods and the home is the place where these foods are consumed. Among hazards, consumers rated biological hazards as the highest perceived food safety risk. In details, microbial contamination was the food safety risk perceived as most significant by consumers. Young people (under 30) were found to be less concerned about food safety than older people (over 50 years old). Moreover, knowledge of food safety issues increases with age. Finally, 43.8% of consumers had heard of Anisakis spp. Also it was identified that the most common food practices mistakes were cooling and storage of raw fish foods in inappropriate ways. A better understanding of Italian consumers' perceptions of food-related risks associated with raw fish food would help improve effective communication about food hazards. In conclusion, data obtained from the survey indicated the need for much more consumer education regarding freezing, as a preventive treatment, as expressly required by law.

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BIOFILM-FORMING ABILITY OF STAPHYLOCOCCUS AUREUS FROM FOOD ENVIRONMENT

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Staphylococcus aureus (S.aureus) is one of the well known pathogens that can live in a wide variety of environments. It also has an inherent ability to form biofilms on biotic and a-biotic surfaces (1). Biofilm formation is important for survival of S.aureus in the food industry (2). Currently, little is known about the ability of food-related S.aureus strains to form biofilms (3).

The aim was to evaluate the biofilm-forming ability of S.aureus on food processing surfaces at 12 and 37°C. Biofilm assays were performed on n.26 wild isolates. Biofilm was allowed to develop on polystyrene and stainless steel at selected temperatures. After 24-h incubation, the amount of biofilm was determined spectrophotometrically and results expressed as 'Biofilm Production Index' (BPI) = [O.D.meanbiofilm surface (mm2)-1] x 100. Biofilm was compared with reference strains: S.aureus ATCC 35556 (positive control - BPIPC), S.aureus ATCC 12600 (reference strain - BPI12600) and S.epidermidis ATCC 12228 (negative control - BPINC) for each isolate. All isolates were defined into different categories on the basis of their BPIs values. The cutoff point for the biofilm production was the BPI value obtained by negative control on polystyrene (BPINC = 0.294) and stainless steel (BPINC = 0.149). S.aureus strains showing ability to produce biofilms were classified as weak (BPINC $\leq$ S.aureus BPIs < BPI12600), moderate (BPI12600 $\geq$ S.aureus BPIs < BPIPC) or strong (S.aureus BPIs âL'ě BPIPC). Finally, in order to evaluate the architecture of the biofilms, the SEM analysis was carried out.

A strain-specific variation in biofilm formation within S.aureus strains was observed. At 37°C, n.17/26 (65.3%) of strains were biofilm producer in at least one tested surface. A total of n.13/26 (50%) of strains were biofilm producer on polystyrene whereas n.10/26 (38.4%) were biofilm producer on stainless steel. Moreover, n.6/26 (23%) of strains were biofilm producers on both surfaces. In details, n.2/17 (11.7%) were classified as moderate biofilm producer on polystyrene whereas they were no biofilm producer on stainless steel. At 12Â°C, all strains were no biofilm producer.

Our results suggest that the biofilm formation of S.aureus is influenced by environmental conditions relevant for the food industry. This study attempted to investigate the biofilm formation by wild S.aureus isolates and to correlate the BPI values with the SEM images.

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POLYBROMINATED DIPHENYL ETHERS (PBDES) IN ITALIAN MUSSELS FROM MIDDLE ADRIATIC SEA

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Polybrominated diphenyl ethers (PBDEs) are a class of Brominated Flame Retardants (BFRs) used worldwide as additive to inhibit or slow down the ignition of fire in electronic equipments and various other consumer products. PBDEs are persistent, have very low water solubility, high binding affinity to particles and tendency to accumulate in the various environmental compartments and in biota. Accordingly in 2008 the European Union banned their use and in 2010 PBDEs have been included in the Stockholm Convention Persistent Organic Pollutants List. In 2011 EFSA (European Food Safety Authority) requested an update of occurrence data of PBDEs in food. Recommendation UE 118/2014 issued in March asked Member States to monitor different classes of BFRs in food, including PBDEs, aiming to collect sufficient data to define possible residue limits. In 2013 the IZS dell'Umbria e delle Marche started a project founded by the Italian Ministry of Health, with the aim to improve the know-how on PBDEs food contamination and to investigate their toxicological properties. An analytical method for the detection of 15 PBDE congeners (28, 47, 49, 66, 77, 85, 99, 100, 138, 153, 154, 183, 197, 206, 209) at sub-ppb levels in mussels was developed. Final extracts were obtained applying a QuEChERS-like extraction followed by two purification steps. The analysis was then performed injecting 10 microliters in an Agilent Thecnologies 7890A coupled to a GC 7000-QQQ-MS. The validation study demonstrated good analytical performances of the developed method. Since PBDEs are ubiquitous pollutants widely dispersed in the environment, a strict control of laboratory contamination had to be implemented. The contamination of procedural blanks becomes more relevant as the PBDEs concentrations in real samples decrease (< 100 pg/g), requiring a severe quality controls. Specific decontamination procedures had to be implemented before performing the analysis to obtain negligible concentrations of procedural blanks. The method was used to collect information on the PBDEs levels in mussels harvested along the Marche coasts. To the best of our knowledge, a similar monitoring plan was never undergone before in the central Adriatic sea and still today very few are the data available on PBDEs food contamination in Italy. From April till November 2013 an overall of 134 mussel samples (Mytilus Galloprovincialis) were collected in 21 breeding or reefs along the Marche coasts. Only four congeners were detected: BDE-49 (mean=15 min=5.0 max=42 pg/g), BDE-47 (mean=73 min=16 max=186 pg/g), BDE-100 (mean=18 min=4.1 max=68 pg/g) and BDE-99 (mean=30 min=4.4 max =91 pg/g). All the other 11 PBDEs were below the LOQs. The results are in agreement with the data published by Bianco et al. (2010) and Giandomenico et al. (2013) on Apulia mussels. Also Bianco et al. reported the 47, 99 and 100 to be the predominant congeners; they didn't analyze the BDE-49. Generally PBDEs levels measured in north America and Asia are much higher than the ones found in mussels from Adriatic Sea.

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UNIVERSAL EXHIBITION "EXPO MILANO 2015": THE OFFICIAL CONTROL OF THE NEW FOODS TO GUARANTEE PUBLIC AND ANIMAL HEALTH

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The Universal Exhibition "EXPO Milano 2015", is dedicated to the theme "Feeding the Planet, Energy for Life". Its first deal is the sustainable supply worldwide. There is a big question in EU, and for the same reason in Italy, in Lombardia and in the local health system (ASL), about the import regulation. Import regulation of food of animal origin, unknown or prohibited by Eu food law. However we are talking about traditional food in the populations of extra EU Countries. Italy and the EU must address health legislation to animal health and food safety, to ensure the import of 'novel food'.

In order to ensure food security during distribution and administration of 'novel food' from extra EU Countries, the EU Regulation, March 2, 2015, n. 329, regulates specifically import, storage, marketing, administration and establishes as well the official controls, precautionary measures and the system certification of food introduced in Lombardia with EXPO.

"EXPO Milano 2015" is a starting point for sure import and distribution of 'novel food' in Europe.

The offer of ethnic food, will not only be allocated to individual communities of immigrants: all the European Citizens have, in addition to their own eating habits, the opportunity to learn about different food traditions.

Another question, strictly national, is to decide how manage - only within 'EXPO' - food from animals in Italy prohibited by national law for ethical reasons (dogs and cats) or for food safety (dangerous fishes for the EC Reg. no. 853/2004).

We are talking about food traditionally and commonly used in certain Countries. The authors analyze laws and regulations which can be applied on unknown food and not popular food in Western countries (Europe).

Food actually named 'novel food', as already described above.

The authors analyze as well the tendence to import or produce directly in the EU 'novel food' as new habit. One example for all: what happened few years ago for the preparation with raw fish -sushi- of ancient Japanese tradition.

DEVELOPMENT AND VALIDATION OF AN ELISA SCREENING METHOD FOR THE DETERMINATION OF RACTOPAMINE IN URINE SAMPLES

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Beta-Agonists (BAs) are synthetic drugs currently used as illegal growth promoters in animals (1). Ractopamine is particularly known to increase muscle fiber size and the rate of weight gain. The route of exposure to Ractopamine in humans is through the consumption of animal source products. This drug is included in the group "A" by European Commission, and the Minimun Required Performance Limit (MRPL) for this residue in urine samples is 3.0 ng ml-1 (2). In this work, an ELISA screening method has been developed and validated in order to determine Ractopamine in urine samples.

The main objectives of the present work were to optimise a rapid technique, by exploiting a simple diluition of samples and to develop an ELISA screening method for the detection and identification of Ractopamine in urine samples at low levels. The performances of this method were studied by validation parameters such as specificity, precision, ruggedness and capability of detection (CCβ), in compliance with 657/2002/EC.

The ELISA is based on the antigen-antibody reaction. The measurement is made photometrically at 450 nm. The absorption is inversely proportional to the Ractopamine concentration in the sample. The urine samples, previously filtered, were centrifuged for 20 minutes at 3000 rpm. The clear supernatant was diluted 5 times with dilution buffer and it was used directly in the enzyme immunoassay. A validation protocol was carried out in order to establish the performances of this method which ensure the correct identification and quantification of Ractopamine in urine samples.

Validation parameters such as specificity, precision, recovery, CCβ and ruggedness were determined, resulting in compliance with the requirements of Decision 2002/657/EC (3), for banned substances of category A, and according to the "Linea Guida per la validazione intra-laboratorio di metodi di prova immunochimici di screening gruppo di lavoro II.ZZ.SS" (4). The results obtained demonstrated that the method developed is rugged and specific with a false compliant rate < 5% (β-error) at the level of interest and that it can be used for screening purposes in compliance with Decision 657/2002/EC. This simple method allows to analyze simultaneously a lot of samples in the routine analysis, and it significantly reduces the time and the cost of clean up.

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UPDATE AND IMPLEMENTATION OF FLOW DIAGRAM OF GMO METHODS IN FAST REAL-TIME PCR

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In the last years, the number of GM events commercialised worldwide has been increasing steadily and lot of them are not authorised in European Union (EU) (1). Current legislations in the EU foresee zero tolerance for unauthorised GMOs, a real and pragmatic problem of chain food contamination, and stringent requirements for GMO approval and labelling (2; 3; 4). Traceability is a key element in the implementation of EU Regulations and relies on the availability of validated analytical methods for sensitive and accurate determination of GMO content. Therefore the aim of this work was the optimization and the validation in real-time PCR mode fast of endogenous and transgenic systems of three crop species, respectively: cruciferine A gene (CruA) and Ms8 line for canola, Acyl carrier protein gene (Acp1) and MON1445 line for cotton and finally glutamine synthetase gene (GS) and H7-1 line for sugar beet. As certified reference materials (CRM) purchased from AOCS, 0% and 100% MON1445 cotton powdered, 0% and 100% H7-1 ground sugar beet seed and 0% and 100% Ms8 canola leaf tissue DNA were used for the validation. DNA of CRMs was purified with the NucleoSpin gDNA Clean-up starting with CTAB extraction (ISO 21571:2005). PCR inhibitors absence was tested by means of fast monitor run, a taxon-specific PCR carried out on undiluted DNA and its 1:4 dilution. Primers optimization was carried out testing three different concentrations of forward and reverse primer combined each other, obtaining 9 combinations, each one verified in 4 replicates. The best combination was identified depending on the lowest mean threshold cycle (Ct), the lowest standard deviation and a high ΔRn. Probe optimization, based on the lowest mean Ct, was obtained testing in 4 replicates, 5 different and increasing concentrations, with primers concentration previously chosen. For each specific system, primers and probe optimal concentration were identified. The PCR was performed on 7900HT Fast Real-Time PCR System using the following cycling conditions in fast mode: 95°C for 20 s, 40 cycles with 95°C for 3 s and 60°C for 30 s. For validation of qualitative methods, sensibility, specificity, robustness and limit of detection (LOD) were tested in accordance with MPR (5). Sensibility and specificity of all systems resulted of 100%. LOD was estimated 10 haploid genome equivalents (HGE) for Acp1, GS and MON1445, 20 HGE for H7-1 and finally 60 HGE for CruA and Ms8. To investigate method reliability and robustness, the same experiment as for LOD determination, was repeated with a different real-time PCR instrument, showing 100% concordance for all systems. This work proved that methods in fast real-time PCR are efficient to detect single endogenous systems and single events considered. Furthermore, the adoption of this technology in fast mode allows us to obtain good data in less than half the time compared to standard mode and this approach is very important for a laboratory assigned to official control of GMO on food and feed. Finally this work allows our laboratory to update and to implement the flow diagram of GMO methods, in particular from 4 to 7 endogenous genes and from 15 transgenic events to 18.

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A RAPID DNA EXTRACTION PROTOCOL FROM HONEY AND BEE POLLEN DEVELOPED AND VALIDATED BY DETECTION OF THE PLANT SPECIFIC ACTIN GENE SEQUENCE IN FAST REAL-TIME PCR

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Honey, a natural food produced by Apis mellifera bees, is highly consumed for its appreciated taste and also for its potential health benefits and biological properties. However honey is also a potential risk for human health due to environmental contaminants like pesticides, antibiotics, microbes, heavy metals and also allergens (1). Furthermore, in recent years, a case of GMO contamination with pollen derived from transgenic maize MON810 was registered in Bavaria (Germany). For this reason, the aim of this work was to find an innovative, efficient, practical, less expensive and more rapid method of pollen DNA extraction than those already existing in literature. To evaluate extracted DNA, a fast real-time PCR system, using the plant specific actin gene sequence, was optimized and validated. In the process of validation, a pool of seven crops interested by genetically modification and eventually present in honey was used (in particular, certified reference materials, CRMs: maize, soybean, rice, potato, cotton, canola, sugar beet). To develop a new DNA extraction protocol two already published procedures (2; 3; 4) were combined and tested with some improvements, in particular the pre-treatment was carried out without beads used to grind pollen. The best approach found in terms of quantity and quality of extracted DNA was a combination between the pre-treatment and the extraction method described in the German guideline. The protocol was modified and the DNA purification kit NucleoSpin gDNA Clean-up (Macherey-Nagel, Duren, Germany) was used. For validation of extraction method the inhibition test was carried out on 18 DNA extracted both from honey and from bee pollen, satisfying acceptance criterions. The universal plant DNA sequences, actin and tRNA-Leu, were evaluated, but tRNA-Leu was not considered appropriate for this study. On the other hand, the actin system was verified in fast real-time PCR, demonstrating 100% specificity and robustness and obtaining a limit of detection of about 5 haploid genome equivalents. The new protocol of DNA extraction from pollen proved to be efficient, rapid and also suitable and useful for routine analysis. Therefore the results obtained from this work could be used for other types of analysis like molecular species characterization, study of traceability relative to origin, determination of allergic components as well as detection of authorised and unauthorised GMO and environmental monitoring, considering pollen also an indicator of coexistence and cross-contamination.

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EFFECTS OF PHYTO-ESTROGENS ON PROGESTERONE RECEPTOR EXPRESSION IN VEAL CALVES PROSTATE

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Phyto-estrogens are natural estrogenic compounds present in plants and they may exert both estrogenic and anti-estrogenic activity (Vitale et al., 2013). The presence of phyto-estrogens in the pasture has been related to fertility disorders in sheep and cattle (Adams, 1995). They are classified as isoflavones, flavones and lignans. Isoflavones are the most common compounds and genistein and daidzein are the most important, mainly found in soybeans. Secoiisolariciresinol is the most frequent lignan and it is commonly found in cereals and grains (Dixon, 2004). Soybean, wheat, corn and potatoes are commonly used as protein sources in milk replacer for veal calves and their estrogenic activity on prostate histology has been studied by Groot (2006). Phyto-estrogens are able to induce mild histologic changes similar to those induced by estrogens even if to a lower extent; in particular, prostate of calves fed with soy-derived protein-supplemented milk replacer showed few hyperplastic foci and slightly dilated tubules. No increased basal cell proliferation was detected but an unusual cytoplasmic elongation of basal cells was noticed (Groot, 2006). Moreover, it is well known that estrogens induce histological changes and progesterone receptor (PR) over-expression in prostate of treated veal calves (De Maria et al., 2010). Aim of the work was to verify if the employ of milk replacer with plant-based proteins, which could contain phyto-estrogens, alters PR expression in prostate.

Five groups of 6 veal-calves were fed with different milk-replacers from 20 to 24 weeks of age. Group A received a dairy-based control feed, group B: 5% soy concentrate (Sojcomill, 5%), group C: 5% soy isolate (Nurish, 5%), group D: 5% wheat gluten protein (Kalpro 5%), group E: 2% potato protein (Mysamine 2%). Animals were then put back onto dairy-based milk replacer and slaughtered at 26 weeks of age. Prostate tissue was collected and processed to obtain paraffin sections. Immunohistochemical stain for PR (PgR Ab-2 clone hPRa2, Bioptica) (1:50) was performed as described earlier (De Maria et al., 2010). As a positive control, prostate sections from veal calves treated with 190mg/animal of 17βestradiol i.m. over a 6 weeks period were selected. In animals fed with soy concentrate and soy isolate based milk, only scattered glandular epithelial cells exhibited nuclear staining for PR, compared with the strong and diffuse positivity of the 17βestradiol treated prostate. Animals fed with wheat or potatoes-derived protein based milk showed respectively occasional positive cells or absolutely none reaction, as control animals did.

In conclusion, weak PR positivity was detected in animals administered with phyto-estrogens. Therefore, since PR expression is not affected from the administration of phyto-estrogens in the diet, its role as a biomarker to detect the abuse of estrogens as growth promoters in cattle is confirmed.

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IDENTIFICATION, CHARACTERIZATION AND VANCOMYCIN-RESISTANCE OF ENTEROCOCCI IN PECORINO DI FARINDOLA CHEESE

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Enterococci are typical opportunistic pathogens and can cause nosocomial infections such as endocarditis, bacteremia and urinary tract infections (1). They have also natural and acquired antibiotic resistance and efficient mechanisms for genetic material transfer (2).

The aim of this paper was to identify and characterize Enterococcus species throughout the ripening process of Pecorino di Farindola cheese and to investigate the presence of vancomycin genes encoding for the phenotypes vanA, vanB and vanC.

Samples of raw milk, curd and Pecorino di Farindola cheese were analyzed at different ripening stages (3). The average values of plate counts were about 10^3 CFU/ml/g in raw milk and curd. The presence of enterococci in Pecorino di Farindola ranged from 10^4 to 10^5 CFU/g at the beginning of ripening and reached 10^7 CFU/g in ripened cheeses. A total of 90 isolates of enterococci were identified by Vitek 2 ®BioMérieux system. To identify four clinically relevant species of enterococci (E. faecalis, E. faecium, E. gallinarum, and E. casseliflavus) and to detect four glycopeptide resistance genotypes (vanA, vanB, vanC1, and vanC2/C3) a multiplex PCR was performed (4). Unidentified species strains were subject to 16S rRNA gene sequencing (5). Among the Enterococcus populations, were identified at different rates: Enterococcus faecalis (72.2%), Enterococcus faecium (13.3%), Enterococcus casseliflavus/gallinarum (11.1%), Enterococcus hirae (3.4%). None of the strains carried the vanA and vanB genes. Nine E. gallinarum strains showed vanC1 intrinsic resistance genotype and just only E. casseliflavus strain showed vanC2/C3 genetic determinant. The vanC intrinsic resistance genotype is associated with several enterococcal species: E. gallinarum (vanC1), E. casseliflavus (vanC2) and E. flavescens (vanC3) (6).

The chromosomal location of vanC genes makes them presumably non-transferable, conferring an intermediate resistance level to vancomycin and sensitivity to teicoplanin (3). In this study the most isolated strains (E. faecalis) showed no vancomycin-resistance genes. The analyzed samples of Pecorino di Farindola cheese had a low pathogenic potential and the health human risk linked to these bacteria seemed to be absent or minimal.

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PRELIMINARY STUDY OF LACTIC ACID BACTERIA IN PECORINO DI FARINDOLA CHEESE

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Pecorino di Farindola is a traditional cheese produced from ewes raw milk and pig rennet without the addition of selected starter cultures (1) and therefore the acidification and the ripening process depend entirely on the autochthonous lactic acid bacteria (LAB) population. The natural microbiota of Pecorino cheeses is mainly composed of mesophilic lactobacilli that largely contribute to the determination of the typical flavor and texture of these traditional cheeses (2, 3).

The aim of this study was to identify and characterize LAB present throughout the ripening process of Pecorino di Farindola cheese.

Samples of raw milk, curd and Pecorino di Farindola cheese were analyzed at different ripening stages (7, 14, 21, 35, 49, 63, 91, 121 and 150 days) (4). The average values of plate counts were about 103 CFU/ml in raw milk and the presence of LAB in Pecorino di Farindola reached about 108 CFU/g at the end of ripening. A total of 90 isolates of LAB were identified using the API 50 CH ® BioMérieux test strips and 16S rRNA gene sequencing (5). Among the *Lactococcus* populations we found *Lactococcus lactis* subsp. *lactis* (41.1%), *Lactococcus lactis* subsp. *cremoris* (27.8%) and *Leuconostoc pseudomesenteroides* (8.9%). Regarding to *Lactobacillus* genera we detected *L. plantarum* (5.5%), *L. paracasei* subsp. *paracasei* (12.2%) and *Lactobacillus brevis* (4.5%).

Lactic acid bacteria of Pecorino di Farindola cheese were isolated and identified to assess the biodiversity within this wild population and to select starter cultures suitable for the local dairy industry in the production of Pecorino cheeses.

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SALMONELLA ENTERICA SUBSP. DIARIZONAE IIIB SEROVAR 61:K:1,5,(7) IN PECORINO CHEESE IN UMBRIA REGION

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S. IIIb 61:k:1,5,(7) is considered as adapted to sheep, however few human cases have been described in the last decades (1). In Italy, eight human cases of S. IIIb 61:k:1,5,(7) infection has been reported during the years 2011-2014 (2,3). This serovar includes many lactose-positive strains (75%-99%), which show atypical growth characteristics on differential media, resulting in underdiagnoses (4). Because of the limited epidemiologic data of this serovar in human beings and the lack of information about the route of transmission, the potential role of S. IIIb 61:k:1,5,(7) as foodborne pathogen cannot be excluded (5).

In Italy and in other countries, S. IIIb 61:k:1,5,(7) has been reported to be found in sheep as agent of rhinitis, orchitis and abortion or sub-clinical infections (5,6). The presence of S. diarizonae in food producing animals represents a possible source of human infection (1, 5).This study aims to investigate the presence of this serovar in pecorino cheese in Umbria and analyze the molecular epidemiology of S. IIIb 61:k:1,5,(7) isolates using Pulsed Field Gel Electrophoresis (PFGE).

From 2010 to 2014, 13 strains of S.IIIb 61:k:1,5,(7) were obtained from cheese samples derived from unpasteurized sheep milk. Samples were collected from eight different dairies in Umbria. The detection of Salmonella spp. was performed using AFNOR BIO 12/16-09/05 ELFA-based method (VIDAS SLM-Biomérieux). Each ELFA-positive broth was confirmed in accordance to UNI EN ISO 6579:2008. Lactose-fermenting and non-fermenting strains were confirmed by biochemical tests and serotyped, according to Kauffmann-White- Le Minor scheme. Antibiotic resistance of isolates was evaluated using Kirby-Bauer test, and molecular typing of isolates was performed by PFGE according to the CDC's PulseNet protocol (7) and analyzed using BioNumerics software.

ELFA-based screening tests for Salmonella spp. have proved useful for detection of atypical strains. All isolates showed high levels of susceptibility to tested antibiotics. Data concerning PFGE profiles are showed in Fig.1. Most of profiles showed a similarity index between 80% and 100%. In some samples collected in different years from the same cheese factory or from the same geographic area, high similarity levels of isolated strains have been found. These preliminary data underline the importance of further investigations about the epidemiology of S. IIIb 61:k:1,5,(7), from the primary production to the food consumption. Even if pathogenicity of S. IIIb 61:k:1,5,(7) is not fully elucidated, proper application of Good Hygiene Practices and HACCP system in whole production chain is particularly important for foods, such as pecorino cheese, which do not undergo a heat treatment.

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REAL-TIME PCR AND LATERAL FLOW TEST, TWO DIFFERENT APPROACHES TO DETECT AND TO QUANTIFY RESPECTIVELY DNA TRANSGENIC MAIZE MON810 AND CRY1AB ENCODED PROTEIN IN CORN GRAIN SAMPLES

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Real-time PCR is considered the gold standard for the control of genetically modified organism (GMO) and unauthorized GMO (UGM) for a wide range of matrices, because DNA is a molecule unlikely degradable and it is present in all the cells of an organism. At the same time, some techniques allow a rapid analysis and evaluation of GM proteins content and their presence also in field. The aim of this work was to compare qualitative and quantitative results, using two different approaches to analyze corn grain samples. The one based on DNA detection of MON810 in fast real-time PCR, the other one, Lateral Flow Test (LFT), based on detection of Cry1Ab encoded protein derived from Bacillus thuringensis. The LFT is a rapid immunoassay, easy to interpret and to use also by personnel not highly specialized. In this study 18 corn grain samples (ID 1-18) were accurately grinded and mixed. For DNA analysis, a test portion corresponds to a weight of 2 g Âś 0.2 g was used, while for protein analysis 50 g were necessary. DNA was extracted in duplicate using CTAB protocol (1), purified with QIAamp DNA Mini kit and estimated photometrically. All the samples were verified in fast real-time PCR, using Alcohol Dehydrogenase 1 (ADH1) endogenous gene for maize. MON810 event was identified in 10 samples out of 18 (ID 1-8, 10, 18), previously resulted positive to 35S promoter (P35S) of Cauliflower Mosaic Virus (CaMV). A relative quantification (qPCR) between MON810 and ADH1 was carried out in order to quantify GMO percentage. Quickscan instrument (2), based on LFT, detected in the same samples the content of Cry1Ab transgenic protein. Cry1Ab protein reacted with the specific antibody in the 10 samples (ID 1-8, 10, 18). Two red strips were observed, one corresponding to control line and the other one corresponding to test line: greater is GMO protein content, greater is the colour intensity. Qualitative results were comparable, differently from those quantitative. Where MON810 was quantified in qPCR, resulting superior to 5%, also encoded protein was superior to 5%. Where MON810 maize was quantified in qPCR, resulting lower to limit of quantification (LOQ, estimated 20 haploid genome equivalents for MON810), with values corresponding to 0.30%, 0.15%, 0.05%, 0.04%, Quickscan instrument measured a higher concentration of encoded protein, respectively with values of 1%, 1.7%, 1.7%, 2.9%. This is because DNA is constant in terms of quantity in all the plant genome, while protein changes expression level both during plant growth and in the different tissues. Furthermore, it is evident from molecular biology that transcription of DNA to RNA could be enhanced both by strong promoter, like P35S in this case, and by enhancer. In conclusion, for raw materials it is possible to adopt also the LFT in order to identify the protein content or for a rapid screening in routine analysis, always to be confirmed by real-time PCR, the only method recognized by legislation in force (3, 4, 5).

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SPIROLIDES IN MUSSELS FROM MARCHE REGION

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Spirolides (SPXs) are marine biotoxins belonging to the Cyclic Imines group, considered âĂIJemergingâĂİ toxins as ever more often encountered in different geographical areas of the world (1). SPXs are produced by the dinoflagellate Alexandrium ostenfeldii and can accumulate in filter-feeding seafood. These compounds are able to bind and block acetylcholine receptors in the central and peripheral nervous system, causing neurological symptoms and death in vivo animal models, while no cases of human intoxications have been reported, therefore no regulatory limits are established to date (1, 2). The aim of this work was to evaluate the SPXs contamination in mussels farmed along the coast of Marche Region and its seasonal and geographical trend during 2014. For this purpose a LC-MS/MS method for the detection of SPXs, based on the EU Official protocol for the determination of Marine Lipophilic Toxins (3), was implemented. 80 mussel samples, collected as part of the bivalve molluscs monitoring plan from 6 breeding areas along the Marche coast, were analysed. Mussel tissue homogenate was extracted with methanol and analysed in multiple reaction monitoring (MRM) mode by means of a 3200 Q Trap triple quadrupole LC-MS/MS system. Two transitions were monitored for the analogues 13-desMeSPX C, 13,19-didesMe SPX C, 27-OH 13,19-didesMe SPX C and 20-Me SPX G. Dilutions of the certified 13-desMeSPXC standard solution (CRM-SPX1, NRC, CANADA) were used as calibrants for the quantification of all analogues. 13-desMeSPX C and 13,19-didesMeSPX C were detected in trace amounts in analysed samples, with decreasing trend from the beginning to the end of the year, while 27-OH 13,19-didesMe SPX C and 20-Me SPX G were never detected. 13,19-didesMeSPX C appeared to be the predominant analogue during the winter, but rapidly disappeared within the first half of the year. Amounts of 13-desMeSPX C were detected near to the level of quantification for a longer period of time, probably due to a lower detoxification rate in mussels. The geographical trend of SPXs contamination showed the highest levels in the Southern areas, with maximum concentrations of about 20 μg sum of SPXs/kg shellfish meat, this reveals the predominant role of the local sources of eutrophication, than the Adriatic most important input of nutrients represented by Po River. However the low levels of SPXs found in mussels, compared to the guidance level of 400 μg sum of SPXs/kg shellfish meat, proposed by the EU Community Reference Laboratory for marine biotoxins (1), don't yet represent a risk for the public health. The implemented method was suitable for the detection of both SPXs and regulated toxins, so its application can be useful to fully describe the North-Central Adriatic toxicological scenario, continuously changing in terms of amount and kind of toxins in mussels. The identification of the specific biogenic origin of SPXs would be helpful to better assess the phenomenon.

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PARASITIC LESIONS OF FOWL PLUMAGE

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On the bird plumage, ectoparasites may produce slight lesions, with small alterations, invisible to the naked eye, or more serious and visible alterations, with consequent loss of the ability of thermal and mechanical insulation of the feathers. The severity of the lesions depends on the parasite load, but also on the capability of some parasites to cause itching and then to stimulate the bird to peck itself, completing the detrimental action on the feathers, that so end to crack at various points [1].

Aim of this work was to get a comprehensive overview of the alterations of plumage of parasitic origin and to classify them depending on the location on the animal and on the feathers.

In the Urania Centre of Research (Perugia, Italy), material stored in the last 30 years, resulting from the scientific collaboration with the Parasitology Section-Dept. of Veterinary Medicine of Perugia and Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, was observed by stereomicroscope. The arthropods present were isolated, mounted on glass slides with the solution of Berlese and identificated by optical microscope.

The anatomical areas most frequently affected were: the wings and axillary areas, tail, chest, neck, head and periorbital areas. The lesions of the feathers may be distincted, in relation to their distribution, as:

1) Lesions by Siringophilus inside the calamus [2], which assume a typical orange colour for the presence of these mites.

2) Lesions by Knemidokoptes outside the calamus with intracorneal galleries.

3) Lesions by Knemidokoptes on the calamus in the outer follicular area, manifested by a crusty collar in the basal area of rachis and in the apical area of the quill.

4) Punctiform lesions between the barbules caused by feather mites, such as Proctophyllodes, which are located in long lines along the barbs.

5) Linear lesions by the rescission of individual barbs due to the action of the jaws of Mallophaga. They are the most frequent and the most obvious ones.

6) Lesions of feathers for shredding of barbs by Mallophaga with adhesion of the eggs. As a result the feathers stick together to form large blocks, sometimes in the underaxillary area, but also in the head and periorbital areas.

7) Lesions between the barbs due to grafting of eggs laid with silky threads; typical lesions are those produced by Neocheyletiella (=Ornithocheyla) megaphallos in small birds.

8) Linear lesions by the rescission of bundles of barbs due to the action of the jaws of Dermestidae beetles (Dermestes), which can also affect the rachis of the feathers, especially in the tail and the wings, causing their breakage. These insects attack the animals during the night, causing, for example, the pathology known as "broken tail" in pheasants in breeding.

9) Irregular lesions on the entire feather that ends up breaking spontaneously, caused by the action of Dermestidae beetles (Anthrenus, Attagenus, Trogoderma) on the feathers, that touch the ground during the night.

Ectoparasites of birds are able to cause lesions to the plumage that not only affect the health of animals with loss of waterproofing of the feathers, of fundamental importance for migratory birds and swimmers, but also at the economic level, determining the general decline of the animal and its depreciation in the market.

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CONTROL OF DERMANYSSUS GALLINAE (ACARI: DERMANYSSIDAE): EXPERIMENTAL TRIALS WITH INERT POWDERS

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Introduction Dermanyssus gallinae is the most common and feared pest in poultry. At the present, the main method of control of the infestation is the use of chemical acaricides [1]. The onset of resistance phenomena and their toxicity have required further studies to find alternative methods for D. gallinae control; encouraging results were obtained through the use of essential oils [2], biological control [3] or physical treatments [4].

The aim of this study was to investigate the effectiveness and persistence against D. gallinae of two products, based on inert powders, already marketed and used in poultry farms as sanitizers: Bi-Protec ® (15% silicon-based and 85% sodium bicarbonate) and Diatom® (based on diatomaceous fossil powder). Additional objective was to test the association of their active ingredients to verify the possibility of obtaining a better, faster and more effective acaricide.

Field trial: it was conducted in a layer hen breeding in Forlì (Italy) infested with D. gallinae, in May-October 2013. Bi-Protec ® (shed A) and Diatom ® (shed B) were applied as a single dose, according to the manufacturer instructions, by a high pressure automatic pump. The monitoring was carried out every 15 days for 4 months, removing 10 samples of substrate (4 grams) for each shed. At the âĂIJIstituto Zooprofilattico della Lombardia e dell'Emilia Romagna the samples were kept in an incubator (T: 30°C, RH: 70%) for 3 days to allow the hatching of eggs, and then observed by stereomicroscope to count the mites.

The first test was carried out at the Urania Centre of Research (Perugia, Italy) using Petri dishes containing 20 adults of D. gallinae; for each test (Bi-Protec ® diluted and in powder, Diatom ® diluted and in powder, test control) three replications were set up. Plates were observed daily by stereomicroscope until the death of all individuals, counting alive mites. In December 2013 a second test was carried out at the Lab. of Entomology Dept. of Veterinary Medicine (Perugia, Italy); the active ingredients in powder form, amorphous precipitated silica and Diatom powder, were tested together in proportions 3:7.

In the field trial, a considerable reduction of the infestation occurred with both products, even if the treatment with Bi-Protec ® was more lasting (> 4 months) compared to treatment with Diatom® (3 months). In addition, farmers reported a reduction in visual mites, the absence of blood stains on the eggs and a reduction of itching in the staff. In the first laboratory test Bi-Protec® showed a better result. Both products diluted showed less effective, probably because the powder diluted in water is compacted when it dries and mites easily walk over it without getting dirty; in the field trial this does not happen, because the movement of the birds disrupts the compacted powder. The last test, with both the active ingredients of the two products, gave good result, because in 6 hours' time there was 100% mortality of mites.

The data emerging from our tests are very significant, as it give us hope in the possibility of formulating new effective antiparasitic products with less environmental impact and less toxic to animals and operators.

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PREVALENCE OF CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI IN RAW RETAIL CHICKEN MEAT PRODUCTS

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In the European Union, campylobacteriosis is the most commonly reported zoonotic disease in humans (1) and the consumption of undercooked poultry meat is considered a major risk factor for sporadic infections in humans (2). A recently published scientific opinion (3) estimates that the handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases. The prevalence of Campylobacter spp. in broiler meat at retail level varies among Countries, but an average contamination of 50% has been reported (4) indicating a high probability of exposure to Campylobacter spp.

The aim of the present study was to determine the occurrence of C. jejuni and C. coli on raw retail chicken meat products (breast and hamburger). Materials and methods - 60 chicken hamburger and 60 chicken breast samples were collected and screened for the presence of Campylobacter jejuni and Campylobacter coli. The analyses were conducted as described in the ISO 10272-1:2006 method. PCR protocols were used to identify Campylobacter spp. (5, 6) and to further differentiate between C. jejuni (589 bp mapA gene fragment) and C. coli (462 ceuE gene fragment bp) (7, 8).

The number of samples positive to thermotolerant Campylobacter in chicken meat products was 41 and 25 out of 60 in hamburger and breast meat respectively. The prevalence of positive samples was higher in hamburger than in breast meat (P=0.006). On the other hand there was no significant difference in the isolation frequency between C. jejuni and C. coli in the chicken products considered (P=0.248 for hamburgers and P=0.369 for breast). Furthermore, co-infection with both C. jejuni and C. coli was detected in 9 hamburgers and in 7 breasts. The prevalence found in our study is in agreement with those reported by other authors regarding the breast (9), while there is no data in literature concerning Campylobacter prevalence in chicken meat preparations such as hamburgers. However, some authors have noted a large variety of Campylobacter PFGE types in these products due to the presence of meat from different flocks (10). This could explain the different prevalence found between hamburger and breast meat samples in our study. Furthermore it is possible that blending and further manipulation of the minced chicken meat could increase the levels of thermotolerant Campylobacter in hamburger.

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EFFICACY OF ESSENTIAL OILS ON RABBIT MANGE

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Some of the currently known active principles against rabbit mange are certainly effective, but they have a more or less pronounced toxicity to humans and animals and, recently, some of them showed phenomena of drug resistance.

The aim of the study is to test the efficacy of some essential oils against the rabbit mange, caused by Psoroptes equi var. cunicoli (Acarina: Psoroptidae), to formulate some compounds without toxicity for topical and environmental treatments.

A) Laboratory tests with Tyrophagus putrescentiae. This mite, provided by the Urania Center of Research (Perugia, Italy), was used as a model for his resistance to biocides. N°20 essential oils were tested in the Sec. of Parasitology, Dept. of Veterinary Medicine of Perugia (Italy). For the test of toxicity, the mites were introduced on Petri Dish and sprayed with essential oils diluted with grapeseed oil (1:1, 1:2, 1:3) through a manual nebulizer from the distance of about 20 cm. For detecting the state of vitality, the mites were kept under observation, with the stereomicroscope, for a day (every 30' for 3 h and, subsequently, after 6 h and 24 h). Each test was repeated three times for each dilution, using N°10 mites for replication; for the control only grapeseed oil was used. The results of this phase were analyzed in order to detect the two essential oils more effective to use in subsequent trials.

B) Field tests: these tests were carried out in N°3 rabbit farms in South-Central Italy, on animals suffering from auricolar mange, using essential oils of Mentha piperita and Thymus vulgaris. Three compounds were tested: two different liquid formulations with essential oils in liquid and microencapsulated form and with grapeseed oil as excipient (one to be used topically on animals and one for environmental treatment by spraying) and one formulation in solid form for topical use, whose active principle was present only in the microencapsulated form and with talcum powder as excipient.

-Liquid form for topical use: rabbits were treated by introduction of 2 ml of solution in the ear with a disposable pipette. This formulation had an efficacy of 100%, as in the first 24 h post-treatment all adult mites, nymphs and larvae died; devitalization of eggs occurred within 48 h post-treatment. The feedback parasitological examination lasted 8-12 days, while a progressive clinical recovery was observed.

-Liquid form for spraying: the samples, collected by scotch-test on the backs of the animals, revealed a reduction of about 60% of mites; this shows that the periodic use of environmental treatment by nebulization may help to decrease the population of mites in the farm, reducing the risk of new infestations.

-Solid form for topical use: the results were good, but there was not a complete cure. The lesions almost entirely disappeared, but not all mites died deeply. This led to a recurrence of the mange after about 20 days.

Our tests confirm the efficacy of some natural products made from essential oils for the treatment of rabbit mange by P. equi var. cunicoli [1, 2]. The use of these compounds in livestock farming, as in the rabbit farms, would certainly lead to significant benefits, especially because, due to the non-toxicity, they can be used for environmental sanitization carried out in the presence of animals.

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USE OF OVINE AS ECOSYSTEM SERVICE: SAFEGUARDING BIODIVERSITY TO IMPLEMENT THE FARM'S INCOME ALSO THREATENED BY CLIMATE CHANGE

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Given the importance of rangeland resources for the provision of forage for livestock grazing, as well as for biodiversity conservation, the definition of management strategies for semi-extensive farming systems has both economic and environmental consequences. Maintenance of extensive farming is, in turn, the main tool to preserve the valuable biodiversity of these ecosystems. In fact, grazing has major impacts on species composition and forage feed value, because livestock foraging strategy, disturbance intensity and grazing history strongly influence the competitive relationships among species. Thus, grazing management has major impacts on ecosystem services provided by semi-natural grasslands, such as aesthetic value (mainly determining the level of touristic attraction of pastoral landscapes), soil conservation, carbon storage, cultural heritage, etc. However, from the farmers' point of view, low-intensity farming often means intensive human labor and small yield. Therefore, current socio-economic changes such as abandonment of farms and reduction in the number of people working in mountain agriculture are causing severe land use changes, which led to a dramatic decline of biodiversity in grassland area. The farmer's income is a key question in grassland biodiversity conservation. The sustainability of extensive farming depends on the conservation of forage resources, but also on the ability to promote animal welfare, which, in turn, is influenced by the amount and quality of food, that produces morphological and functional modifications at different levels of the digestive apparatus. In addition, in Mediterranean areas, trends in climate change mainly cause greater aridity during summer and likely lead to the worsening of extensive farming sustainability. As a consequence of this, the multi-tasking use of grazing activities is a key tool in supporting the extensive farming. Firstly, it has to be considered that the conservation of these open ecosystems is a key element within the European Agricultural Policies, particularly since the 92/43/EEC Directive stated that the conservation of grasslands is a high priority for European farmers, that are largely helped in this direction by financial supports aimed to aware management actions. To this regards, there is a good example represented by the agro-environmental agreement of Marche Region, devoted to a bottom-up setup of strategies for grassland conservation. Farmers that operate following these guidelines receive a financial compensation in addition to those normally made available by the European Agricultural Policies. Moreover, this new task of mountain farmers could be inserted in a wide range of ecosystem services (carbon storage, conservation of aesthetic values, fire prevention, integration of touristic attractors, etc) that might be recognized for the Payment for Ecosystem Services (PES) that is one of the key actions inside the UE policies for nature conservation.

Part VI

XV CONVEGNO SO.FI.VET

MODULATION OF IMMEDIATE EARLY GENES IN MAMMARY EPITHELIAL CELLS

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In the mammary gland, during the different phases of the reproductive cycle, moments of tissue growth with increased cellular proliferation are followed by moments of tissue involution, with augmented cellular apoptosis. These phases are regulated both by endocrine hormones (estrogens, progesterone, etc) and by local acting growth factors (EGFs, IGFs, etc). The downstream cellular signaling of these factors induces the expression of immediate early genes (IEGs) and late genes that monitor different steps within the cell cycle. An altered cellular response to these signals may cause deregulated proliferation within this tissue, with increased cancer susceptibility.

The aim of our work is to understand, in mammary epithelial cells, the expression kinetics of three important IEGs (EGR1, FOS, JUN) in response to different stimuli. We are also focusing our research on which intracellular signaling pathway, involved in mammary growth or differentiation, is able to modulate the expression of EGR1, FOS and JUN.

Mammary epithelial cells obtained from different species of domestic animals are grown in a starving medium in order to obtain a synchronization in the G0/G1 phase of the cell cycle. Subsequently cells are stimulated with various stimuli (growth medium, estrogen, progesterone, EGF, IGFs, etc.) at different time points and IEGs expression is analyzed by real-time PCR and/or western-blot. Treatment with UO126 (ERK 1/2 inhibitor) and/or Wortmannin (PI3K-akt inhibitor) in combination with different stimuli are used to investigate the relative importance of these two important signaling pathways on IEGs RNA and protein expression.

We have observed that expression of EGR1 and FOS is strongly upregulated following stimulation with growth medium, EGF and phorbol 12-myristate 13-acetate. JUN is also upregulated, but to a lower levels. Following addition of growth medium we have observed an increase in EGR1 protein expression associated with ERK1/2 and PI3K-akt signaling pathway activation. When ERK1/2 inhibitor is added together to stimuli that upregulate IEGs we observe that UO126 abolishes EGR1 and FOS upregulation leaving JUN expression mostly unaltered. Western blot analysis suggests a similar trend in EGR1 protein expression. This result indicates that IEGs upregulation following phorbol 12-myristate 13-acetate (an activator of protein kinase C which is downstream G-protein coupled receptors) stimulus is driven exclusively by ERK1/2 phosphorilation. PI3K-Akt pathway inhibition with Wortmannin does not influence IEGs RNA expression and EGR1 protein levels. We conclude that the ERK1/2, but not the PI3K-akt pathway, is the most important signaling event that drives the modulation of both EGR1 and FOS in mammary epithelial cells of domestic animals. On the other hand, JUN Expression is probably controlled through different signaling pathways.

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TEMPORAL EXPRESSION OF MIRNAS AND TRANSCRIPTION FACTORS INVOLVED IN BOVINE SKELETAL MUSCLE DIFFERENTIATION

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Satellite cells are adult stem cells located between the basal lamina and sarcolemma of muscle fibers. In physiological conditions, satellite cells are quiescent but maintain a strong proliferation and differentiation attitude, responsible for muscle preservation and growth[1]. MiRNAs play essential roles during animal development and in stem cell self-renewal and differentiation regulation. MiR1, miR133 and miR206 are closely muscle specifically related thus defined as myomiRNAs[2]. MyomiRNAs are integrated into myogenic regulatory networks: their expression is under the transcriptional and post-transcriptional control of myogenic factors, and in turn they have widespread control of muscle gene expression[3].

Information available from large farm animals about satellite cells, their regulation and behavior during differentiation is quite limited. Here, we show bovine satellite cells (BoSC) in differentiation conditions and the expression pattern of genes and miRNAs involved in this process.

Muscle samples were collected from Holstein male animals (18-23 months). Muscles samples were processed to collect satellite cells. Total RNA from cultured cells was extracted and cDNA was synthesized. To determine the relative amount of specific transcription factors, real-time quantitative PCR (qPCR) was performed. GAPDH was used as reference gene. To quantify expression of mature miR1, miR133a and miR206, RNA were reverse transcribed by TaqmanTM miRNAs reverse transcription kit (Applied Biosystems) and subjected to qPCR. MiR16 was used to normalize the results[4]. Immunoflorescence was performed on cultures to analyze the expression of Ki67, MyoG and MYH in proliferating and differentiating conditions.

All satellite cell preparations have demonstrated myotube differentiation. To characterize the dynamics of transcription factors expression in BoSC, we performed qPCR in growth medium (GM) and in differentiation medium (DM) for 4 days. In GM condition, BoSC express the satellite cell lineage markers as well as transcripts for the myogenic regulatory factors. Pax7 mRNA was up-regulated at 1d DM ($p<0.05$) and decreased after d2. MyoD mRNA levels increase at 1d DM ($p<0.05$), showing a gradual activation of myogenic gene program. During 4 days of culture in DM, several tested genes such as MRF4, MyoG, Mef2C, Des, MyH and TMEM8C have early increased their mRNA expression at d1 DM ($p<0.05$) and these levels remained at high levels along the culture. Meanwhile the genes expression involved in differentiation process increased, also miR1, miR133a and miR206 were strongly up-regulated at d1 DM ($p<0.05$). Spearman's test showed a highly significant positive correlation between myomiRNAs expression and Mef2C, MRF4, TMEM8c, Des and MyH genes ($p<0.01$). Determination of the proportion of differentiating satellite cells, as assessed by MYH and MyoG staining, was performed on BoSC culture under the two different medium conditions. All muscle samples differentiated into MHY and MyoG positive multinucleated myotubes within 2 days in DM. Understanding the processes involved in the regulation of muscle differentiation is necessary to develop strategies for improving the efficiency of meat production.

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EVALUATION OF THE EFFECTS OF DIET LEPIDIUM MEYENII (MACA) SUPPLEMENTATION ON STALLION SEMEN LIPOXIDATION

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The stallion for reproductive aims is chosen on the basis of its appearance, performance, pedigree and lineage. The ideal stallion is dominant in the transmission of its genetic heritage. Sometimes, for ageing or overexploitation, the reproductive performances of good stallions get worse. It often occurs the necessity, in these cases, to improve the fertilizing capacity aimed to the conservation of semen for assisted reproductive technologies (ART) with safe and effective methods. The sperm cells, especially the damaged ones, produce reactive oxygen species (ROS). ROS include radicals like hydroxyl ions, superoxide, peroxyls and others (1). A certain, still low, concentration of ROS is necessary for the sperm function like capacitation, hyperactivation, acrosome integrity and sperm-oocyte fusion (2), but ROS become detrimental at excessive amounts or if it occurs a low antioxidant status or both. In the case of metabolic oxidative stress, however, provoked by imbalance between ROS production and antioxidant barrier, impaired sperm function may occur due to the rising of a series of chain reactions, in the course of which the radicalic sites can be transferred to the biomacromolecules, e.g. lipids, that compose the cell structures. The sperm is very sensitive to lipid peroxidation because its plasma membrane is rich in polyunsaturated fatty acids (PUFA), especially in the long-chain PUFA docosahexaenoic acid and docosapentaenoic acid (3). Lepidium meyenii (Maca) is a traditional Andean crop that grows best at high altitudes in the Peruvian Highlands. It owns aphrodisiac and fertility-enhancing properties. In the present study, we evaluated the effects of Maca diet supplementation on lipid peroxidation of horse ejaculates. In the experimental group of 2 hypofertiles (H) and 2 fertiles (F) stallions, the hypofertile (HM) one and the fertile (FM) one were administered Maca for 60 days, 20 g/day in the food. The remaining two stallions were the control ones (H and F). Ejaculate was withdrawn from each horse at day 0 and every 15 days for four times and processed for cooling at 5°C and stored up to 72h. For each sample the degree of semen lipoxidation (LP sperm) was assessed. The results show a reduction of semen lipoxidation in both HM and FM, respect to H and F, and the same effect was observed during the storage in treated stallions too. In particular, we found a more steady decrease of lipoxidation in HM respect to H. Further, Maca showed benefit effects in sexual behavior, in fact HM was able to ejaculate during the first jump of the last withdrawal. In conclusion, the data obtained suggest a beneficial effect of Maca on reproductive function and performances.

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MICROFILTERED SEMINAL PLASMA PRESERVES THE MORPHOFUNCTIONAL CHARACTERISTICS OF PORCINE SPERMATOZOA IN THE ABSENCE OF ANTIBIOTICS FOR A LONG TIME IN LIQUID STORAGE

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Boar ejaculates usually contain 104 to 105 bacteria per ml (Morrell & Wallgren, 2011), some environmental contaminants and only few potential pathogens (Althouse & Rossow, 2011). The risk for disease transmission following Artificial Insemination (AI) is minimal, but the impact of bacteriospermia can be enormous on spermatozoa quality and function (Althouse et al., 2000). To reduce the growth of bacteria in swine AI doses, antibiotics are added under direction of the EU (Council Directive 90/429/EEC). The increasing phenomenon of antibiotic resistance requires urgent action (Leung et al., 2011). The European Commission requested scientific advice from the European Medicines Agency in April 2013 on the impact of the use of antibiotics in animals on public and veterinary health, and measures to manage the possible risk to humans. Therefore the need of alternative methods to the use of antibiotics in porcine semen extenders is mandatory.

Evaluation of the quality of spermatozoa in AI doses with antibiotic-deprived semen extender obtained by seminal plasma microfiltration.

We utilized 3 ejaculates from 3 LW adult boars of proven fertility. Sperm rich fraction spermatozoa were separated from seminal plasma (SP), which was splitted in two part, and then one μfiltered with a 0.22 μm syringe filter (μfiltered SP). Four AI sample doses were prepared using Swine Fertilization Medium (SFM) extender (Fantinati et al., 2009) adding or not 6gr/L of Ampicilly (Ab+/Ab-): A (SP/SFM Ab+), B (SP/ SFM Ab-), C (μfiltered SP/SFM Ab+) and D (μfiltered SP/SFM Ab-). Objective Motility, Viability, Mitochondrial Membrane Potential, Acrosome Reaction, pH and Total Antioxidant Capacity were evaluated on fresh ejaculate and on doses the day of collection, after 4 and 7 days of liquid bath (16° C) storage.

This study demonstrates that AI doses set with antibiotic-deprived semen extender and μfiltered seminal plasma doesn't affect negatively spermatozoa condition, but seems to improve it.

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FEATHER PICKING IN COMPANION PARROTS: SENSIBLE SPECIES, RISK FACTORS AND ETHOLOGICAL EVIDENCE

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Feather Picking (FP) is a behavioral disorder that is frequently observed in captive parrots. FP includes plucking, chewing, fraying and/or biting, resulting in loss of or damage to the feather (van Zeeland et al., 2013). The prevalence FP reported by McDonald Kinkaid et al. (2013) in a sample of 538 parrots was 15.8%. Despite several studies, the mechanisms of this pathological condition still remain to be unequivocally elucidated.

The goals of this study were to estimate the prevalence in Italian pet parrots and evaluate the risk factors and possible ethological correlation.

A web survey was created through the Google Drive application. It was addressed to owners of all species of companion parrots and was distributed through on line parrots association websites, social networks and by mail. The survey was available for compilation from June to October 2014. The 31 questions were divided in two sections: one addressed to all parrots' owners; the second limited to owners of FP parrots. The diagnosis of FP was confirmed by a veterinarian who excluded other possible pathological conditions.

A total of 335 survey was obtained, of which 292 (82.9%) were useful for the statistical analysis. Forty-one different species of parrots were indicated. The most popular species kept as pets were Psittacus spp. (24.3%), Agapornis spp. (19.5%), Nymphicus hollandicus (17.8%) and Amazona spp. (8.9%). Our study showed a FP prevalence rate of 17.6%. The highest prevalence were reported in Psittacus spp. (31.4%) and Agapornis spp. (25%). Although several authors refer Psittacus spp. and Cacatua spp. as the main species affected by FP (Chitty, 2003), in our sample Cacatua spp. was under-represented (1.7% of the whole population). Our data show that also Agapornis spp. is affected by FP; in the last years, in Italy, this genus was increasingly adopted as pet. The percentage of FP in parrots that live with other parrots was significantly higher (62.7%) than in animals living alone (37.2%; $p < 0.05$). Among hand-reared parrots (88.2% of our sample) 52.1% were fed in neonatal age by the breeder and weaned by the final owner; 47.8% was fed in neonatal age and weaned by the farmer and sold when weaning was completed. FP prevalence was significantly higher in parrots fed in neonatal age and weaned by the breeder and sold when weaning was completed (81.8%), than those fed in neonatal age by the breeder and weaned by the final owner (18.2%) ($p < 0.001$). This finding suggests that different hand-rearing techniques could influence the prevalence of FP. Fifty percent of parrots affected by FP showed behavioral stereotypes (i.e. altered sexual behavior, aggressiveness against humans, etc.). As suggested by several authors, FP may be considered a multi-factorial behavioral pathology in which factors of different origin (i.e. hand-rearing techniques, sexual frustration) may cause behavioral disorders associated to self-injuries.

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EVALUATION OF THE IMPACT OF NOVEL ANCIENT FLOUR BAKERY PRODUCTS ON THE INTESTINAL MICROBIOTA, OXIDATIVE AND INFLAMMATORY STATUS AND ON THE GLYCEMIC INDEX

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Epidemiological studies find that whole-grain intake is protective against cancer, cardiovascular disease, diabetes and obesity (Slavin, 2004). In comparison to modern wheat, einkorn (Triticum monococcum L. subsp. Monococcum) whole meal flour "Ancient Flourâ" have a higher concentration of some bioactive compounds especially proteins and antioxidants (Brandolini et al., 2008). Although whole-grain bakery products and cereals are valuable sources of dietary fiber, vitamins and trace elements, the presence of phytate, could decreases mineral bioavailability due to its chelating properties, these problem could be reduce by sourdough fermentation (Lactic Acid Bacteria) (Frontela et al., 2009).

Measurement in animal model of the effect of sourdough fermentation on glycemic index, anti-inflammatory effects and microbiota ant-inflammatory characteristic, in order to better understand the potential benefit of innovative breads obtained with einkorn whole meal flour. The study was performed as part of the European Project Bake4Fun (http://www.bake4fun.eu/)

Experiment 1: 36 hybrid pigs of 30Kg, 18 female and 18 males were divided in six groups and fed with six different experimental diet for 30 days. The diets were: : A) STDF-B (Standard flour - bread); B) AF-B (Ancient flour bread); C) STD-B+STDF sourdough fermentation; D) AF-B+STDF sourdough fermentation; E) AF-B+AF sourdough fermentation; F) STD-B+AF sourdough fermentation. The animals were weight at the beginning of the trial and every week to the end of it. Blood samples (EDTA) were collected at the beginning and at the end of the trial. Experiment 2: 6 hybrid pigs of 50Kg were cannulated in the jugular vein under general anesthesia and stabled in single boxes for a week to recover before the beginning of the trial. In the morning, after 18 hours of fasting, pigs were feed with 50gr of one of the six experimental diet (Experiment 1 diets). Each diet was repeated three times. Blood samples were collected from jugular vein 15 minute before the administration of the diet and every 15 minute for 2 hours to measure glucose and insulin level by standard clinical chemistry analysis.

None of the pigs feed with the experimental diet had any macroscopic and hematologic inflammatory status. The sourdough fermentation seems to play a role on the glycemic index of the bread, indeed the breads obtained with AF sourdough fermentation have a lower and delayed peak blood glucose. Aknowledgements Granted by the European Project Bake4Fun (grant agreement n. 606476)

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A "POTENTIAL" PHYSIOLOGICAL ROLE OF BETA-AMYLOID IN THE MODULATION OF HIPPOCAMPAL SYNAPTIC PLASTICITY

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Introduction Beta-amyloid (Aβ) is normally produced in the brain throughout life and it has been estimated to be in the picomolar range. Its role is not well defined but some studies suggest a possible physiological role of Aβ in learning and memory. We demonstrated that picomolar concentrations of Aβ increase long-term potentiation (LTP) and enhance hippocampal-dependent memory (1). At nanomolar and higher concentrations Aβ leads to inhibition of LTP, loss of spines and eventually neuronal death. We have shown that the toxic effects of Aβ are mediated by activation of caspase-2, despite concurrent activation of caspase-3 that is neither necessary nor sufficient for toxicity (2). Here we investigate the involvement of caspase-3 and its regulators in the enhancement of synaptic plasticity induced by picomolar Aβ. Caspase-3 has been identified as a relevant enzyme in physiological processes that do not result in cell death and are localized in a specific neuronal compartment: the synapse. Methods Primary hippocampal neurons were cultured from E18 rat embryos. β-amyloid were prepared as described in (3). Caspase-3 activity assay was used to measure caspase-3 activity using the caspase affinity ligand bVAD-fmk (3). siRNA were conjugated to Penetratin 1(4). Mouse hippocampal Aβ infusion. Aβ and vehicle were infused into dentate gyrus (3).Results Our result found that treatment of primary hippocampal neurons with pM Aβ leads to a rapid increase in spine density that is accompanied by a rapid increase in caspase-3 activity in purified synaptosomes. Effects are seen within 30 minutes of treatment of the cultures; injection of pM Aβ in vivo also causes an increase in spines. As this effect is rapid, we posited that the synapses contain cleaved (activated) caspase-3 that is inhibited by endogenous inhibitor of apoptosis proteins, IAPs. We found that the synaptosomal fraction contains cIAP1 and XIAP, and co-immunoprecipitation shows that there are complexes of cIAP1-cleaved caspase-3 and XIAP-cleaved caspase-3. Treatment of cultures with pM Aβ induces a decrease in the cIAP1-cleaved caspase-3 interaction, but no change in the XIAP-cleaved caspase-3 interaction, suggesting that the increase in caspase-3 activity is modulated by cIAP1. Actin, the major component of spines, is a substrate of caspase-3, and we find that pM Aβ leads to an increase in cleaved actin. siRNA knockdown of caspase-3 prevents the effects of pM Aβ and siRNA knockdown of cIAP1 potentiates the effects of pM Aβ on spine density. Surprisingly we found that siRNA knockdown of XIAP prevented the effects of pM Aβ. A recent study has shown that XIAP can bind to RhoGDI (Rho GDP dissociation inhibitor), leading to an increase in f-actin. Co-IP shows that pM Aβ increases the XIAP-RhoGDI interaction. Our data show that spine dynamics can be regulated by pM Aβ through both induction of caspase-3 activity and sequestration of RhoGDI by XIAP.

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HEMATOLOGIC AND BIOCHEMICAL REFERENCE INTERVALS FOR NEWBORN AND YOUNG EUROPEAN BREED PIGS

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The strong similarities between the swine and the human in both physiological and genomic patterns [1,2], and the great correlation in size and anatomy, make the pig an extremely accurate preclinical model for translational medicine [3]. In its early stages of life, it represents an accurate analogue to newborn child for the study of congenital and genetic diseases [4]. It is very well known that, unless given iron supplements, piglets develop iron-deficiency anaemia (IDA) few days after birth [5]. IDA occurs regardless of the breed and the management system, and is the results of the interaction of several factors including low levels of stores, increased requirements, poor exogenous supply and immaturity of absorption mechanisms [6,7]. It is therefore necessary to administer piglets with exogenous iron to prevent dangerous deficiency [8]. This procedure may interfere with several parameters of complete blood count (CBC) and clinical biochemistry [9], and it is extremely important to acknowledge this when interpreting blood analyses results in a research context.

The aim of the present study is to determinate specific age-related reference intervals for hematologic and biochemical parameters in specific genetic backgrounds we use for research purposes (pure Large White and commercial hybrids LW x Duroc or LW x Landrace), including reticulocytes and platelets indices.

We collected blood samples from 5 days old (P5), 30 days old (P30) and 90 days old (P90) pigs enrolled as negative controls in experimental protocols run at our institution. In order to rule out any possible variation in genetic line and management, both pregnant sows and pigs were born and raised in the same Italian farm. Samplings for CBC and clinical biochemistry were performed under general anesthesia and analyzed by our veterinary clinical pathology service. Reference ranges were assessed using a scientific statistics program; comparisons between values at the three ages were made by the Kruskal-Wallis and Mann-Whitney tests for non-parametric values.

Our findings are valuable reference data at precise ages and could be used in the future as historical control improving the Reduction in animal experiments.

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YOU ARE WHAT YOU ATE: LONG TERM EFFECTS OF PHYTOESTROGENS EXPOSURE IN EARLY POSTNATAL LIFE

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Phytoestrogens (e.g. genistein) are nutraceuticals, highly present in edible plants such as soy, widely used in animal feed and nutritional supplements. They may interfere with endocrine system with a beneficial or detrimental effect according to the administration paradigm, sex and age of exposure. In particular they may affect specific neural circuits, especially sensible to estrogens during pre- and/or postnatal critical periods of their development [1], leading to irreversible behavioral and morphological alterations in adults even at low doses. Among them we focused on the estrogenic effect genistein (GEN) [2,3] which may interfere with hypothalamic circuits controlling sexual behavior, energetic homeostasis and anxiety.

GEN exposure through mothers resulted in an anxiolytic effect and a concurrent significant decrease in the number of nNOS+ cells in the basolateral amygdala in male progeny compared to controls [4], consistently with the role of NOS system in anxiety regulation and its sensitivity to gonadal hormones [5].

Here we analyzed, morphological and behavioral effects in mice directly exposed to GEN in a narrow time window critical for development. CD1 mice (Harlan) were treated orally with 10 ul/g of vehicle, Estradiol or GEN from birth (P0) to P8. Biological measures were collected daily until P21, then weekly until sacrifice. Behavioral test were conducted at P60. Females were tested in estrous. Tests were analyzed and measured with Ethovision software. One month later mice were perfused. Coronal serial cryosections (40 um thick) of the brains were prepared. Slices were processed for immunohistochemistry against nNos (Diasorin, Sillwater, MN,USA). The number of labeled cells was counted in ROI with ImageJ software. System controlling energetic homeostasis, fertility and anxiety were affected by early postnatal administration of GEN, to a certain extent. An increase in the body weight, more evident in pups was observed only in GEN treated males. Fertility was affected by the treatment in both males and females through different mechanisms. In females estrous cycles are disrupted and the ratios between uterus weight and total body weight is impaired. In males prostate/body weight ratio is decreased. Moreover, preference T maze-test indicates that males had a minor interest in the sexual cue than the food stimulus, while females have a significant delay in the time of vaginal opening (puberty landmark).

Open field and elevated plus maze tests showed that GEN early administration during postnatal development have a dichotomic effect on anxiety: it have an anxiolitic effect on females and an anxiogenic effect on males. Interestingly, a significant increase of number of nNOS cells in the PaAP subdivision of PVN in males parallels the increase in anxiety behavior. These results have important economic fallout in livestock and on animal welfare, since, soy based supplements are largely used for farm animals like in pigs. Meanwhile hypo fertility is a common problem in those animals and the soy phytoestrogens could be one of possible causes.

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HOW TEACHERS AND STUDENTS FEEL THE HUMAN-ANIMAL BOND. ANIMAL REFERENCE, COGNITION AND EMOTION

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In recent years the interest in animal emotions is improved. The central point is whether and in what circumstances the animals suffer for example, after strong experiences or persistent negative emotions. If animals have fear and pain experiences and if their frustration experience is the result of being unable to perform their natural behavior patterns, then this has legal and ethical importance and in turn may have major economic consequences (1). Some humans claim to be able to read emotional facial expressions in dogs, whereas others are skeptical of such a human ability. Recently, it was investigated if dog facial expressions can be identified accurately in photographs of a dog face (2). Humans were able to accurately, but not perfectly, identify at least one dog facial expression, anchored in behavioral situations that were expected to produce specific emotions. The basic question remains if this ability results from a common mammalian lineage or has been enhanced by our long shared history (3). The aim of our trial was to evaluate how teachers and students of Veterinary Medicine in Italy feel the human-animal bond about animal reference, cognition and emotion. Therefore, we organized a questionnaire according to the analytical framework based on zooanthropological predicate references: subjectivity, diversity and uniqueness; part of the work is also based on the theme of the emotions of animals and on their importance in the processes of interaction with the human. The questionnaire were administered to 935 units of the Veterinary Medicine Faculty of Italy. The data obtained express interpretable results at multiple levels, interconnected among them: experimental, educational and communicative. Experimentally it shows an awareness of the active role of the animal in structure of the report, but the data are connected to the direct experience and the species of animal than the understanding of cognitive processes related to their subjectivity: cognition is recognized as basis of animal behavior only 11.6% of the sample. Very interesting is the comparison of data between favorite animal / what is an animal / cognition, which highlights the correlation between the concept of active relationship and mental processes. Another significant result is expressed by the recognition of the socialization activities as more important for the well-being animal, respect to the health care. The results indicate a trend to anthropomorphism, especially to the animal companions, linked to the lack of adequate tools for interaction, especially at communicational and ethological level. Anyway, all categories of participants consider the emotions that animals feel a focal point for their behavior and for relationship with the humans.

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GLUCOCORTICOIDS IN HAIR, FECES AND URINE OF FARMED REPRODUCING EUROPEAN BROWN HARES

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Included within minor farmed species, hares started to be farmed by '60s but rearing systems are still not standardized and few information is available on the stress level of animals in captivity. In general, chronically elevated stress levels affect metabolism, immune response, reproduction and/or survival (Boonstra et al., 1998; Sheriff et al., 2009). The present study aimed at measuring corticosterone and cortisol concentrations as stress indicators in hair, feces and urine of farmed reproducing hares in different moment during farming. The pairs were housed in a commercial farm (Venice, Italy),in outside roofed cages (1 m long, 1.60 m wide, 70-80 cm high). Ten pairs at their first reproductive cycle were used: five with dams previously kept in mixed-sex groups and five with dams kept in female groups. Feces, urines and hair of all pairs were collected 1, 7 and 14 days after pair forming, 1 day after partum and after weaning offspring (at 25 d). Feces and urines were collected by a net and a bowl put under the cage 24 hr before sampling while hair was individually collected by gentle pulling hair from the hare back. Corticosterone and cortisol were measured by specific microtitre radioimmunoassays (RIAs)) as detailed by Bertotto et al. (2011) upon steroid extraction by diethyl ether (urine and hair) or ethanol (faeces). To validate RIAs, parallelism and intra-assay tests were performed. The data were analyzed by PROC MIXED (SAS, 2013) with sex composition of origin group (mixed-sex or only females), sampling time and their interaction as fixed effects. RIA validation tests showed acceptable parallelism for extracts in all matrices and steroids and the intra-assay coefficients of variation were always below 10%. The sex composition of the origin group had no effect on glucocorticoid levels in various matrices, whereas cortisol (124, 29, 37, 86, 39 ng/g at 1, 7, 14 day post pair forming and in post partum and post weaning, respectively; $P<0.001$) and corticosterone (86, 54, 59, 69, 78 ng/g at 1, 7, 14 day post pair forming and in post partum and post weaning, respectively; $P<0.10$) concentrations in feces changed with the sampling time. In the present study, both cortisol and corticosterone concentrations were successfully measured in all matrices. Pair forming resulted as the highest stressful time, but the glucocorticoid decrease in feces after 7 d indicates that the stress occurred for a relatively short period. Feces resulted an optimal non invasive matrix for measuring stress in hares, while hair did not both for the lack of differences in glucocorticoid levels and the invasiveness of sampling.

Acknowledgment

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WHAT ARE THE EXPECTATIONS OF A PERSON WHO ADOPT A DOG? A SURVEY AMONG ITALIAN RESIDENTS FOR PROMOTING THE ADOPTION OF SHELTER DOGS

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Prevailing feeling of Italians towards animals is positive and based on respect, and 20% of Italians own at least one dog(1). Reasons to adopt a pet are not utilitarian but rather related to a process of interrelationship, which Marchesini(2) classified in 6 macro-areas: Affective, Recreational, Hedonic, Social, Epistemic, and Affiliative. However, each year more than 100000 dogs enter shelter in Italy(3), and a number of these animals return after adoption. This results in high public costs and compromised dogs' welfare. Dog's behavioural problems and lack of owner fulfilment are cause of relinquishment and abandonment(4). Identification of âĂIJideal dogâĂİ can be the first step to link people's expectations and shelter dogs to increase adoptions and reduce relinquishments.

The aim was to identify characteristics important to the Italian residents in their ideal dog.

Two sections (C and E) of a questionnaire developed by King et al.(4) were administered to 770 volunteers residents in Italy, aged 18-64 years. Most of the participants were women (74%) and dog owners (66%). In addition to demographic data, participants were asked to indicate 5 characteristics important in their ideal dog with an open-ended question. Answers were classified in macro-areas according to the relational dimensions proposed by Marchesini(2). Data were analysed by Mann-Whitney or Kruskal-Wallis test.

The highest number of desirable characteristics were included in Affiliative (33%) and Affective macro-area (27%), like "loving", "looking for me", "obedient", and "faithful". People want from their dog mainly an interchange similar to a parental relationship, based on loyalty and constant presence, reciprocal protection and reassurance. Previous researches(4,6) indicated that training courses emphasize these desired traits increasing obedience, sociability, owner-dog attachment and reducing behavioural problems. Conversely, answers included in Epistemic motivations, like "communicative", were poorly represented (5%). In Epistemic area, human see the pet as âĂIJcognitive activatorâĂİ(2), implying empathy and knowledge. Lack of understanding of dog behaviour can contribute to relationship breakdowns(7) while, in our opinion, knowledge of canine communication and physiology can help owners to establish a better-balanced bond with their dogs. Finally, our study confirms the gender difference in the attitude to animals: women most indicated Affective ($P < 0.01$), Social ($P < 0.001$), and Affiliative features ($P < 0.05$) while men preferred Hedonistic ($P < 0.05$) and Epistemic ($P < 0.001$) traits. A pre-adoption education for owners and housetraining of the dog, proved to reduce the gap between ideal and real dog, improving owner satisfaction then reducing relinquishment of shelter dogs. In Umbria, the RandAgiamo project(8) is successfully implementing these aspects, increasing shelter dog's adoption rates and post-adoption owner fulfilment.

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EVALUATION OF ADRENAL ACTIVITY MONITORED IN HAIR, FAECES AND PLASMA IN SCHUTZHUND DOGS DURING TRAINING

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Dogs perform many tasks that require training according to the purpose to be achieved; certain types of training require intense physical effort subordinate to a lower psychic concentration, while others training methods subject the dogs to physical efforts of medium-low intensity, but at the same time require great attention. In training, as well as in coaching, physiological, endocrine and behavioral changes are realized; when the neuroendocrine activation exceeds a given gateway there is a stress reaction and consequent behavioral and physical disorders (Beerda et al., 1999).

The object of the study was to determine the cortisol levels in schutzhund dogs during training, looking for a connection between different working programs and the adrenal cortex activity.

The study involved 15 dogs (12♂and 3♀), aged between 2 and 7 years, 9 dogs have the highest IPO license (IPO-3) whereas 6 have no license (IPO-0). The dogs training programs were different in relation to their preparation's level: dogs IPO-3: two months of strong work (P1) in preparation for the Italian specialties championship and next 2 months (P2) for the reduction of work's intensity; dogs IPO-0: 4 months of constant intensity work equal to that of dogs IPO-3 in P2. From each dog, daily collection of feces has been carried out and, always by the same anatomical site, the collection of hair at the beginning and at the end of each training period was carried out. On these samples, by RIA, the cortisol levels (C) were assayed (Tamanini et al., 1983).

The psycho-physical exercise in dogs of both groups lead to a fecal C increasing ($P < 0.01$) compared with the observed levels when at rest. The dogs IPO-3 presented, both at rest and during the training, greater fecal C levels ($P < 0.01$) compared to animals IPO-0. The dogs IPO 3, show a fecal C reduction ($P < 0.01$) in P2, both at rest and in training. The highest C levels in the hair were observed in association with the national championship; these levels are higher ($P < 0.01$) than those observed at the beginning and at the end of the experimental period. The dogs IPO-0 had similar levels of C follicles at the beginning and at the end of the test.

The mental and physical activity produce a fecal and hairy cortisol levels increasing with relation to the intensity of the work done. The neuroendocrine activation persistence, even when at rest, can affect the animal's welfare.

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Part VII

XV CONVEGNO A.I.P.Vet

THE PRESENCE OF SHORT FORM OF RON/STK TRANSCRIPT COULD BE A PREDICTOR OF POOR OUTCOME IN FELINE MAMMARY CARCINOMA

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Since 1980, feline mammary carcinoma (FMC) has been recognized as a suitable animal model for studying human breast cancer because it shares epidemiological, morphologic and prognostic features with human breast carcinoma(1). RON/stk tyrosinase receptor, identified in cat as feline-stk (2), is activated by Macrophage Stimulating Protein (MSP) and over-expressed in human breast cancer (3). Human RON gene is able to generate both the full length (fl) and the short forms (sf) of the transcripts. Sf-RON is generated from an alternative transcriptional start from a second promoter, within the intron 10 (4). The sf-RON lacks the N-terminus of the protein, including most of the extracellular domain, but conserves the kinase activity of the COOH terminus after heterodimerization. In human the short form of RON plays an important role in breast cancer and its expression is correlated to invasive capability in vitro (5,6,7).

The aim of this research was to investigate the expression of both RON and MSP and to identify the presence of the sf-RON transcript in feline mammary carcinomas (FMCs) in relation to clinico-pathological findings.

Tissue samples of spontaneous mammary tumours were collected from 50 queens. All the animals underwent a complete clinical staging and were surgically treated with surgery and then they were followed until the recurrence of the neoplasm or death. All the samples were histologically evaluated and immunohistochemically tested for RON and MSP. Histological and immunohistochemical results were evaluated in relation to clinico-pathological data. RNA was extracted from each formalin fixed paraffin embedded case and RT-PCR was performed to detect sf-RON, with primers annealing on exon 10 and exon 11.

IHC expression of RON and MSP was observed in the 68% and 58% of FMCs respectively while the 52% of the cases co-expressed both proteins. IHC expression of RON, MSP or both in FMC was not correlated with clinical outcome.

RON protein is associated to MSP by IHC suggesting a similar interaction in feline as seen in human as well as a possible involvement of RON in tumor progression For the first time, RT-PCR performed on FMC tissues, revealed the presence of the short-form in the 51% of feline mammary carcinomas. This form originates, as in humans, from the alternative promoter (P2) and codifies the proper feline short form (sf-RON). sf-RON resulted statistically associated with the poorly differentiated histological grade, with a shorter disease free (DFI) period and a shorter survival (OS). These results confirm FMC as suitable model in comparative oncology and identify sf-RON expression as new predictor of outcome for this disease.

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EXPRESSION OF PDGFRS RECEPTORS IN CANINE MAMMARY CARCINOMAS: PATHOLOGICAL AND CLINICAL IMPLICATIONS

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Canine mammary tumors (CMT) are the most frequent tumors in bitches (1) and several similarity between CMT and human breast cancer have been found (2). Platelet growth factor receptors (PDGFR α and β) are tyrosine kinases receptors over-expressed in several human and canine cancers (5) and play an important role in the tumoral transformation as well as in tumoral-stromal interaction.

The aim of this study was to evaluate the immunohistochemical expression (IHC) of α and β PDGFRs in tumoral and stromal compartments, and to correlate their expression with grade, histological type and clinical follow-up.

IHC against α and β isoforms of PDGFRs was performed on 83 CMT (42 simple, 25 complex and 16 mixed carcinomas) samples. Immunohistochemical expression of PDGFR α and β was evaluated both in tumoral and stromal compartment and relation with histological type, grade and clinical follow-up was investigated. cDNA from 11 fresh CMT surgical samples was subjected to q-PCR and quantitative expression (2 - $\Delta\Delta$Ct) was determined.

IHC and q-PCR revealed that PDGFR α and β are expressed in 88% and 78% of tumors respectively, and that their expression are significantly more frequent in mixed and complex tumors compared to simple carcinomas (p=0.0262 and p=0.0447). PDGFR α and β loss of expression is significantly correlated to high grade in mixed CMT and simple CMT, respectively. Over-expression of α isoform confers a better prognosis in all CMT, while β isoform only in mixed CMT. Moreover, PDGFR β loss of expression in the stromal compartment was significantly correlate with high grade of malignancy in CMT (p=0,05) and in simple carcinoma compared with mixed and complex carcinoma (p=0,019). PDGFR α and β transcripts are expressed respectively in 8/11 and 5/11 fresh tissues. PDGFRs have a different role in the pathogenesis and histological differentiation of CMTs. As in humans also in CMTs, the loss of beta isoform had shown to be correlate with a high grade phenotype (3,4). Collectively, these data suggest that continued characterization of PDGFR expression in CMTs should present opportunities for improved accuracy in prognosis and also to assess or not the efficacy of PDGFR-directed tumor therapy.

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SOMATOSTATIN RECEPTOR (SSTR2A) IN CANINE MENINGIOMA: PRELIMINARY RESULTS OF IMMUNOHISTOCHEMICAL AND QRT-PCR INVESTIGATIONS

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The neuropeptide somatostatin (SST) plays an important regulatory role in the proliferation of both normal and neoplastic cells. Five subtypes of somatostatin receptors (SSTRs) have been identified in several human tumors. Among human brain tumors, meningiomas show the highest incidence of somatostatin receptor expression by either immunohistochemical (IHC) or RT-PCR analyses. The receptor most commonly identified is the SSTR2a subtype. Although the exact functional role remains unclear, in vitro studies indicate that the activation of SSTRs may result in cytostatic effects on neoplastic cells. Long half-life somatostatin analogues (i.e. octreotide) are today included in chemiotherapy schedules for unresectable or radiation-refractory recurrent human meningiomas.

The aim of this study is to test the expression of SSTR2a in canine meningioma by immunohistochemical and qRT-PCR analyses. The presence of SSTRs may be predictive of a positive response of canine meningioma to somatostatin analogue therapy, especially for cases that cannot receive conventional treatments.

Twenty one FFPE meningiomas were used for IHC investigations performed with rabbit anti-human Somatostatin Receptor Type 2a antibody (1:500, Alomone Labs, Jerusalem, Israel) and avidin-biotin-peroxidase complex method. FFPE canine pancreas and gastric wall were used as positive controls. For each tumour, area of labeling was assessed in five grades, ranging from (-) = absent to (++++) = > 75% of tumor. Twenty four cases including the main histotypes were also submitted to qRT-PCR investigations performed with Taqman probe (Life Technologies). Total RNA was extracted from 5 μm sections of FFPE tissue with FFPE-RNA Purification Kit (Norgen), and mRNA was reverse-transcribed with iScript cDNA synthesis (Bio-rad).

At IHC, SSTR2a was expressed in 18/21 cases (86%) showing a diffuse cytoplasmic immunoreaction pattern. The most common histotypes, including meningothelial, fibroblastic, transizional, and psmammomatous meningiomas as well as papillary meningioma were positive, ranging from (+) to (++++). Anaplastic type (grade III) did not show any immunoreaction. In all positively stained tumors, SSTR2a immunoreactivity was uniformly present on nearly all tumor cells. The PCR-amplification tests of the extracted and reverse transcribed RNA gave positive results confirming the expression of SSTR2a in the majority of the canine meningiomas. Therefore, these preliminary results encourage continuing this study aimed to find new chemiotherapeutic protocols for dogs, usable as additional or as an alternative to the most traditional. We expect these preliminary results to be supported by further biomolecular and functional studies. The absence of somatostatin receptor (SSTR2a) in the anaplastic meningiomas remains to be confirmed.

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GLOMERULOID MICROVASCULAR PROLIFERATION IN CANINE CHOROID PLEXUS TUMORS

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Choroid plexus tumors (CPT) are intraventricular neoplasms, accounting for 7% of all canine primary brain tumors.1 Canine CPTs are classified according to the human WHO classification of central nervous system tumors (2007)2 into choroid plexus papilloma (CPP, grade I), atypical choroid plexus papilloma (aCPP, grade II) and choroid plexus carcinoma (CPC, grade III).3 Tumor-related microvascular proliferation (MVP) is morphologically defined as vessels lined by multilayered and mitotically active endothelial cells, pericytes and smooth muscle cells and occurs classically in glial tumors.2 In these tumors, MVP appears as glomeruloid bodies and is associated with higher grades and poor prognostic outcome in human patients. We have observed the occurrence of glomeruloid MVP in a subset of canine CPT. The aim of this study was to characterize MVP in canine CPT and to investigate whether the appearance of glomeruloid MVP is associated with high histological grades. Fifty-five cases of canine CPT were included in this study. Tumors were graded according to the human WHO 2007 classification on HE stained sections and the presence/absence of MVP were recorded. Immunohistochemistry with antibodies against aSMA, vWF, and Ki67 was performed on 25 canine CPT. Labelling was recorded as either positive or negative. The Ki67 labeling index was obtained from the evaluation of 10 HPF, using an image analysis program (ImageJ). Statistical analysis was performed using t test (P value < 0. 05). According to the human WHO-classification2, 32/55 tumors were CPC and 23/55 CPP. The Ki 67 proliferation index was 1.065 % in the CPPs (range of 0.593-1.644) and 9.44 % in the CPCs (range of 1.86-24.75). MVP was observed in 33/55 tumors. Two types of tumor-associated MVP occurred: simple hypervascularity and glomeruloid bodies. Simple hypervascularity was characterized by increased density of vessels with a single vascular lumen lined by vWF+ endothelial cells and bordered by plump aSMA+ pericytes. This type of MVP occurred in 8/23 CPPs and 11/32 CPCs. Glomeruloid bodies resembled aberrant glomerular structures and were characterized by exuberant proliferation of plump and disorganized aSMA+ pericytes. These inconsistently surrounded multiple poorly defined vascular lumina, lined by weakly vWF+ endothelial cells. Glomeruloid bodies occurred in 13/32 CPCs and only one of 23 CPPs. In conclusion, our data provide evidence that histological grade of canine CPTs statistically correlates with Ki67 proliferation index. Even though glomeruloid MVP occurs only in a proportion (40%) of CPC, its occurrence clearly correlates with high grade tumors (CPC). No relationship was found between the simple hypervascularity and tumor grade. These data suggest that glomeruloid MVP may serve as histological marker of malignancy in canine CPT. Obviously, this hypothesis needs to be confirmed by prospective studies as it is currently not clear whether the human classification system 2 reflects the biological behavior of canine CPTs. Pathogenetic mechanisms of MVP in canine CPTs have to be identified.

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PDGF RECEPTORS AS PREDICTOR OF OUTCOME IN CANINE ORAL MELANOMAS

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Malignant melanoma represents one of the most frequently diagnosed oral neoplasm of dogs(1-2). Canine Malignant Melanoma (CMM) has an aggressive behaviour and poor surviving rate. The Platelet-derived growth factors (PDGFs) are involved in some physiologic processes and to several diseases, including cancer, in which these factors promote angiogenesis and autocrine stimulation of tumour cells (1). PDGFRα and PDGFRβ are tyrosine kinases receptors (TKR) whose dysfunction are identified in some human cancers and a lot of studies have observed similar expression in canine and feline cancers(3-4). Tyrosine kinase inhibitors (TKI) specific for PDGFRs receptors and others are currently studied in veterinary oncology and used in the treatment of canine mast cell tumours(5). The aim of this research is to evaluate the expression of PDGFR-α and -β in CMM, in order to identify their role in the tumour pathogenesis and their correlation with prognosis.

Tissue samples were collected from 36 dogs with stage II-III surgically resected CMM followed for at least 6, and up to 24 months. All the samples were histologically evaluated and immunohistochemically tested for PDGFRα and β and Ki-67 (as prognostic factor) and PNL-2 (to confirm the diagnosis). Histological and immunohistochemical results were evaluated in relation to clinic-pathological data.

All samples analyzed showed positivity to PNL-2 antigen. PDGFR-α and -β positivity were observed in 50% and 44.4% of cases respectively, while they were co-expressed in 38.9% of cases. The positivity to each receptor as well as their co-expression were associated to shorter disease free period (Log-rank test, P<0.001) and a shorter survival (Log-rank test, P<0.001), suggesting that they can be considered good prognostic indicator and good target for specific therapies. The majority of cases (75%) had an high Ki67 index (associated to a poor prognosis according to literature). These results emphasize the importance of PDGFRs in improving the accuracy of the prognostic evaluation of CMM and pose the basis for the potential use of PDGFR-directed tumour therapy.

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EFFECTS OF HSP90 INHIBITOR 17-AAG ON VASCULOGENIC MIMICRY IN D22 AND D17 CANINE OSTEOSARCOMA CELL LINES

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Vasculogenic mimicry (VM) is a neovascularization pattern of aggressive malignancies and an unfavorable prognostic factor in human osteosarcoma (OSA) (1). VM differs from traditional tumor angiogenesis, since neoformed microcirculatory channels are lined by nonendothelial pluripotent embryonic-like and highly invasive tumor cells (2). VM was observed in three-dimensional (3D) collagen cell cultures of several cancer models (3,4). Key factors involved in neovascularisation, such as VEGF, HIF-1α, FGF, PDGFα, PDGFβ, TGFβ-1, are all overexpressed in OSA tissue and cell cultures (5-8) and VEGF and HIF-1α involvement in VM has been confirmed (3,9). The functional folding of these factors and their receptors is guaranteed by Hsp90. Anti-cancer treatments based on Hsp90 inhibition could negatively influence the action of these factors and the consequent VM process in metastatic OSA.

Aim of the study was to evaluate the capability of two canine OSA cell lines, D22, derived from a primary bone tumour and D17, isolated from a metastatic site, to form tubular networks (VM marker), when grown in 3D cultures, as well as the efficacy of the Hsp90 inhibitor 17-AAG in preventing the formation of VM structures.

The ability of OSA cell lines to form vascular channels was assessed in 3D cultures on Collagen Rat Tail Type 1. D22 and D17 cells were seeded on solidified gel and maintained in culture hood for 3 week. VM marker was morphologically evaluated after 24, 36, 48 h and 5-8 days of cultures. After 3 weeks, cultures were fixed, paraffin embedded, sliced in 5 μm serial sections and haematoxylin and eosin (H&E) stained. Once established the ability to form endothelial-like structures, cells were treated with 0,5 μM 17-AAG for 24 and 48 h and principal VM features (number of junctions, meshes, segment and branches) were quantified by an ImageJ macro Angiogenesis Analyzer. Results were analyzed through Univariate Analysis of Variance.

D22 cells formed isolated clusters with round shape, but they did not show VM pattern, probably being unable to grow on collagen. On the contrary, D17 cell line exhibited tubular structure formation after 24 h of culture and tubular network surrounded by clusters of tumor cells, losing their typical epithelioid shape and extending cytoplasm, after 36 h. H&E stained serial sections showed the presence of tubular cavities, supposing a longitudinal stretch longer than 25 μm, surrounded by endothelial-like flat cells. 17-AAG-treated D17 cells also showed a significant decrease ($p<0.05$) of junctions, meshes, segment and branches number after 48 h. The results of this study give further confirmation to the involvement of VM on cancer cell malignancy and the possibility to impair VM process by Hsp90 inhibition. The latter result confirms the ability of 17-AAG to intervene on the common molecular pathways that induce both VM and classical neoangiogenesis.

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CHLAMYDIA SPP. INFECTION AND MALE REPRODUCTIVE PATHOLOGY IN KOALAS (PHASCOLARCTOS CINEREUS)

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Koala chlamydiosis presents with a range of clinical signs resulting in hundreds of animals being admitted to koala hospitals in South-east Queensland each year. The most common impact of this disease is infertility in the female, associated with ovarian cysts, salpingitis and/or metritis [1]. Unlike the female, male koala infertility from chlamydiosis is not well defined and its impact on semen quality has yet to be documented. Therefore, the aim of this study is to assess the incidence and aetiology of male reproductive pathology associated with Chlamydia infection in koalas submitted to koala hospitals in South-east Queensland. Thirty koalas referred for suspected clinical chalmydiosis and presented for necropsy at the Moggill Koala Hospital were examined. Chlamydia spp. was detected in the semen and/or urogenital swabs by real-time PCR. The effects of Chlamydia infection on the reproductive tract was evaluated through the histopathological examination of selected tissues. The sperm DNA fragmentation (SDF) was analysed by means of the sperm chromatin dispersion test (SCDt), recently developed and validated for koala spermatozoa [2]. Twenty-six koalas showed evidence of histological lesions suggestive of Chlamydia infection. Chronic lymphoplasmacytic inflammation has been observed in the kidney 12 times, the urinary bladder 16 times, the prostate and prostatic urethra 24 times, the membranous urethra on 13 occasions, the bulbourethral glands 8 times, and the testis and epididymis on 1 and 2 occasions, respectively. Twenty-nine out of 30 samples were positive for Chlamydia spp. (14 semen, 7 swabs, 8 semen and swabs). The mean SDF of the semen samples was approximately 30% and compared to only 5% SDF from a captive koala population that showed no clinical signs of the disease [3]. Chlamydia spp. may be therefore able to induce inflammatory and/or degenerative lesions in the reproductive tract of the male koala and our results underline the potential adverse impact of Chlamydia spp. infection on male koala reproduction. The increased SDF may represent how Chlamydia can potentially interact with and affect sperm morphology and function and ultimately fertility.

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HEALTH STATUS OF GREY SQUIRREL POPULATIONS (SCIURUS CAROLINENSIS) IN PIEDMONT: ANATOMO-PATHOLOGICAL AND MICROBIOLOGICAL INVESTIGATIONS

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The grey squirrel (Sciurus carolinensis), an American species introduced in Europe, is considered a pest with high potential for widespread, and represents a serious threat to the red squirrel (Sciurus vulgaris) because of their interspecific competition (MARTINOLI et al., 2010). Since 2010 the north regions of Italy (Liguria, Lombardy and Piedmont) have joined the LIFE + EC-SQUARE European Project, acting for the protection of the red squirrel through the control of the grey squirrel (BERTOLINO et al., 2012).

The aims of this study were to increase the knowledge of the viral, bacterial, parasitic and fungal diseases affecting a grey squirrel population captured following a containment programme, and to assess possible risks to public health and for the transmission of pathogens to the red squirrel.

Forty grey squirrels (21 males and 19 females) were captured and euthanized in 2013 and 2014 in the province of Cuneo (Piedmont Region, north-west Italy). Each squirrel was recorded, the main biometric measures were registered, samples of the body surface were collected to detect dermatophytic fungi, and age was determined by the dry weight of the crystalline. At necropsy, samples were collected and fixed in 10% buffered formalin (pH7) for histological investigations, and frozen at -20°C for microbiological and biomolecular investigations. Fixed tissues were routinely processed and stained with haematoxylin-eosin and additional stains were performed. Investigations for Hepatitis E virus (qPCR), Francisella spp.(PCR), Salmonella spp.(bacteriological cultures), Squirrel Poxvirus (nested PCR), Toxoplasma gondii (nested PCR) and dermatophytic fungi (fungal cultures and PCR) were also carried out. All data were statistically analysed with the software GraphPad InStat (vers. 3:05; GraphPad Software, California, USA).

Lesions were found in the lungs (n=28), heart (n=28) and skin (n=10). All the squirrels resulted negative for viral, bacteriological and parasitological analyses, except for the presence of bacteraemia in three squirrels showing positive for Staphylococcus spp. and Streptococcus spp. Mycological and biomolecular investigations revealed 14 squirrels positive to keratinophilic fungi belonging to 9 different genera. In Europe the main cause of extinction for the red squirrel is the competition for food with the grey squirrel; the competition is also mediated in UK and Ireland by a squirrel poxvirus, which so far is not found in Italy. However, other bacterial or viral pneumonia, fungal infections or parasites might be involved in the competition and should be investigated in the two species. Moreover, the role of the grey squirrel as a zoonotic carrier for mycotic diseases should not be underestimated.

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TEHV3 OUTBREAK CHRACTERIZATION IN CAPTIVE TESTUDO SP

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Tortoises represent a popular non-conventional pet in Italy. Of these, several species are either considered endangered (Testudo hermanni) or near threatened (T. marginata and T. graeca) according to the Italian commission of the International Union for Conservation of Nature. When pet tortoises are abandoned or found injured or seized following illegal detention, they are sent to wildlife rehabilitation centers. Starting from 2008, the Testudo spp. population housed in the WWF Vanzago's oasis exhibited clinical signs of variably severe lethargy, nasal discharge, conjunctivitis and diphtheronecrotic glossitis. During that period of time 50 tortoises died with reported mortality peaks during March and October. In Spring 2012, the Vanzago center population was composed of 9 T. marginata, 7 T. hermanni and 2 T. graeca, still variably exhibiting the same abovementioned clinical signs. By the end of 2012 all Testudo species had died. Based on these findings, Testudinid herpesvirus 3 (TeHV3) infection was suspected. The presence of TeHV3 was investigated by molecular biology and anatomical pathology. All the tortoises housed in Vanzago were tested for the presence of anti-TeHV3 antibodies by ELISA and they all resulted positive but one T. hermanni. Of these, 3 T. marginata, 2 T. graeca and 7 T. hermanni died and were all necropsied. Lesion frequency distribution was lingual and oral diphtheric plaques (15.4%), serous atrophy of the fat (23.1%), hepatic lipidosis (15.4%) ulcerative stomatitis and/or glossitis (7.7%), pneumonia with emphysema (43%), focal intralesional bacterial aggregates (17%), intravascular bacterial thrombi (25%), hepatic granulomas (28.6%), necrotizing tracheobronchitis (25%) and intranuclear amphophilic/eosinophilic inclusion bodies (8.33%). PCR confirmed the presence of the virus in 8/12 tortoises. To better complement the epidemiological evaluation of TeHV3 distribution in northern Italy tortoises, 20 retrospective cases were selected from the archive of the University of Milan. Selection criteria were the presence of inclusion bodies or necrotizing lesions of the respiratory or gastrointestinal tract. Of the 20 cases, 5 were TeHV3 PCR positive tortoises. Lesions closely resembled those of the Vanzago's population but there were more cases with diphtero-necrotic glossitis and stomatitis and inclusions bodies. These results are consistent with a high prevalence of TeHV3 in northern Italy tortoises household population. The finding of intranuclear inclusion bodies was specific but did not represent a sensitive diagnostic tool. TeHV3 diagnostic gross and microscopic lesions have been reported to vary according with the host immune response and by the viral replicative status, and can be obscured by autolytic changes, thus gross and microscopic findings are not always diagnostic and the support of additional techniques is often necessary to confirm viral infection. In the current caseload, TeHV3 infection was often associated with secondary lesions suggestive of immunodepression that can be attributed to virus itself associated with abnormal hibernation. According to the literature and to our findings, T. hermanni spp. seems the species with higher mortality and lower antibody concentrations when infected with TeHV3

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DIETARY INSECT MEAL INCLUSION IN CHICKENS: PRELIMINARY RESULTS ABOUT ANATOMOPATHOLOGICAL INVESTIGATIONS

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Insects are being considered as a novel protein source for poultry feed, because they have high quality and quantity of protein, show low competitiveness with human feed and reduce the environmental contamination (1, 2). Previous studies found that chickens fed diets containing insect meal can improve growth performances in terms of feed intake, body weight gain and feed conversion efficiency (2), but limited anatomopathological data are available. The present study aims to investigate the anatomopathological findings in different chickens strains fed with standard or experimental diets including insect meal.

160 male broiler (group 1) and 100 female medium-growing hybrid chickens (group 2) were divided in 4 (basic feed, 5%, 10% and 15% Tenebrio molitor inclusion) and 2 (basic feed and 7.5% Tenebrio molitor inclusion) dietary treatments, respectively. For each experiment birds were distributed over 5 replicates for each dietary treatment. Diets were isoenergetic and isonitrogenous. At the age of 53 (male) and 100 (female) days the animals were slaughtered and 2 birds for each replicate were taken for analysis. 5 animals for each dietary treatment in the group 1 and 10 animals for each dietary treatment in the group 2 were submitted to anatomopathological investigations. Samples of liver, spleen, thymus, bursa of Fabricius, kidney, heart, glandular stomach and intestine (4 standardized segments of duodenum, jejunum, ileum and caecum) were collected, fixed in 10% buffered formalin solution and paraffin embedded to obtain 5μm histological sections stained with Haematoxylin & Eosin. Histopathological lesions were scored using a semiquantitative scoring system as follows: absent or minimal (score 0), mild (score 1) and severe (score 2). Data were compared by Kruskal-Wallis and Mann-Whitney U tests (GraphPad Prism® software, P value < 0.05).

Histopathological findings were similar in both groups and were not significantly different (P > 0.05) between broilers fed with standard diet and with dietary insect meal inclusion. Spleen, thymus, bursa of Fabricius, liver and glandular stomach were the most frequently affected organs, while heart and kidney showed no significant alterations. Spleen showed white pulp hyperplasia or depletion. In thymus there was cortical depletion. Bursa of Fabricius showed follicular depletion with or without intrafollicular cysts. In liver there was lymphoid tissue activation. The etiopathogenesis of the lymphoid tissue activation and/or depletion remains to be elucidated. Glandular stomach showed lymphoplasmacytic flogosis with lymphoid tissue activation and epithelial squamous metaplasia. Interestingly, glandular stomach of medium-growing hybrids was affected by more severe alterations (P = 0.0008) than broilers. This finding could be related to the free range farming of this group. These preliminary results suggest how insect meal could be included in chickens diet without inducing histopathological changes. Studies are in progress to evaluate the effects of dietary insect meal inclusion on intestinal mucin composition and morphometry.

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HISTOLOGICAL FINDINGS IN LUNGWORM INFECTION IN ROE DEER (CAPREOLUS CAPREOLUS)

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Introduction: Roe deer (Capreolus capreolus) is the most common wild ungulate living in Italy; despite this abundance, little is known about the sanitary status of this species. As in many wild animals, parasitism is one of the most common and dangerous sanitary problem and, among parasites, lungworms are relatively common in roe deer. The most commonly reported lungworms in roe deer are Dictyocaulus eckerti and Varestrongylus capreoli1,2.

Aim: to investigate distribution presence of parasites and associated lesions from roe deer

Materials and methods: organs from roe deers culled in three areas of the province of Arezzo, Tuscany, during the hunting seasons 2009-2011.

Results: The lesions found with greater frequency were those of parasitic origin due to Sarcosporydium spp in myocardium and skeletal muscle, gastrointestinal nematodes and lungworms. In particular 94 samples of lung were examined, 42 of which were histologically normal, while 42 showed histological lesions consisting in diffuse interstitial pneumonia (24 cases), bronchitis-peribronchitis of medium-sized bronchi (22 cases), hyperplasia of BALT (25 cases), smooth muscle hypertrophy (20 cases) and lymphomonocytic to granulomatous pneumonia (15 cases), seldom associated with intralesional nematodes (12 cases). Parasites were seen in 16 cases; in 2 cases a single section of an adult nematode was present in medium-sized bronchi, while in 14 cases rare adults and variably numerous ova and larvae were present in alveolar spaces. Intrabronchial parasites, morphologically consistent with Dictyocaulus spp, were associated with severe catarrhal bronchitis, characterized by infiltration of lymphocytes and eosinophils. Bronchioloalveolar parasites were associated to a wide range of histological changes: in 2 cases there were scattered ova and larvae associated with minimal inflammatory infiltrate; in 12 cases the parasites were associated with lymphomonocytic infiltrates with multinucleated giant cells. The number of parasites ranged from scarce to very abundant: the parasites were less abundant in samples with marked granulomatous and eosinophilic infiltrates, while more numerous where the inflammation was mainly lymphoplasmacytic. A very suggestive change seen in 20 cases was mild to severe hyperplasia of smooth muscle from small and terminal bronchioles, as well as medial hypertrophy of arterioles; in 10 cases this lesion was associated with the presence of parasites, while they were not evident in the remainder of the cases. In 50 cases parasitological examination of lung tissue or faeces was performed; Dictyocaulus eckerti was found in 33/50 cases (66%), Varestrongylus capreoli in 22/50 (44%) cases, and a dual infection with both species in 6/50 cases (12%). The results of this observational study showed a great degree of lesions in the pathological picture of verminous pneumonia in roe deer, similarly to the great variability also observed in domestic small ruminants, probably reflecting different status of immune system and host natural resistance3; furthermore they can suggest a possible interspecies ecological influence in areas shared between roe deer and other wild animals (especially wildboar) or domestic ruminants.

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WHAT CAN WE LEARN FROM DEAD ANIMALS IN ZOOS?

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During the years 2004-2014, 287 cases of necropsies performed on zoo animals referred to the Department of Veterinary Science of the University of Turin (Italy) from four different Italian zoos were reviewed. Aim of the study was to evaluate the mortality rate due to inadequate veterinary care or zoo management. Macroscopical and/or microscopical necropsy reports have been classified according to the cause of death, including spontaneous pathology, veterinary and management errors. Every year in each zoo under investigation from 0% to 100% of mammals died for inadequate veterinary care and management for a total of 78 cases during the eleven-year period. Causes of death included: trauma (38 cases; 48.7%), inbreeding (13 cases; 16.7%), diagnostic (11 cases; 14.1%) and management mistakes (16 cases; 20.5%).

The investigation reveals how poor management and lack of knowledge about wildlife behavior and medicine are crucial factors responsible for zoo animal mortality. Future studies will include other zoo animal classes and will enquire by questionnaire other Italian and European zoos to estimate how much the human influence can affect the mortality rate of wild animals under human control.

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A COMPARATIVE APPROACH TO FORENSIC NECROPSY PROCEDURES: INTRIGUING SELECTED CASES

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Forensic veterinary science concerns the use of technical and scientific methods to answer questions posed by courts of law in order to establish crimes against animals1. Necropsy is a cornerstone of forensic practice and it has the task to answer questions on cause, mechanism and manner of death. Furthermore, other objectives of a forensic necropsy are: the accurate and complete inspection of crime scene, establishment of time elapsed since death, the proper sampling and handling of evidences, the collection of photographs at any stage of necropsy and the edition of a clear report useful to support judicial investigations. Thus, forensic necropsy should be performed by highly skilled pathologists, avoiding to ignore any details considering that many procedures are unrepeatable.

The aim of the study is to compare the forensic necropsy procedures to support judicial investigations in cases of suspected crime against animals.

In this study we present five cases of crimes against wild and companion animals. Necropsy were requested by magistrates or owners and were performed at the Department of Veterinary Medicine of University of Naples "Federico II" and Experimental Zooprophylactic Institute of Lazio and Tuscany. Results of investigation showed that first dog died for air gun multiple shoots: in this case radiographs were useful to identify and localize bullets. The second dog died for starvation: the investigation on crime scene helped the authorities to confirm the crime of neglect. Last dog died for severe infected wounds due to illegal dogfight: in this case the accurate external examination of cadaver allowed to highlight bite wounds. One bear was shot and the ballistic study was crucial to identify the culprit. Lastly, a wolf was found victim of poaching.

Forensic necropsy requires special and most complex technical procedures, special organization, and, for its legal relevance, should be performed following accurate standard procedures. For this purpose, the definition of "guide lines" must be considered mandatory to ensure the accuracy and efficacy of necropsy and definition of "good quality standards in forensic necropsy".

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PATHOLOGICAL FINDINGS IN WOLF PREY

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The wolf is a skilled and extremely efficient predator. Its predatory behaviour consists of a number of complex and integrated sequences, which are instinctive (so called "fixed action patterns", such as aggression, killing and consumption of the prey) or learned during the "hunting school" (e.g. location of the prey and ambush). Such behaviors deeply influence the pathological findings observed in wolf prey (1,2).

Aims are to report the main lesions observed in domestic and wild animal species preyed on by wolves. Such findings are of functional relevance and can be useful for differential diagnosis.

The present study has been carried out in Gran Sasso & Monti della Laga national park, a large protected area in central Italy. Wolf predation was confirmed in 716 small ruminants (sheep and goats), 83 cattle, 73 equids, 6 roe deers and 1 red deer.

Small ruminants and roe deers always showed characteristic, single (smaller animals, <30 Kgs) or multiple (rams, large meat breed), bite injuries at the parotidean region. In cattle and red deer, bilateral and symmetrical bite injuries severely affected thighs and shoulders. After skinning, wide hemorrhages and lacerations of the following muscles were commonly observed: semitendinosus, semimembranosus, quadriceps femoris, latissimus dorsi, triceps brachialis. Furthermore, bite injuries usually involved the parotidean region, the muzzle and the nose. Due to the skin thickness, bite injuries affecting the head and the neck were mild and superficial. In equids, bite injuries were always seen on thighs and on the parotidean region, their pathological features largely overlapping those observed in cattle and small ruminants, respectively.

Our data confirm that predation result from a number of features of the prey (e.g. size, neck diameter, skin thickness), as well as of the predator (size, bite efficacy, predatory behaviour, experience, "risk analysis"). In small ruminants, bites at the upper neck play a key role for a successful predation, by stimulating the vagus nerve and the carotid baroreceptors, while the laceration of specific muscles (crucial for standing) is a strategic point to prey on larger animals. In equids, bite injuries of the parotidean region have functional significance closely resembling those reported in small ruminants. On the contrary, biting on the head and the upper neck seem useful to immobilize and beat down cattle and calves only by virtue of leverage effects. Finally, pathological findings reported herein are of diagnostic relevance to confirm/rule out predation, and to correctly identify the predator.

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ASSOCIATION BETWEEN KIT EXPRESSION PATTERNS AND EFFICACY OF TREATMENT WITH TYROSINE KINASE INHIBITORS IN CANINE MAST CELL TUMORS

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Canine mast cell tumor (MCT) is a common neoplastic disease in dogs that shows a variable biologic behavior [1]. Several studies [2,3] have shown that canine MCTs express a mutated form of KIT, a receptor tyrosine kinase involved in the control of mast cell growth and differentiation. Three KIT immunohistochemical expression patterns have been identified in MCTs: Pattern I (membrane-associated), Pattern II (cytoplasmic focal) and Pattern III (cytoplasmic diffuse). Patterns II and III have been found to correlate with higher histological grade and with a worsened post-surgical prognosis. However, to our knowledge, there is no study investigating the correlation of KIT staining pattern and efficacy of the treatment with tyrosine kinase inhibitors.

The aim of our study is to address the role of KIT in canine mast cell tumours by studying the correlation between KIT expression patterns and the overall survival in dogs postoperatively treated with tyrosine kinase inhibitors toceranib phosphate (Palladia) and masitinib mesylate (Masivet).

We selected thirty cases of canine cutaneous MCTs submitted to the Pathology Service of the Veterinary Medicine University of Naples between 2011 and 2014. Case selection criteria included 1) original diagnosis of a MCT, 2) immunohistochemical analysis of KIT expression 3) post-surgical chemoterapeutic treatment with a tyrosine kinase inhibitor and 4) complete history and follow-up data. Statistical analysis was performed in order to compare the overall survival times (OS) of dogs postoperatively treated with chemotherapy and the KIT pattern staining.

All dogs with KIT pattern 1 MCT were still alive at the end of the study period, without evidence of tumor recurrence or metastasis. On the contrary, eight out of twelve dogs (66%) with KIT pattern 3 MCT died for recurrence and metastasis, with a mean survival time of 6 months. Two out of twelve dogs (16%) with KIT pattern 2 MCT died for recurrence and metastasis. Our data confirm the key role of KIT in the biopathology of canine MCTs and suggest that the aberrant cytoplasmatic distribution of KIT is negatively related to the efficacy of tyrosine kinase inhibitors giving also a significant prognostic information about the treatment outcome. Further studies are necessary to unravel the cellular mechanisms underlying focal and diffuse cytoplasmic KIT staining patterns and their respective pathologic relevance.

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IMMUNOHISTOCHEMICAL EXPRESSION OF MACROPHAGE MARKER, MAST CELL TRYPTASE, CD 79, IGA, IGG AND IGM IN CANINE HEPATOID GLAND TUMORS

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Introduction: According to the 'field of the organization of tissues' (Toft) theory, cancer arises from the deregulation of the interactions between cells and their stromal microenvironment6. Immune cells that infiltrate tumors play decisive roles at different stages of tumor development2. Among canine neoplasms, tumors of perianal glands are common and their causes and biological behaviour are still not well known.

Aim of the paper: Contribute to the knowledge of the canine hepatoid gland tumors through the assessment of stromal cell population such as macrophages, plasm cells and mast cells.

Material and methods: An immunohistochemical study for Mast cell Tryptase, Macrophage Marker, CD79, IgA, IgM, IgG on 25 lesions classified, according to the WHO classification, in adenoma (10), epithelioma (5) and carcinoma (10) was performed.

Results: Cell markers revealed the number and localization of plasm cells (PC), mast cells (MC) and macrophages (MA). These cells increased in number in benign lesions and progressively decreased in carcinomas. In addition, in normal glands and hyperplasia/adenoma CD79 epithelial positive cells were found inside the glandular lobules. Positive epithelial cells were scattered in carcinomas and epitheliomas. Anticanine IgA, IgM and IgG were localized in PC and hepatoid cells in normal, hyperplastic/adenomatous and cancerous cells.

Discussion and conclusion: Stromal immune components support cancer initiation, progression and metastasis1. We found an increased number of MC, PC and MA in neoplastic lesions of hepatoid gland. Moreover, we found Igs and CD79 both in normal and neoplastic hepatoid glands. In the normal epithelium of human skin, IgA and IgG have a potential antimicrobial activity4. In dog, the expression of antimicrobial substances and the potential involvement of hepatoid glands in local defensive mechanism of the skin, has been suggested5. CD79 and Igs have been found in human cancerous cells and their role in cancerogenesis has raising interest3. On the basis of our preliminary results and literature data, we suggest that such cells and molecules could have a role in local immune responses and could be directly involved in the biology of hepatoid gland tumor.

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CORONARY ARTERIOSCLEROSIS IN VEAL CALVES AND BEEF CATTLE: POTENTIAL RELATION TO HOUSING CONDITIONS

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Arteriosclerosis, defined as chronic arterial change consisting of hardening, loss of elasticity and luminal narrowing, is greater in older animals. However, adverse socio-environmental factors have been reported to be a major stimulus to the development of arteriosclerosis of the intramural coronary arteries in young chicken (1), swine (2), mice (3) and monkeys (4). The present study aims to investigate the prevalence of coronary arteriosclerosis in regularly slaughtered veal calves and beef cattle.

From January 2013 to March 2015 a systematic macroscopic and histological study of 42 bovine hearts was performed. Animals were 25, 6/9-months-old veal calves (60%) and 17, 10/24 months-old beef cattle (40%) housed in intensive livestock farming. Samples of interventricular septum, left and right papillary muscle, left and right ventricular free wall and left and right atrium were collected, fixed in 10% buffered formalin solution and paraffin embedded to obtain 5μm histological sections stained with Haematoxylin & Eosin, Weigert Van Gieson and Alcian Blue. Pathological intramural coronary arteries were manually counted in every localization. Data were compared by Kruskal-Wallis and Mann-Whitney U tests (GraphPad Prism ® software, P value < 0.05). Selected paraffin embedded samples were also submitted to ultrastructural investigations.

Arteriosclerosis of the intramural coronary arteries was observed in all calves and cattle (100%). Intimal hyperplasia, degenerative changes of the media tunica and medial hypertrophy/hyperplasia were the most important observed lesions. In calves there was a greater percentage of intimal hyperplasia (92% vs 88%) and degenerative changes of the media tunica (76% vs 71%). The medial hypertrophy/hyperplasia increased in cattle (59% vs 44%). This finding could reflect a temporal evolution of the arteriosclerotic disease. In calves the interventricular septum and the papillary muscles were significantly more affected ($P < 0.0001$), while in cattle the interventricular septum and the left papillary muscle only showed greater coronary arteriosclerosis ($P < 0.0001$). In cattle there was a greater percentage of stenotic intramural coronary arteries (45%) than in calves (23%), even if there was no significant difference between them ($P > 0.05$). Anitschkow cells, confirmed by ultrastructural investigations, were detected in both calves (60%) and cattle (76%). They were localized almost exclusively in coronary walls, suggesting a potential role in arteriosclerosis development. The preliminary results here described suggest a potential relationship between the development of coronary arteriosclerosis and intensive livestock farming of veal calves and beef cattle. A comparative study on free range cattle is in progress.

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DEVELOPMENT OF A DIAGNOSTIC PROTOCOL FOR MASTITIS IN GOATS

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Mastitis is an inflammation of the mammary gland that can lead to alterations in milk produced and composition. Therefore mastitis represents an important problem for animal welfare and public health (1). A wide range of infectious agents is known to cause mastitis. Contagious pathogens such as Staphylococcus aureus and Streptococcus agalactiae are transmitted among animals. Environmental pathogens such as Streptococcus uberis and Streptococcus dysgalactiae are opportunistic invaders of the gland (2). Among viral agents Small ruminant lentiviruses (SRLV) can cause subclinical mastitis (3). Aim of this research was to develop a diagnostic protocol for goat's mastitis correlating the pathologic findings with the isolated microorganisms.

25 udders with macroscopically suspected mastitis were collected in a small slaughterhouse in northern Italy from regularly slaughtered dairy goats and sent to the Department of Veterinary Science, Torino University. Anamnestic data about the animals (age, breed, characteristics of the farm) were also reported. After gross examination, 2 samples were removed: one, ïň?xed in 10 neutral buffered formalin for histological investigations and the other one, frozen at -20Â°C as tissue bank. The remaining tissue was sent to the Istituto Zooprofilattico Sperimentale of Torino to perform bacteriological, virological (PCR for ecthyma and SRLV) and mycological investigations. Antibiogram and research of inhibitory substances were also made.

According to the literature, the pathological lesions and the bacteria isolated revealed a high prevalence of suppurative-chronic infections. In fact histologically most of the udders (80%) showed chronic mastitis characterized by mixed or suppurative infiltrates. This result was supported by bacterial isolates reporting in particular pyogenic agents. Nevertheless the histological findings were difficult to correlate with the microbiological data because often co-infection of several microbial species were isolated in the same udder(84%). Staphylococcus spp.(37%) - especially S. caprae, S. xylosus and S. aureus- and Streptococcus spp.(9%) -especially S. agalactiae and S. uberis- were the most frequently isolated bacteria. Mycological survey was positive only in one udder (Aspergillus spp.) probably as a contaminant. 20 udders were positive for SRLV (genotype A), only one sample was PCR positive for the contagious ecthyma virus. 22 samples were negative for the research of inhibitors. No particular antimicrobial resistances were observed to routine texted antibiotics. This diagnostic protocol was easy to perform and relatively quickly. It provides a large number of data and it is also applicable to the ovine species whose agents of mastitis are similar. In the absence of an histological classification of small ruminant mastitis, this protocol can be useful to define a specific classification in these species. Moreover it can represents a useful epidemiological tool. In particular antibiotic resistance data can be used to select a correct therapy in the farm. To correlate the pathologic findings with the isolated microorganisms a greater number of samples have to be carefully investigated.

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ELLIS-VAN CREVELD SYNDROME IN GREY ALPINE CATTLE: IMMUNOPHENOTYPIC AND MOLECULAR CHARACTERIZATION.

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Ellis-van Creveld (EvC) syndrome is an autosomal recessive disorder due to a mutation in one of two genes, EVC or EVC2, described in human medicine. A whole genome re-sequencing study revealed a single candidate causal mutation in EVC2 and Sanger sequencing confirmed the deletion of 2bp in exon 19 as the cause of dwarfism in Grey Alpine cattle. Chondrodysplasia is characterized by histological changes affecting the growth plate. Physeal chondrocytes are subjected to a plethora of extracellular factors. When chondrocytes hypertrophy occurs the synthesis of collagen II, one of the major components of the cartilage extracellular matrix, is down regulated, and the synthesis of Collagen X is initiated. Collagen X is found in the hypertrophic zone and its role is to facilitate the deposition of calcium in the matrix. Sonic Hedgehog (SHH) and fibroblast growth factor (FGF) are molecules involved in skeletal development. Transgenic mice overexpressing SHH are affected by disarray of the physis in the tibia and absence of the femur and humerus. FGF signaling is essential for endochondral bone formation, and mutations in FGFR3 cause achondroplasia in humans. The aims of this study were to: 1) evaluate by immunohistochemistry (IHC) the degree of differentiation and proliferation index of the physes in order to better elucidate the pathogenesis of this EvC syndrome in Grey alpine cattle; 2) determine the level of expression of EVC and EVC2 mRNA in affected bones. Five Grey Alpine calves, with a known mutation in the EVC2 gene, were autopsied. Two calves, Grey Alpine breed, not affected by EVC2 gene deletion, were used as controls. IHC was performed on bone sections using anti-Collagen II, -Collagen X, -SHH, -FGF2, and -Ki67 antibodies. RT-PCR was performed using the primers for EVC1 and EVC2 on tissue samples of bone, heart, trachea, testicle and tooth of one affected calf and one control calf. Collagen II labelled diffusely the resting, proliferative, hypertrophic zones, primary and secondary spongiosa in controls, with a loss of labeling in the resting zone of two dwarfs. In the controls Collagen X was expressed in hypertrophic zone in the matrix around hypertrophic chondrocytes, but it was absent in all five chondrodysplastic cases. SHH labeled hypertrophic chondrocytes, and the primary and secondary spongiosa similarly in controls and affected animals. In both controls FGF2 was expressed in the chondrocytes of all growth plate zones, but it was completely lost in 3 of 5 cases, and had scattered expression in 2 of 5 dwarfs. The Ki67 index was lower in dwarf calves compared with controls. Unexpectedly, Both EVC and EVC2 transcripts were detected in affected and healthy calves, in contrast to what inferred in previous works. The premature collagen II degradation, abnormal collagen X expression, and the low proliferation index together with loss of expression of FGF2 are all findings that

suggest the pathogenesis of EvC syndrome in Grey Alpine cattle may involve reduced proliferation, and early hypertrophy of physeal chondrocytes with accelerated differentiation leading to early ossification.

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BLUETONGUE VIRUS SEROTYPE 1 IN THE GENITAL APPARATUS OF AFFECTED SARDA RAMS

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During the 2013 Bluetongue virus serotype 1 (BTV 1)Sardinian epidemic, severe hyperthermia and edema of the scrotum characterized the clinical signs of the infected rams, while histologically, evidences of testicular degeneration were observed.

This study aims to investigate the pathogenesis of the histological changes in the testis of naturally BTV affected rams.

Thirteen rams were collected from different flocks, in which clinical Bluetongue (BT) infection was observed. Rams were serially euthanized from 5 to 140 days after the onset of the disease. At the necropsy blood, spleen, testis, epididymis, accessory glands and regional lymph nodes, tongue and scrotal skin were adequately sampled for viral RNA quantification by Real-Time qRT PCR as well as for viral VP7 and NS2 proteins detection by immunohistochemestry (IHC).

BTV RNA was detected in blood, spleen and regional lymph nodes up to 140 days after the onset of the disease, whereas in accessory glands and testis it was detected up to 30 and 60 days, respectively. By IHC, BTV was found in the endothelial cells of the testicular, epididymal and scrotal skin capillaries only in the early stage of the disease. On the contrary, severe testicular degeneration with oligospermia or azoospermia was observed in the testis by histology up to 60 days, with a partial recovery being evident only after 100 days. Hypofertility has been reported in rams vaccinated with BTV 2 live modified vaccine[1] or naturally affected by BTV 8[2], whereas BTV 1 and BTV 8 field strains did not cause any lesion in the reproductive tracts of experimentally infected rams [3]. In this study, we observed that rams naturally infected with BTV 1 displayed severe degeneration of spermatic epithelial cells. Results obtained by IHC associated to histopatological findings indicate that the degeneration of the germinative epithelium during BTV infection might be ascribed to endothelial damage of the intertubular capillaries of the testis.

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ORAL CONGENITAL FIBROPAPILLOMATOSIS IN LAMBS

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Papillomavirus (PV) are oncogenic, double stranded viruses responsible for tumors in humans as well as in domestic and wild animals1.Congenital papillomatosis or fibropapillomatosis have been reported in piglets2, horses3 and cattle4. In these cases, a viral etiology was suspected but not confirmed. In humans there are controversial data about the vertical transmission of PV; on the contrary, in cattle such an infection has been documented5.

In sheep, the association of PVs with tumors and other disorders has been poorly investigated. Two ovine papillomavirus genotypes OaPV1 e OaPV2 are known to occur in sheep and are responsible for fibropapillomas; more recently, OaPV3 has been found in squamous cell carcinoma and in skin of healthy sheep6. To our knowledge, no previous reports about congenital fibropapillomatosis in lambs are known to occur. The aim of the present study is to report preliminary findings observed in lambs of Sarda breed sheep affected by congenital fibropapillomatosis in the gengiva, palate and muzzle skin.

Lesions were macroscopically observed just few days after the birth. In gengival and palate mucosa lesions were characterized by the presence of a proliferative tissue, white-reddish in color which makes lambs unable to suckle. Muzzle proliferative lesions appeared to be rather greyish in color. The animals died at about one month of age as they were not able to feed.

Tissues from two lambs were collected for histopathological, molecular and electron microscopic investigations.

Histologically, a mixture of epithelial (keratinocytes) and mesenchymal cells was seen. Numerous mitoses were observed in both cell types. Many mitoses appeared to be atypical. Ultrastructurally, the lesions appeared to be composed of heterogeneous epithelial and mesenchymal cells showing severe alterations of nuclei such as deep meandering invaginations which give nuclei a bizarre and lobulated appearance often containing nucleoli located peripherically. Electron dense particles, 40 nm in diameter, consistent with virus particles were scattered in some nuclei. In sheep, lesions induced by PV were only described in adult animals7,8. No lesions caused by vertical PV infections have been described so far in sheep. Here, we report mucosal and skin lesions in lambs. Our preliminary findings (molecular investigations are in progress) seem to indicate, for the first time, the presence of virus particles responsible for congenital fibropapillomatosis of lambs. It has been suggested that Bovine Deltapapillomavirus infect trophoblastic cells and are responsible for reproductive disorders in cattle and buffalo5. OaPV1 and OaPV2 are classified as ovine Deltapapillomavirus9,10. They are characterized, like bovine Deltapapillomavirus, by a tropism for epithelial and mesenchymal cells. It is conceivable to think that, as already shown for bovine Deltapapillomavirus11, ovine Deltapapillomavirus can join the genital apparatus via bloodstream. The role, if any, of ovine Deltapapillomavirus in reproductive disorders in sheep warrants further studies in an attempt to improve our knowledge in molecular pathways responsible for virus infection leading to neoplastic and no-neoplastic events.

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2 Nishiyama et al., 2010
3 White et al., 2004
4 Desrochers et al., 1994
5 Roperto et al., 2012
6 Alberti et al., 2010
7 Trenfield et al., 1990
8 Tilbrook et al., 1992
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LYMPHOPLASMACYTIC MYOSITIS AND SARCOLEMMAL EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I AND II ASSOCIATED WITH MUSCULAR SARCOCYSTOSIS IN SHEEP

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Muscular sarcocystosis in sheep is an extraordinarily worldwide common affection caused by Sarcocystis spp., protozoan parasites with an obligatory two-host predator-prey (definitive-intermediate) lifecycle. Sheep is the intermediate host of four species of Sarcocystis: S. gigantea, S. medusiformis, S. tenella and S. arieticanis, whose definitive hosts are felids or canids (1). The aim of this study was to investigate if parasitized muscle fibers could play a role in immune-stimulation, as sporadically described in accidental muscular sarcocystosis in definitive hosts (2).

Skeletal muscle samples from 80 sheep of mixed breed 4 - 5 years old were collected at the slaughterhouse and snap frozen in liquid nitrogen. Cryosection were processed with a standard panel of histological and histoenzymatic stains; immunohistochemical (HRP method) detection of MHC I, MHC II, CD3, CD4, CD8, CD79Îś, CD45RA. Part of the samples was fixed in 2,5% glutaraldehyde for ultrastructural examination. Frozen samples were collected for species identification by PCR.

A moderate to high number of intra-sarcoplasmic cysts with thin wall and internal compartments were detected in 97,2 % of cases examined. 69% of cases were characterized by inflammatory changes scored as mild (58,1%), moderate (36,3%) or severe (5,4%). Inflammation consisted of a mixed mononuclear infiltrate of small lymphocytes and plasma cells mostly located in the perivascular connective tissue or in the endomysium in a scattered fashion, with attendant myofiber degeneration and necrosis. The predominant populations were CD3+, CD8+ with lesser numbers of CD4+ and CD79Îś+ cells. Eosinophils were constantly absent. Notably, moderate to strong sarcolemmal and cytoplasmic labeling to MHC I and II was found both in biopsies with evident inflammatory infiltrate and in cases without inflammation. The wall of the cysts resulted strongly positive to MHC II and occasionally positive to MHC I. Our data suggest that muscle fibers respond to the presence of cysts by expression of MHC I and II that can play a role in stimulating and maintaining the lymphoplasmacellular inflammation. This findings underline the importance of sarcocystosis in the differential diagnosis of idiopathic inflammatory myopathy in all species (humans included) and raises interesting questions about meat consumption safety.

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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NORMAL SERTOLI AND GERM CELLS IN POST-NATAL RABBIT TESTES, FROM NEONATAL TO ADULT AGE

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In the last decades, human male reproductive pathologies and testicular cancer have increased. During the physiological maturation of the testis, from foetal to adult age, both Sertoli (SCs) and germ cells (GCs) switch from an immature to a mature immunophenotype. The re-expression of markers of immaturity in adult has been reported in numerous pathological conditions affecting the testis, in man as in animal species. Rabbits have been commonly employed for scientific research of human male reproductive system, but reports on rabbit testicular cells markers are few and data about the immunophenotype of normal postnatal SCs and GCs are lacking.

The aim of this study was to evaluate the immunohistochemical expression of anti Müllerian Hormone (AMH), Vimentin (VIM), CKAE1/AE3 (CKs), Desmin (DES), Inhibin-α (INH-α) and Placental Alkaline Phosphatase (PLAP) on normal rabbit testes, from the neonatal to adult age.

Twelve neonatal, 17 prepubertal and 7 adult testes were considered in the study. VIM was constantly expressed by SCs independently from the age of the rabbits, AMH and CKs expression in SCs was limited to the neonatal and prepubertal age, and DES and INH-α were never expressed by SCs as well as PLAP in GCs. This latter finding indicates the absence of early GCs (gonocytes) in postnatal rabbit testes.

The immunolabelling of normal GSc and SCs from rabbit testes revealed analogies with the human testicular phenotype in the different stages of testicular postnatal development. In fact, during maturation, in SCs some markers are maintained, as VIM, while other markers are lost as CKs and AMH. Considering GCs, the absence of early, undeveloped, GCs also parallels with findings in human species. The data obtained from this pivotal study suggest that rabbit could a potential good animal model for human testicular pathologies and encourage further investigations focusing on the immunohistochemical phenotype on rabbit testicular neoplasms.

IMMUNOHISTOCHEMICAL ASSESSMENT OF FOLLICULAR DENDRITIC CELLS IN HEALTHY AND PMWS-AFFECTED PIGS

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Post-weaning multisystemic wasting syndrome (PMWS) is caused by porcine circovirus type 2 (PCV2). The pathogenesis of PMWS is largely unknown. Macrophages are considered the main target for PCV2, since viral antigens and genome can be easily detected in those cells. Notwithstanding this, whether PCV2 replicated in monocytes/macrophages is still controversial (5). Some data suggest that follicular dendritic cells (FDCs) could be also infected by PCV2, thus likely playing a role in the pathogenesis of PMWS (1,2,4).

The aims are to quali-quantitatively assess FDCs in tonsils of healthy (n = 8) and PMWS-affected (n = 10) pigs.

Tissue samples were routinely processed for histopathology. Consecutive tissue sections were tested by immunohistochemistry to detect PCV2, FDCs (S-100 and CNA.42) and macrophages (lysozyme). FDCs and PCV2 antigens were quantified by means of the Image J software, and data submitted to statistical analysis.

In healthy pigs, lymphoid follicles appeared normal with well-developed FDC networks. The immunohistochemical pattern markedly differed between S-100 (mainly nuclear) and CNA.42 (mainly cytoplasmic and dendritic). Lymphocytic depletion, infiltration of histiocytes and/or syncytia were confirmed in PMWS cases; large amounts of PCV2 antigens were seen within macrophages, syncytia, and/or showing a "FDC-like" pattern. Both S-100 and CNA.42 immunoreactivity were significantly reduced in PMWS-affected pigs. A positive correlation was seen between S-100 and CNA.42 , while a negative correlation was observed between S-100 and PCV2, as well as between CNA.42 and PCV2.

Our results demonstrate a significant reduction of FDCs in PMWS-affected pigs, which goes âĂIJhand in handâĂİ with the severity of lymphocytic depletion and with the infiltration of macrophages and syncytia. The reduction of FDCs likely compromises the immune response and enhances the occurrence and the severity of secondary infections, which are relevant for the expression of PMWS (3).

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PERFORMANCES COMPARISON OF DIFFERENT ELECTRODES FOR RADIOFREQUENCY THERMAL ABLATION (RTA) ON EX-VIVO BOVINE AND PORCINE LIVER

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Radiofrequency thermal ablation (RTA) is a consolidated, safe and minimally invasive approach for the treatment of hepatic nodular lesions and neoplasm of different organs (kidney, spleen, prostate, breast, lung, bone, and recently thyroid nodules) (Deandrea et al., 2008). Tissue necrosis is achieved around the needle tip, through the heating induced by rapid ion movement, in a controlled fashion.

Aim of the work was to compare the ablation characteristics of the moving-shot technique (MST) and the fixed electrode technique (FET) for radiofrequency (RF) ablation in an ex-vivo bovine and porcine liver tissue model.

In the first pilot experiment different conditions (technique, ablation time, electrode type) were investigated on bovine livers, under ultrasound guidance. Subsequently, MST (both perfused or not) with single or double ablation, and FET were applied on excised porcine livers. The efficacy of induced necrosis was evaluated by monitoring tissue impedance. Following ablation the liver was macroscopically and histologically examined, and morphometrical techniques were applied in order to measure the parameters of each type of ablation (diameters, perimeter and area of surfaces, and morphology of the necrotized tissue). The liver was cut along the longitudinal plane through the longitudinal axis of the electrode and then cut transversely and perpendicular at the center of the ablation area.

Differently from similar studies (Ha et al., 2014), the FET achieved a significantly larger ablation zone than the MST (confirmed by each of the measured parameters). Moreover a double passage with both normal and perfused moving shot electrodes reached values similar to the ones obtained with FET. The non perfused electrode for MST, with a single passage of ablation, induced a smaller ablation zone.

The evaluation of the performances of different electrodes and conditions of application in an ex-vivo model is very useful in order to manage with nodules of different shapes and location in vivo. As the application of ex-vivo data to in-vivo situations could result in different outcomes, further investigations in an in vivo model is fundamental in order to better evaluate the effects of the best combinations identified in the ex-vivo model, before the application on patients. The mechanism underlying the differences detected by our study and other investigations is an important issue to be further investigated.

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MYOPATHY OF THE PIPPING MUSCLES, HEPATOSIS DIETETICA, AND CATARACTS IN EMU CHICKS (DROMAIUS NOVAEHOLLANDIAE)

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Vitamin E is considered one of the key factors in ratites nutrition and management, since its absorption is limited in newly hatched birds and thus the embryonic stores may be depleted before the onset of an efficient dietary assimilation (Tully et al., 1996). Few cases of encephalomalacia and nutritional myopathy caused by vitamin E deficiency have been described in adult emus (Aye et al., 1991; Rae et al., 1992), while reports on similar lesions in embryos or newly hatched chicks are lacking. Seven emu chicks from a farm with poor hatchability (16-18%) and increased neonatal mortality were presented for necropsy with a history of death at or within few days after hatching. Macroscopic examination revealed subcutaneous oedema and haemorrhages and swelling of the pipping muscles in the proximal neck (71.4%), pale liver with haemorrhages (57.1%), non-internalised residual yolk sac (85.7%) and anasarca (14.3%). Histologically, the most remarkable findings were degeneration and loss of cross striations of the musculus complexus (pipping muscle) (100%), as well as myocardial degeneration and mineralisation (14.3%). Liver contained multifocal severe hepatocellular necrosis and haemorrhages (57.1%) and both eyes exhibited swollen and vacuolated lenticular fibres in 5 c hicks in which the eyes were examined. The lesions observed here are suggestive of a nutritional deficiency. The deficiency was confirmed by finding low levels of vitamins E and A in the livers and feed. Although vitamin E deficiency is considered the primary aetiological factor of the lesions observed in our cases, management factors (such as a high relative humidity during incubation) may be potentially considered additional contributing factors. The involvement of the muscles in the neck region, and specifically the musculus complexus - whose contraction at the end of the incubation elevates the head of the chick and therewith the beak (Fisher, 1958) - could have compromised the hatching process.

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EPHRIN A3 RECEPTOR AS TARGET FOR CANCER THERAPY: PRELIMINARY IMMUNOHISTOCHEMICAL RESULTS IN THREE CANINE TUMOURS

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With 16 members, the Ephrin (EPH) receptor family is the largest family of the receptor tyrosine kinases and it is of increasing interest in developmental therapeutics. Recent findings on the elevated expression of EPH in human malignancies as well as in stem cells are of particular interest [1]. The most promising, EPHA3 is highly expressed at various stages of embryonic development [2] while its expression declines, usually being low, if detectable at all, in adults. However, EPHA3 re-expresses in cancers and plays important roles in a variety of biological functions, such as tumour cellular proliferation, angiogenesis and tumour progression [3]. The aim of this study is to evaluate the expression of EPHA3 in three significant cancers of dogs (cutaneous haemangiosarcoma, osteosarcoma and prostate carcinoma) as an important prelude for evaluating an EPHA3-targeting antibody as a potential therapy. Twenty-four cutaneous haemangiosarcoma (HSA), 29 osteosarcoma (OSA) (18/29 osteoblastic, 6 mixed type fibroblastic and osteoblastic, 3 telangiectatic, 2 mixed chondroblastic and osteoblastic), and 22 prostate carcinoma (PC) (13/22 cribriform with comedonecrosis, 5/22 solid, 3/22 small acinar/ductal and 1/22 papillary) cases were studied. For immunohistochemistry, tissue sections were labelled by the avidin-biotin-peroxidase complex (ABC) procedure with a commercial immunoperoxidase kit. The sections were incubated with a primary rabbit polyclonal anti-EPHA3 antibody (Santa Cruz Biotech., dilution 1:800 for HSA and PC, 1:1500 for OSA). In 13 out of 24 haemangiosarcoma samples, a strong cytoplasmic expression was detected in more than 80% of neoplastic cells, while the remaining 11 samples contained 50-60% positive cells. In all osteosarcoma samples, the antibody showed diffuse and strong cytoplasmic labelling with a mean of 86% of cells staining positively. Moderate to strong diffuse cytoplasmic expression was observed in 90-100% of prostate carcinoma cells. Labelling pattern was similar in all histological types of all three tumours. Most normal prostate tissues display weak cytoplasmic positivity. In normal prostate cells, moderate granular cytoplasmic expression was observed in 20-30% of cells, mainly in the basal layer. The present study demonstrated strong immunohistochemical labelling for EPHA3 in neoplastic haemangiosarcoma, osteosarcoma and prostate carcinoma cells suggesting that EPHA3 may play a role in the carcinogenesis of the three entities under consideration and putatively in other canine tumours. Further studies are required to clarify whether EPHA3 overexpression is correlated with survival and could be used as a predictor of disease-free survival time or as a new therapeutic target for these neoplasms.

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FIRST REPORT OF MYCOBACTERIUM BOVIS INFECTION IN A FREE-RANGING MARSICAN BROWN BEAR (URSUS ARCTOS MARSICANUS)

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Mycobacterium bovis (MB),causative agent of bovine tuberculosis (boTB),has a wide host range and is often maintained in complex transmission cycles[1]. Diagnosis of tuberculosis in wildlife relies on necropsy,histopathology and microbiology. MB was cultured from a black bear in absence of lesions[2]. M. avium paratuberculosis was reported as cause of disease in 2 brown bears[3]. To the authors' knowledge this is the first report of MB in a brown bear. Aim This study describes a case of MB tuberculosis in a free-ranging brown bear.

An adult female brown bear (U. arctos marsicanus) died in Abruzzo in 2014.The carcass was submitted for postmortem examination to ascertain the cause of death. Tissue samples for histopathology were formalin-fixed,embedded in paraffin wax,sectioned at 4 μm and stained with HE and Ziehl-Neelsen. Samples were also submitted to cultures for bacterial pathogens including Mycobacteria,viruses isolation (cell cultures) and toxicological analysis(GC-MS).Bacterial identification was obtained by molecular techniques (PCR,sequencing) of selected target genes.

The bear was in fair body condition. Gross findings included peritonitis, enlarged necrotic mesenteric lymphnodes, thickening of intestinal wall, hepatosplenomegaly, rhinopharyngeal exudate, pulmonary edema, subpleural petechiae and meningeal hyperemia. A presumptive diagnosis of mycobacterial infection was made. Histology showed large necrotic foci in the peritoneum and intestinal mucosa, massive necrosis of mesenteric lymphnodes, granulomatous hepatitis, perisplenitis, membranoproliferative glomerulonephritis and granulomatous meningitis. In all the examined organs acid-fast bacilli were observed in macrophages and extracellularly. Slow-growing Mycobacterium sp,identified as M.bovis by molecular methods,was isolated from multiple organs. Staphylococcus schleiferi subsp coagulans was isolated from intracardial clot and peritoneal fluid. Virological tests and toxicology were negative.

The bear was diagnosed with a chronic severe systemic MB infection,with both pathological and microbiological aspects suggesting ongoing generalization. The cause of death is likely to be attributed to MB and a concurrent opportunistic infection with S. schleiferi coagulans. Due to the main gastrointestinal localization of lesions MB infection was thought to be acquired by ingestion. MB infected cattle have been known to graze in the home range of the bear in 2012:some bovines died on pastures and were consumed by scavengers. Gross lesions in cattle are typically caseous and mineralised with histology showing central necrosis surrounded by granulomatous reaction and fibrosis, but lesions in wildlife may differ[4].The case herein described presented with poorly organised lesions,similarly to what have been observed in other carnivores. Bears are thought to be spillover hosts and are likely to play no significant role in the maintenance of boTB.Nonetheless,spillover from cattle to bears may have serious implications for the conservation of this species. Stricter application of health regulations in force is warranted along with wildlife monitoring to assess presence of infection in other scavengers. We underline the importance of personal protection measures when dealing with wildlife forensic cases as zoonotic infections cannot be ruled out based on external findings.

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GLEASON-LIKE GROWTH PATTERNS OF CANINE PROSTATIC CARCINOMA AND THE POTENTIAL APPLICATION OF A MODIFIED GLEASON GRADING IN THE PRACTICE SETTING

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Human prostatic carcinomas (PCs) are graded by pathologists using the Gleason system [1], which remains one of the most powerful prognostic indicators in PC [2], assigning numerical grades (1-5) based upon the architectural patterns of the tumour. Gleason grading was updated at the 2005 consensus conference by the International Society of Urological Pathology in response to evolving clinical practice and understanding of prostate cancer pathology [3]. Since we have recently recognised Gleason-like growth patterns in dogs [4], this study aimed to apply the modified Gleason grading to score the aggressiveness of 45 canine prostate carcinomas. Specimens were represented by tissue samples collected during necropsy (n=20), prostatectomy (n=4) or biopsy (by ultrasound or exploratory laparotomy; n =20). Gleason score (GS) was obtained by adding the primary and secondary grades together. A tertiary pattern higher than the primary and secondary grades has been included in the final GS as the secondary grade. Any amount of Gleason pattern 5 - predicting a worse outcome in men [5] - was considered significant and included for analysis. A single primary growth pattern was observed in 28 cases, a secondary pattern in 11 cases and a tertiary pattern in 6 cases. Cribriform, solid and small acinar/ductal were the most common primary, secondary and tertiary morphological patterns, respectively. Seven (15.6%) dogs were classified as Gleason score 3+3 = 6; 2 (4.4%), 4+3 = 7; 7 (15.6%), 4+4 = 8; 2 (4.4%), 5 +3 =8; 4 (8.9%), 5 +4 = 9; 2 (4.4%), 4 + 5 = 9. The highest Gleason score (GS10) was obtained in 46.7% of cases (n = 21). Nine of 14 metastasising cases were classified as GS10. The most common score observed in tissues collected during necropsy and prostatectomy was GS 10, while GS8 in biopsy samples. Gleason pattern 5 was present in 35 of cases. This study suggests that canine PC may show variable morphological features and Gleason-like growth patterns that would aid in the acceptance of the modified GS as a grading system for histopathology. As expected due to the aggressive biological behaviour of canine PC, the most common GS is 10 and the highest GS was observed in metastasing PCs. We suggest that once carcinoma is detected and the different morphological patterns recognised, the Gleason grading system may be potentially applied in the practice settings in order to complete the clinical assessment for the best management of the patient.

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PATHOLOGICAL FINDINGS IN A FATAL OUTBREAK OF ORTHOPOXVIRUS INFECTION IN TONKEAN MACAQUES (MACACA TONKEANA)

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Orthopoxviruses are known to naturally infect a broad range of host species such as ruminants, cats, rodents, various zoo and exotic animals and humans. Human cowpoxvirus usually causes self-limiting skin lesions but severe complications and fatal disease can occur in immunocompromised people. The cowpox infection in nonhuman primates in Europe is reported with fatal course in an outbreak. As asymptomatic carriers small rodents are considered the most responsible for the spread of infection.

Aim: Describe the pathological findings observed following an Orthopoxvirus infection in a group of macaques (Macaca tonkeana).

In January 2015, twelve macaques of a group of 18 animals, housed in a sanctuary in a wooded area of Central Italy, died between 48 hours and 7-8 days from the beginning of symptoms. Severe respiratory distress, depression and in most of cases skin lesions were observed. Animals were submitted for post-mortem examination. Samples from the major organs and from the skin lesions, were routinely processed for histology and virological investigations. Data relative to animal introduction and movements were recorded to identify a probable source of the infection.

All monkeys presented a good body condition. Animals dead within 48 hours (N=2) showed severe lung congestion and hepatosplenomegaly; erythematous papular and pustular lesions on the oral and tongue mucosa and at the inguinal region, in some cases diffuse, were evident in subjects dead in 7-8 days (N=10). In the latter animals, several lymph nodes were enlarged and haemorrhagic and hepatosplenomegaly and liver degeneration were observed. Histologically, cutaneous lesions were characterized by focal epidermal necrosis, acanthosis and acantholysis and early vesiculation with eosinophilic intracytoplasmic inclusion bodies in enlarged degenerate cells. The liver showed moderate steatosis and scattered foci of necrosis. Splenitis occurred as foci of necrosis of the lymphoid follicles and histiocytosis. Affected lymph nodes showed a severe necrotising lymphadenitis associated with haemorrhages and histiocytosis. In some cases a mild interstitial pneumonia was associated with focal necrosis of bronchial epithelium. Transmission electron microscopy detected Orthopoxvirus particles in all tested animals. The preliminary characterization of virus isolates ruled out the presence of Monkeypoxvirus and lead to suspect a Cowpox infection. Deeper molecular investigation are still ongoing. The introduction of susceptible species in the last year was excluded, but free ranging cats and rodents are present in the area.

Even if Orthopoxvirus infections in Macaca tonkeana had not been previously described, pathological findings observed were similar to those described in New World monkeys in a fatal outbreak occurring in Germany. Epidemiological investigations to define the source of the infection are in progress.

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'BRAIN-ONLY' FORM OF DOLPHIN MORBILLIVIRUS INFECTION IN STRIPED DOLPHINS (STENELLA COERULEOALBA): PATHOGENETIC INSIGHTS

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Dolphin Morbillivirus (DMV), a highly pathogenic agent, can give rise to peculiar, 'brain-only' forms of infection (BOFDI), in which evidence of viral antigen and/or genome can be found exclusively in the brain tissue from striped dolphins (Stenella coeruleoalba)1-4 and, far less commonly, from bottlenose dolphins (Tursiops truncatus)4. These BOFDIs show morphopathological and neuropathogenetic similarities with subacute sclerosing panencephalitis and old dog encephalitis, which are known to occur in Measles Virus (MeV)-infected patients and in Canine Distemper Virus (CDV)-infected dogs, respectively5. We investigated in the brain tissue of 3 BOFDI-affected, male striped dolphins, 2 adults and 1 newborn, the expression levels of 5-lipoxygenase (5-LOX), along with the ultrastructural damage and the neuronal and non-neuronal cell populations colonized by the viral pathogen. The expression levels of 5-LOX, which were evaluated by means of Western Blot (WB) analysis, were significantly ($P \leq 0.05$) higher in the brain parenchyma of the 3 aforementioned cetaceans, when compared with those of 3 additional striped dolphins showing no direct nor indirect evidence of DMV infection. Furthermore, alongside with a number of nuclear (chromatin) and cytoplasmic (mitochondrial) ultrastructural changes, double labeling-indirect immunofluorescence (DL-IIF) microscopy revealed different degrees of viral colonization of calbindin (CALB)-immunoreactive (IR) and nitric oxide synthase (NOS)-IR neurons, but not of (GFAP-IR) astrocytes, within the brain tissue from the two DMV-affected adults as compared to the DMV-affected newborn. Albeit preliminary, this is the first study addressing the ultrastructural pathology and the neuropathogenesis of BOFDI, with special emphasis

on the neuronal and non-neuronal cell populations colonized by DMV in the striped dolphin's brain. Further studies aimed at characterizing the virus- and the host-related factors involved in BOFDI pathogenesis are warranted.

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ELODONTOMA AND CEMENTIFYING FIBROMA IN A DEGU (OCTODON DEGUS)

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Degus are diurnal, caviomorph, small rodents belonging to the family of Octodontidae, characterized by complete elodont (continuously growing) dentition. They are used in research studies and have been increasingly popular as pet animals. Reports of dental diseases are common in hystricomorphs, while in degus there are few descriptions (1; 2). This report aim at describe a case of multiple dental disease in an adult degu. A 4.5 years old intact male degu, kept as pet, was evaluated clinically because of a history of respiratory disease, dysorexia, progressive weight loss, and deformation of the left mandible ventral profile. Necropsy was performed after the death of the subject. The degu was in poor body condition and its bowel was severely dilated by gas content. The examination of the left mandibular branch revealed two ventral lumps at the level of the first and the third molar, respectively of 0.4 and 0.3 mm in diameter. The entire jaw was collected, fixed in 10% neutral buffered formalin, decalcified for 24 hours in a commercial solution, cut transversally, processed routinely and stained with hematoxylin and eosin. Histologic examination revealed two space-occupying lesions. The first molar was affected by a neoplasia composed of conglomerates of haphazardly arranged dental tissue, including cementum directly associated with columnar odontogenic epithelium and fibrous connective tissue. The second lump was a neoplasia surrounding the inferior incisor root, composed by fibroblast-like spindle cells within a collagen matrix, admixed with foci of cementum. An heterophilic gingivitis centered around a plant fragment and bacterial aggregates was also evident controlaterally. The histologic diagnoses were elodontoma of the first molar tooth, cementifying fibroma of the incisor root, and focal heterophilic gingivitis with foreign body. Elodontomas are space-occupying lesions of continuously developing odontogenic tissue. They are considered by many authors hamartomas rather than true neoplasm (3). In 2006 Boy and Steenkamp (3) proposed this term to replace the term "odontoma", aiming at distinguishing hamartomas of continuously growing elodont teeth, from those of anelodonts, detectable only in developing teeth of younger animals. Cementifying fibroma is a rare tumour, already seen in horses and dogs. Its matrix component has features of cemental differentiation including complex basophilic lines growing in a mosaic or lamellar pattern typical of cementum (4). Both these neoformations are locally destructive, disturb normal odontogenesis and require complete excision but have no metastatic potential (4). Molar elodontoma and inflammation of the mouth soft tissue have been bound to molar malocclusion-related laceration and food impaction (1). Maxillary elodontoma can be highly disruptive to the sinuses and nasal cavity, causing severe respiratory deficiency, whereas mandibular elodontoma is generally less symptomatic, frequently causing lumps along the ventral mandible and making eating difficult (1; 3). As dental diseases are frequent in elodont captive animals, it is important to develop a good understanding of these conditions for effective prevention and treatment.

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LIPOMATOSIS OF A MANDIBULAR SALIVARY GLAND IN A DOG

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Salivary gland enlargements in dogs are rare and they include inflammation (sialoadenitis), salivary mucoceles, infarction and neoplasia. Tumors are predominantly carcinomas or adenocarcinomas (3). Another rare condition causing salivary gland enlargement is lipomatous infiltration (lipomatosis of the salivary gland) (1,2,3).

This report describes a case of lipomatosis of the salivary gland in a dog. Material and methods: Cytological smears were obtained by fine needle aspirate, air dried and stained with May-Grünwald Giemsa. Histological sample were formalin fixed-paraffin embedded and stained with Hematoxylin and Eosin.

An 8 year-old, male viszla dog was presented with a 2 years history of a slowly growing mass in the right submandibular region. The dog was asymptomatic. The physical examination revealed a soft, not movable and not painful retromandibular mass. The regional lymph node was not palpable. The laboratory tests, including complete blood count, chemistry profile and coagulation profile were unremarkable. CT examination revealed a soft tissue density mass in the right submandibular space, measuring 10 x 7 cm. The mass had an irregular shape and extended from the temporomandibular joint to the 3rd vertebral body. Based on the CT images, the mass apparently arose from the mandibular/parotid gland. Cytological smears revealed lipid droplets and two types of cells: rare mature adipocytes alone or in small groups and clusters of epithelial cells with acinar structures. The epithelial cells were round to cuboidal, with dark blue to clear foamy cytoplasm and single round nucleus with a small nucleolus. Occasional spindle cells were also present. The cytological features were suggestive of an epithelial glandular neoplasm. The mass was excised and the dog completely recovered after the surgical procedure.

Histologically the lesion was composed by a well differentiated salivary gland, preserving its lobular architecture, and severely expanded by the presence of abundant well differentiated adipose tissue infiltrating the interlobular and intralobular septa. Adipose tissue separated the salivary acini and excretory ducts. The histological features were consistent with lipomatosis of the salivary gland.

Lipomatosis of salivary gland is a rare condition characterized by fatty infiltration of the salivary gland (1,2,3). It can be differentiated from true neoplasm of adipose tissue based on the presence of salivary gland cells scattered throughout the adipose tissue (3). As previously reported in the literature, in this case the lesion was slowly growing and monolateral (1,2,3). Lipomatosis should be considered in the differential diagnoses of monolateral enlargement of the salivary gland in the dog.

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IMMUNOHISTOCHEMICAL EVALUATION OF P62 IN CANINE MAMMARY TUMORS

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In the last years in human and veterinary oncology most of the pathogenetic studies regarding mammary tumors has been paid to apoptosis and autophagy mechanisms. P62 can be considered the crossroad molecule of autophagy and apoptosis (2).

The p62 protein, also called sequestosome 1, is a ubiquitin-binding scaffold protein that polymerizes via an N-terminal PB1 domain and can interact with ubiquitinated proteins via the C-terminal UBA domain. P62 is found in cellular inclusion bodies and in cytosolic protein aggregates that accumulate in various chronic, toxic, and degenerative diseases(1).

In veterinary medicine, the role of p62 in tumors is poorly understood. A recent study has been performed in canine cutaneous mast cell tumors (3). The aim of this study is to evaluate the immunohistochemical expression of p62 in normal mammary tissue, in adenomas and carcinomas of the dog.

The immunohistochemical analysis were performed on thirty-six mammary tumors and eight normal mammary tissues present in archive of Laboratory of Animal Pathology - University of Camerino. The samples were histologically classified according to criteria of WHO. When present, the regional lymph nodes were analyzed too.

Immunohistochemistry was carried out by the Streptavidin-Biotin-Peroxidase method using as primary antibody an anti-p62 antibody (Sigma- Aldrich). Immunohistochemically, we have found specific reaction to p62 in epithelial cells of normal and neoplastic tissues. All normal mammary tissues, normal, and hyperplastic lobules exhibited a strong, homogeneous positiveness towards p62. Almost all epithelial cells showed a brown granular stain in the cytoplasm while the nucleus was negative. Only 5% of myoepithelial cells were immunostained while the stroma was always negative. In all adenomas immunostain to p62 was enough intense but the percentage of epithelial positive cells was lower (65%).

In malignant tumors, the immunoreaction appeared heterogeneous both between samples and within the same sample. In fact, 19 tumors (68%) showed little areas strongly positive close to others hardly negative while 9 tumors (32%) exhibited a diffuse weak stain. Only two of 7 high-grade carcinomas appeared positive to p62. Metastatic cells in lymph nodes were p62 positive in 50% of cases. These data could suggest a correlation between p62 expression and neoplastic progression because in carcinomas p62 overexpression is not observed. To date, as the paucity of samples examined and the complex role of p62 in autophagy and apoptosis, we believe that is not possible to consider p62 a progression marker in canine mammary tumors.

In the future will be interesting to compare these results with data obtained from breast cancer studies where a few authors hypothesize a negative correlation between p62 expression and neoplastic progression while most authors believe that p62 play a role in the interactions between epithelial neoplastic cells and stroma.

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CHRONIC INTESTINAL PSEUDO-OBSTRUCTION WITH SEVERE MYOPATHY AND FIBROSIS IN A YOUNG MINIATURE BULL TERRIER

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Chronic intestinal pseudo-obstruction (CIPO) is a rare clinical syndrome in veterinary medicine defined by severe intestinal dysmotility without evidence of mechanical occlusion of the intestinal lumen. A few canine cases of CIPO have been reported and most have been related to an idiopathic sclerosing enteropathy or fibrosing gastrointestinal leiomyositis, less frequently to dysautonomia (3). In human medicine CIPO can be caused by different gastrointestinal neuromuscular diseases (GINMDs) including primary visceral neuropathies, interstitial cell diseases and myopathies (3). A one-year-old male miniature bull terrier dog was presented with chronic weight loss, regurgitation, vomiting and diarrhoea. On exploratory laparotomy the small intestine was not obstructed but appeared markedly distended with fluid and gas and the wall was thinned. Full thickness intestinal biopsies of small intestine were obtained. Due to the persistent clinical signs of dysmotility the dog's clinical condition severely deteriorated thus euthanasia was elected. Necropsy confirmed that small intestine was severely dilated and filled by a moderate amount of greenish fluid content. The wall was diffusely thinned and atonic. A complete set of tissue was taken for histopathology, including various portions of intestinal tract. Sections of intestinal tract were also stained with periodic acid-Schiff (PAS) and Masson trichrome and were submitted to immunohistochemistry using antibodies to alpha-smooth muscle actin (α-sma), neurofilament, synaptophysin, neuron specific enolase (NSE), CD117, glial fibrillary acid protein (GFAP), CD3 and CD79. Histological findings of the small and large intestines consisted of severe diffuse atrophy of the tunica muscularis and severe locally-extensive to diffuse fibrosis of submucosa as demonstrated by Masson trichrome stain. Additionally intestinal mucosa appear multifocally eroded. The myenteric and submucosal nerve plexuses had intact neurons confirmed by immunohistochemistry for NSE, neurofilament and synaptophysin without inflammatory infiltrates. Also interstitial cells of Cajal were preserved and were strongly stained for CD117. However α-sma immunoreactivity was markedly reduced in the muscular layers of all the different intestinal sections examined with foci of complete loss. Loss of α-sma expression is recognized as a marker for intestinal dysmotility and myopathy causing CIPO in human medicine. Recently a case of CIPO associated with deficient expression of α-sma in the muscular layer and loss of myofibrils has been described in a Bengal cat and a leiomyopathy has been hypothesized (1). The clinical, histopathological and immunohistochemical findings of this rare case is consistent with enteric myopathy and fibrosis and could be referred to a GINMDs as in human medicine.

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CASE REPORT: DIAGNOSTIC APPROACH FOR IDENTIFICATION OF A ZINC PHOSPHIDE POISONING IN A BADGER (MELES MELES)

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Zinc phosphide is a dark grey, crystalline compound used as a rodenticide but in Italy it is frequently used for malicious poisoning of domestic and wild animals. Identification of the cause of death represents a key element of the investigation process aimed at identifying criminals as well as preventing further risks for poisoning in both animals and humans. In the present work we report a case of poisoning in a badger (Meles meles) found in the countryside area of Grosseto, Tuscany, Italy. The animal was discovered by Provincial Police while showing convulsions and foam at the mouth and soon after it died. Based on suspicious of animal poisoning, the carcass was delivered to the National Reference Center for Veterinary Forensic Medicine, located in Grosseto.

The aim of the study is to describe the diagnostic approach adopted in a case of suspected poisoning and to describe the post-mortem and laboratory findings observed in a badger dead for zinc phosphide poisoning.

Post-mortem analyses were carried out using the forensic approach thus, all steps of necropsy were documented by photos taken using a metric reference system (ABFO). Based on anamnestic information and post-mortem findings, brain samples were collected for virological investigation; samples of oesophagus, stomach, intestine, brain, liver, spleen, pancreas, myocardium, lung and kidney were submitted for histological examination. The contents of the stomach and samples of luminal contents of small and large intestine were collected for toxicology.

Soon after death the carcass showed a rectal temperature of 43,4°C. At necropsy, carried out twenty hours after death, the rigor mortis was still present. A generalised subcutaneous congestion was observed. Blood of dark appearance was present in both pleural and peritoneal cavities. Lungs were congested and moderately oedematous. Hydropericardium and congestion of pericardium were recorded at cardiac level. Myocardium was atonic and interested by focal areas of tissue degeneration. Hazel (Prunus spp.) and some dark grey granules with characteristic garlic smell were found in the contents of the stomach; the gastric and intestinal mucosa and the pancreas were congested. The liver was moderately increased of volume, congested and friable. Congestion of meninges was also observed. Histology mainly revealed: multifocal necrosis of myocardium; mild multifocal fatty changes associated with central venous congestion and sinusoidal dilatation in liver; multifocal tubular cloudy swelling and congestion in kidney. All virological tests carried out as differential diagnosis turned out negatives, whereas gastric-enteric contents resulted positive for zinc phosphide.

Following ingestion of zinc phosphide, in presence of gastric acids, it is hydrolysed in phosphine gas that is rapidly adsorbed by gastric mucosa. Once phosphine enters the circulatory system, it causes major metabolic acidosis with systemic consequences. Both post-mortem lesions and hystopathological findings reported in our case reflect those described in the literature and mainly in humans.

Bay et al. 1980
Bildfell et al. 2013
Bumbrah et al. 2012
Dogan et al. 2014
Gray et al. 2011

Krishnakumari et al. 1980
Link 1953
Murphy 2002
Poppenga et al. 2005
Stephenson 1967

TORSION OF THE URINARY BLADDER IN A DOG

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Diseases of the lower urinary tract frequently occur in dogs. In particular, cystitis, urinary incontinence and urolithiasis are the most commonly reported disorders affecting the lower urinary tract in that animal species (1). On the contrary, urinary bladder torsion represents a very rare condition in dogs.

The aim is to describe a case of torsion of the urinary bladder in a dog.

An adult, female, neutered German Shepherd showed depression and was very painful on abdominal palpation. According to what reported by the owner, the dog lived outdoor in a large garden, clinical signs suddenly appeared and rapidly worsened. The dog was referred to a veterinary clinic, where it died few minutes later. Therefore, the carcass was submitted to diagnostic investigations at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" (Teramo, Italy).

At necropsy, the urinary bladder was extremely expanded and occupied a major part of the abdominal cavity. Both ureters were also distended, while blood vessels of the urinary bladder wall, as well as of the renal capsule, appeared markedly congested. A bladder torsion of approximately 360° was evident at the level of the trigone. On cut section, the bladder wall was thickened and congested, while the mucosal surface was wrinkled, congested and hemorrhagic. No relevant lesion was observed elsewhere. On the basis of the gross findings, the torsion of the urinary bladder was diagnosed.

The torsion of the urinary bladder is occasionally observed in sows and in cattle affected by the torsion of the uterus (2). To the best of our knowledge, only two cases of urinary bladder torsion have been previously described in dogs, as a complication after ovariohysterectomy (3) or of presumable traumatic origin (4). In the present case report, the presence of any reasonable predisposing factor remained unknown.

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WOLF PREDATION ON LIVESTOCK: TRUTHS, SIMULATIONS AND COSTS IN A PROTECTED AREA IN CENTRAL ITALY

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The peaceful cohabitation between large carnivores and livestock is almost impossible. However, various and complementary measures can be carried out to manage such conflict, including compensation for damage to livestock. Compensation should integrate other preventive strategies, without becoming an additional tool for agricultural subsidies. In this respect, veterinary inspections are crucial to evaluate the real impact of predation and to minimize fraudulent behaviors (1).

To report data collected from 2004 to 2014 within a protected area (Gran Sasso Monti della Laga National Park, GSLNP) in Central Italy, in order to confirm/rule out wolf predation.

In total, 1,774 small ruminants, 376 cattle and 230 equids were reported, and therefore investigated as presumed cases of wolf predation. A special emphasis was placed on pathological features useful to differentiate wolf predation from other non-compensable events (e.g. predation by free-ranging dogs, fraudulent simulations, accidental wounds). Data about the costs for compensation were also collected and provided herein.

Wolf predation was confirmed in 1,326 (74.7%) small ruminants, 172 (45.7%) cattle and 125 (54.3%) equids, while fraudulent simulations of wolf attack were detected in 60 small ruminants (3.3%), 19 cattle (5.0%) and 3 horses (1.3%). The ratio wolf predations/total reports progressively increased during the period under study. A drastic drop of costs was observed between 2003 and 2004, with the beginning of veterinary inspections.

Our data indicate that the wolf is the major liable for livestock damage caused by predators in GSLNP. Wolf predation might be overestimated due to the presence of free-ranging dogs. However, wolves and dogs show different predatory behaviors, which deeply influence the pathological findings and could be extremely useful to correctly identify the predator (2).

Fraudulent simulations of wolf predation are relatively few and decreasing within the GSLNP; at the same time, illegal killing of wolves has been never reported during the last 15 years. Taken together, our data suggest that the conflict between wolves and human activities is efficiently managed. The progressive increase of the ratio between wolf predations and total reports - i.e. the reduction of reports due to other causes - further supports such belief. In conclusion, the correct identification of wolf predation, along with the implementation of complementary strategies, seem useful to manage the cohabitation between predators and livestock and, as a consequence, for the conservation of endangered predators.

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ETHYLENE GLYCOL TOXICITY: A RETROSPECTIVE PATHOLOGICAL STUDY IN CATS

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Ethylene glycol (EG) is an organic compound responsible for intoxication by ingestion in humans and animals. EG has in itself a low toxicity, but is rapidly metabolised in toxic compounds that determine severe acidosis, deposition of calcium oxalate crystals with acute, severe and often fatal renal, cardio-respiratory or nervous clinicopathological alteration (1, 2).

Few reports are available on gross pathological signs in animals and are limited to renal changes (3, 4). Aim of this study is to describe anatomohistopathological changes in cats with ethylene glycol intoxication (EGI).

From 2011 to 2014, 637 cats were submitted to necropsy to confirm suspected poisoning, in the framework of a national surveillance program on poisoning (Ministerial Decree "Norme sul divieto di utilizzo e di detenzione di esche o di bocconi avvelenati" 08.12.2008).

If necropsy was sufficient to diagnose the cause of death, no further analysis were performed. Otherwise samples of organs were processed to assess anticoagulant or pesticides poisoning, or other causes of death (i.e infectious agents or degenerative diseases). When renal gross findings were compatible with EGI, histopathology with hemathoxylin-eosin and Pizzolato stain were applied to highlight calcium oxalate deposits

In 452 (71%) cases necropsy alone defined the cause of death. Six cases got a direct suspect of EGI on the basis of medical history and renal changes (bilateral nephromegaly). On the remaining cases (29%) histopathology and/or ancillary exams on lesioned organs were necessary to confirm the presumptive diagnosis. Seventeen of this cases (3%), submitted to histopathology either with vacant diagnosis or with suspect of feline infectious peritonitis (FIP), had diffuse, severe tubulonephrosis with dilation of proximal tubules, flattening, vacuolization and necrosis of the epithelium and intratubular or interstitial deposition of moderate to high amount of lightly yellow, refringent, round-shaped, radially disposed crystals, consistent with calcium oxalate deposits; no other relevant changes were noted in the other organs examined. In all the 23 aforementioned cases the presence of calcium oxalate crystals was confirmed by Pizzolato stain.

Gross findings in the 6 EGI-suspected and in the newly 17 detected cases were respectively: thoracic and/or peritoneal sero haemorrhagic effusion (5/6 and 12/17), hyperemia of lungs (5/6 and 9/17), hyperemia of liver (3/6 and 8/17), enlarged (6/6) or pale/degenerated (7/17) kidneys, and hepatic degeneration (1/6 and 5/17). This retrospective study on cats points out that in EGI, gross findings are not limited to renal changes (nephromegaly and/or pale kidney): serohaemorrhagic cavitary effusions, lungs hyperemia, liver degeneration or hyperemia and degenerated kidney are frequent gross findings, as already described in humans (with the exception of effusions) (1).

EGI should not be ruled out in case of these macroscopic picture, even in the absence of typical renal changes, and especially when medical history is absent or with animals found dead. Microscopic examination, which is the unique postmortem method available, would allow to diagnose otherwise missed EGI cases.

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SOX9 EXPRESSION IN FELINE FIBROADENOMATOUS CHANGE

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Fibroadenomatous change (FAC) is a progesterone-responsive non-neoplastic proliferation of the mammary gland of the cat characterized by sudden, rapid onset. High Ki-67 proliferative index has been reported in FAC in several studies (Millanta et al., 2002) and an important role of autocrine and/or paracrine production of growth hormone and insulin-like growth factor has been suggested in its pathogenesis (Ordás et al. 2004) however, many pathogenetic aspects have not been clearly elucidated yet. Recent studies indicated that SOX9 transcription factor, in cooperation with Slug, controls the mammary stem cell state (Guo et al., 2012) and that increased ductal branching may be observed in transgenic mice overexpressing SOX9 in mammary epithelium (Wang et al., 2013).

To gain insight into the role of SOX9 in FAC development, we analyzed by immunohistochemistry SOX9 expression in FAC, non-FAC hyperplastic/dysplastic mammary lesions and normal mammary tissue of cat.

Materials and methods: Sections from FFPE tissue blocks of surgical biopsy samples of 10 FAC, 6 non-FAC hyperplastic/dysplastic mammary lesions and 3 normal mammary gland from female cats were examined for SOX9 expression by ABC immunostaining method, using a polyclonal rabbit serum produced with a polypeptide with 96% homology with Felis catus Sox9. Intensity was scored as negative, weak, moderate, strong. Percentage of reacting cells was evaluated by counting 1000 cells.

Positive SOX9 immunostaining of variable intensity and percentage was seen in all the samples analyzed and was essentially nuclear or in a few cases nuclear and weakly cytoplasmic. In normal mammary gland SOX9 was detected both in epithelial and in myoepithelial cells. Percentage of positive cells ranged from 33.2% to 55.4% (mean value 40.5%). Sox9 positive staining was seen only in a few stromal cells. Intensity was strong in one case and moderate and in 2 cases. In non-FAC hyperplastic/dysplastic lesions staining was intense and percentage of positive cells varied from 60.3% to 71.9% (mean value 66.4%); Sox9 positive staining was infrequent in stromal fibrocytes. All FAC samples showed moderate to strong SOX9 expression both in glandular and in stromal tissue; positivity ranged from 81.6% and 94.5% (mean value 86.5%) in ductal cells and from 51.4% and 86% (mean value 75.1%) in stromal fibrocytes.

This study provides evidence that in feline mammary FAC both glandular and stromal cells express high levels of SOX9. A possible role of SOX9 in tumor development and progression has been suggested however FAC is a non-neoplastic, benign mammary disease and the high-level expression of SOX9 observed in this condition should not be regarded as indicative of neoplastic transformation. Various studies suggest now that SOX9 plays multiple important roles in branching morphogenesis of several organs (Furuyama et al., 2011; Reginensi et al., 2011; Rockich et al., 2013), we hypothesize therefore that also in FAC SOX9 drives branching morphogenesis by controlling proper balance between proliferation and differentiation.

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ROLE OF NECTIN-4 IN CANINE PROSTATE CANCER

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Aggressive cancer cells are characterized by the ability to self-aggregate in order to survive and proliferate when an appropriate matrix anchorage is lacking. PVRL4 (poliovirus-receptor-like 4), also called Nectin-4, is a component of the E-cadherin-based adherens junctions in epithelial cells and potent mediator of the anchorage-independent colony formation in normal epithelial as well as cancer cells. Clusters of circulating tumour cells (CTCs) have been identified in blood samples of several tumours including prostate cancer (1,2). Targeted therapies aiming to block such cell-cell contacts may represent a novel anticancer treatment approach.

In dog, Nectin-4 expression has been only evaluated in relation to the Morbillivirus infection (3). Since Nectin-4 is a well-known tumour-associated histological and serological marker for several types of adenocarcinoma (lung, breast and ovary) (3) and it is expressed in a human prostate carcinoma cell line derived from a lymph node metastasis (4), we evaluated its expression pattern in canine prostate tissue to understand Nectin-4 role in prostate cancer pathogenesis.

The study was carried on formalin-fixed, paraffin-embedded samples from 42 canine prostate tissues including 2 normal prostates, 10 benign prostatic hyperplasia (BPH), 28 prostatic carcinomas (PCa), 1 pulmonary and 1 lymph node metastatic lesions. Immunohistochemistry was performed using a primary antibody specific for Nectin-4 (1:70). Nectin-4 expression was classified as membranous or cytoplasmic; samples were grouped in four categories based on the number of positive epithelial cells : absent (0%), low (0-30%); moderate (30-80%), high (>80%). The labelling intensity was recorded as weak, moderate,or strong.

Nectin-4 expression pattern showed a progressive loss during malignant progression and a switch in distribution from membranous to cytoplasmic. No immunostaining was observed in solid undifferentiated tumours. In particular, normal and BPH prostates showed high membranous distribution associated with low cytoplasmic positivity in BPH. In PCa samples, a low to high membranous distribution and low to high cytoplasmic positivity was observed, whereas metastatic cells, both in the lymph node and in the lung, exhibited a moderate/high membranous distribution and moderate cytoplasmic positivity. These results suggest the involvement of Nectin-4 in the CTC migration and maintenance during prostate cancer metastatization; furthermore, its loss of function in primary PCa may support the presence of the clivated form of the protein in canine serum (5) and thus its possible application as a serological marker in dog.

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ANGIOSTRONGYLUS VASORUM IN DOGS FROM CENTRAL ITALY, WITH THE DESCRIPTION OF THREE CASES OF DISSEMINATED INFECTION

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Angiostrongylus vasorum is a worldwide distributed nematode living in the pulmonary arteries and right heart of dogs and wild carnivores (1), with red fox considered its reservoir (2). Infection in dogs can be totally asymptomatic or cause respiratory and circulatory disorders (3). Migration of first stage larvae with dissemination to different organs can occur (2, 4).

In the last few years many clinical and pathological cases have been described in central Italy. Due to this increasing number of reports, a retrospective study on dogs from central Italy was performed, focusing on its prevalence and on the occourrence of disseminated infection.

Between January 2009 and February 2015, among dogs coming from Lazio region and submitted to IZSLT for necropsy, 433 cases were selected according to the following features: gross examination of heart and lungs and subsequent histopathology at least on lungs.

At necropsy a chronic, moderate to severe, locally extensive or diffuse pneumonia was noted in 8 cases (1.85%); histopathology revealed adults and larvae of A. vasorum in all lungs. In 4 cases (50%), pneumonia had a clear granulomatous appearance suggesting parasitic etiology, while in the remaining cases it was described as hardening of locally extensive areas of tissue with reddish discoloration, or with aspects of suppurative infection. Three dogs (30%) had adult parasites in the right ventricle .

In 3 cases histology confirmed the disseminated nature of infection, revealing larvae in multiple tissues (3/3 brain; 2/3 kidney and liver; 2/2 heart; 1/2 spleen). In 2 out of 3 cases (66%), adults in the right ventricle were associated with disseminated infection and in the third case, a focal hemorrhage with trombosis in the cerebral ventricles, with no evident intralesional larvae, was observed. In 6 cases (75%) gross pulmonary findings were severe and considered the cause of death; in remaining cases a severe hemoperitoneum due to traumatic liver rupture or cerebral hemorrhage occurred. Histopathology detected no A. vasorum parasites in other lungs of dogs with pneumonia.

Anatomohistopathological examinations revealed 8 cases of A. vasorum infection, with a prevalence of 1.85%, comparable to those described in other works on asymptomatic animals (5, 6) and higher compared to what described in northern Europe (7, 8). Gross pulmonary lesions observed on dogs were severe and frequently considered as cause of death. A. vasorum has not be observed without gross pulmonary findings, conversely to what described in wild foxes (9) Interestingly, disseminated infection was found in a high prevalence of cases (37.5%); this finding can be related to a different host/parasite relation, either for the increasing presence of the latter in specific areas, for changes in its virulence (10) or in case of host immune depression.

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HSP90 IMMUNOEXPRESSION IN CANINE CUTANEOUS EPITHELIAL AND MELANOCYTIC TUMOURS

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Heat shock protein 90 (Hsp90) is involved in the regulation of several biological processes such as cell signaling, proliferation and survival and guarantees the correct folding, functions and localization of numerous key proteins. Thus, Hsp90 inhibition has the potential to affect multiple signaling pathways that frequently contribute to the tumour development and progression2, explaining the recent and increasing interest in this molecule as a potential target for cancer therapy.1 Aim of the present study was to investigate the immunohistochemical patterns and levels of expression of Hsp90 in normal canine skin and cutaneous neoplasms, in order to understand the potential therapeutic application of a Hsp90 inhibitor in these types of tumour. Formalin-fixed, paraffin-embedded samples of 11 squamous cell carcinomas (SCCs), 30 follicular tumours, 8 melanocytomas and 10 melanomas were analysed using a streptavidin-biotin-peroxidase method. A semi-quantitative analysis of the immunoreactivity and Fisher's exact test were used to evaluate the associations between the examined parameters.

SCCs showed an increased cytoplasmic staining of neoplastic cells compared with surrounding normal epidermis, more intense in the outermost layers, with rare nuclear staining. Most of the follicular tumours showed an intense cytoplasmic staining, that was drastically reduced in the infiltrating cords and small clusters of neoplastic cells in the malignant cases analysed (pilomatricoma, trichoepithelioma), where it was associated with an increased nuclear staining. Half of the cases of melanocytoma showed a complete absence of immunostaining, while in most of the melanomas cytoplasmic Hsp90 was highly expressed, with a low to moderate nuclear expression. High levels of cytoplasmic Hsp90 immunostaining (>50% of positive neoplastic cells) were significantly related with malignancy in canine melanocytic tumours.

The present work demonstrates the expression of Hsp90 in the majority of the cases evaluated, indicating a role of the molecule in the development of canine cutaneous SCCs, hair follicle and melanocytic tumours. The diffuse and intense immunostaining observed in SCCs and the significant correlation of Hsp90 expression with malignancy in melanocytic neoplasms, would indicate Hsp90 as a possible molecular targets in the anti-cancer therapy, as suggested by recent experimental studies on non-melanoma skin cancer in murine models3,4, as well as in human melanoma cell lines.5 Interestingly, a partial response to the therapy, with marked decrease in size of the mass in an aggressive oral malignant melanoma of a dog was observed following treatment with the Hsp90 inhibitor STA-1474.6 These data and our results suggest Hsp90 as a potential effective target in canine anti-cancer therapy.

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UNUSUAL MULTIFOCAL PULMONARY NEOPLASTIC LESIONS IN A CAT

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An 8 years old castrated male stray cat, daily fed and looked after by feral cat caretakers was found death near the feline colony where it lived. The body was referred to the University of Milan for the necropsy. The cat was severely dehydrated, had lost incisives with severe gingivitis and abundant tartar accumulation and presented a shrunken, reduced in volume, left eye (phthisis bulbi). The most relevant alterations, affecting the abdominal and thoracic organs, were renal papillary erosion and necrosis associated to irregular renal profile with severe scarring and yellowish, pale cortex with numerous cortico-medullary strikes, and severe pulmonary atelectasis of the caudal lobes. Two pearly white, rounded, flat lesions, less than 1 cm in diameter, were detected in the left and right caudal lobes. Histologically, pulmonary lesions consisted of numerous, well circumscribed, not encapsulated nodules composed of irregularly arranged, tubular and/or dilated acinar structures, lined by a single layer of tall, columnar epithelial cells with abundant clear cytoplasm and basally located nuclei. Mitosis were less than 1 for HPF and anisokaryosis and anisocytosis were mild. Renal lesions were bilateral and diffuse. Histologically, they consisted of ulceration and necrosis of the papilla, numerous perivascular to interstitial aggregates of lymphocytes and plasmacells associated to severe interstitial fibrosis, glomerular synechiae/sclerosis and tubular degeneration, necrosis and mineralization. A diagnosis of end stage kidney, the most likely cause of death, was posed.

Immunohistochemical investigation for thyroid transcription factor-1 (TTF-1), AE1/AE3 cytokeratins (CKs), CK5, smooth muscle actin (α-SMA) and histochemical staining with PAS and alcian blue (AB) (pH 2.5) was performed. Neoplastic glands were diffusely positive for CKAE1/AE3, CK5 and PAS. AB staining was faint and multifocal, while TTF-1 and α-SMA were negative. Based on histological and immunohistochemical findings, a diagnosis of mucus gland adenoma was formulated. Lung tumors, namely bronchial gland carcinoma, bronchiolo-alveolar tumors and squamous cell carcinoma, have been extensively reported in cats, even though they are overall considered rare tumors. Conversely, to the authors' best knowledge, pulmonary mucus gland adenoma has not been reported to date in the feline species. In human beings, mucus gland adenomas are extremely rare tumors, arising mostly within the main, lobar or segmental bronchi and more rarely in the lung periphery. They are often endobronchial and multicystic, causing signs and symptoms of obstruction.

The present report described the first case of peripheral lung nodules arising from the submucosal mucinous gland in a peripheral small airway in a cat, an unusual and rare benign lesion that shares many similarities with the human counterpart.

NASAL CARCINOSARCOMA IN TWO DOGS

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Carcinosarcoma (CS) is a rare tumor composed by two cell types, epithelial and mesenchymal, both showing features of malignancy. It is rarely reported in animals, mostly in canine mammary gland1and more rarely in the head2, thyroid gland3and other organs. Aim of the work is to describe two cases of nasal carcinosarcoma, never previously reported in the dog. Meterials and methods: CASE 1: a 7 years-old, male, crossbred dog was examined clinically for persistent bloody nasal discharge. X-ray examination and computerized tomography scans showed a mass lesion in the right nasal cavity. The cytological exam revealed two types of cells: huge clusters of epithelial cells and single spindle cells admixed with inflammatory cells and scattered osteoclasts. Histological examination revealed a biphasic neoplasm composed by solid trabeculae of epithelial cells with moderately abundant eosinophilic cytoplasm, oval nuclei and multiple prominent nucleoli; the second tumor type was composed by polygonal cells occasionally embedded in a eosinophilic amorphous extracellular osteoid matrix with multifocal mineralization and bone remodelling. Anisocytosis and anisokaryosis were marked in both the populations and the mitotic activity was high. A final diagnosis of carcinosarcoma was done (transitional cell carcinoma and osteosarcoma). The dog was euthanized and submitted to complete necropsy, that failed to show any secondary neoplastic lesion. CASE 2: a 6 years-old, male neutered, crossbred dog was referred for a catarrhal-hemorrhagic nasal discharge with sneezing and noisy breathing. Rhinoscopy revealed an exophytic tumour filling the left nasal cavity and extending till choanae. Histological examination of endoscopic biopsies revealed a biphasic tumor composed by solid lobules of epithelial cells admixed with a more undifferentiated tumor composed by polygonal to spindle cells with multiple areas of osteoid deposition and mineralization. Anisocytosis and anisokaryosis were moderate in the epithelial component, marked in the mesenchymal part of the tumor. A final diagnosis of carcinosarcoma (undifferentiated carcinoma and osteosarcoma) was made. The owner refused any therapy other than palliative treatment with FANS and prednisone, prolonged until euthanasia 3 month after the diagnosis. No secondary lesions was suspected based on clinical examination, but necropsy was not done. In both cases the biphasic nature of the tumor was confirmed by immunohistochemical examination with cytokeratin and vimentin, that stained respectively the epithelial (carcinomatous) and mesenchymal (osteosarcomatous) portion of the tumor. Carcinosarcoma in dogs is rarely reported; mammary carcinosarcomas are highly malignant with reported metastatic rates up to 100%1; other sites are thyroid gland3 and head (frontal skull and maxilla): in this latter the tumour appears to be less aggressive, with no metastases in 4 cases reported2. None of these two cases of nasal carcinosarcoma were associated with evident metastatic disease, even if complete necropsy was done only in one case. Further studies are needed to assess the biological behaviour of nasal carcinosarcoma in the dog.

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Part of this work was performed during the activity of "Registro Tumori Animali" of Umbria region

CANINE ORBITAL PSEUDOTUMORS: A REVIEW OF 9 CASES

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The term orbital pseudotumor was introduced by Birch-Hirschefeld (1905) to describe a space-occupying orbital mass grossly consistent with a neoplasia but histologically composed of a mixed inflammatory infiltration. More recently the definition idiopathic orbital inflammation -(IOI) hasbeen proposed for this entity. Aim of this study is to review and immunohistochemically characterize 9 cases of canine orbital lesions previously diagnosed as orbital pseudotumors.

Six cases were from Veterinary Pathology Archive (University of Milan), 4 cases were provided by Dr Dubielzig (COPLOW, US). Microtomic section were Hematoxylin and eosin stained and immunolabelled with antibodies anti vimentin, alpha smooth muscle actin (α-SMA), MHCII, lysozyme, CD3 and CD20.

Affected dog were aged 4.5 to 13 years (mean 7.7); 5 males/4 females. Different breeds were represented. Three pseudotumors affected the dorso-lateral portion of the orbita with lacrimal gland infiltration in 2/3. Six pseudotumors were located deep in the orbita without connection with any specific structures. A nodular mass, with focal infiltration of surrounding tissues, was surgically removed preserving the eye in 6/9 cases. In 3/9 cases the mass required orbital exenteration. Histologically, all lesions were composed of a mixed inflammatory cell population: in 6/9 macrophages/histiocytes predominate, with a variable number of lymphocytes, scattered plasmacells, neutrophils, large fibroblasts and occasional eosinophils (granulomatous pattern). In 3/9 cases, macrophages/ histiocytes, lymphocytes and plasmacells were almost equally represented, with fewer granulocytes. In 2/3 cases, affecting the lacrimal gland, large bundles of dense, collagen rich fibrous tissue, were evident. These cases were histologically consistent with the sclerosing form of human IOI. Immunohistochemically, histiocytes were consistently MHCII stained, scattered macrophages were lysozyme positive . Lymphocytes T (CD3) and B (CD20) were always present, CD3+ T cells predominating in 5/9 cases. In 2 cases sclerosing IOI-like pseudotumors, fibroblasts were α-SMA and vimentin stained (myofibroblast), fibroblast in granulomatous pseudotumors were α-SMA negative/vimentin positive.

In the present review of canine orbital pseudotumors, all lesions were consistent with an idiopathic inflammation of orbital soft tissues. The authors propose that, consistently with human medicine, the definition "idiopathic orbital inflammation (IOI)" is adopted. Granulomatous inflammation was the most common histological-type in dogs. This pattern was histologically and immunohistochemically strikingly similar to another idiopathic orbital condition: canine nodular granulomatous episcleritis (NGE). Further study could elucidate if NGE and granulomatous-IOI are actually the same disease in different locations. In 2 cases pseudotumors affected the lacrimal and were associated with prominent fibroblastc/myofibroblastic proliferation. In men it has been suggested that these cases could represent chronic dacryoadenitis and that the release of lacrimal secretion could induce fibroblast/myofibroblast proliferation.

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INTRANUCLEAR GLYCOGEN IN OXYNTIC CELLS IN CANINE GASTRIC BIOPSIES

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Foreign material within the nuclear contour seen in histological sections is referred as inclusion (I) or pseudoinclusion (PI), depending on the absence or presence, respectively, of an infolding of the nuclear membrane around the material. Intranuclear glycogen is un uncommon finding described for the first time by Ehrlich in 1883 in hepatocytes of diabetic patients; afterwards this finding was reported in many other diseases (1,2), as well as in the liver of healthy individuals (3).

In animals intranuclear glycogen has been observed in liver of cows(4), of animals with chronic pyrrolizidine alkaloid poisoning(5), of western barred bandicoot with papillomatosis and carcinomatosis syndrome(3) and in few other conditions. Pathological significance of this lesion is unclear. To describe, to investigate the nature and to speculate about the pathological meaning of an histological finding observed in stomach of dogs during routine diagnostics, morphologically consistent with nuclear glycogen inclusions/pseudoinclusions (I/PI) in oxyntic (parietal) cells, never reported in literature in our knowledge.

Samples from 107 dogs submitted to endoscopy because of gastrointestinal clinical signs were routinely processed and evaluated at light microscopy for histological lesions. The samples with nuclear lesions in parietal cells, and an equal number of samples without histological lesions, were stained with PAS with and without diastase pre-treatment. Data were used to evaluate the associations between the presence of nuclear I/PI and signalment / histological diagnosis.

We found nuclear lesions in parietal cells consisting in enlargement of the nucleus with chromatin margination and central pale or slightly eosinophilic area with sharp contours; these nuclear I/PI were observed in scattered cells ranging from occasional (0-1/hpf) to numerous (4-5/hpf). The lesion was detected in 24 dogs and in 19 cases this finding was associated with gastritis, mainly lymphoplasmacytic, of mild severity, while in 5 cases there were no lesions. The nuclear I/PI showed PAS positivity (10/13) and diastase sensitivity (5/10), consistently with the typical pattern features of glycogen. In samples with nuclear lesions generally the cytoplasms were slightly PAS-positive, but did not show diastase sensitivity. We did not find a statistically significant association between gastritis and the presence of nuclear I/PI ($P > 0.05$). Possible pathogenetic mechanisms of glycogen accumulation within the nucleus are discussed: some authors stated that glycogen could be translocated from the cytoplasm through nuclear pores, others suggest the possibility of a pseudoinclusion; otherwise glycogen could also be synthesized in interchromatin regions of the nucleus. Further studies are needed (specifically, TEM examination is ongoing) to better determine the nature, pathological and functional significance of this finding.

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STEM CELL MARKERS IMMUNOEXPRESSION IN CANINE CUTANEOUS MELANOCYTIC TUMOURS

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Nestin and SOX9 were identified as specific markers of melanocytic stem cells. Nestin is a class IV intermediate filament, mainly expressed in the cytoplasm of neuroepithelial stem cells and in developing yet not differentiated endothelial cells of blood vessels. Nestin expression was increased in numerous cancers. In many tumors, such as melanoma, nestin has been identified as a prognostic factor. Transcription factors belonging to the SOX family have been shown to have a role in the survival and migration of oligodendrocyte precursors. A SOX family protein, SOX9, has been demonstrated to interact with SOX10 and BRN2 in melanocytic differentiation and to be strongly expressed in pigmented cells of cultured melanomas. Nestin, SOX9, BRN2 and SOX10 were found to be strongly expressed in primary and metastatic melanomas in humans, while the levels of expression of these molecules in melanocytic nevi were much lower. In particular nestin and SOX9, respectively, were associated with the presence of ulcerations in primary tumors and with a more advanced stage of disease progression and therefore considered as negative prognostic markers.

The aim of our study was to investigate whether these two markers could have a similar prognostic significance, through the correlation of their immunohistochemical expression levels and histologic features of malignancy. A total of 31 melanocytic tumors were included in the present study: 8 melanocytomas and 23 melanomas (4 metastatic melanomas; 9 amelanotic melanomas; 10 pigmented melanomas). Tumors were investigated by immunohistochemistry using a specific rabbit polyclonal anti-human SOX9 and a mouse monoclonal anti-human nestin antibody. SOX9 antibody is reactive with the dog, according to manufacturer's instructions and nestin antibody has already been used in two studies in the dog. With few exceptions, almost all melanocytic neoplasms investigated showed an absent or very low reactivity of neoplastic cells for both markers used. However the positivity of both external and internal positive controls used (hair follicles) confirmed the validity of these markers as putative stem cell markers in the dog, similar to what is described in humans. In particular, SOX-9 positivity was present in <5% of neoplastic cells in only three cases, while nestin immunoreactivity was noted in 5 cases. Three of these five cases, all represented by melanomas, were characterized by a percentage of positive neoplastic cells ranging from 40 to 60%. In general, the nestin-positive cells were located at the periphery (the invasive front) of the tumor. In one of these cases (metastatic melanoma), the reactivity was present both in the primary tumor as well as its metastases (lung, pancreas, adrenal). None of melanocytomas was positive for nestin. In conclusion, the results of this study in the dog, different from what is described in human medicine, dampen the use of nestin and SOX9 as valid prognostic negative markers in canine melanocytic tumors. However, the positivity for nestin in rare cases of melanoma, in the face of a total negativity for this marker in melanocytomas, would lead to speculate that this molecule may be involved in the process of malignant transformation of melanocytic cells. Further studies are needed to investigate this hypothesis.

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EFFECT OF GROWTH PROMOTERS ON APOPTOSIS PATHWAY OF VEAL CALVES TESTIS

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During spermatogenesis, apoptosis is an essential physiological event that controls the number of germ cells. Androgens and estrogens play an important role in the regulation of homeostasis and cell death in testicular cells [1-2], even if a long-term exposure to these molecules may alter the balance between cell survival and apoptosis. There are two main pathways involved in the apoptosis: the extrinsic pathway triggered by the activation of death receptors on the cell surface and the intrinsic pathway activated in response to signals originated from the inside of the cell. The intrinsic pathway involves molecules belonging to the BCL2 family. Both these pathways converge at the level of caspases. The aim of this study was to investigate the expression of some genes, involved in the apoptosis pathway, in testis of veal calves experimentally treated with growth promoters (GPs).

Forty Friesian veal calves, 6 months old, were randomly assigned to 5 experimental groups: group A (n=8) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.25 mg/die of brotizolam for 31 days; group B (n=6) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.4 mg/die of dexamethasone (DEX) for 31 days; group C (n=8) treated with 150 mg/2 weeks of Nandrosol for 4 weeks and 80 mg/die of ractopamine for 31 days; group D (n=8) treated with 15 mg/die of prednisolone (PRD) for 31 days; group K (n=8) was untreated. The animals were slaughtered at 3 days after the last treatment.

Samples of the testis were collected from each animal. Quantitative PCR (qPCR) of APAF1 (apoptotic peptidase activating factor 1), AVEN (apoptosis, caspase activation inhibitor), BAX, BCL2 and CASP3 (caspase 3) mRNA was performed. Statistical differences were determined by ANOVA, followed by Dunnett's post test. AVEN expression was significantly up-regulated in group A ($P<0.01$), whereas CASP3 expression was up-regulated both in group A ($P<0.05$) and in group C ($P<0.05$). No effect on APAF1, BAX and BCL2 expression and BAX/BCL2 ratio has been detected.

Estrogens and androgens are known to play a critical role in preventing apoptosis in a wide range of cell types. It has been reported that estradiol or DHT (5Îś-dihydrotestosterone) treatment increases the anti-apoptotic BCL2 levels and decreases the expression of both BAX and CASP3, two pro-apoptotic proteins [3]. Conversely, the hormone deprivation causes an increase of BAX expression, a decrease of BCL2 expression and the activation of caspases [4]. Our results point out a balance between BAX and BCL2 expression, whose ratio is often used as a biomarker for the apoptosis detection. Thus, the intrinsic pathway seems to be not involved, but the increase of CASP3 expression following estrogen or androgen administration suggests an increment in the apoptotic signalling mediated by extrinsic factors. AVEN is known to inhibit the apoptosis pathway [5]. The AVEN up-regulation observed in this study could be due to the interruption of the hormonal treatment. Therefore, these findings may be explained considering the withdrawal time. Indeed, in this period the estrogen and androgen protective effect probably declines, resulting in the increase of the downstream effectors of apoptosis.

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A CASE OF WARTY DYSKERATOMA IN A DOG

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Warty dyskeratoma is an uncommon human benign tumour described for the first time in two dogs by Hill (1987). It is a dermal mass with a nodular, cup-shaped or cystic architecture1, with an umbilicated center opening on the skin surface; when cystic, it is filled with keratin and cellular debris. Multiple lesions are rare2. Histologically, nodules are lined by squamous mature epithelium with foci of acantholysis and dyskeratosis3, and the lower portion shows dermal papillae resembling intestinal villi2. Some authors proposed a follicular origin because of its positivity to anti-keratin antibodies for cortex and inner root sheath of normal hair follicle4, but the localization of lesions in oral and genital human mucosa makes the pathogenesis confused5. An association with viruses (i.e. Papillomavirus) failed to be demonstrated2. The main differential diagnosis is acantholitic squamous cell carcinoma1,3. As warty dyskeratoma is a rare disease in dogs, with only 5 descriptions in veterinary literature, the aim of our work is to describe a new case. A 4-year-old crossbreed dog was presented to the clinician for a partially exophytic, dermal mass of 16 x 13 x 5 cm at scapula-humeral joint, without bone invasion. The mass was sampled in two different locations and the biopsies were submitted for histopathologic examination. Samples were routinely processed and observed at light microscopy; special stains were used (i.e. PAS, Gomori's trichrome and Giemsa) and IHC techniques (anti-keratin and anti-laminin antibodies) were applied to characterize the lesion. Histologic examinations showed the presence of multiple dermal cystic structures lined by a mature squamous epithelium with foci of acantholysis, often creating suprabasal clefts, as well as keratinocytes apoptosis and dyskeratosis; the lower portion showed dermal papillae lined by a single layer of basal cells resembling intestinal villi. Cystic structures were surrounded by an abundant fibrous stroma. Numerous neutrophils and multiple foci of mineralization were observed in these structures; occasionally, cystic rupture was associated with macrophagic and neutrophilic inflammation around epithelial elements. Anisocytosis and anisokaryosis were mild and mitotic index was low (4-5/10 hpf). PAS staining for fungal elements was negative. Immunohistochemistry with anti-cytokeratin and anti-laminin antibodies confirmed the epithelial nature of the lesion and the integrity of the basement membrane, respectively.

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COMPARATIVE ASSESSMENT OF CYTOLOGICAL VERSUS HISTOPATHOLOGICAL BIOPSIES IN THE DIAGNOSIS OF CANINE OSTEOLYTIC BONE LESIONS

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Primary bone tumors account for 2-5% of canine malignancies. Affected dogs often present with typical radiographic changes including cortical bone lysis, sclerosis and periosteal reaction. Osteosarcoma is the most common primary bone tumor in dogs. Being an extremely aggressive tumor, it should be differentiated from other less common tumor and from tumor-like lesions, such as fibrosarcoma, chondrosarcoma, hemangiosarcoma and osteomyelitis, as therapy and prognosis can vary greatly. Accordingly, a histological diagnosis is generally preferred before surgery, requiring general anaesthesia and collection of bone samples, with possible complications such as pathological fractures. An early and accurate diagnosis obtained by lesser invasive methods should be important to decrease patient discomfort and allow owners to make informed treatment decisions. Nevertheless, there is a paucity of information regarding the utility and accuracy of aspirate cytology of bone lesions in dogs.

Aim of the work/Objectives - The purpose of this study was to compare the diagnostic accuracy of cytological and histopathological biopsies of bone lesions in dogs with the definitive diagnoses performed by the histology on surgical samples.

A computer search of canine medical records at the Department of Veterinary Medical Sciences, University of Bologna, from January 2000 to present identified 41 cases of bone lesions that were sampled for cytology by fine needle aspiration (n = 21) or by incisional biopsy for histology (n = 20). Seven cases were sampled by both methods. The accuracy of both methods was assessed by comparing the former diagnosis with the final histological diagnosis on surgical samples or post mortem samples, when applicable.

The examined case series included 18 primary bone tumors, including osteosarcomas, chondrosarcomas, giant cell tumors, 4 carcinoma metastases and 12 non-neoplastic lesions, including osteomyelitis, osteonecrosis and reactive bone. Accuracy was 85% for cytology (86% for tumor lesions and 83% for non-tumor lesions) and 80% for histology (75% for tumor lesions and 87.5% for non-tumor lesions). Cytology correctly identified the tumor histotype in 6 out of 11 cases (54.5%).

The results of this study indicate that fine needle aspiration cytology is a reliable technique in the diagnostic work up of bone lesions in dogs. Accuracy is higher for neoplastic lesions compared with non-neoplastic lesions. Among bone tumors, cytology is moderately effective in distinguishing osteosarcoma from other tumors. Being an efficient, inexpensive and minimally invasive technique, cytology should be further considered to aid decision making in the preoperative setting of aggressive bone lesions.

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CLINICAL AND RADIOGRAPHICAL ASSESSMENT OF A SINGLE INTRA-ARTICULAR INJECTION OF PRP ON OSTEOARTHRITIC JOINT IN DOGS: PRELIMINARY STUDY

Erika Bianchini, Elisabetta Chiaradia, Antonello Bufalari, Francesco Mancini, Giulia Moretti, Daniele Mari and Antonio Di Meo

INTRODUCTION - Osteoarthritis and articular degeneration are the most important causes leading to a poor life quality both for patients and the owners. Many approaches had been developed in veterinary medicine and in the last decades clinicians are paying attention to infiltrative substances such as non steroid anti-inflammatory or steroid drugs, hyaluronic acid and blood derivates. One of the latter is platelet rich plasma (PRP), whose use is increasing but lacking of evidences and standardized procedures of preparation and administration. Its regenerative potential had already been established in human medicine, both in vitro and in vivo, and numerous experimental animal in vitro studies had been performed suggesting a promising outcome (1). Therefore in vivo studies are required in order to assess a real efficacy of PRP, a safety and relatively easy clinical application (2). AIM-The aim of this study is to point out the effect of a single PRP injection in osteoarthritic joints of dogs in a period of three months examining clinical and radiographical scores, other than owners satisfactory grade. MATHERIALS AND METODS-Autologous PRP preparation - Autologous anticoagulated venous blood samples were centrifuged twice, and platelet pellet was than resuspended in platelet poor plasma (PPP) at a final concentration ranging from 7 to 10 folds above whole blood platelet count under aseptic conditions .Patients - Six dogs (3.6 Âś 2.56 years old, body weight 35.05Âś16 Kg) with osteoarthritis involving a single joint were enrolled. All patients underwent a general visit and serum blood analysis. After sedation and intravenous anaesthesia, the autologous PRP was injected intra-articularly, after a clinical evaluation of synovial fluid aspect. Clinical and radiographical evaluations - Patients underwent a clinical evaluation (lameness, clinical objective assessement, response to manipulation) using standard tables with three grades at 0, 15, 30, 60 and 90 days from the infiltration. Radiographs of both the affected joint and the normal one were made at day 0, 30, 60 and 90 from injection. Projections for elbows were lateral standard at 130Â°,at 90Â°, at 45Â°, at maximum extension, and standard antero-posterior and antero-posterior with 15Â° of pronation; for other joints, standard lateral antero-posterior and postero-anterior were made. Same radiographical parameters were maintained during the study. Radiographs were evaluated following the International Elbow Working Group graduation by two radiologist blinded to the study. Satisfactory grade of the owners were assessed using the Liverpool Osteoarthritis in dogs questionnaire at the first visit and at day 90 of the follow up. CONCLUSIONS -An overall positive effect of the autologous treatment was observed in all dogs. Radiographical scores showed no statistical difference between all time points of the study, suggesting a stability of both affected and normal joints. Clinical lameness scores at 90 days were significantly different from those observed at 0 and 15 days ($p<0,036$); while clinical objective assessements and manipulation response were significant between day 0 and 90 days ($p<0,05$). All owners' questionnaires indicate a reduction in lameness and pain. BIBLIOGRAPHY - 1) Everts PA,et al. J Extra Corpor Technol. 2006 Jun;38(2):174-87. 2) Fahie MA et al. JAVMA, 2013;,243: 1291-97.

A PATHOLOGICAL SURVEY ON SICILIAN RAPTORS

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This study was carried out on 20 raptors obtained from some regional recovery centres. Birds have been collected during the years 2013 and 2014 and were stored frozen at -20 C. Later specimens were subdivided per species as follows: 8 buzzards, 3 kestrels, 3 honey buzzards, 1 marsh harrier, 1 lesser kestrel, 1 red-footed falcon, 1 owl, 1 barn owl and 1 horned owl. Necropsies were performed and tissue samples were obtained from all organs and tissues for histology and molecular biology. At necropsy undigested food with abnormal dilation of oesophagus and stomach was found in 8 subjects. In a buzzard, several granulomatous changes with a cavernous core were detected at the abdominal and thoracic air sacs, as well as in different coelomic organs. Grocott's staining showed several dichotomous and septated black hyphae; molecular exam confirmed perfect homology with Aspergillus niger. A metallic foreign body (steel nail, 20mm x 3mm) was found driven in an abdominal air sac. A lung mycetoma due to Candida sp. was found in a lesser kestrel. In another buzzard, 3 parasites identified as acanthocephala belonging to the species Centrorhynchus globocaudatus. Several trematodes belonging to the species Physaloptera alata were found in gizzard of a kestrel. 4 nematodes belonging to the species Dispharynx nasuta were found in a horned owl, fixed to the gizzard cuticle which showed erosion and inflammation. Finally, in another buzzard a carpus-metacarpus join luxation with ulnar epiphyseal fracture was found; histological exam performed on lungs and liver showed several cartilaginous emboli in the blood vessels and some free in the parenchyma. Pathological findings here reported provide useful information considering the lack of data available in literature. The application of molecular exam to identify specific pathogens confirms the meaning of this diagnostic tool in routine pathological examination. The unusual localization of D. nasuta in the gizzard must be underlined. Finally, the presence of huge amount of undigested food within the entire gastro-intestinal tract of 8/20 raptors probably suggests the need of an improvement of animal care and animal welfare, as well as of health management in recovery centres, considering also the difficulties related to the various interspecific differences among the wild species present in the Sicilian area.

Part VIII

II CONVEGNO R.N.I.V.

CHARACTERIZATION OF THE INTERACTION OF AFRICAN SWINE FEVER VIRUS WITH PORCINE MONOCYTES AND MACROPHAGES

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African Swine Fever (ASF) is a devastating viral disease that poses one of the greatest risks to the swine industry worldwide. It is currently endemic in Sardinia and there is no vaccine or treatment available (Costard et al., 2013). The aetiological agent is a large double-stranded DNA virus and mainly targets cells of the myeloid lineage (Sánchez-Cordón et al., 2008). Virulent ASFV isolates have developed strategies to avoid apoptosis, so that infected cells survive and further disseminate the virus (Sánchez et al., 2013). ASFV-infected monocyte/macrophages also produce monokines, such as TNF-α and IL-1α, which may trigger lymphocytes to undergo apoptosis and thus impair the induction of adaptive immune response (Fernandez de Marco et al., 2007).

Considering the importance of monocytes and macrophages in the pathogenesis of ASF, this study aims to examine in vitro the differences in response of porcine monocyte/macrophages to infection with an avirulent ASFV strain (BA71V) and a virulent Sardinian field strain (22653). In addition, the differences in the responses to ASFV infection of unactivated macrophages is directly compared to those of classically (M1) and alternatively (M2) activated macrophages.

Monocytes isolated from pig blood were infected immediately with ASFV using a multiplicity of infection (MOI) of 3 or were further differentiated into macrophages. Macrophages were left untreated or activated for 24 hours and then infected using an MOI of 1. Mock-treated monocytes/macrophages were included as controls. The effect of ASFV on the expression of surface markers (CD16, CD14, MHC II, MHC I, CD163) and the release of inflammatory cytokine TNF-α is under investigation. Using multiparameter flow cytometry, the phenotypic differences between infected and bystander cells can be characterised. The ability of both isolates (BA71V and 22653) to replicate in the different populations have been assessed with flow cytometry.

Our preliminary data show that for both isolates, infected monocytes presented a lower expression of CD14 and CD16 and an equivalent expression of MHC I compared to uninfected bystander cells. Thus, ASFV infected monocytes had a more active phenotype suggesting that infection induces differentiation of these cells towards a macrophage phenotype. Although, infected cells does not have a higher expression of CD163 than un-infected cells. It is hoped that data generated by this study will aid our understanding of the immunomodulation of host cell responses by ASFV and support the development of a vaccine or treatment.

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ATTENUATED MUTANT STRAIN OF SALMONELLA TYPHIMURIUM (STM) CONTRASTS TUMOR GROWTH AND PROMOTES ANTITUMOR IMMUNE PATTERNS

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Cancer is the second cause of death in the western world and within a few years will become the leading one in developing countries (1). The incidence of cancer, as well as the biologic behavior, pathologic expression, and recognized risk factors, in pet population are similar to what is observed in humans (i.e. non-Hodgkin's lymphoma, prostate, head and neck, and mammary carcinoma, melanoma, soft tissue sarcoma, and osteosarcoma) (2). The use of conventional treatment modalities (surgical removal, chemo and radiotherapy) has considerable limitations, including toxicity, poor tumor targeting, inadequate tissue penetration, which together often result in incomplete destruction of the tumors. Therefore, prevention and control of cancer diseases is an important task for today's medicine, due to either the possible implications of such diseases on public health or to their economic consequences. Therefore, the development of new therapeutic strategies to fight cancer is now a priority for research. In this regard, the use of bacteria as alternative cancer therapeutics, in particular their potential of bacteria to selectively target cancer cells has been studied for more than a century (3).

The aim of the study was to investigate the anti-cancer potential of an attenuated mutant strain of Salmonella Typhimurium (STM) devoid of the operon znuABC, coding for the high-affinity zinc transporter, which is important for the bacteria growth in environments poor in zinc and for the virulence of different gram-negative pathogens (4-6).

4T1 mammary adenocarcinoma cells were injected subcutaneously in immunocompetent Balb/c mice. Then, tumor and non-tumor bearing animals was injected or not (control) subcutaneously with STM. Tumor progression was monitored at different time points after treatment through size measurements and differences in mortality rates were observed among the groups. Phenotype and functional capacity of different immune parameters were explored by FACS and histological analysis of the peritumoral zone and spleen, and by assessing cytokine production through ELISA assay.

We showed that STM was able to penetrate and replicate into tumor cells in in vitro and in vivo models. The STM administration in mammary adenocarcinoma mouse model resulted in a significant reduction of tumor growth with a marked increase of life expectancy of STM treated mice. Finally, we provided evidence that STM promotes antitumor immune patterns, capable to influence clinical outcomes. On the whole, our results support the potential of STM as a promising anti-cancer therapy (5,6).

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SALMONELLA TYPHIMURIUM EXPLOITS GUT INFLAMMATION IN PIGLETS

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Salmonella Typhimurium (STM) is responsible for foodborne zoonotic infections. The human disease is characterized by self-limiting gastroenteritis that occasionally can cause fever and severe gut inflammation (1). Most of the current studies about STM infection have been conducted on murine models that naturally do not develop gastroenteritis. These models are also based on the lack of an intact microbiota, caused by antibiotic treatment, which limits a comprehensive evaluation of the complex interactions of STM within the gastrointestinal environment (2). STM acquires an evolutionary adaptation to overcome antimicrobial defences in the lumen of the inflamed intestine and to exploit inflammation in order to outcompete the intestinal microbiota in mice (3). Since STM is able to naturally infect pigs, inducing a disease closely resemble those in humans, it is reasonable to hypothesize that pigs can be proposed as the model of choice for salmonellosis and gastrointestinal research.

The aim of the study was to evaluate whether STM is able to exploit inflammation, favoring an active infection, using an experimental model of infection in piglets, as a paradigm of the pathogenic mechanisms occurring in humans during salmonellosis.

For in vitro studies macrophage cells were isolated by adherence from porcine peripheral blood mononuclear cells. Macrophages and porcine intestinal columnar epithelial cells (IPEC-J2 cell line) were primed overnight with LPS and/or the antagonist of TLR-4/MD-2 complex (RS-LPS), the natural ligand of LPS, infected with wild type S. Typhimurium (STM14028), and then analyzed for intracellular colonization. For in vivo studies piglets, pre-treated with LPS, were orally infected with STM14028 and euthanized 24-48 hours. Innate immune response was assessed by FACS and ELISA; STM14028 colonization was evaluated in ceca, spleens and tonsils.

We showed that STM14028 was able to efficiently colonize mono-macrophages and IPEC-J2 cells. However, a pre-treatment with LPS makes these cells more susceptible to infection with STM14028 resulting in a significant increase of the STM14028 colonization compared to the LPS-untreated group. This result was confirmed and strengthened by the use of the LPS-antagonist, which inhibited the LPS stimulation and significantly reduced the STM14028-intracellular colonization, restoring them to the values of the STM14028 colonization alone. Moreover, in vivo study indicated that, after infection of piglets by oral route with STM14028, the immune response rapidly react involving the innate compartment with a marked increase of granulocytes, mono-macrophages and neutrophils populations. Finally, the induction of inflammation,

by LPS-treated piglets, influenced the colonization of STM14028 inducing a significant increase of the production of the pro-inflammatory IL-1beta and TNF-alpha cytokines in the blood, accompanied by a marked increase of STM14028-colonization in tonsils, cecum and spleens, compared to the control groups. As a whole, these findings suggest that STM is able to exploit inflammation for its own benefit in a model of porcine gastroenteritidis.

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EXPRESSION AND IMMUNO-MODULATORY ACTIVITIES OF SWINE INTERFERON-α SUBTYPES

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Porcine Type I IFNs are a heterogeneous group including several distinct families like IFN-α, consisting of 17 subtypes with different antiviral activity and expression profiles. IFN-α subtypes are characterized by some structural differences resulting in distinct antiviral anti-inflammatory, and immuno-regulatory properties. Indeed, these proteins play an important role in the host's immune response and other homeostatic control actions. The characterization of their immuno-modulatory activities could be useful for evaluating their prophylactic and therapeutical potential for diseases sustained by infectious and non-infectious stressors (1).

Owing to the above, the aim of our study was to characterize the immuno-modulatory and anti-inflammatory potential of each swine IFN-α subtype.

IFN-α subtypes were cloned and expressed in CHO cells. The supernatant of mock plasmid-treated CHO cells was used as negative control (2). Swine intestinal epithelial cells (IPEC-J2) were treated with 10 IU/ml of each IFN-α subtype for 18 hours. Total RNA was extracted and the expression of IL-1β, IL-6, IL-8, IL-4, IL-10, TNF-α, IFN-β, βD1, βD2, βD3, βD4, NF-kB1, IL-18, TLR4, SOCS1 was determined by RT-Real-time PCR (3).

The addition of mock plasmid to cells produced a significant increase of βD4 (P=0.016), TNF-α (P=0.032) and IL-8 (P=0.0147) gene expression. The addition of rIFN-α1 or IFN-α6 determined a significant reduction of TNF-α (P=0.0018 and P=0.007, respectively) and IL-8 (P<0.0001, P=0.0012, respectively) gene expression. Moreover, IL-8 was significantly down-regulated by suIFN-α8 (P=0.0001), suIFN-α9 (P=0.0025), IFN-α10 (P=0.01) and IFN-α11 (P=0.014) with respect to mock plasmid control. The expression of βD4 was up-regulated after incubation of IPEC-J2 with IFN-α9 (P=0.0129), IFN-α10 (P=0.0028) or IFN-α11 (P=0.0028). Concerning the effects of IFN treatments on the expression of other cytokines, we obtained the following results. rIFN-α1: TLR4 and βD1 gene expression were down-regulated (P=0.06 and P=0.0123, respectively) after stimulation. IFN-α2 significantly (P=0.0366) decreased the expression of TLR4 and NF-kB1 (P=0.02). IFN-α8: it down-regulated βD1 (P=0.043) and NF-kB (P=0.04) gene expression. IFN-α9: treatment with this IFN modulated gene expression; in particular caused increase of IFN-β (P=0.09, tendency) and down regulation of NF-kB1 (P=0.049), IL-1β (P=0.0018) and βD1 (P=0.014). IFN-α10: IL-1β (P=0.0018) and IFN-β (P=0.012) gene expression were up-regulated after IFN treatment; the same effect was observed after suIFN-α11 treatment (IL-1β, P=0.0018 and IFN-β, P=0.046); in addiction, also βD2 gene expression showed a significant increase (P=0.014). Our study highlighted different profiles of immunological activity of each IFN-α subtypes. In particular, our results confirm an anti-inflammatory control action in vitro and outline the ability to modulate antimicrobial peptides (3). This study also suggests the possibility to employ some IFN-α subtypes to improve the expression of βD2 and βD4, known for their antimicrobial activity versus Gram positive and negative bacteria, and to regulate the inflammatory response underlying the occurrence of several opportunistic microbial infections of farm animals.

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SALMONELLA SEROVAR SPECIFIC MODULATION OF INFLAMMATION IN A JEJUNAL EPITELIAL CELL LINE

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Salmonella infections are an important source of food-borne illnesses and therefore a major public health concern. In Liguria, this pathogen have a prevalence of 9.8% in wild boars liver and the 85.1% of isolated were characterized by resistance of antibiotic(1). These evidence outline a possible risk for public health. However, no all these strain are associated to salmonellosis in animals and/or humans (2).

Owing to the above, the aim of our study was to evaluate the effect of Salmonella infection on inflammatory regulation in the porcine GI-tract using IPEC-J2 cell line, that represent a good model to study salmonella infection (3).

An overnight culture of 6 different Salmonella enterica strain: S. Coeln, S. Ablogame, S. Enterica subspecie Diarizonae (Strain 1), S. Veneziana, S. Enterica sub-specie Diarizonae (strain 2) and S. Thompson was sub-cultured for 2 h at 37Â°C in BHI. Each Bacteria cultured, re-suspended at 100 million CFU/ml in DMEM/F12 medium (4) was used to treated IPEC-J2 cell; untreated wells were employed as negative control. After 1 hours of incubation at 37Â°C with 5% of CO2 monolayers were washed three time; than 2 ml/wells of plain medium were added and cell were incubate for 4 hours at 37Â°C with 5% of CO2. Supernatants were harvested to evaluate IL-8 release by ELISA and the gene expression of IL-8 and NF-Kb1 was investigated by RT Real-time PCR (4). A Kolmogorov-Smirnov test was conducted to check Gaussian distributions, than differences between data sets were checked for significant differences by Kruskal-Wallis test, followed by a Dunn's test. The significance threshold was set at $P < 0.05$ with a correction for multiple comparisons.

Porcine intestinal epithelial cells more closely mimic human physiology than analogous rodent cell lines, which is important in studies of zoonotic infections. Moreover this cell line give information about pathogenicity of salmonella spp. (3). In our study we demonstrated that S. Coeln determine a significant increase both of IL-8 release (P=0.005; +527 pg/ml) that a gene expression (P<0.0001) with respect to control cells; also NF-Kb1 was up-regulated by this strain (P=0.059). S. Veneziana, like S. Coeln, determine a significant increase both of IL-8 release (P=0.0037; + 2898 pg/ml) that a gene expression (P<0.04) with respect to control cells; also NF-Kb1 was up-regulated by this strain (P=0.0168). Regarding S. Enterica sub-specie Diarizonae (strain 2) and S. Thompson caused a significant increase of IL-8 secretion (P=0.0021 + 3148 pg/ml and P=0.0007 + 3374 pg/ml; respectively) and up-regulation of IL-8 gene expression (P=0.0098 and P=0.0010); whilst, S. Ablogame and S. Enterica sub-specie Diarizonae (Strain 1) no modulated significantly any parameter under study. These data suggest a potential pathogenic of S. Coeln, S. Veneziana, S. Enterica sub-specie Diarizonae (strain 2) and S. Thompson. In particular, S. Coeln and S. Thompson data are in according with EFSA report (2). Moreover, S. Enterica sub-specie Diarizonae are usually found the environment and only occasionally are associated with human disease; however, one of our strain may be associated with disease. These evidence outline a possible risk for public health associated with the consumption of wild boars livers.

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INNATE IMMUNITY PARAMETERS IN MARCHIGIANA BREED: PRELIMINARY DATA

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Originating specifically in the Marche Region, the Marchigiana is a large breed kept for beef today. Marchigiana makes up 45% of the beef herd in Italy and have been exported internationally to the United States and elsewhere. They occasionally exhibit double muscling. The Marchigiana breeding is, in some farm rural and other industrial but has to respond always to the Community rules on animal welfare. Some pathologies in modern intensive production units are of conditioned type since their onset is influenced by a wide variety of environmental and managerial factors and the evaluation of non-specific immunity parameters does provide precise indications of animal welfare related to his living environment. In this study we evaluated the coping effort and the welfare situation in some rural or industrial Marchigiana breeding. The aim of this study is to investigate some innate immunity parameters Marchigiana under different environmental and clinical conditions

The study was performed in 36 farms and 270 blood samples were tested for the followers parameters.Serum lysozyme, serum bactericidal activity, total haemolytic complement , haptoglobin and serum-amyloid A.Serum lysozyme was assessed by the lyso-plate assay (Osserman and Lawlor, 1966). Total Haemolytic Complement was evaluated following the procedure described Seyfarth, (1976) and serum bactericidal activity (SBA) was performed in a assay in microtitre format (Amadori et al., 1997). Haptoglobin and Serum-amyliod A were measured by using commercial kit (Tridelta Development Ltd, Kildare, Ireland).

A questionnaire was used to categorize farms in three levels ("good", "intermediate", "bad"). The questionnaire takes into account three big aspects of the breeding: clinical and sanitary situation, structural and management issues and the management of the drug. The association between farms and innate immunity parameters was evaluated using Odds Ratio (OR) and relative confidence interval. A P-value < 0.05 was considered significant.

Data on the innate immune system (bactericidal activity, serum lysozyme and complement) and acute phase proteins (haptoglobin and serum-amyloid A) showed an excellent adaptation to all tipes of breeding systems applied in the controlled farms. The animals don't shown significant differences from the cattle normal range reported in literature. If stress can be defined as an environmental effect on an individual that over- taxes its control systems and reduces its fitness or seems likely to do so and animal welfare is "the status of an individual as regards its attempts to adapt to the environment" (Broom 1993), we can believe that the animals under study are able to cope with environmental stressors without developing disease.

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EVALUATION OF OXIDATIVE STRESS IN HEALTHY DOGS

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Inflammation represents a universal defense response triggered by both innate and adaptive immune mechanisms. Inflammation is also involved in tumorigenesis, playing a pivotal role in initiation, promotion, growth, invasion and metastasis processes. A wide range of mediators (e.g. cytokines, free radicals, prostaglandins)synergically contribute to a favorable microenvironment for tumor development. Thus, these molecules have been proposed as prognostic factors in many kind of tumors (e.g. colorectal carcinoma and breast cancer). The oxidative reactions are an integral part of the inflammatory response; in veterinary medicine, free radicals have been associated to several pathologies, such as cardiovascular diseases, lymphosarcoma, mammary carcinoma (1-2). More recently, it has been reported that oxidative stress seems to be related to tumor pathogenesis and prognosis in dogs(4). However, only few bibliographic data on the physiological values of oxidant and antioxidants species in domestic animals are currently available. Objective: The objective of this study was to evaluate the oxidative balance in healthy dogs. Two parameters were investigated: the reactive oxygen metabolites (ROMs), an important class of Reactive Oxygen Species (ROS) derivatives, and the biological antioxidant potential (BAP). The aim was to define a reference range, useful for comparison with both ROMs and BAP levels in tumor-bearing animals. Materials and Methods: 117 healthy, client-owned dogs were enrolled in this study by the Italian Reference Center for Veterinary and Comparative Oncology (CEROVEC). The analyses were performed on serum samples using a dedicated photometer and 2 specific commercial kits: d-ROMs and BAP tests(Diacron). Sex, age, sterilization, size, dietary conditions and daily life style were considered as variables. Analytical data are expressed in conventional units, i.e. 1 CARR U=0.08 mg hydrogen peroxide/dL (d-ROMs) and μmol/L of reduced ferric ions (BAP). Data were analyzed using SPSS v.21 software (IBM Corp.); the significance threshold was set at $P< 0.05$. Conclusions: The BAP ranged between 1255 and 5782 μmol/L, with a median value of 1936 μmol/L and a mean of 1994.16 ± 567.81 μmol/L. The d-ROMs ranged between 45 and 298 CARR U, with a median value of 89 CARR U and a mean of 94.37 ± 33.90 CARR U. With respect to data of previous studies in dogs, the BAP results are similar, whereas the d-ROMs values are slightly higher(5). Moreover, no significant differences were observed among the variables under study, with the exception of gender. The values measured in this study are lower than the BAP and d-ROMs normal values in humans, 2200 micromol/L and 250- 300 U CARR respectively (panel Carratelli, Diacron). It is possible to conclude that, although oxidative stress does not induce any clinical sign, it is important to evaluate the oxidative balance in healthy individuals under physiological conditions, to be offset against the values measured over and after a pathological status (6).

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INNATE IMMUNE RESPONSES IN BRONCHO-ALVEOLAR FLUIDS AFTER INFECTION WITH SWINE-ADAPTED OR NON-ADAPTED INFLUENZA VIRUS STRAINS

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Three IV subtypes (H1N1, H1N2 and H3N2) are currently circulating in swine herds in Europe (1), and have been associated with disease occurrence and gross lesions in swine (2). Also, pigs are susceptible to infection with low pathogenic and high pathogenic avian influenza viruses (LPAIV and HPAIV, respectively) (3). However, pigs have been shown to be susceptible to other IV strains that are able to cross the species barrier. Besides, it is well known that some influenza viruses are able to infect humans and pigs, as it was the case in the last H1N1 2009 pandemic infection (1). Additionally, the pig has been proposed as an animal model for human influenza as the anatomy and physiology of the porcine respiratory tract exhibit more similar features to humans than those of rodents (4).

Owing to the above, the aim of our study was to investigate the modulation of innate immune responses in bronchoalveolar fluids (BALF) after infection with swine-adapted or non-adapted IV strains.

6 groups of healthy pigs each were set up. One was the uninfected control group (group 1, six pigs). 12 other pigs were infected with a H3N2 Swine IV (Group 2). Four different H3N8 IV strains circulating in different animal species (dogs, horses, wild aquatic birds and seals) were administered to groups 3 to 6 (12 pigs each). At day 0, each pig in groups 2, 3, 4, 5, 6 was intratracheally infected with 2 ml of virus, containing 200,000 Doses (EID)50 of the corresponding virus strain. Animals were clinically inspected on a daily basis. Four pigs of each virus-infected group were euthanized at day 3, 6 and 21 post infection (p.i.), respectively. BALF were harvest to perform Real time PCR to evaluate the expression of the following genes: porcine IFN-α, IL-8, IL-6, bD1, bD2, IL-1b, TNF-α, IFN-α5/6, IFN-α7/11, IFN-α9, IFN-α13 and IFN-α16 (5-6).

All infected groups exhibited Ab responses to Influenza virus NP protein. Also, no antibody response was observed in mock-infected animals. The gene expression profile was the following. MOCK group: there was no significant difference between the different sampling times for each cytokine gene under study. SWINE group: only tendencies ($P< 0.10$) were shown for IL-8, IL-10 and IFN-γ genes. EQUINE group: significant differences were shown for IFN-α9, IFN-γ and IL-10 genes. CANINE group: significant differences were shown for IFN-α5/6, IFN-α7/11 and IFN-γ genes. AVIAN group: there were significant differences for IFN-α5/6, IFN-α13, IFN-α16 and IFN-γ gene expression, and tendencies for IFN-α7/11 and IFN-α9 genes. SEAL group: significant differences were observed for IFN-α5/6, IFN-γ and IL-10 genes. In all groups

infected with non-adapted strains there was a significant modulation of IFN- γ gene expression characterized by down regulation at day 21 after infection. Moreover, different influenza virus strains activated different IFN-α genes; in particular, genes of IFN-α subtypes with little if any antiviral activity were activated by the canine and seal strains. Also, each virus strain could be associated to an expression pattern of cytokine genes in BALF cells.

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BOVINE PARATUBERCULOSIS AND GAMMA INTERFERON TEST

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Traditional diagnosis of bovine Paratuberculosis (PTB), due to M. avium subsp. paratuberculosis (MAP), is based on serology and fecal culture. Gamma Interferon (γ -IFN) test, already used for ante-mortem diagnosis of bovine Tuberculosis (bTB) (1), is able to detect cytokine production by T lymphocytes after stimulation with mycobacterial antigens. γ -IFN test could be also useful for PTB diagnosis (2) and, at the same time, for detection of animals exposed to other mycobacteria belonging to the Mycobacterium avium complex. Istituto Zooprofilattico Sperimentale of Umbria and Marche, by permission of Italian Ministry of Health, since 1981 produces purified protein derivatives (PPDs) from Mycobacterium bovis (PPDB), from Mycobacterium avium (PPDA) and recently, for experimental use only, from MAP (PPDJ). In order to identify animals infected by mycobacteria, and particularly for early detection of subjects exposed to MAP infection, in the last decade we performed, in different cattle herds, a γ -IFN assay which provides the use of PPDB, PPDA and experimental PPDJs in the lymphocyte stimulation phase. At first, 391 sera of cattle older than 24 months, from officially bTB-free herds in Central Italy, collected during monitoring programs, were processed with PTB ELISA (IDVet), as screening test and then confirmed by PCR and cultural assays. For the γ -IFN test, 1 ml whole blood aliquots were stimulated respectively with PBS (blank), PPDs supplied by BOVIGAM kit (Prionics) and Italian PPDs. At the later time 3 new PPDJs extracted from culture of MAP field isolates were produced and compared to classic PPDs in the γ -IFN test. In this second study, 68 cattle, older than 12 months, from officially bTB-free herds, with previous PTB clinical cases, were included. All cattle were screened as previously described twice a year. PPDJs were added in γ -IFN test at dilutions 1:5 and 1:10. Samples have been considered positive when Optical Density (OD) value was, at least, twice the OD obtained after stimulation with PBS alone (blank). In the first study, 50/391 sera reacted in the screening test and 48 cattle were confirmed PTB positive by PCR and/or fecal cultures; out of these 48 PTB positive animals, Avian PPDs identified 43 subjects as M. avium infected cattle. In the later study, 55/68 animals were PTB positive, and 46 reacted to both PPDA and PPDJ, while only four to PPDJ. The sensitivity in PTB diagnosis of PPDJs was around 78%, but this value could be influenced by the poor sensitivity of the tests used as gold standard, which are able to detect only advanced stages of disease. In fact four of nine subjects, previously classified as PTB negative, but positive to PPDJ, became positive eight months later to serology and culture. Our preliminary results highlight the ability of γ -IFN test to avoid false positivity for bTB; for detection of MAP infection we obtained encouraging results, but more subjects should be included in the study to increase the robustness of γ -IFN with PPDJ, especially young animals with a suitable follow up.

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EFFECT OF POLYUNSATURATED FATTY ACIDS IN THE DIET ON SHEEP IMMUNE PROFILE AROUND PARTURITION

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The objective of the study was to characterize the immune profile of healthy dairy ewes fed flaxseed, rich in polyunsaturated fatty acids (PUFA), around parturition. The hypothesis to be verified was that a physiological stressor, such as parturition, could be overcome with a nutritional manipulation in the diet of the animal in order to decrease the incidence of diseases and mortality connected to post partum, and to guarantee animal welfare. Twenty Comisana ewes were divided in two groups (10 ewes/group), and fed a supplementation of whole flaxseed in the diet or not. All groups were individually fed twice daily and received 1.8 kg/ewe/d of oat hay. Control group (CON) received 1 kg/d of pelleted concentrate; flaxseed group (FS) was fed a supplementation of whole flaxseed, and received 750 g/ewe/d of pelleted concentrate, and 250 g/ewe/d of whole flaxseed. The animals were immunised with the antigen chicken egg albumin (Ova, 6 mg emulsified in 1:1 of sterile saline solution and incomplete Freund's adjuvant) at parturition, followed by a booster immunisation without incomplete Freund's adjuvant 7 days later. Blood samples were collected at parturition and then at 7, 14, 21, 28, and 42 d post partum in heparinized tubes; plasma samples were collected after centrifugation and stored at -20°C to assess the humoral immune response. At parturition, at 14 d, and 42 d post partum the level of plasma cytokines (IL) was assessed on plasma samples by sandwich ELISA test. All the data were analysed using ANOVA for mixed models using the MIXED procedure of SAS (2013), having the diet, the time of sampling and their interactions as fixed effects and the animal as a random factor nested in the treatment. At 21 d post partum, the anti-OVA IgG titer was higher in the ewes supplemented with flaxseed than in control ewes ($P < 0.05$). The IL-6 level in control ewes decreased starting from parturition to 42 d post partum; whereas, in flaxseed-supplemented ewes, the IL-6 level remained unchanged until 14 d post partum and then decreased from 14 d to 42 d post partum ($P < 0.01$). IL-10 level was higher in control ewes than in flaxseed supplemented ewes at parturition, then at 14 d the IL-10 level was higher in flaxseed supplemented ewes than in control ewes ($P < 0.01$). IL-1β level was found lower in flaxseed supplemented ewes than in control ewes at parturition. On average, IL-1β level was lower at 42 d post partum than at parturition and at 14 d post partum ($P < 0.01$). The level of TNF-α in plasma increased at 42 d in control ewes compared with TNF-α level registered in flaxseed supplemented ewes. In conclusion, PUFA from flaxseed, as supplement in the diet of ewes around parturition, can modulate sheep immune reactivity by influencing cytokine production.

S. AUREUS MODULATES TIR8 EXPRESSION DURING INTRAMAMMARY INFECTION IN GOAT

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Mastitis is a mammary gland inflammation with a bacterial aetiology. Invading pathogens activate the immune response engaging leukocytes and epithelial cells of the udder. To better understand the innate immune response in goat mammary gland, a model of S. aureus intramammary infection has been established(1). We investigated the expression of TIR8 in blood and milk cells and in mammary tissue. TIR8 is an important molecule of the innate immunity involved in the early response to invading pathogens. TIR8 is a regulatory receptor, able to inhibit ILRs and TLRs signaling, preventing damages caused by inflammation(2).

The aim of this study was to elucidate the role of TIR8 in the immune response of mammary gland. Given that no data are available on TIR8 in goat, we first studied its pattern of expression in a panel of goat healthy organs and tissues. Then we investigated the role of TIR8 during IMI, comparing its expression in healthy and infected blood, milk and tissues.

Six goats were infused with PBS in the right udder and with S. aureus in the left udder. Mammary biopsies from both udders were collected 30 hours postinfection, formalin fixed and routinely processed for microscopic evaluation or immediately stored in RNAlater(1). As controls, tissue samples from various non-lesional organs were collected at the slaughterhouse from healthy goats and immediately stored in RNAlater. Blood and milk were collected from healthy and infected goats; blood and milk cells were isolated and processed for RNA extraction or for cytospin; milk fat globules were obtained through milk centrifugation and immediately processed for RNA extraction. Total RNA from different organs, blood and milk cells, milk fat globules and mammary infected tissues was extracted and used as template in qPCR for TIR8. TIR8 protein expression pattern was investigated by immunohistochemistry on formalin fixed, paraffin embedded tissue samples from normal and infected mammary glands and on cytospin of isolated goat blood and milk cells.

TIR8 messenger was ubiquitously expressed with very high levels in pancreas, mammary gland, spleen and lymph nodes. High levels were also present in kidney, salivary gland, thymus, gastrointestinal tract, liver and lung. Given the high expression in the mammary gland, we investigated which cell population expressed TIR8. TIR8 was mainly expressed by ductal epithelial cells. During S. aureus infection the receptor was down-modulated in ductal cells, but up-regulated in antigen presenting cells (APCs). PMNs didn't show any modulation. Moreover, the secretum increased its TIR8 positivity during infection. We demonstrated that after infection TIR8 was down-modulated in lymphocytes and PMNs, but up-regulated in monocytes and in mammary secretum.

TIR8 is ubiquitously expressed in goat tissues, with higher levels in organs with a predominant epithelial component and in lymphoid tissues. This pattern of expression is very similar to other mammals(3). TIR8 down-modulation after S. aureus infection by ductal cells could lead to an exaggerated inflammatory response, whereas the TIR8 up-regulation by APCs could impair pathogen clearance by infiltrating immune cells. Further experiments are needed to elucidate the role of TIR8 in the pathogenesis of S. aureus infection.

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PTX3 IS UP-REGULATED IN EPITHELIAL MAMMARY CELLS DURING S. AUREUS INTRAMAMMARY INFECTION IN GOAT

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Pentraxins are a superfamily of conserved molecules with immune functions such as complement activation and opsonization. PTX3 is the prototypic long pentraxin and is produced by different cell populations (DCs, monocytes/macrophages, endothelium, fibroblasts) after proinflammatory stimuli (TLR ligands, IL-1b, TNFa)(1). Different studies have demonstrated the up-regulation of PTX3 during ruminant mastitis, but its role is still unknown(2,3). To better understand the role of PTX3 we investigated its pattern of expression in a model of S. aureus intramammary infection (IMI).

The aim of this study was to elucidate the role of PTX3 in the immune response to S. aureus IMI. Given that no data are available on PTX3 expression in goat tissues, we first studied its pattern of expression in goat normal tissues. Then we investigated the role of PTX3 during mammary infection, comparing its expression in healthy and infected blood, milk and tissues.

Six healthy goats were infused with PBS in the right udder and with S. aureus in the left udder. Mammary biopsies from udders were collected 30 h post infection, formalin fixed and routinely processed for microscopic evaluation or immediately stored in RNAlater(2). Tissue samples were collected at the slaughterhouse from healthy goats and were immediately stored in RNAlater. Blood and milk were collected from healthy and infected goats; cells from blood and milk were isolated and processed for RNA extraction or for cytospins; milk fat globules were obtained through milk centrifugation and immediately processed for RNA extraction. Total RNA from different organs, blood or milk cells, milk fat globules and mammary tissues was extracted and used as template in qPCR for PTX3. PTX3 expression was investigated by immunohistochemistry on formalin fixed paraffin embedded mammary tissue samples and on cytospins of isolated goat blood and milk cells.

PTX3 mRNA was expressed with very high levels in bone marrow, mammary gland, aorta, pancreas, skin and lung. In other organs low levels of mRNA were observed. Given the high expression in the mammary gland, we investigated which cell population expressed PTX3. PTX3 was mainly expressed in the apical cytoplasmic portion of mammary gland epithelial cells, and in macrophages. During S. aureus infection PTX3 was up-regulated by epithelial cells. Macrophages and mammary secretum didn't show PTX3 modulation, but PMNs recruited during infection were variably intensely positive. Moreover, in peripheral blood, following infection, PTX3 was up-regulated mainly in lymphocytes and PMNs, whereas in monocytes it was not modulated.

PTX3 mRNA expression was low in healthy organs and tissues of goats as has been reported indeed the molecule is commonly induced after proinflammatory stimulation(1). As expected, PTX3 was constitutively expressed in bone marrow, rich in PMNs and monocytes, in aorta covered by endothelium and in the skin. PTX3 was up-regulated in epithelial mammary cells and in milk cells after S. aureus infection, demonstrating that it represents a first line of immune defense in goat udder. No modulation was observed in macrophages, in the secretum and in the ductal epithelial cells. Further experiments are needed to elucidate the role of PTX3 in the pathogenesis of S. aureus infection.

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USE OF PROTEOMIC ANALYSES TO IMPROVE IMMUNOBLOTTING TEST FOR THE SEROLOGICAL DIAGNOSIS OF CONTAGIOUS BOVINE PLEUROPENUMONIA

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Contagious Bovine Pleuropneumonia (CBPP) is a highly contagious disease of cattle caused by Mycoplasma mycoides subsp. mycoides small colony (MmmSC) that causes high morbidity and mortality in affected herds (1). Diagnosis of CBPP is based on isolation and identification of the causal agent, post-mortem examination of lungs of affected animals and serological methods as complement-fixation test (CFT), c-ELISA and immunoblotting (IB) (2). Despite being considered highly sensitive and specific, due to its technicality, IB test is not suitable for mass screening and it is mainly used as a confirmatory test to solve doubtful results obtained with CFT or ELISA. The differences in the electrophoretic protein profile among MmmSC strains (3) and the use of the whole cell made IB difficult to standardize; moreover the separation of bands with a very close molecular weight is not optimal and results interpretation may be difficult. Improvement of test would simplify IB standardization and result interpretation.

Our study aimed to better investigate the composition of the 5 common immunogenic bands (110, 98, 95, 62/60 and 48 kDa) in IB protein profile of MmmSC in order to set up a new generation IB test based on specific recombinant antigens.

SDS-PAGE of MmmSC was carried out according to OIE procedure (2) and the gel was stained with Comassie. The 5 immunogenic bands (110, 98, 95, 62/60 and 48 kDa) recognised in IB by immunoglobulins of CBPP infected animals were characterised by mass spectrometry (LC-ESI-MS/MS). Three of the identified proteins, reported as immunogenic, were expressed as recombinant antigens using E. coli as expression vector and tested by IB against positive and negative CBPP reference sera.

Mass spectrometry analyses of the 5 immunogenic bands identified a total of 35 different proteins; 15 of them were detected in more than one band. Twelve proteins were found in the 110 kDa band, 16 in the 98 kDa band, 15 in the 95 kDa band, 14 in the 62-60 kDa band and 12 in the 48 kDa band. Among the 35 proteins, 17 have been reported in the literature as immunogenic. IB analyses performed on the three proteins expressed as recombinant antigens (Ribose/galactose ABC transporter, substrate-binding component, MSC_0011, 61 kDa; Translation elongation factor Tu, MSC_0160, 43 kDa; Pyruvate dehydrogenase -lipoamide-, beta chain, MSC_0266, 36 kDa) showed clear differences between CBPP positive and negative sera. Our preliminary results demonstrated that using MmmSC recombinant proteins of different molecular weights, selected in order to make them clearly distinguishable, it would be possible to optimize the IB test and to improve CBPP serological diagnosis.

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DIFFERENTIAL APOPTOTIC EFFECTS OF BVDV AND BHV-1 ON PBMCS COMPARING ANIMALS FROM BVDV SERONEGATIVE AND IMMUNIZED DAIRY HERDS

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Apoptosis can prevent the replication and spread of viral infections, therefore many viruses have developed strategies to prevent this phenomenon. However, in a variety of acute viral infections, there is an increasing evidence that immunodepression is directly associated with the induction of apoptosis in immune cells in order to facilitate viral dissemination. Bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BHV-1) infections give rise to acute and persistent or latent infections, predisposing to development of the Bovine Respiratory Disease Complex as a result of their immunosuppressive features. Since synergic interactions have been observed in vivo between BVDV and BHV1, bovine peripheral blood mononuclear cells (PBMCs) are infected with both viruses to characterize the susceptibility of animals in contact with an immunotolerant calf to BVDV for apoptotic processes following a secondary infection. PBMCs were collected from eight animals, four from a seronegative dairy herd and other four suffering an ongoing infection with a persistently infected (PI) animal to BVDV in Lecco and Como provinces. PBMCs were separated into 4 groups of infection: mock infected, infected with noncytopathic BVDV-1a, infected with BHV-1.1 and infected with both viruses, being incubated for 18, 24, 48 and 72 hours. To quantify BVDV and BHV-1 in PBMC cultures, a quantitative PCR method was devised. Dual staining with Annexin V-FITC and Propidium Iodide was used to discriminate by flow cytometry early apoptotic, necrotic/late apoptotic and live cells (MBL MEBCYTO Apoptosis kit).Caspases 3/7, a common complex where the two apoptotic routes converge, were determined by an Apo-ONE©Homogeneous Caspase-3/7 kit. Statistical analyses were generated using GraphPad Prism. At the beginning of study, animals from the free-herd were confirmed to be BVDV and BHV-1 antigen and antibody free, while animals exposed to the PI calf were confirmed to be BVDV and BHV-1 antigen free and with BVDV neutralization antibody titres >128. Total leukocyte and platelet counts from the animals used in this study had values included within the normal range for the bovine species, except for the PI animal that suffered a marked lymphocytosis. In vitro infection with BVDV and/or BHV-1 induces an apoptotic effect in up to 70% of PBMCs primary cultures at 72 hpi. BVDV pre-infected animals exposure to the PI animal played an important role in the development of an increased susceptibility of PBMCs against secondary infections, observing less cell survival at the in vitro conditions tested and, in particular, after viral infections. Cell death was associated with a significative activation of Caspases-3/7, which appears to be related to massive PBMCs apoptosis, being the progression from early to late apoptosis/necrosis more intense and of sooner appearance in the animals in contact with the PI calf. These evidences could suggest a possible association between BVDV pre-infection and the apoptotic mechanisms by which this virus would establish immunosuppression in cattle. BVDV and BHV-1 ability to induce apoptosis in vitro on T lymphocytes, B lymphocytes and monocytes might also have important implications in vivo, by affecting cell cytotoxicity, cytokine and antibody production, as well as having an inhibitory effect on the lymphocyte proliferative response.

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CONSEQUENCES OF EXPOSURE TO CATTLE PERSISTENTLY INFECTED WITH BVDV ON VIRAL REPLICATION AND SURFACE EXPRESSION OF BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS INFECTED IN VITRO WITH BVDV AND BHV-1

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Cattle industry is of great importance for the Italian and Spanish economy, being considered one of the most important economic sectors related to livestock production in these countries, and in productive importance within the European Union. Consequently, a wide knowledge of the diseases affecting this industry becomes critical in order to improve the control, and thus, the productivity. The aim of this study was to develop an experimental model for understanding the mechanisms of immunomodulation responsible for the state of immunosuppression induced by bovine viral diarrhea virus (BVDV) in the herds and its role in the establishment of the Bovine Respiratory Disease Complex, assessing the responsiveness of peripheral blood mononuclear cells (PBMCs) from BVDV-free animals in comparison with animals in contact with an immunotolerant calf to BVDV, both subjected to in vitro infections with BVDV and/or bovine herpesvirus (BHV-1). For this work, sera and PBMCs were collected from eight heifers, four from a seronegative dairy herd and four from other suffering an ongoing infection due to contact with a persistently infected (PI) animal to BVDV in Lecco and Como provinces. PBMCs were separated into 4 groups of infection: Uninfected control, infected with noncytopathogenic BVDV-1a, infected with BHV-1.1 and infected with both viruses, being incubated for 18, 24, 48, and 72 hours. To quantify intracellular and extracellular BVDV and BHV-1 in PBMC cultures, a sensitive quantitative PCR method was devised. Replicative capacity of viruses and their elimination to the extracellular environment were evaluated by measuring extracellular viral titres. To determine the effect of these infections on the expression of different leukocyte differentiation antigens, flow cytometry analyses of PBMCs were carried out. At the moment of study, animals from the free-herd were confirmed to be BVDV and BHV-1 antigen and antibody free, while animals exposed to the PI calf were confirmed to be BVDV and BHV-1 antigen free and with BVDV neutralization antibody titres >128. Results showed that PBMCs of all animals were efficiently infected with BVDV and/or BHV1 in the in vitro conditions tested, being observed that animals in contact with the PI calf in the herd were more susceptible to the infections, especially due to BHV-1 elimination to the extracellular environment, thus favouring the infection of other cell populations. This study revealed an absence of changes in the percentage of T lymphocytes despite the viral infections in both groups of animals, being observed only a CD25 up-regulation of the mean fluorescence intensity that affects animals in contact with the PI calf, which possibly was due to the process of immunization itself. The main findings in the PBMCs subpopulations included the reduced percentage of monocytes in the animals in contact with the PI animal after in vitro infection with BHV-1, which also produces a detrimental effect on CD11b expression in these cells, reflecting synergic mechanisms that undermine the response of monocytes-macrophages and, in turn, the innate immune response to these viruses. Monocytes also seemed to down-regulate CD80 expression in response to BHV-1 infection, a fact that may be responsible for an impaired process of antigen presentation and activation of lymphocytes.

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Part IX

XIII CONVEGNO S.I.R.A.

IN VITRO EFFECTS OF PLATELET-RICH PLASMA ON BOVINE ENDOMETRIAL-DERIVED CELLS

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Endometritis is one of the most important causes of reduced fertility in cows as it might affect endometrial function and impair future pregnancy rates. Regenerative medicine by platelet rich plasma (PRP) treatment may offer a new therapy strategy. PRP is defined as the plasma portion with the highest platelet concentration. It contains several growth factors (i.e PDGF, TGF- , PDEGF, PDAF, IGF-1) that have been proven to have beneficial effects on tissue regeneration and anti-inflammatory properties (Marx, 2001). Despite its broad-range applications, few papers have been focusing on the biological effects and the action mechanisms of PRP at cellular and molecular levels. In this pre-clinical study, in view of its in vivo application, the effect of PRP on the expression of genes involved in the regulation of oestrous cycles and fetal-maternal interaction, such as cycloxygenase 2 (COX2), tumor protein p53 (TP53), oestrogen receptors (ER and ER) and progesterone receptor (PR) was evaluated on bovine endometrial cells. The expression of the transcription factor c-Myc was also investigated to evaluate the potential mitogenic effect of PRP. To produce PRP, whole blood from the mammary vein of different cows were collected into bags containing CPDA-1. The whole blood was centrifuged at 100xg for 30 min and then at 1500xg for 10 min. The resulting platelet pellet was diluted to obtain a concentration of 1x109 platelet/ml. The PRP was frozen at -70°C and thawed at room temperature for three times to promote the release of platelet-derived growth factors. Endometrial cells were obtained from endometrium tissue of normal-cycling cows by digestion with 1mg/ml of collagenase type I for 3h. Endometrial-derived cells were cultured in vitro until passage (P) 10 with two different concentrations of PRP (5% and 10%) and mRNA levels of examined genes were quantified by qPCR. The results were compared to those of control cells, cultured with 10% fetal calf serum (FCS) only. This in vitro study showed a significant increases in the expression of all studied genes in cells at P5 cultured in presence of 5% PRP compared to 10% PRP or 10% FBS. In particular, PR expression increased 5.44-fold and ER expression increased 250-fold. At P10, decrease in the expression of all the evaluated genes was observed, with the only exception of TP53, whose expression remained constant. These data indicate that PRP determines an up-regulation of genes that play an important role in reproduction. It is conceivable that PRP derived growth factors are involved in this mechanism. Indeed, c-Myc, that was up-regulated in this study and is involved in cell proliferation and growth, is activated by EGF that is a component of PRP (Anitua et al., 2004). In future, it will be interesting explore the anti-inflammatory properties of PRP by in vivo studies.

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CAN LIPOPROTEINS AND METABOLIC PROFILES IN THE DRY PERIOD ASSESS THE SUSCEPTIBILITY OF TRANSITION DAIRY COWS TO POSTPARTUM REPRODUCTIVE AND METABOLIC DISORDERS?

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Transition dairy cows undergo negative energy balance and mobilize adipose tissue; very low-density lipoproteins (VLDL) play a pivotal role in hepatic fat metabolism, but dairy cows physiologically show limited rate of VLDL synthesis (1). This increases hepatic lipidosis during the transition period and the animal susceptibility to periparturient disorders such as ketosis, metritis and immune dysfunction (2). The aim of this study was to check whether there is a relation between energetic and lipoprotein metabolism in Italian Friesian cows during the dry-calving interval and postpartum reproductive and metabolic disorders. For this purpose, 14 Holstein Friesian cows were included; BCS and blood samples from coccygeal vein were performed at dry-off, repeated 30 days before the expected calving date and at calving. Biochemical profile, serum non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), lipoprotein electrophoresis and hematocrit were assessed. Animals were classified in 3 groups (8 healthy cows, 2 cows with left abomasal displacement (LDA), 6 cows with reproductive diseases) based on postpartum clinical features, which were checked twice weekly until 60 days in milk. Data of postpartum reproductive performance were collected from the herd management software, except for cows undergoing LDA which were early culled. Butyric acid concentration was evaluated in silage and total mixed ration. Mean BCS ranged between 3.42±0.38 and 3.75±0.35 at dry-off, and between 3.08±0.29 and 3.38±0.18 at calving; without difference among groups. Mean BHB levels were greater than 1.1±0.11 mMol/L in all groups throughout the study, with differences (PâL'd'0.05) at dry-off: the third group reached the greater value (2.09±0.13 mMol/L). Butyric acid levels varied between 0.03 and 0.01 g/100g both in silage and in total mixed ration, so no food-borne hyperketonemia could be suspected. Cows with LDA showed higher glucose levels ($P \leq 0.05$) 30 days before calving (80.5±2.12 mg/dL); mean glucose and NEFAs levels in other groups were near the upper reference values (3). Low lipomobilization together with physiological glucose levels and persistent hyperketonemia led to the suspect of hepatic insulin resistance (4). Healthy cows showed at calving lower mean VLDL (2.86±1.28%) compared to those with reproductive disorders (3.13±1.49%); this apparently contrasting result could be related to greater VLDL peripheral catabolism and lesser hepatic lipidosis. However, the lack of reference values made the interpretation difficult and more studies are necessary to identify pathological thresholds. Healthy cows showed lower calving-first AI interval and days open (88.25±49.74 vs 108.5±57.68 and 142.83±60.00 vs 182.67±84.13 days, respectively).This preliminary study, although the limited statistical significance due to the small number of animal included, seem to show that homeorhetic control in energetic metabolism can lead to insulin-resistance and hyperketonemia yet in early prepartum period. In conclusion, peripheral catabolism of VLDL and LDL seems to be important in protecting liver from lipidosis, decreasing the animal susceptibility to periparturient disorders and enhancing postpartum reproductive performance.

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LH RECEPTOR CONTENT IN BOVINE LUTEAL CELLS DURING THE ESTRUS CYCLE

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Corpus luteum (CL) dysfunction represents one of the main causes of embryonic mortality in cattle. This may be related either to the origin of the gland or to the activity of other glands/tissues which are responsible for maintenance or regression of the CL. Luteinizing hormone (LH) is considered among the main factors in maintaining the CL in cattle. The dynamics of changes in the content of the LH receptor (LHR) in the bovine CL during the estrous cycle was evaluated in a previous study (Ostuni et al., 2014). The purpose of the present study was to evaluate the presence of LHR in CL cells obtained in different phases of the estrous cycle. Bovine ovaries were individually collected from slaughterhouse and stored at 4°C. After a gross morphological in situ evaluation (Ireland et al., 1980), the CLs found were classified in relation to the estrus cycle stage (estrus= day 0); in particular, Stage I, II, III and IV included intervals between days 3-5, 6-9, 10-15 and 16-19, respectively. CL slices were obtained by cutting along the sagittal axis of the gland; they were finely minced and shaked at 1500 rpm at 37°C for 90 min in PBS containing 0.1% PVA, Collagenase II, DNAse I, penicillin and streptomycin. The dissociated cells were filtered through a 50 μm nylon mesh to remove undigested tissue fragments. Cell suspensions were added with 20% FCS in PBS, centrifuged and then fixed with 2% paraformaldehyde in PBS for 60 min. After blocking for 30 min in 5% bovine serum albumin in PBS and double washing, cells were incubated for 90 min with 1:200 goat anti-LHR antibody (Santa Cruz Biotechnology) and then for 60 min with 1:100 donkey anti-goat IgG-FITC antibody (Santa Cruz Biotechnology) and DAPI. Cells were analyzed by either laser confocal or fluorescence microscope in order to test results by using two different and independent equipments. Background-subtracted fluorescence intensity (FI) data were analyzed by ANOVA (Systat 11.0) and related to the estimated estrus cycle stage and the donor age. The estrus cycle day played a significant role ($P<0.001$) on LHR content in CL cells In particular, FI increased from I to II stage and dropped down in cells belonging to CL older than 15 days. A significant difference of FI was also found between CL cells collected from cows and heifers ($P<0.01$). A very high correlation ($R=0.962$; $P<0.01$) was found between confocal and fluorescence microscopy evaluation. It was difficult to discriminate between large and small luteal cells, since dissociated LHR positive cells ranged from a small to a large size without any clear size discontinuity. Assuming that small and large CL cells represent 88.5% and 11.5% of the total steroidogenic cells, respectively (Wildbank, 1994) and considering the level of these threshold values, we did not found differences between these two cell categories. In conclusion, LHR content in luteal cells follows the same pattern already found in luteal tissue in toto; this further supports LHR as a reliable marker for evaluating CL quality.

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THE EFFECT OF GLUTATHIONE PEROXIDASES GPX SUPPLEMENTATION AS ANTIOXIDANT OF IN VITRO FERTILIZATION MEDIUM ON THE CAT EMBRYOS PRODUCTION

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The domestic cat (Felis catus) is an excellent model both in biomedical research and in rare wild feline species studies. Assisted Reproductive Techniques (ART) are invaluable tools for the spread and management of genetically valuable domestic and endangered nondomestic cat population (Gomez et al. 2004; Pope et al. 1997). The oxidative factors are known to be physiological mediators of fertilization (Griveau et al., 1997), but sperm oxidative damage is also a potential cause of fertilization failure (Aitken, 1995). A fine balance between the useful and the excessive peroxidation exists and requires a complex control before spermatozoa reach female gametes. This phenomenon is emphasized during in vitro fertilization (IVF), when a high number of sperm is placed into fertilization medium (Noblanc et al., 2011). GPx is an endogenous antiossidant and its activity was demonstrated the in mammalian epididymal tract and in coltured oocytes (Noblanc et al., 2011). In the present study we hypothesized that the GPx supplementation of IVF medium (SOFaaBSA + 25UI GPX) could provide better fertilization performances after IVF respect to medium without GPx. To this aim we evaluated the impact of GPx supplementation in IVF medium on the percentage of clivage after IVF and the blastocyst formation rate. The experiment was performed in 20 replication from may 2014 to november 2014. 476 Cat oocytes grade 1 or 2 were collected from 84 fresh ovaries excised from 42 female cat ovariectomized at the Naples university veterinary hospital (OVUD). Collected oocyte were cultured in maturation medium (SOFaaBSA; 20COC/500ul maturation medium) in 5%CO2 at 38∘C for 24 h. They were then fertilized in vitro in SOF aa BSA without or with 25UI/ML of GPx (control vs experiment respectively) for 12h with 1x106 epididimal fresh spermatozoa. After in vitro fertilization, the zygotes were cultured in synthetic oviduct fluid (SOFaaBSA) medium for 8 days. All data were recorded using a computerized spreadsheet (Microsoft ® Excel ® 2011 for Mac, Microsoft Corporation, USA) and imported into a program for statistical analysis (JMP ® 8.0.2,SAS Institute Inc., USA). Normality was tested with a Shapiro-Wilk's W test. As not all data were normally distributed non-parametrical tests were used throughout. The Kruskal-Wallis one-way analysis of variance by rank was applied to compare the two groups for developmental data. Statistical significance was set at $p \leq 0.05$. The results show a statistical difference in clivage rate (5,27 $\pm$ 1,2748 vs 10,63 $\pm$ 2,15 in experimental vs control group respectively; $p \leq 0.0002$) and in blastocysts development (2,36 $\pm$0,92 vs 4,63 $\pm$ 0,80 in experimental vs control group respectively; $p \leq 0.0002$). Conclusion: Our findings indicate that supplementation of GPx into fertilization medium may improve blastocyst formation of cat oocytes after in vitro fertilization.

ISOLATION OF MRSA FROM CANINE FOREMILK: A CASE OF HUMAN CONTAMINATION

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Introduction- Staphylococci are commonly isolated from canine milk, both in case of healthy mammary glands and in case of infection (1,2). Among the coagulase-positive, potentially pathogen staphylococci, Staphylococcus pseudintermedius is the most represented species (1,3,4) while S. aureus has been only occasionally isolated (5). Case Report- We report the case of the isolation of a single S. aureus strain out of 145 bacteriological cultures of bitches foremilk and milk, compared to 66 isolates of S. pseudintermedius. S. aureus was isolated from the foremilk of a miniature poodle, privately owned, that had been subjected to Caesarean section. The bitch and her three puppies were healthy both at the moment of sampling and in the following days, growing normally until weaning. The isolated strain resulted methicillin-resistant and mecA positive (MRSA); besides being resistant to all beta-lactam antimicrobials it also showed resistance towards erythromycin and tetracycline. By genetic typing it resulted belonging to clonal complex 1 and showed the presence of SCCmec IVa. Following swabs from milk samples of the same bitch, collected at D7 and D15, did not yield S. aureus. Due to what we considered an anomalous isolation, we thought to investigate the operator who had sampled the dog, discovering that had suffered from recurrent tonsillitis: we asked him to collect a nasal and a pharyngeal swab: from the nasal swab, a MRSA, with the same clonal complex and SCCmec-type, was isolated. After the end of lactation, the bitch was sampled from axillary skin, mouth, perineal region, but no S. aureus was detected. Resistance to methicillin appeared in S. aureus in 1960 and originated because of widespread use of beta-lactam antibiotics in nosocomial settings. The CC1-SCCmec IV-MRSA isolates have become predominant since the 90s and are defined 'community-acquired' MRSA: they spread outside the hospitals, involving not only patients but also healthy contact persons. These methicillin-resistant strains often carry other genes associated with antimicrobial resistance, virulence-factors e.g. PVL and enterotoxin genes. The first reported PVL-positive SCCmec IV belonged to Clonal Complex 1 and caused fatal infections in children in the late 90s in the USA (6). The strain isolated in our investigation was negative for PVL genes. Conclusions- This case confirms the low prevalence of S. aureus in canine mammary secretions and it also shows that bacteriological samples have to be collected very accurately to avoid contamination.

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POSSIBILITIES AND LIMITS OF HEMATIC INDICATORS AS MARKER OF INFLAMMATORY UTERINE PATHOLOGIES IN PIEDMONTESE COWS.

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Acute phase proteins are blood proteins synthesized by hepatocytes as part of response of the early-defense or innate immune system, which is triggered by different stimuli (trauma, infection, stress, and inflammation). Many studies, both in cattle and sheep, have reported an association between circulating concentrations of haptoglobin (APT), an acute phase protein, and uterine infection after calving (2). Creatine kinase (CK) is considered a muscle specific enzyme. CK activity is especially high in tissues with high-energy transfer such as muscle and brain (1). In abdominal organs, there are also high activities of CK particularly in the uterine tissue which has the third highest CK concentration after skeletal and cardiac muscle. There are relatively few studies about CK in cows and none in beef cattle.

The aim of this paper is to evaluate the accuracy of APT and CK as infertility marker in postpartum Piedmontese cows.

In this study 73 apparently healthy cows were enrolled (50-60 days post partum, dpp), for each cow one blood sample was collected with venoject system (jugular vein) and evaluated respectively with IFCC method (CK) and Immunoturbidimetric method (APT): CK >100 U/l was considered the cut off for the antibiotic therapy, and 15 days after therapy a second blood sample was collected. For each cow pregnancy status, partum to conception (PC), were recorded. For PC a 150 dpp was used as cut off to evaluate pathological cows.

17% of cows were open and 82% were pregnant (CK 153 vs 129 U/l), 67% of cows were CK>100 and from these 18% were open and 49% were pregnant (P=0.01), Treated versus not treated cows shows a different CK concentration (57 U/l vs 168 U/l), P=0.003. Only 5 cows with PC>150 days show a CK<100 U/L and were not treated. APT was measured only in 24 cows showing a trend in difference between open and pregnant cows (15.65 vs 11.80 mg/dl, P=0.06) but no difference was shown for PC groups. After therapy 49% of cows with CK>100 U/l, were pregnant. Higher CK was shown in cows who received therapy than others either in groups PC<150 dpp(189 vs 89 U/l; P<0,01) than PC>150 dpp (171 vs 78; U/l; P<0,01). Neither CK nor APT were different in concentration from first and second blood sample (P>0,05). Receiver operating characteristic curvee show a CK cut-off for pregnancy of 115 U/l (AUC 0,63).

CK and APT seems to be good markers to evaluate fertility even in Piedomentese cows on 50-60 days postpartum, antibiotic therapy seems to be effective to cure animals mainly if they show a PC>150 days.

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VALIDATION OF EQUINE OOCYTE IN VITRO MATURATION USING POLARIZED LIGHT MICROSCOPY (PLM)

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In vitro maturation (IVM) of equine oocytes is a well-known procedure used in many assisted reproductive technologies. The occurred maturation is usually verified by morphological examination using a stereomicroscope, but this evaluation has an important limitation consisting in subjectivity. Classification as mature or immature is based on the presence/absence of some oocyte structures and characteristics such as: presence of polar body (PB, marker of nuclear maturation) and cytoplasm with light and dark areas (marker of cytoplasmic maturation). The complete maturation is reached when nuclear and cytoplasmic maturations occurred together. PLM is a computerized analysis of the images observed using polarized light, which is utilized for other mammalian species and provides magnitude of the ZP thickness and molecular order.

The purpose of this study was to compare these subjective morphological parameters to the values obtained applying the PLM for the oocyte evaluation, in order to confirm the occurrence of IVM by an objective analysis. The data obtained using a Polscope (a polarized light microscope which use an image analysis software) were compared with the morphological evaluation of the oocyte maturation parameters.

454 COCs (Cumulus-Oocyte Complex) were collected from ovaries obtained from an abattoir. After retrieval the gametes were randomly divided into 3 groups (g1-127, g2-169 and g3-158 COCs) and each group was submitted to a different incubation time (24h, 36h and 45h) in standard maturation media. At the end of the IVM period the oocytes were classified by morphological features and analyzed using PLM.

The average percentages of mature and immature oocytes classified by morphological criteria were 28,38$\pm$10,94% and 32,56$\pm$14,97% (g1), 49,67$\pm$4,39% and 7,10$\pm$5,05% (g2), 36,80$\pm$6,07% and 24,74$\pm$7,48% (g3). The differences between the g2 and the other groups were statistically significant ($p<0,05$). The IVM protocol with 36h of incubation (g2) showed the best output and 127 oocytes (36 immature and 91 mature with PB extrusion) of this group were analyzed using PLM. The zona pellucida (ZP) birefringence parameters, grouped by oocyte maturation stage (mature or immature), were compared. Another birefringent oocyte structure is the meiotic spindle but in the horse the cortical granules often cover it. The average ZP thickness was significantly increased ($p<0,001$) in immature (20,07$\pm$2,93μm), compared to mature (17,90$\pm$2,53μm) oocytes. The average retardance showed a statistically significant increase ($p<0,01$) in immature (2,71$\pm$1,06nm) compared with mature (2,12$\pm$0,54nm) oocytes. Currently there is a lack of information concerning the application of PLM for equine oocyte evaluation, so we referred to studies regarding bovine species for comparison with our work. Based on our results, the application of PLM for oocyte evaluation has confirmed that 36h are effectively a suitable time for IVM and that the PLM technique is an appropriate maturation control method as referred for other species. In the future it could be necessary to increase the number of analyzed oocytes in order to choose appropriate cutoff values for this technique.

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ADDITION OF SEMINAL PLASMA TO FROZEN SEMEN OR UTERINE INFUSION OF SEMINAL PLASMA AND INRA96 24 HOURS BEFORE INSEMINATION HAD NO EFFECT ON UTERINE INFLAMMATORY RESPONSE AND PREGNANCY RATE IN MARES

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Removal of seminal plasma (SP) before cryopreservation or its addition after thawing affected pregnancy rates and uterine reaction after artificial insemination [1-4]. Aims of this study were to evaluate the differences in pregnancy rates and uterine inflammatory response in mares submitted to frozen semen AI: 1. in the body of the uterus (AI) 2. by deep uterine insemination (DUI) or 3. by AI 24 hours after uterine infusion of SP and INRA96® The ovarian activity of 8 healthy cyclic Trotter mares was monitored by ultrasound (US) for 3 cycles. During each estrus at the evidence of a growing follicle $\geq$35mm ovulation was induced by 6.6 mg buserelin acetate (Suprefact® Sanofi-Aventis, Milan, Italy; hour 0). Each cycle, mares were included in a different insemination group: AI - DUI - Uterine infusion with 7.5 ml of equine SP + 15 ml of INRA96 24 hours before AI (UI+AI).

All the mares were submitted to AI/DUI at hour 40 from induction, using 1 ml of frozen/thawed semen. Uterine inflammatory response (IR) was evaluated by US 6 and 20 hours after AI/DUI, and scored 0, 1 or 2 (no intrauterine fluid, fluid $\leq$2 cm or fluid >2 cm in height). Proportion and concentration of PMN was determined 6 hours after AI/DUI on a small volume uterine lavage performed with 60 ml of Lactated Ringer [5]. Pregnancies were diagnosed by embryo recovery (ER) at day 8 after ovulation. Differences between AI groups in embryo recovery rate were analysed by Fisher's exact test. Differences in uterine score, % of PMN and PMN concentration were analysed by repeated measures Friedman's test and by Dunn's Multiple Comparison as post-hoc test. Differences between AI groups for pregnancy rates and IR were:

- Uterine score 6h after AI/DUI; median (IQ range): AI, 1 (0-1); DUI, 0 (0-0); UI+AI, 0 (0-0) (P<0.05).
- Uterine score 20h after AI/DUI; median (IQ range): AI, 0 (0-1); DUI, 0 (0-0); UI+AI, 0 (0-1) (ns).
- % of PMN: AI, 94% (90-99%); median (IQ range); DUI, 72% (49-99%); UI+AI, 91% (84-99%) (ns).
- PMN/ml (x106); median (IQ range): AI, 2525 (1700-16875)a; DUI, 1113 (16-3988); UI+AI, 688 (297-1488)b ($^a \neq$b: P<0.05).
- ER rate/cycle: AI, 4/8 (50%); DUI, 7/8 (88%); UI+AI, 4/8 (50%) (ns).
- ER rate/ovulation: AI, 4/11 (37%); DUI, 7/12 (62%); UI+AI, 4/8 (50%) (ns).

The removal of SP before stallion semen cryopreservation seems to reduce pregnancy rates in mares and it's shown that SP could modulate post-mating induced endometritis [1-2]. The post-thaw addition of SP to frozen semen increased fertility [3,5] and modulated the PMN-influx into the uterine lumen [4,5] in mares and jennies. In the present study UI+AI mares, had a significantly lower PMN concentration as previously described [2] even though there was a tendence of an higher uterine score 6 hours after AI/DUI. Pregnancy rates, however, where not improved compared to DUI and AI ones.

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NGF/TRKA SYSTEM IN RABBIT UTERUS: IMMUNOPRESENCE AND ENDOCRINE FUNCTION

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The nerve growth factor (NGF) is a neurotrophin that plays an essential role in both central and peripheral nervous system (1). The biological actions of NGF are mediated mainly by its cognate receptor tyrosine kinase A (TrkA) (2). Several studies have shown that NGF also influences the reproductive function of both males and females (3). With regard to this, the expression of both NGF and its receptors has been detected in different organs of the male reproductive tract (4). NGF has been detected in the seminal fluid of several species (5) and recent studies indicate that NGF is an ovulation-inducing factor in both llamas and alpacas (6). In rabbits, as is well known, ovulation is induced by coitus (7) but it is possible that an ovulation-inducing factor may exist in the seminal plasma of this species (8). Therefore, the aim of this study was to investigate the presence of NGF and its cognate receptor TrkA in rabbit uterus, as well as the in vitro effects of NGF on the production of prostaglandin E2 (PGE2) and PGF2α, as potential mediators of the NGF-depended ovulatory effects.

Sexually mature New Zealand white female rabbits were used. The animals were sacrificed by cervical dislocation. Immunohistochemical investigation was performed using mouse monoclonal anti-NGF and TrkA primary antibodies. Western blotting was performed using the same immunohistochemistry primary antibodies. In in vitro experiments, uterus pieces were incubated with NGF and TrkA inhibitor (GW 441756). PGE2 and PGF2α were assessed by RIA.

A strong positivity for NGF was localized in the nucleus and cytoplasm of the uterine epithelial, glandular, stromal, miometrial, and endothelial cells, whereas the TrkA immunopresence was evidenced only in the cytoplasm of epithelial, glandular, and stromal cells. The Western blotting data confirm the specificity of the antisera used for the immunohistochemical detection of NGF and TrkA in the uterus. In uterine samples, a single band for each protein was found at approximately 55 kDa for NGF and 42 kDa for TrkA. NGF (30 nM minimum effective dose) increased the in vitro uterine release of PGF2α, whereas this neurotrophin did not affect that of PGE2.

The present data evidence that the NGF/TrkA system is present in rabbit uterus and that it affects the uterine synthesis of PGF2α. Since this uterine prostaglandin has a role in the modulation of the ovarian activity in mammals, these results shed a new light on the possible direct involvement of NGF/TrkA system in the physiological mechanisms regulating mammalian reproduction.

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EFFECT OF DIET SUPPLEMENTATION WITH LEPIDIUM MEYENII ON STALLION FERTILITY: PRELIMINARY REPORT

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Different studies on dietary intake of antioxidants are reported in human and showed the improvement of semen quality not only in hypofertile patient but also in healthy men. The reason behind the usage of antioxidant supplements in the treatment of male infertility lies in the fact that the spermatozoa are very much susceptible to oxidative stress-induced damage.(1) The effect of dietary supplementation with nutriceuticals plants is receiving increasing attention in the scientific community but little or no data are available on the potential influence on sperm quality in horses. In others mammals, feeding of maca (Lepydium Meyenii) has been demonstrated to positively affect sperm production and quality. (2) In the present study, we investigated the effects of adding maca to the diet of breeding stallions on the motility and acrosome integrity of semen. Starting in May 2014 the diet of two stallions (1 fertile and 1 hypofertile) but not of two-control stallions (1 fertile and 1 hypofertile) was supplemented with maca (20 g/day) for a total of 60 days. Ejaculates were collected every fifteen days (day 0; 15; 30; 45 and 60). Collected semen were processed for cooling at 5Â°C and stored for 72h. Cooled semen was sent to the laboratory for semen analysis of total motility, progressive motility, acrosome integrity at 0 (T0), 24 (T1), 48 (T2) and 72 hours (T3) after the collection. Fluorescent-labeled peanut lectin agglutinin (PNA-FITC conjugated staining) was employed to evaluate acrosome integrity. The percentage of total and progressive motility resulted 80-60%and 50-10% at T0 of the day0 (fertile and hypofertile respectively) and 85-80% and 80-65% at T0 of the day60, respectively in fertile and hypofertile treated stallions. Data from acrosomal status of treated stallions (hypofertile and fertile respectively) showed that the percentage of cells with reacted acrosome to T0 day0 was 18-10% and at T0 day60 was 5-4%. This study was preliminary performed in order to standardize the protocol. The low animals number tested not allow a comparative analysis of the results. Nevertheless, we performed a mathematical time stack series data analysis as a preliminary data evaluation. However, the increase of number of animals tested it is necessary to conferme the efficiency of our treatment with a statistical method. Furthermore, the treatment with maca could improve the percentages of motility not only in hypofertile but also in fertile stallion. The preliminary results are very interesting and the hypothesis is very much worth investigating, as a maca-supplemented diet might be very useful in many cases in equine practice. In conclusion, if the nutrition requirement of maca could increase the ability of spermatozoa to contrast reactive oxygen species, we can optimize the fertility of cooled-shipped semen only with dietary supplementation.

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EFFECT OF DIFFERENT CONCENTRATIONS OF TROLOX ® IN A CANINE SEMEN FREEZING EXTENDER ON POST THAW SPERM CHARACTERISTICS

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A Frozen semen can be preserved for a potentially indefinite time, however sperm cells fertilizing ability is lower, partially due to the increased levels of Reactive Oxygen Species, which cause a damage to spermatozoal membranes, proteins and DNA, and reduce motility (1). In dogs, several anti-oxidants were tested (e.g. 2). Trolox® a water-soluble synthetic tocopherol analogue, has been evaluated for canine semen preservation only at 200μM (3). The aim of this study was to evaluate if Trolox® at different concentrations in the freezing extender would improve post-thaw motility and plasma membrane integrity.

Semen from 8 dogs was frozen in two steps in a Tris-citrate-fructose extender with a final concentration of 5% glycerol and 0.5% Equex STM paste (CONTR) to which 100, 200 or 400 μM Trolox ® were added (T100, T200 and T400, respectively). Post thaw, motility was evaluated at hours 0, 1, 2 and 3 of incubation at 37°C by a Computer Assisted Sperm Analyser; while plasma membrane integrity (HOS-test) was evaluated at hours 0 and 2. Motility data were evaluated by Friedman test within each evaluation time. HOS-test data were analyzed by one way ANOVA.

There was no difference between treatments at hour 0 and 1 (mean total motility h0: CONTR: 50.9%, T100: 46.9%, T200: 49.2%, T400: 47.2%), while T400 had a statistically significant lower total, progressive and rapid motility than CONTR at hours 2 (e.g. mean total motility CONTR: 17.0%, T100: 12.3%, T200: 13.4%, T400: 10.1%) and a significantly lower mean total motility at h3 (CONTR: 11.2%, T100: 9.1%, T200: 8.1%, T400: 4.7%). The proportion of intact plasma membranes was never significantly different between groups (h0: CONTR: 51.0%, T100: 43.4%, T200: 47.9%, T400: 42.5%; h2: CONTR: 42.1%, T100: 37.8%, T200: 39.3%, T400: 38.7%).

Trolox ® was able to improve post-thaw semen quality in swine (200 μM, 4), ram (60-120 μM, 5), and human (40 μM, 6) species. The present results disagree with those data and confirm what observed at 200 μM (3). It cannot be excluded that lower Trolox ® concentrations may have positive effects, however the inclusion of 100 to 400μM in the freezing extender did not improve post-thaw canine semen characteristics, on the contrary Trolox ® 400 μM worsened motility parameters.

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OVERCOMING THE CHALLENGES OF ENDOCRINOLOGICAL RESEARCH ON BELUGA WHALES (DELPHINAPTERUS LEUCAS): HOW TO COLLECT AND PROCESS RESPIRATORY SAMPLES FOR STEROID AND ADRENAL HORMONES QUANTIFICATION

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The blood sampling procedure conventionally used on marine mammals to monitor health status and reproduction presents many limits; plasma hormonal concentrations may be affected by coercive handling procedures (Buholzer et al. 2007), blood collection requires appropriate training of staff members, it may cause a painful response and it may lead to the formation of abscesses. Goals of this study were to standardize a method to collect and process the droplets of condensed respiratory vapour exhaled by beluga whales (blow samples) and to prove this as a suitable substratum to quantify cortisol (D), progesterone (P) and testosterone (T). Subsequent goals were to achieve physiological and biological validation of this method for P and D. Two beluga whales, housed at the Oceanographic Park (Valencia, E) were used. Sampling (♀n=40, ♂n=34) was performed in the early morning three times a week, using routine husbandry training and on a voluntary basis. To achieve the biological validation of blow D, additionally samples were performed before and after some known stressful events (e.g. blood collection, artificial insemination) and, to physiologically validate the method for P, during the AI process (Robeck et al. 2010) witch included estrus synchronization (0.044 mg/kg po Regu-Mate® Intervet Inc., Millsboro, DE, USA) and ovulation induction (3 x 250g IV q1.5-2 h Cystorelin® Merial, Duluth, GA, USA). Blow samples were collected with polypropylene jars, stored at -20°C and tested via Radioimmunoassay using petroleum ether extraction for P and ethyl ether extraction for T and D, analysis was performed in duplicate (Seren et al. 1974; Gaiani et al. 1984; Tamanini et al. 1983). Weekly means resulted as follows: ♀D 19,57±10,24 - 45,35±24,79 pg/ml blow; ♂D 2,99±0,67 - 26,40±6,92 pg/ml; ♀P 42,83±8,42 - 422,4 pg/m1 blow; ♂T 2,42±35,03 - 26,03 pg/ml. An estrous cycle was monitored, follicular ultrasonography confirmed that the female had a dominant pre-ovulatory follicle (circumference 6,54 cm, diameter1 2,18 cm, diameter2 1,98 cm) and was in estrus during the study period. Weekly P concentration following ovulation induction via exogenous GnRH administration increased and a luteal phase was monitored. Higher cortisol concentrations were detected from blow samples obtained post-blood collection (i.e. 9.94 vs 18.97pg/ml, 11.07 vs 19.77 pg/ml blow). Physiological validation was obtained for P and biological validation for D confirming blow samples as a viable alternative substratum to quantify reproductive and adrenal hormones of beluga whales. Blow analysis offers unique potential for expanding conservation physiology research of beluga whales and possibly other cetacean endangered species since there is tremendous potential in applying this technique to in situ and ex situ populations. Further study is needed to clarify the relationship between blood and blow hormonal

concentrations, to identify all possible influences on adrenal activity and to describe profiles of respiratory samples in relation to the aforementioned hormones.

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DETERMINATION OF SERUM PROGESTERONE IN THE BITCH COMPARING TWO ASSAYS: ELFA VS CLIA.

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Veterinarians dealing with canine reproduction need the efficient quantification of serum progesterone, which is mainly used to identify the appropriate time of insemination and to predict the parturition date (1), but has many other applications. The Radioimmunoassay (RIA) technique has been considered the "gold standard" for progesterone quantification (2), but nowadays its replacement by other analytical techniques is prompted by the necessity to avoid the hazardous radioactive material handling.

The aim of the present study was to compare in the dog the accuracy of serum progesterone level detected by enzyme-linked fluorescence assay (ELFA) technique and by chemiluminescence immunoassay (CLIA).

For the study, 19 bitches were enrolled during oestrus and pregnancy clinical monitoring. Serum progesterone was assayed in double with ELFA (MiniVidas, Biomèrieux) and CLIA (Liaison, Diasorin), designed for human species but validated for dogs (3, 4). Some samples were used to calculate inter and intra-assay variations. Pearson's correlation and Passing Bablock tests were employed to analyse obtained data. In addition, an endoscopic-assisted transcervical insemination with a single dose of frozen semen was performed in 11 bitches.

A total of 70 blood samples were evaluated. Pearson's correlation and Passing Bablock tests demonstrated the linear correlation of ELFA and CLIA values, but ELFA values were significantly above CLIA values. The 11 transcervical inseminations were performed when progesterone was 18-38 ng/ml in ELFA (10-20 ng/ml in CLIA) and 10 bitches were diagnosed as pregnant. Conclusions. Serum progesterone assay gives different values if an ELFA or CLIA method is employed and this may lead to different clinical decisions, especially during calculation of the fertile window. In a previous study (3), comparing ELFA and RIA, major deviations were registered under 2 ng/ml and over 10 ng/ml, but authors concluded that the aberration was not significant by a clinical point of view. Veterinarians have to consider the employed method when they assay canine progesterone or receive data from laboratories or colleagues, which necessarily should include the employed assay. ELFA method is a powerful, rapid, safe and not expensive system but may give overestimated values when compared to other techniques such as CLIA.

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RETAINED PLACENTA FOLLOWING AGLEPRISTONE TREATMENT IN A 25-DAY PREGNANT QUEEN

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Feline pregnancy and its management are challenging because of several aspects, which are not fully comparable with those known in the bitch. Progesterone profile is different and parturition is not always preceded by a detectable fall in progesterone levels (1). In many countries, including Italy, the use of antiprogestins, prostaglandins and anti-prolactinic drugs to terminate feline pregnancy is considered off-label.

The clinical findings, treatment and outcome of a queen that underwent pregnancy termination using the progesterone antagonist aglepristone are described.

A one-year-old Maine Coon queen was evaluated 25 days after last mating. On ultrasound examination, pregnancy was diagnosed. Fetal hearth rates were in the normal range, but cloudy fluid was found in about half vesicles. Clinical examination of the queen, including haematological and biochemical profiles, were not indicative of a general impairment. Moreover, a progressive mammary hyperplasia was evident. The owner decided to terminate pregnancy. A treatment with aglepristone (10 mg/kg on days 1, 2, 8 and 15) was administered subcutaneously in conjunction with antibiotics (amoxicillin plus clavulanic acid). A bloody vaginal discharge was constantly observed starting from treatment day 2. At 45 days post mating, the queen was re-evaluated by ultrasound. Four uterine enlargements compatible with placental remnants were seen. Serum progesterone was 9 ng/ml. A further administration of aglepristone (15 mg/kg on days 1 and 2) and cloprostenol (1.5 mcg/kg on day 3 and 4, 2 mcg/kg on day 5) was administered subcutaneously. On day 5, four placental remnants (subsequently confirmed by histology) were expelled and ecbolic treatment was discontinued. On the same day, serum progesterone was 1.9 ng/ml, dropping to 0.5 ng/ml 2 days after. The discharge was not more evident 3 days later when ultrasound examination revealed an involuting uterus with a constant diameter of 0.4 cm.

In this case report, a protocol of aglepristone at the 10 mg/kg dose was used to treat the mammary hyperplasia and to terminate pregnancy, associating an antibiotic to prevent or treat the suspected uterine infection. The drug was effective to cause abortion and mammary involution but was ineffective to cause total expulsion of placentas. The retained placentas were the primary cause of the persistent bloody discharge. The new therapeutic approach was realized using a higher dosage (1) associating synthetic prostaglandins and was effective to remove placental remnants and to cause luteolysis. The efficacy of aglepristone treatment in the queen seems lower than that observed in the bitch and may lead to retained dead foetuses in uterus (2), sequelae of endometritis (3) and retained placenta. On the other hand, spontaneous retention of placenta is extremely rare in the queen.

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VERBASCOSIDE SUPPLEMENTATION DURING IVM IMPROVES EMBRYO DEVELOPMENT OF PREPUBERTAL OVINE OOCYTES THROUGH MITOCHONDRIAL ACTIVATION

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Verbascoside (VB), a bioactive polyphenol from olive mill wastewater with known antioxidant activity, was shown to act as a prooxidant molecule, by impairing energy/redox status and embryo developmental competence of prepubertal ovine oocytes when added at microM concentrations in a continuative 24 hours in vitro maturation (IVM) exposure protocol (1). The aim of the study was to determine whether a lower (nanoM) VB concentration during IVM may improve: a) the IVM rates of prepubertal ovine oocytes and their subsequent embryo development in vitro; b)âĂĺthe mitochondrial (mt) activity at ooplasm and/or cumulus cell (CC) level.

Oocyte-cumulus complexes (OCCs) from the ovaries of slaughtered one-month old prepubertal sheep underwent IVM in TCM 199 with 10% oestrus sheep serum, 0.1 IU/ml of FSH/LH and 100μM cysteamine, in 5% CO2 in air at 38.5Â°C for 24h. Based on our previous results (1), 1.03 nM VB was added in IVM medium for 24h. Oocytes cultured in absence of VB were used as controls. A first group of matured (MII) oocytes was fertilized with frozen-thawed ram semen in SOF medium for 22h and zygotes were cultured in vitro for 8 days. Cleavage and blastocyst rates were analyzed (Chi-square test). Embryo quality was evaluated by staining and total cell count of the blastocyst (ANOVA). A second group of MII oocytes was assessed for mt distribution pattern (Chi-square test), mt activity, intracellular reactive oxygen species (ROS) levels and mt/ROS colocalization (ANOVA and Tukey's post hoc test). All methods were reported previously (1).

Compared to controls, VB treatment at 1.03 nM and 24h exposure significantly increased the rates of cleaved embryos/MII oocytes (156/196, 80% vs 165/226, 73%; P<0.05), blastocyst yield/cleaved embryos (59/156, 38% vs 45/165, 27%; P<0.05) and total blastocyst cell number (108.62Âś19.87 vs 89.61Âś26.32; P<0.05). VB did not affect ooplasmic mt distribution pattern (80%, 4/5 of VB-treated OCCs with small mt aggregates diffused throughout the ooplasm vs 100%, 7/7 in control oocytes, not significant). However, it increased mt activity and ROS levels (arbitrary densitometric units) in ooplasms (mt: 204.88Âś47.04 vs 114.24Âś21.11, P<0.01; ROS: 688.20Âś296.54 vs 303.56Âś63.95; P<0.05) and CCs (mt: 334.60Âś137.14 vs 183.59Âś57.85, P<0.001; ROS: 136.69Âś45.21 vs 104.08Âś37.58; P<0.01) of treated OCCs. Ooplasmic mt/ROS colocalization (overlap coefficient) did not vary upon VB treatment in ooplasms (0.67Âś0.16 vs 0.60Âś0.13) and CCs (0.55Âś0.16 vs 0.56Âś0.12).

In conclusion, VB treatment at 1.03 nM during 24h IVM increased ovine blastocyst yield and quality. Increased mt activity and ROS levels in treated OCCs sustain the hypothesis that VB at nanoM concentrations may improve OCC energy/redox status.

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NON-INVASIVE MONITORING OF THE REPRODUCTIVE STATUS IN TURSIOPS TRUNCATUS FEMALES: IMPORTANCE IN THE MANAGEMENT OF ANIMALS HOUSED IN CONTROLLED ENVIRONMENT

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The monitoring of the reproductive status of marine mammals is very important for their management in a controlled environment. The endocrine status of these animals is normally monitored by collecting blood. However the operation repeated over time can be stressful for the animals and can predispose to tissue inflammation and microbial infections (Pedernera et al, 2006).. Scientific research is oriented towards the use of non-invasive techniques for collecting saliva, feces and recently expired air for routine investigations (Tizzi et al, 2010 - Turner et al, 2008).

The aim of this study was to monitor and comparing Progesterone (P4) values in Tursiops truncatus females, using expired air (Tizzi et al, 2010) and serum samples and evaluate the correspondence with clinical findings during the routine health check of these animals in order to optimize their reproductive management (Biancani et al, 2009).

This study was carried out on 4 females hosted in the Oltremare Park (Riccione Italy) during a 4 months period. Systematic withdrawals of expired air and blood were made through operant conditioning (Brando, 2010). Meanwhile these animals underwent reproductive ultrasonographic evaluations. The blood and expired samples P4 concentration were determined using immunoenzymatic assay method.

Due to the small number of samples it has not been possible to obtain a representative statistical support; and a significant correlation was not observed between expired and serum P4. However, data obtained are supported by clinical investigation results. To high values of P4 on blow corresponding to ultrasound confirmation of pregnancy. Further studies should be conducted in order to improve this innovative method and find a statistically significant correlation. And introduce the use of breath as a source of frequent, quick and invasiveness, biological sample during the routine procedures. (Pedernera-Romano et al, 2006 - Brando, 2010). It may also be used in the future to determine other steroid hormones in order to improve the monitoring of these animals welfare.

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BLOOD CONCENTRATION OF ANTI-MÜLLERIAN HORMONE IN ITALIAN SIMMENTAL COWS IS POSITIVELY ASSOCIATED WITH REPRODUCTIVE HERD LIFE: PRELIMINARY RESULTS

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Heritability estimates of herd longevity are very low, ranging from 0.03 to 0.07 [1]. The discovery of biomarkers that are moderately to highly heritable and highly correlated with longevity would be of benefit both for enhanced future herd reproductive performance, choosing heifers with potentially high longevity, and for sire selection to genetically improve survival rates and thus productive herd life of dairy cows and profitability of the dairy industry [2]. There is mounting evidence that a single dosage of anti-Müllerian hormone (AMH) is a good biomarker of antral follicle count (AFC) and ultimately of bovine fertility and longevity [3].

The objective of the present study was to evaluate possible relationships between AHM blood levels and reproductive indexes in Italian Simmental cows. Materials and methods: Animals were selected from the database of Regional Breeder Association (AAFVG); only cows that had completed at least one lactation without any recorded diseases were selected. A total of 481 cows from 6 different dairy farms were selected for blood sampling and AMH analysis (Beckman-Coulter, AMH Gen II ELISA, Pantec, TO). The mean data relative to age at first calving, parity, calving to conception interval (CCI), calving interval (CI) and number of services per conception were obtained from AAFVG database. Data were analysed by ANOVA. Data were also analysed subdividing animals based on AMH blood levels (Low = <130, Medium = from 131 to 200 and High = > 200, pg/mL).

Mean age at first calving ranged from 26.1±2.0 to 30.0±3.2 months in farms 2 and 5, respectively. The farms 5 and 6 presented the higher values compared to the other farms (P<0.05). Parity ranged from 3.1±1.2 to 4.1±1.6, and farms 2 and 3 showed the highest values compared to the other farms (P<0.05). The CCI ranged from 167±89 to 95±44 days, with farms 1 and 6 needing higher number of days for impregnating the cows (P<0.05). The number of services per conception ranged from 4.1±1.5 to 2.0±1.1, and farms 1 and 6 needed a higher number of services per conception compared to the others (P<0.05). The AMH levels were 169±94, 171±83, 267±180, 168±180, 282±187 and 147±59 pg/mL of blood, for farms 1, 2, 3, 4, 5 and 6, respectively. Interestingly, farms 3 and 5 showed the higher values for blood AMH (P<0.05). The cows in the group Low (n = 195) and Medium AMH (n = 135) showed a lower number of mean calving (3.4±1.5 and 3.5±1.5, respectively) compared to the High AMH group (3.9±1.7; P<0.05). The CCI also showed a progressive decrease with the decreasing AMH levels (129±73.6, 121.1±66.1 and 117.1±59.7 days for low, medium and high AMH groups, respectively). The AMH blood levels were also significantly (P<0.01) correlated with the total number of calvings.

Although preliminary, our results suggest that a single determination of AMH concentration may be a simple diagnostic method to predict herd longevity thus highlighting the need for further research on this topic. :

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CADMIUM SUPPLEMENTATION DURING IN VITRO MATURATION IMPAIRS MITOCHONDRIA DISTRIBUTION PATTERN OF PREPUBERTAL OVINE OOCYTES

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Cadmium (Cd) is highly toxic, and one of the most important environmental pollutants in industrialized countries. Cadmium supplementation, at micromolar concentrations, during in vitro maturation (IVM) was reported to affect oocyte maturation, fertilization and embryo development in adult sheep (1) but its action mechanisms need to be further investigated. The aim of the present study was to determine whether Cd, added at nano or microM concentrations during IVM may affect the maturation rates of prepubertal lamb oocytes. The hypothesis that Cd may affect oocyte maturation by impairing mitochondrial (mt) function was explored.

Oocyte-cumulus complexes (OCCs) from the ovaries of slaughtered two/four-months old prepubertal lambs underwent IVM (n=3 repetitions) in a TCM199-based medium under 5% CO2 in air at 38.5°C for 24h (1). Cadmium, at the concentrations of 1 and 100 nM and 10 microM was added in IVM medium for 24h. Oocytes cultured in absence of Cd were used as controls. At the end of the culture time, oocytes underwent cumulus cell removal and nuclear chromatin and mitochondria labeling with specific fluorescent probes (Hoechst 33258 and MitoTracker Orange CMTM ROS). Oocytes were assessed for meiotic stage and those showing the metaphase II (MII) plate with the first polar body were analyzed for ooplamic mt distribution pattern (Chi-square test) by 3D confocal microscopy. All methods were reported previously (2-3).

Oocyte maturation rates did not differ between Cd-treated and control oocytes (46.2%, 18/39, 38.7%, 12/31 and 58.6%, 17/29 for 1 nM, 100 nM and 1 microM respectively vs 56.3%, 27/48; not significant). Overall, reduced percentages of oocytes showing signs of cytoplasmic maturity (3), such as perinuclear and/or pericortical distribution of mt aggregates, were observed in Cd-treated oocytes compared with controls (53.8%, 14/26 vs 25% 12/48; $P<0.05$). All other oocytes were classified as having homogeneous mt distribution pattern. Comparisons between each concentration group and controls did not statistically differ (26.3%, 5/19; 25%, 3/12 and 23.5%, 4/17 for 1 nM, 100 nM and 1 microM, respectively vs 53.8%, 14/26; not significant).

In conclusion, Cd treatment during 24h IVM did not affect the maturation rates of prepubertal lamb oocytes. However, it seemed to affect oocyte cytoplasmic maturity, as higher rates of treated oocytes showed homogeneous distribution of small mt aggregates diffused throughout the cytoplasm. Further studies are needed to increase the data body, to evaluate Cd effects on mt activity and to elucidate mechanisms leading to impaired mt migration and compartimentalization.

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CONTRAST-ENHANCED-ULTRASONOGRAPHIC CHARACTERIZATION IN A DOG AFFECTED BY PROSTATIC LYMPHOMA: A CASE REPORT

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Primary lymphoma has been described in human patients (1), while in dogs only few reports are present (2,3) and no data on the use of Contrast enhanced ultrasound (CEUS) in this pathology is recorded. The use of microbubble contrast-enhanced ultrasound agents and the development of contrast-specific imaging techniques has overcome the limitations of Doppler Ultrasonography, due to the ability of the contrast agent to reach even very small vessels (4). Angiogenesis is known to play a key role in the growth and development of prostate cancer (5); CEUS has been recently used in veterinary medicine to study normal and pathological prostatic conditions (6-9).

The aim of this study was to describe the prostate perfusion kinetics in a dog with prostatic lymphoma by means of CEUS

An eight-year-old intact male American bulldog, affected by prostatic lymphoma, was subjected to clinical examination, serum chemistry profile, urinalysis, ultrasound and CEUS scanning of the prostate gland. The parameters examined were: peak intensity of perfusion (Peak %), arrival time of the contrast medium to its maximum value of video intensity (TTP), regional blood volume (RBV), regional blood flow (RBF) and mean time transit (MTT). Finally, a guided TruCut biopsy of the prostate was performed.

During the clinical examination the subject appeared alert, extremely thin and frequently postured to urinate without elimination of urine. Explored mucous membranes appeared slight pallor. Rectal examination evidenced a firm, non painful and symmetrically enlarged prostate. There was a mild normocytic, normochromic anemia (HCT 34%, Hb 11,1 g/dl), hyperazotemia (30 mg/dl), high creatinine level (2,3 mg/dl), alkaline phosphatase (294 U/l). The urine sample, collected via catheterization, showed a urine specific gravity of 1.025 and the presence of erythrocytes and leukocytes in the sediment. The ultrasound exam of the prostate showed a symmetrically increase in volume and a heterogeneous echo texture. In particular the cranial portion of the prostate (sagittal scanning) appeared with poor-defined margins. An increase in volume of the medial iliac lymph-node was also detected. To our knowledge, this is the first report using CEUS to describe perfusion of a prostatic lymphoma in a dog. The CEUS of the gland showed earlier wash-out characterized by a typical appearance of faint echo pollution, as already reported in human spleens with lymphoma (10). The value of TTP was lowest and PPI was higher than reported in normal prostate condition (6-9). The histological examination of the gland's biopsy confirmed the diagnosis of lymphoma. The hemodynamic indices and the vascular pattern evidenced that CEUS could be a valuable, non invasive, collateral tool to improve the diagnostic accuracy of prostatic lymphoma in a dog. Further investigations on a greater number of animals are needed to confirm the usefulness of CEUS for the identification of this pathology in dogs.

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LEPTIN RESISTANCE DURING PREGNANCY IN BITCH: PRELIMINARY RESULTS

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Leptin, a peptide secreted primarily by adipose tissue, is a key molecule for the regulation of energy balance through hypothalamic centers influence appetite. In non-pregnant animals its concentrations are positively correlated with body weight (BW)(1). Leptin plays an important role also in reproductive process, such as steroidogenesis and ovulation (2), and patterns of leptin during pregnancy were reported in human and in different animals species (3-5). As far as we know, there is a lack of information regarding leptin concentrations during pregnancy of bitches.

The aims of this study were to evaluate i) the plasma leptin concentrations and ii) the relationship between leptin, BW, and food intake throughout the canine gestation.

Blood sample were collected biweekly (at 10 am) from the day of mating (D0) until delivery from 7 German shepherd bitches, aged 4-9 years and weighting 30.2$\pm$ 0.7 kg. Individual BW was recorded biweekly and food intake was assessed daily by weighing food prior and after feeding. All bitches were submitted to ultrasonographic diagnosis of pregnancy (D25). Plasma leptin concentrations were determined by double antibody RIA using the multi-species leptin kit as previously reported 5. Data were analyzed by RM ANOVA and Pearson's r test. Comparisons against day 0 were done by Least Significant Difference test.

All bitches delivered naturally healthy puppies. Plasma leptin concentrations increased early (+15% at D15 compared to D0, $P < 0.05$) peaking at D45 (+22%, $P < 0.05$) followed by a decline before parturition. This increase was lower than that observed in other species (3-5). The increase in BW was delayed with respect to leptin rise (from D45 $P < 0.01$) and continued until the end of pregnancy. The correlation between leptin concentrations and BW previously reported1 were not found in our study during pregnancy ($r = 0.033$, $P = 0.849$). Moreover, hyperleptinemia did not decreased food intake which increased from D40 until the end of pregnancy ($P < 0.001$). This adaptive phenomenon, called "leptin resistance", is reported in other species (3-5) and maintains a positive energy balance during pregnancy to support fetal growth and subsequent lactation. Further studies are required to elucidate (i) the origin of leptin during canine pregnancy together with the endocrine factors that increase its steady-state levels, and (ii) the mechanisms involved in leptin resistance.

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THORACIC SPINE MALFORMATION IN PUPPY DOG A CASE REPORT

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Thoracic vertebral anomalies are common in English Bulldogs, Pugs, Boston Terriers and other brachycephalic breeds and most often involve the mid thoracic spine. Complex spinal malformation involving hemivertebrae, butterfly vertebrae, block vertebrae result from abnormal development, ossification and fusion of the vertebral ossification centers. These malformations often lead to some combination of scoliosis, kyphosis ,lordosis and spinal canal stenosis. Although alterations in the spinal canal may prevent normal spinal cord development, adaptation may initially minimize the extent of neurological compromise. Because the stability of the spine may also be affected, patients with complex vertebral anomalies often develop clinically appreciable neurologic deficits as are result of what might be considered relatively minor trauma (1,2,3,4).

The purpose of this work is to show as a careful neurological exam may show signs of a serious malformation that can be confirmed by radiological examinations.

A male pug of 4 months was brought to consultation for difficulties in the movement of the hind limbs. The puppy was subjected to neurological examination and radiographic evaluation of the spine. The patient was alert. Neurological examination of the patient shown bilateral strabismus, ataxia, normal reflexes of the front limbs, slowed reflexes in the hind limbs. Absence of proprioception of the hind limbs; test bounce slightly slowed to the right and all the other neurological tests were normal. Neurological examination showed a suspicious of lesion of thoracolumbar tract so we proceeded at radiographic examination To 'X-ray examination is observed congenital malformation of T7 that determines lordosis and scoliosis extremely serious. No other alteration was evident, the great organic functions of the patient and blood chemistry parameters were normal. ÃŕThe lesion is not typically considered a painful disease. Dogs may carry their head in ventroflexion. Although dogs may sometimes display discomfort when they are walking, they do not often display the degree of pain that is seen in type I disk extrusions, vertebral tumors, and meningitis. Mild discomfort may be representative of meningeal compression, arthritis, or nerve root pain. Dogs that display significant pain may have a massive disk extrusion or radiculopathy from nerve root impingement. Importantly, during the visit of sale of brachycephalic dogs assess the presence of these birth defects. It is also important to perform all diagnostic tests, CT or MRI. With this case report is demonstrate how easy to diagnose in daily practice the presence of these malformations without the use of advanced technology (1,2,3,4).

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LONG DISTANCE IDENTIFICATION IN STRAY DOGS BY EAR TAG APPLICATION DURING NEUTERING: EVALUATION OF PAIN/STRESS RESPONSE

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Herders have marked animals to identify their flocks since the Neolithic period and the practice is strongly associated with the domestication of animals1. In the present day, major concern is to visually identify stray dogs that have undergone sterilization in order to avoid their recapture, which results in wasted time, additional stress to the animal, and the potential for re-treatment. Ear-tagging is promising identification procedure that can also provide specific information about the dog, such as its reproductive status, gender, etc., without compromising health. The aim of this study was to scientifically evaluate the pain/stress response to the presence of ear tags after surgical sterilization in stray dogs.

30 healthy, crossbred dogs were selected. The dogs were around 1 to 8 years of age (mean = 4.1 years, SD= 0.8 years) and weighed 8 to 40 kg (mean 25.4= kg, SD= 5.2 kg). Captured stray dogs were kept in the shelter for 10 days to accustom themselves to captivity, saliva cortisol was evaluated (T0). Good health status was confirmed by routine blood tests and clinical examinations. At day 10 (T1) dogs were checked for saliva cortisol, neutered under anaesthesia and tags (roto or swivel tag) were pierced on the ear pavilion. Dog were under observation for 5 days: two hours after the application (T2) and each day (T3-T6) for Melbourne Pain Scale (MPS) to evaluate pain2, stress indicators (SI), salivary cortisol3, application site status and blood parameters (indicators of infections).

Multiple signs of stress (defecation, urination, pupil enlargement, tachycardia, etc.) and a higher MPS were noticed during handling at T0 and T1. Cortisol level was measured as a stress indicator and showed higher levels during the day of tag application (T1 and T2) as compared to T0 ($p < 0.01$). No inflammatory reactions were noticed in the study period. Video-monitoring indicated that some dogs (with the Roto-tag) scratched their ears using their back legs, shook their head, and rubbed their head against the wall several times during the 30 min taping. A small volume of exudate from the pierced hole was noticed in some dogs: this was more prevalent in long-haired dogs and those with the Roto-tag. Small skin lesions occurred in 6 dogs due to the tag rubbing on skin or the cartilage ridges of the ear. Handling is much more stressful for unowned dogs than the application of the tag and a good application of it reduce side effects.

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PRELIMINARY DATA OF CASA EVALUATION OF EURASIAN EAGLE-OWL (BUBO BUBO) SEMEN

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The Eurasian eagle-owl (Bubo bubo) is a species of eagle-owl that resides in much of Eurasia. Besides being one of the largest living species of owls, it is also one of the more widely diffused. With a total range in Europe and Asia of about 32 million square kilometers and a total population estimated to be between 250 thousand and 2.5 million individuals, the 'International Union for Conservation of Nature' lists the bird conservation status as being of "least concern" (http://www.birdlife.org/datazone/speciesfactsheet.php?id=2215).

Various studies investigated biology or breeding behaviour of this species and reported about reproductive programs in captivity1,2, but there is no information on semen collection and evaluation. The present study aims at improving knowledge about andrological aspects of Bubo bubo.

Seven adult males aged between 7 and 15 years, in full reproductive season, were included in the study. All owls were housed in outdoor pens, in couple with a female, and fed a diet consisting in rabbits, rats and day-old chicks. For semen collection, each bird was physically restrained by an operator by means of a soft towel to contain the front half of the bird body, in order to avoid struggling and stress, and to work safely. Ejaculation was obtained by massaging the dorsal aspect of the abdomen towards the cloaca, with the thumb and index or middle finger, followed by gentle rhythmic squeezing at the base of the cloaca with the same finger of the opposite hand 3. The ejaculate was collected in graduated microcapillary tubes (Microcaps, Drummond Science Company). Macroscopic and microscopic semen parameters were assessed, motility and kinetics characteristics were evaluated through a computer assisted semen analyzer (CEROS Hamilton Thorne Research Inc., version 14, Build 008, IMV Technologies, France)

A total of 8 ejaculates were collected and analyzed. In about ten collection session, at least one ejaculate was obtained only from 5 out of the 7 males. Overall, semen collection had 12% success rate. Semen colour was usually whitish to yellowish and volume was 9.0±4.0 μl (mean±SD). The degree of contamination ranged from grade 1 to 5, with a mean value of 2. Sperm concentration was 37.7± 53.0 sperm x106/ml; total motility was 25.3±18.0 % and progressive motility 13.0±9.6 %. Mean kinetic parameter values were: VAP 31.6±6.3 μm/s; VSL 27.8±5.5 μm/s; VCL 47.0±9.2 μm/s; ALH 2.4±0.8 μm; BCF 26.8±7.8 Hz; STR 88.0±6.1 %; LIN 63.3±9.6 %. The seminal characteristics of Bubo bubo or other owls have never been investigated or reported. If we compare our findings with data from other diurnal raptor species of similar size, we have analogous semen volume, sperm concentration and total motility. Knowledge of some andrological aspects of a bird species represents the first step to develop assisted reproduction programs and may be a useful model for similar endangered species.

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