71°

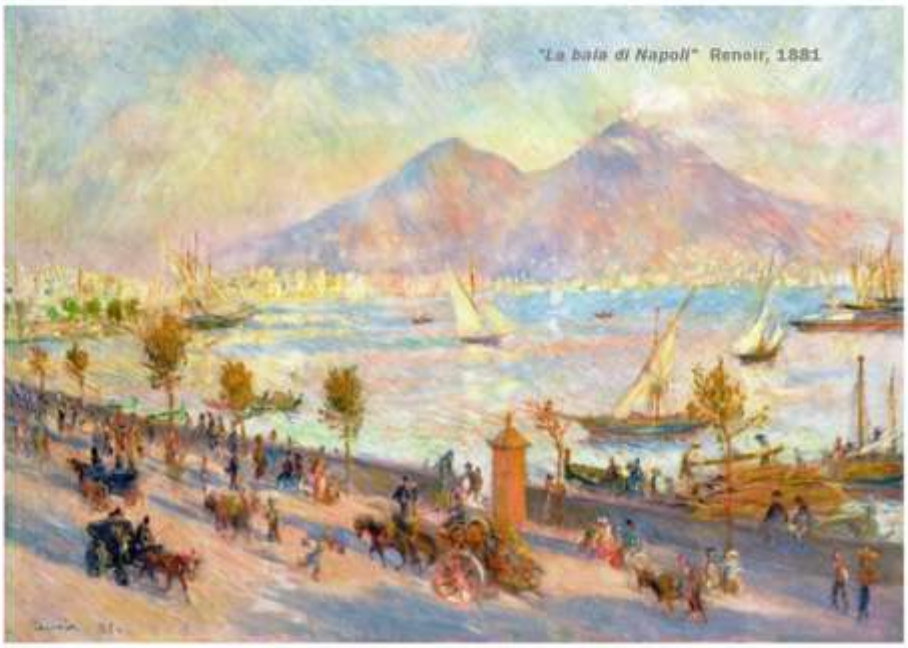
SOCIETÀ ITALIANA DELLE SCIENZE VETERINARIE

CONVEGNO SISVET

In collaborazione con

Dipartimento Medicina Veterinaria Produzioni Animali

Istituto Zooprofilattico Sperimentale del Mezzogiorno



XVII Convegno SICV

XV Convegno SIRA

XIV Convegno AIPVET

XII Convegno SOFIVET

IV Convegno RNIV

I Convegno ANIV

28 Giugno - 1 Luglio 2017

Università degli Studi di Napoli "Federico II"

Corso Umberto I, 40 - 80138 - Napoli

I contributi presenti negli Atti del 71° Convegno SISVet 2017

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Relazione del Presidente

Care colleghe e cari colleghi,

benvenuti al 71° Convegno della SISVet. Quest'anno il Simposio accoglie i convegni della SICV (Società Italiana di Chirurgia Veterinaria), dell'AIPVet (Associazione Italiana dei Patologi Veterinari), della SIRA (Società Italiana di Riproduzione Animale), della SOFIVet (Società di Fisiologia Veterinaria), della RNIV (Rete Nazionale di Immunologia Veterinaria) e della rinnovata ANIV (Associazione Nazionale Infettivologi Veterinari). Anche la neonata Associazione Italiana di Storia della Medicina Veterinaria e della Mascalcia sarà presente al Convegno con la Mostra sulla Medicina Veterinaria nella Prima Guerra Mondiale.

Sono in programma 292 lavori scientifici (rispetto ai 232 dell'anno scorso), sotto forma di comunicazioni orali, poster e *main lectures*, oltre a cinque *workshops*, una tavola rotonda sul "Percorso formativo per la professione veterinaria", un corso di "Neuropatologia veterinaria" per giovani patologi, un evento *pre-congress* in "Patologia Forense Veterinaria" e un *post-congress* organizzato dalla FNOVI in collaborazione con SISVet su "Il rilancio delle piccole produzioni locali". I "*Mystery Cases*", quest'anno in sessione diurna, proporranno casi misteriosi di patologia, clinica e parassitologia.

Anche quest'anno la partecipazione dei più giovani, non strutturati, è agevolata con una quota di iscrizione ridotta del 50%. Il Consiglio Direttivo della SISVet ha inoltre deliberato di bandire, per il Convegno 2017, un premio da 1000 € per ogni sessione scientifica. I premi sono destinati alle comunicazioni orali, presentate da autori under 40, che saranno pubblicate su riviste indicizzate, mentre il miglior poster di ogni sessione sarà premiato con l'iscrizione gratuita al convegno del 2018.

Si riunirà in questa sede anche il Tavolo Tecnico per la costituzione della "Federazione delle Società Scientifiche Veterinarie Italiane", che vedrà la partecipazione dei Presidenti delle principali Società Scientifiche Veterinarie italiane e dei rappresentanti dei SSD che non fanno riferimento a Società Scientifiche.

Riguardo alla divulgazione degli atti dei nostri Convegni, la banca dati CABI Publishing ha già inserito gli atti dei Convegni SISVet 2014, 2015 e 2016, *full text in CAB Abstracts/Full-Text Repository*. Prossimamente saranno inseriti anche gli atti degli anni precedenti.

La cena sociale si svolgerà presso l'Hotel Royal Continental, una delle più belle location di Napoli.

Desidero pertanto ringraziare i membri del Comitato Organizzatore, del Consiglio Direttivo e del Comitato Scientifico, che con il loro impegno hanno dato un contributo fondamentale all'organizzazione del Convegno.

Un doveroso ringraziamento va al Magnifico Rettore dell'Università degli Studi Federico II di Napoli, Prof. Gaetano Manfredi, al Presidente della Regione Campania, On.le Vincenzo De Luca, al Sindaco di Napoli, On.le Luigi de Magistris, al Dipartimento di Medicina Veterinaria e Produzioni Animali di Napoli, all'Istituto Zooprofilattico Sperimentale del Mezzogiorno, ai Servizi Veterinari e agli Ordini Professionali della Regione Campania. Altrettanta gratitudine va alla Conferenza dei Direttori dei Dipartimenti di Scienze Veterinarie e ai rappresentanti del CUN, che con la loro presenza conferiscono autorevolezza al Convegno. Infine un sentito ringraziamento agli Sponsor.

Auguro a tutti i partecipanti una proficua e gradevole permanenza a Napoli, invitandovi a visitare nel tempo libero la città e i magnifici tesori d'arte da essa custoditi.

Benvenuti a Napoli.

Presidente SISVet

Bartolomeo Biolatti

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Alibus collega l'Aeroporto Internazionale di Napoli con la **Stazione Ferroviaria di Piazza Garibaldi** e la **Stazione Marittima di Piazza Municipio**.
Il servizio è effettuato tutti i giorni, festivi compresi, con una frequenza di 20 minuti. Maggiori informazioni: 800 - 63 95 25

Muoversi in metro a Napoli

Napoli ha 2 linee metropolitane e altre linee di treni che dalla città vanno verso la periferia e le città limitrofe. La principale è **la Linea 2** che parte dalla Stazione centrale di Napoli Piazza Garibaldi e taglia la città fino ad arrivare a Pozzuoli, nei Campi Flegrei. **Lungo il percorso ci sono tutte le fermate per raggiungere il centro città**: Montesanto e Piazza Cavour sono le fermate ideali per raggiungere il centro storico, la strada dei Presepi di San Gregorio Armeno, Piazza del Plebiscito e il Molo Beverello per gli imbarchi. **Piazza Amedeo porta alle strade dello shopping** costoso e con pochi passi a piedi al lungomare. Mergellina è la stazione per chi deve prendere gli aliscafi per le isole di Ischia, Capri e Procida. Più avanti ci sono le fermate di Fuorigrotta per lo Stadio, Bagnoli per Città della Scienza e, infine, Pozzuoli. **La Linea 2 funziona tutti i giorni dalle ore 6:15 alle 23:00.** Lo stesso percorso della Linea 2 è seguito dalla Ferrovia Cumana che parte in prossimità della stazione di Montesanto della Linea 2 e raggiunge Pozzuoli e i Campi Flegrei.

La nuova Linea 1

La Linea 1 è quella di recente costruzione, un vero gioiello di tecnologia e arte. Parte da Piazza Garibaldi (Stazione Centrale) e sale verso il Vomero, incrociando la Linea 1 nella stazione di Piazza Cavour. **La Linea 1 è il cosiddetto Metro dell'Arte,** perché ogni fermata è stata costruita insieme a 26 grandi artisti contemporanei. **E' attiva tutti i giorni dalle 6:00 alle 23:00.** Le fermate **Municipio, Università, Toledo, Dante e Cavour** conducono nel cuore storico di Napoli.

Programma Generale

Mercoledì 28 Giugno

9.30 12.30	Pre-Congress Società AIPVet "Patologia Forense Veterinaria" **(Aula Pessina)**
13.00 14.00	APERTURA REGISTRAZIONI (Rettorato C.so Umberto I, 40 – Napoli)
14.15 16.00	WS1: "Attualità e prospettive della citometria a flusso in medicina veterinaria" **(Aula Arcoleo)**
14.15 16.00	WS2/ECM: "Innovazioni zootecniche e sanitarie nell'allevamento asinino" **(Aula Graziani)**
16.00 16.30	**POSTER TOUR n.01** (cfr. Sessioni poster)
16.30 19.15	Sessioni Parallele Comunicazioni Scientifiche e Lezioni Magistrali: SISVet, SICV, SIRA, AIPVet, SoFiVet, RNIV, ANIV (cfr. Comunicazioni Orali)
19.15 19.30	Inaugurazione Mostra itinerante "La Medicina Veterinaria nella Prima Guerra Mondiale"
20.00	***Welcome Party*** ***(Rettorato dell'Università degli Studi di Napoli – Federico II)***

Giovedì 29 Giugno

8.30 10.00	Sessioni Parallele Comunicazioni Scientifiche e Lezioni Magistrali: SISVet, SICV, SIRA, AIPVet, SoFiVet, RNIV, ANIV (cfr. Comunicazioni Orali)
10.00 **10.30**	**POSTER TOUR n.02** (cfr. Sessioni poster) **Coffee Break**
10.45 **11.15**	**Inaugurazione 71° Convegno SISVet** **(Aula Magna Storica – II Piano - Rettorato)**
11.15 **13.30**	TAVOLA ROTONDA: "Un nuovo percorso formativo per la Professione Veterinaria" **(Aula Magna Storica – II Piano - Rettorato)**
13.30 14.30	**PRANZO A BUFFET**
14.30 16.30	Sessioni Parallele Comunicazioni Scientifiche e Lezioni Magistrali: SISVet, SICV, SIRA, AIPVet, SoFiVet, RNIV, ANIV (cfr. Comunicazioni Orali)
16.30 17.00	**POSTER TOUR n.03** (cfr. Sessioni poster) **Coffee Break**
17.00 19.00	WS3: "Uso del farmaco veterinario nelle specie minori (Bufalo mediterraneo e Ovi-caprini)" **(Aula Pessina)**
17.00 19.00	AIPVet – **TEACHING COURSE**: *Basic Approach to Veterinary Neuropathology* **(Aula Gigante)**
19.00 20.00	**ASSEMBLEE DELLE SOCIETÀ SCIENTIFICHE/SSD**
20.30	**CENA SOCIALE** (Hotel Royal Continental)

Venerdì 30 Giugno

8.30 10.00	Sessioni Parallele Comunicazioni Scientifiche e Lezioni Magistrali: SISVet, SICV, SIRA, AIPVet, SoFiVet, RNIV, ANIV (cfr. Comunicazioni Orali)
10.00 10.30	**POSTER TOUR n.04** (cfr. Sessioni poster)
10.30 11.00	**Coffee Break**
11.00 13.30	WS4/ECM: "Il Medico veterinario a tutela delle produzioni tipiche" .**(Aula Marcello Gigante)**
11.00 13.30	Mystery Case **(Aula Pessina)**
13.30 14.30	**PRANZO A BUFFET**
14.30 16.30	Sessioni Parallele Comunicazioni Scientifiche e Lezioni Magistrali: SISVet, SICV, SIRA, AIPVet, SoFiVet, RNIV, ANIV (cfr. Comunicazioni Orali)
16.30 17.00	**POSTER TOUR n.05** (cfr. Sessioni poster) ***Coffee Break***
17.00 19.00	Sessioni Parallele Comunicazioni Scientifiche e Lezioni Magistrali: SISVet, SICV, SIRA, AIPVet, SoFiVet, RNIV, ANIV (cfr. Comunicazioni Orali)
19.00 20.00	**ASSEMBLEA SISVet** **(Aula Pessina)**

Sabato 1 Luglio

09.30 17.00	WS5/ECM: "I sarcomi dei tessuti molli nei piccoli animali. Un approccio multidisciplinare per la cura del paziente" **(Aula Pessina)**

Workshop

WORKSHOP 1

"ATTUALITÀ E PROSPETTIVE DELLA CITOMETRIA A FLUSSO IN MEDICINA VETERINARIA"

FLOW CYTOMETRY IN VETERINARY: CURRENT STATE AND FUTURE PERSPECTIVE

Grandoni Francesco

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria. CREA-ZA (Monterondo-RM)

Flow cytometry is an important and versatile technology: flexibility, accuracy and multiparametric analysis are some of its characteristics [1]. Every year scientific articles number about this technology is constantly increasing and including studies on microbiology, oncology, immunology, hematology, and more recently also nanotechnology. In veterinary contest, we can see this trend over last three decades. At the end of 70's, the first study was published on bull sperm [2] and ten years later it was possible to sort spermatozoa with X or Y chromosome [3]. In the middle 80's the first papers were published that used fluorescent monoclonal antibodies to characterize bovine blood and milk leukocytes [4, 5]. In the following years, flow cytometry has been applied on many other animal species and using different approaches. However, in veterinary this technology hasn't reached the level of sophistication of human sector where it is normal to use panels with 8 or more different markers. The practical problems that limited its use were: availability and cost of reagents, complexity and cost of these instruments [6]. Is it still so today? Something has changed: slowly the amount of monoclonal antibodies is increasing, we can use custom antibodies production or labeling service alternative to use kit for home made labeling. The entry level flow cytometers cost about 40,000€ and they are smaller and simpler than old instruments. So, now what are the limits of its diffusion? First, it is necessary that flow cytometer is used only by specially trained professionals. Secondly, a correct information between clinician and flow cytometrist and to know potential and limits of this technology and these instruments.

For any additional information please contact us at the following e-mail address: francesco.grandoni@crea.gov.it

[1] Shapiro HM. Practical Flow Cytometry. 4th ed. Wiley-Liss, New York, 2003. [2] Van Dilla MA et al. Measurement of mammalian sperm deoxyribonucleic acid by flow cytometry. Problems and approaches. J. Histochem. Cytochem, 25(7): 763-73, 1997. [3] Johnson LA, Clarke RN. Flow sorting of X and Y chromosome-bearing mammalian sperm: activation and pronuclear development of sorted bull, boar, and ram sperm microinjected into hamster oocytes. Gamete Research, 21(4): 335-43, 1988. [4] Lewin HA et al. Monoclonal antibodies that distinguish bovine T and B lymphocytes. Vet Immunol Immunopathol, 9(1): 87-102, 1985. [5] Hageltorn M, Saad MA. Flow cytofluorometric characterization of bovine blood and milk leukocytes. Am J Vet Res, 47(9): 2012-6, 1986. [6] Tarrant JM. The role of flow cytometry in companion animal diagnostic medicine. Veterinary Journal, 170:278–288, 2005.

FLOW CYTOMETRY: GENERAL PRINCIPLES AND FIELDS OF APPLICATION

Claudio Ortolani, Stefano Papa

Università degli Studi di Urbino, Dipartimento di Scienze Biomolecolari

Diagnostics and biomedical research are two evolving fields, and it is particularly difficult to define the boundaries of the techniques, which make their progresses possible.

This is especially true for Flow Cytometry. The difficulty is due to two factors, the first of which is the parallel evolution of Molecular Biology, sister and competitor of Flow Cytometry, and the second is paradoxically the high flexibility of Flow Cytometry itself, whose techniques are often confined in ancillary roles, based on a series of anecdotal applications not coded by guidelines or shared protocols.

Nevertheless, Flow Cytometry applications are of paramount importance everywhere "proteomic" information is needed. In keeping with this assumption, Flow Cytometry has become synonym for immunophenotypization, and is currently used as a diagnostic procedure in every situation in which phenotypic alterations behave as a marker of disease, or are able to cast light on the reactive or causal mechanisms involved in the pathogenesis.

This is especially true in conditions where the population of interest is extremely infrequent, and hided by a highly heterogeneous context, making Flow Cytometry the technique of choice in the assessment of the Minimum Residual Disease.

Moreover, the huge mass of data produced by Cytometry makes this method irreplaceable when it is necessary to manage apparently unrelated information as in the case of cell activation studies, and makes it cooperate with bioinformatic techniques aimed at the identification of pattern not previously known to the operator.

FLOW CYTOMETRY IN VETERINARY ONCOHEMATOLOGY

Fulvio Riondato

Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

In a veterinary diagnostic setting flow cytometry (FC) is mainly used in oncohematology and it is claiming as routine test beside classical cytologic and hematologic evaluation in case of suspected lymphoma or leukemia. In most cases, sampling is minimally invasive and results are provided in a day. FC analysis requires cells in a monodispersed suspension. Peripheral blood and bone marrow blood are therefore 'ready to use' tissues for FC. Lymph nodes are well suited for the analysis as they give separated cells that can be easily collected through fine needle aspiration and used both for cytologic preparation and FC. Other kind of samples can be submitted for FC analysis: effusions, urine, aspirates from organs (spleen, liver, kidney, thymus, etc) and masses with different localization. Samples are conventionally analyzed within 24 hours; peripheral and blood marrow blood are collected in EDTA while lymph node aspirates should be collected in RPMI. The administration of glucocorticoids and chemoterapy can alter results and it is recommended to run the analysis before starting any therapy. In the dog, basic panels for lymph node immunophenotyping include usually CD45 (panleukocytic marker), sCD3, cyCD3, CD5, CD4, CD8 (T lymphocytes), CD21, cyCD79a/b (B lymphocytes). In case of leukemia, markers of myeloid and megakaryocytic lineage (CD11b, CD14, MPO, CD61) and of precursor cells (CD34, CD117) are added. In the cat the availability of antibodies is more limited (cyCD3, CD5, CD4, CD8, CD21, CD11b, CD14).

Diagnosis relies on the combined evaluation of scatter properties and immunophenotype of cells. The presence of a population with a unique immunophenotype (indicative of clonal proliferation) and/or with aberrant antigen expression with respect to the non-neoplastic counterpart is particularly important for the definition of neoplastic condition. In this regard, the use of a multicolour approach can be decisive as in the case of the identification of T-zone lymphoma (1) and the differential diagnosis between thymoma and lymphoma (2). The expression of specific antigens allows the lineage definition both in case of leukemia (myeloid, monocytic, megakaryocytic, lymphocytic) and lymphoma (B vs T). CD34 is usually indicative of acute leukemia; however CD34+ lymphomas are rarely found and acute lymphoid leukemias are often CD34-negative. Acute undifferentiated leukemias are diagnosed when only common (CD45, CD18) and precursor antigens (CD34, CD117) are expressed. Once the antigenic pattern of neoplastic cells is defined, FC is useful to stage the disease allowing the detection of neoplastic infiltration in different tissues. After treatment FC can be used to detect and quantify residual neoplastic cells (minimal residual disease).

FC provides useful informations to classify lymphomas according to the Kiel updated scheme (currently used in cytology). In addition to scatter properties and immunophenotype, Ki67 determination can be used to discriminate between high grade and low grade forms.(3) However, only one specific histologic subtype according to WHO classification can be currently detected basing only on FC. It is the case of T-zone lymphoma characterized by small sized elements that appear CD45-negative and often exhibit CD21 at low intensity.(1) Recently, different studies gave FC a role in providing prognostic indexes for canine lymphomas. Intermediate values of Ki67 (4) and higher values of LMR (lymphocyte to monocyte ratio) (5) have been associated to a better prognosis in diffuse large B cell lymphomas and low levels of class II MHC expression have been reported to predict a poor outcome in B-cell lymphoma. (6)

1) Seelig et al. 2014. J Vet Intern Med. 28:878-86. 2) Lana et al. 2006. J Vet Intern Med. 20:1161-5. 3) Poggi et al. 2015. Vet Comp Oncol. 13:475-80 4) Poggi et al. 2016. Vet Comp Oncol. doi: 10.1111/vco.12184.
5) Marconato et al. 2015. Vet J. 206:226-30 6) Rao et al. 2011. J Vet Intern Med. 25:1097-105

CHARACTERIZATION OF ADULT STEM CELLS IN BOVINE MILK THROUGH FLOW CYTOMETRY

Eugenio Martignani (1), Mario Baratta (1)

(1) Università degli Studi di Torino, Dipartimento di Scienze Veterinarie.

In previous works we demonstrated that a population of adult epithelial stem cells reside in the mammary gland of bovines and other ruminants [1]. While the presence of these cells is important for the cyclic remodelling of the gland itself during subsequent reproductive cycles, it is difficult to use them as an indicator of aging of the tissue or as sensors for alteration of animal welfare, due to the invasiveness of the established techniques for their isolation and purification. There are reports that in human milk, adult mammary stem cells, progenitors and terminally differentiated epithelial cells are shed [2]. In bovines most of the studies done on the cellular fraction of milk take into account only the count of somatic cells or focus mostly on leucocytes [3]. Instead we decided to analyze the somatic cells in bovine milk at different stages of lactation to characterize the different subpopulations of epithelial origin as putative markers for alteration of the mammary gland homeostasis.

For this purpose a 6-color panel for flow cytometry analysis was set up. We used a combination of antibodies directed against surface antigens (CD45 and CD49f), intracellular structural proteins (cytokeratins 14 and 18) and two different nuclear stains. Briefly, milk samples from healthy dairy cows were collected and cells were isolated by diluting milk with PBS 1:1, followed by centrifugation. Cells were then stained for surface markers and the first fixable nuclear stain was added to discriminate for live/dead cells. Then cells were fixed, permeabilized and stained for intracellular markers before analysis.

With this approach we were able to limit the analysis to live cells only, shed in milk, and to quantify the proportion of total epithelial cells in somatic cells. Moreover we assessed the relative frequency of various subpopulations of epithelial cells by detection not only of different markers, but also of their different expression levels, which are associated to specific mammary epithelial phenotypes. We also managed to track variations in the frequencies of epithelial subpopulations throughout lactations stages, since sample collection for this type of analysis is non-invasive and routinely performed during regular milking sessions. Therefore with flow cytometry we were able to quickly analyse a high number of cells and to detect populations that have a very low frequency in milk and which might have gone undetected with other techniques.

We demonstrated that primitive and differentiated epithelial cell subpopulations have a different distribution according to the lactation stage in healthy animals. While many information can be gathered by counting white blood cells that are commonly found in milk and how they associate with pathological conditions of the mammary gland, earlier alterations of the homeostasis of the mammary gland which are not associated with an immune or inflammatory reaction might be detected with more precision by considering alterations in the distribution of the epithelial fraction shed in milk. Hence the importance of a specific and precise characterisation of the epithelial subfraction.

[1] Martignani et al. Human milk protein production in xenografts of genetically engineered bovine mammary epithelial stem cells, PLoS One 5(10) e13372, 2010. [2] Cregan et al. Identification of nestin-positive putative mammary stem cells in human breastmilk, Cell and Tissue Research 329: 129-136, 2007. [3] Li et al. Role of somatic cells on dairy processes and products: a review, Dairy Science and Technology 94:517-538, 2014

WORKSHOP 2

"INNOVAZIONI ZOOTECNICHE E SANITARIE NELL'ALLEVAMENTO ASININO"

UPDATE ON DONKEY INTERNAL MEDICINE

Fulvio Laus (1), Micaela Sgorbini (2)

(1) Università degli Studi di Camerino, Scuola di Bioscienze e Medicina Veterinaria, Italia. (2) Università di Pisa, Dipartimento di Scienze Veterinarie, Italia

Interest in the welfare and diseases of donkeys is constantly increasing in several countries. Despite this, clinical research on donkeys needs to be in continual development since they show different reactions compared to horses in many conditions, including infectious diseases, and need specific clinical and therapeutic approaches. Some update about donkey diseases is here provided. Epidemiology and clinical presentation of piroplasmosis and other TBDs show some differences between horses and donkeys, the latter having a less severe symptoms despite high exposure to the parasites. Also clinical presentation of some respiratory diseases (e.g. guttural pouch mycosis) has peculiarity in this species. Basal data about ultrasound measurement of adrenal glands and ocular structures are described. Results of ocular bacterial and micotic isolation are provided to be used as comparison with infected animals.
Some studies have been published on ultrasonography, endocrinology, hematology and biochemistry in jennies pregnancy and data on semeiological parameters, and hematological and biochemical data in donkey foals have been supplied. Recent studies focussed the attention on diagnostic procedures, such as the use of a single dose of iohexol for the evaluation of GFR and the validation of an ELISA kit for the determination of salivary cortisol for the ealuation of stressful events. Finally, EGUS has been investigated in alive donkeys and the results have been compared to data reported for horses and for dead/euthanized donkeys.

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ANESTHESIA IN DONKEYS

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Although it is tempting to treat donkeys like little horses, there are significant differences in physiology, behavior and drug response which must be taken into account in order to provide successful and minimally stressful anesthesia. The most important differences to keep in mind are: donkeys do not have the same flight response as the horse and they are very stoic making it difficult to assess illness or painful condition; the jugular vein is covered by the cutaneous colli muscle, which is thicker than in the horse and this may make it more difficult to visualize the vein; donkeys require higher dosages of non-steroidal anti-inflammatory drugs or shorter dosing intervals to achieve the same degree of analgesia since they metabolize many drugs more rapidly than horses; standing procedures can be performed with the same drugs and combinations (eg, acepromazine with alfa2-agonosts plus butorphanol) but with increased dosages to achieve an acceptable effects. As with horses, a smooth induction is only achieved when the donkey is adequately sedated before administering induction drugs. A variety of drugs can be used for induction and maintenance. The combination of guaiphenesin, xylazine and ketamine, commonly referred to as “triple drip”, produce smooth induction and can be used for the maintenance, being very careful because donkeys are more sensitive to the respiratory effects of guaiphenesin. Thiopental can also be used with good results for induction but recovery could be slow. Other drugs which have been used in donkeys to induce and maintain general anesthesia are ketamine-benzodiazepine, tiletamine-zolazepam or propofol. Since apnea and desaturation are common problems with propofol, it is not recommended for use unless intubation or oxygen supplementation is available. Endotracheal intubation and maintenance with inhalant anesthetics is preferred for longer procedures. Donkeys have a pharyngeal diverticulum in throat, excess tissue in pharynx, and elongated laryngeal saccules, anatomical differences that make orotracheal intubation more difficult. Depth of anesthesia should be monitored in the same manner as for horses. Careful observation of changes in respiration and eye reflexes are necessary to maintain appropriate depth of anesthesia. Again, donkeys are more stoic and don’t show these differences as much as horses. Recovery from anesthesia is usually not affected by the hysteria which is often seen in horses. However, analgesia should be provided since pain affects the quality of recovery from anesthesia. Donkeys will generally lie quietly until they are able to stand.
Constant rate infusion of alfa2-agonists, opioids, ketamine or lidocaine can be used to provide intraoperative analgesia when needed, but there is no information specific to the use of these drugs in donkeys compared to horses.

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MAIN PARASITIC DISEASES IN DONKEYS

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In some European countries, particularly in Italy, there is an increasing interest on donkey as a pet, in leisure activities, for onotherapy (animal-assisted therapy) and especially for the rediscovery of donkey milk [1]. Donkeys are susceptible to a large range of internal and external parasites. The main internal parasites are intestinal strongyles (large: *Strongylus vulgaris, S. edentatus, S. equinus* and *S. asini*), (small: *Cyathostomidae*), ascarids (*Parascaris* sp.), lungworm (*Dictyocaulus arnfieldi*), pinworm (*Oxyuris equi*), tapeworm (*Anoplocephala* spp.), liver fluke (*Fasciola hepatica*) and protozoa (*Eimeria leuckarti, Babesia caballi, Theileria equi*). All parasites that affect donkeys also infect horses, so animals that co-graze can act as a source of infection for either species. Donkeys with a significant parasite burdens may appear healthy and it is rare to observe clinical signs [2]. In Europe large-scale epidemiological studies on donkey parasites are extremely scarce. Recently, a survey was conducted to determine the prevalence of the principal helmintic infections in Italian donkey farms. Strongyles were the most common parasites found, with a prevalence of 84.9%; 15.1% of donkeys were not infected and strongyle egg count was lower than the donkey cut-off selective therapy (300 eggs per gram) in 28.3% of animals. In all tested farms, coprocoltures revealed the presence of Cyathostomes (100%). Other parasites were: *Dictyocaulus arnfieldi* (6.9%), *Oxyuris equi* (5.8%), *Parascaris* spp. (3.6%), *Anoplocephala* spp. (1.0%) and *Strongyloides westeri* (0.3%). No positivity was detected for *F. hepatica* [3]. Equine piroplasmosis (EPs) are tick-borne diseases affecting horses and donkeys caused by the intraerytrocytic protozoa *B. caballi* and *T. equi*; these diseases are common in the Mediterranean basin. Donkeys generally remain asymptomatic with lower parasitemia. Chronic cases of EP are common in donkeys and clinical signs are usually aspecific, including dysorexia, reduction of work or production performance and weight loss [4]. High seroprevalence values for *B. caballi* (range 36-48%) and *T. equi* (range 41-44%) were reported in donkeys in central and southern Italy [4,5]. A wide range of ectoparasites (insects, mites and ticks) cause disease in donkeys. Lice infestation is rather common in the donkeys, especially during winter and in sick, old or debilitated animals. Donkeys and horses may be infested with two species of louse, the chewing louse (*Mallophaga*), *Werneckiella equi* and the bloodsucking louse (*Anoplura*), *Haematopinus asini*. Louse infections are widespread in donkey farms in Italy. These infestations can be correlated with poor management practices and low frequency of ectoparasite treatments. Lice are highly susceptible to the common pour-on insecticides [6]. Therapeutics, such as antiparasitic compounds, are often administered to donkeys based on dosage and intervals recommended for horses and cattle, because very few drugs have donkey-specific label indications [7]. The frequent use of antiparasitic drugs and sub-optimal dosing could be implicated in the selection of resistant parasites. Indeed the donkeys, have a relative greater capacity than horses to metabolise and eliminate drugs, therefore, should not be regarded as a small horse, but rather recognised and treated as a different species. Therefore it is crucial that veterinary practitioners play an active role in planning and monitoring effective and appropriate parasite control programs considering the donkey as a desert animal like a camel.

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NUTRITIONAL AND NUTRACEUTICAL AMIATINA DONKEY MILK QUALITY

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Recently, a project aimed to the creation of a donkey milk chain from Amiatina native breed has been developed in Tuscany. In a second step of the project, the use of donkey milk in the diet of children with allergy to the cow's milk proteins (CMA) has been evaluated. At the same time, nutritional, nutraceutical, hygienic and safety milk characteristics were tested. Furthermore, another topic of the project was to promote the biodiversity and the economy of Tuscany productions and to provide products having a constant quality. Every month bulk milk samples underwent physical and chemical analysis. The results showed dry matter content of 10 g/100 ml of milk and total protein and fat content of 1.6 and 0.5 g/100 ml, respectively. In donkey milk the protein fraction was more similar to human milk than ruminant milk, with low amounts of casein (0.8 g/100 ml). Whereas whey protein content was about a half of the total proteins (51.1%). The main whey protein fractions were beta-lactoglobulin and alpha-lactalbumin (0.35 and 0.30 g/100 ml, respectively). In addition, the lysozyme content was high (0.15 g/100 ml) and represented about the 9% of the total proteins; this enzyme has antimicrobial activity [1]. Fat content significantly differed from human milk, thus it would require an energetic integration when is used in infant feeding. By contrast, the milk showed a fatty acid composition of nutritional interest, especially for the unsaturated fatty acid content (45 g/100 g of fatty acids). In particular, the unsaturated/saturated fatty acid ratio and the ω6/ω3 ratio were 0.82 and 1.65 respectively. Oleic, linoleic, alpha linolenic and eicosapentanoic acids were respectively 17%, 14%, 8% and 0.13% of the total fatty acids. These polyunsaturated fatty acids are known for their beneficial properties on human health. In addition, milk fat globules showed a mean diameter of 1.92 micron, smaller than the diameter reported for other milks. The smaller globule diameter may promote the digestibility of donkey milk fat [2]. The high lactose content (7.1 g/100 ml) is also of nutritional interest because of its contribution on milk palatability that makes the milk acceptable by children. Ash content was lower than cow milk and was about 0.36 g/100 ml. With regard to individual minerals, the amount of calcium (70 mg/100 ml), phosphorus (60 mg/100 ml), potassium (60 mg/100 ml) and sodium (20 mg/100 ml) were more similar to human milk. The Amiatina donkey milk had also a high content of vitamin D (2.3 µg/100 ml; 92 IU/100 ml on the average), higher in summer than in winter. In conclusion, the similarities with human milk were high, except for the fat content. This finding highlights the need for energy integrations especially when the milk is used in early childhood. Moreover, donkey milk use could be extended to obese people and to the elderly.

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BIOACTIVE PEPTIDES IN DONKEY'S MILK

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Donkey's milk (DM) is considered as a good alternative for children affected by cow's milk protein intolerance (CMPA), when it is not possible breast feeding [1]. DM is very similar to human one, especially considering the similar lactose content, the low casein content and the similar mineral composition [2, 3]. As regards to the casein protein fraction, in DM are present mainly two types of caseins: β- and α_{s1}- caseins whereas the α_{s2}- and k-casein content resulted to be very low. The whey protein fraction of DM is characterized by the presence of proteins/peptides with potential nutraceutical function such as lysozyme, lactoferrin, lactoperoxidase, alpha-lactalbumin and beta-lactoglobulin. Lysozyme (14.4 kDa), is a well know enzyme with antimicrobial properties since it is able to hydrolyze the glyosidic bonds of mucopolysaccarides of the bacterial cell walls. DM lysozyme content (1.0 g/l) is much higher if compared to that one found in bovine (traces), human (0.12 g/l) and goat's milk (traces) [3]. Lysozyme may have therapeutic, antiviral and anti-inflammatory properties, therefore may help to reduce the incidence of gastrointestinal infections in infants. Lactoferrin and lactoperoxidase (0.084 g/l and 0.11 mg/l in DM, respectively) are other two proteins that exhibit several activities. In particular, lactoferrin is an iron binding protein involved in regulation of iron homeostasis, cellular growth, anti-microbial and anti-viral functions and protection against cancer development and metastasis. Furthermore, lactoferrin may have a prebiotic activity, stimulating the growth of beneficial bacteria in the intestinal tract. Alpha-lactalbumin (about 12.0 kDa) is a whey protein with good nutritional value since it provides all essential and branched-chain amino acids to the growing infant, it is able to bind divalent cations facilitating the absorption of essential mineral. In addition, the peptides formed after the digestions of Alpha -lactalbumin may have immunostimulatory and antibacterial activities. Alpha-lactalbumin concentration in DM is 1.8 mg/ml, very close to the content determined in human milk. DM beta-lactoglobulin content resulted to be 3.75 mg/ml, close to values obtained in bovine milk and mare's milk, whereas in human milk this protein is absent. Beta-lactoglobulin is part of the lipocalin family, it is able to bind and to transport small hydrophobic molecules (such as retinol) and lipids. In conclusion, DM possesses a set of nutritional properties especially thanks to the presence of bioactive peptides which have a high nutritional value.

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WORKSHOP 3

"USO DEL FARMACO VETERINARIO NELLE SPECIE MINORI (BUFALO MEDITERRANEO E OVI-CAPRINI)"

ANTIMICROBIAL RESISTANCE, ANTIMICROBIAL STEWARDSHIP AND THE ROLE OF VETERINARIANS IN BUFFALO FARMS

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The growing problem of antimicrobial resistance has become a significant public health concern worldwide in humans and veterinary medicine and it involves all types of bacteria both pathogenic and nonpathogenic (1). Since 1999, there have been numerous international and national reports about correct use of antibiotics in humans and in animals (2-4). The Mediterranean buffalo (*Bubalus bubalis*) plays a major role in the Campania Region where buffalo herds represent over 80% of the national buffalo assets. The economic importance of this animal species, linked to trading of high quality milk and cheese, requires a correct dairy management and a sanitary control program with the purpose to fully protect it.

To our knowledge, until now, there are limited information on antibiotic-resistance in buffalo species, however there seems to be a lower occurrence of the antibiotic-resistance in buffalo respect to bovine species. Some data show that bacterial strains isolated from buffalo present low sensitivity for amoxicillin/clavulanic acid (5-6), a typical antibiotic used to treat a wide variety of bacterial infections both in humans and animals (small and large). Improve antibiotic use to combat antibiotic resistance is a necessary initiative to involve the buffalo herds which, so far, do not show alarming data. The need for preventive and corrective measures is urgent. We suggest to improve the antibiotic use through an antibiotic stewardship (7) which is the systematic attempt to educate and persuade prescribers of antimicrobials to follow evidence-based procedures, in order to stem antibiotic overuse, and thus antimicrobial resistance. For this purpose the role of the veterinarian in buffalo farms is crucial since he assists to construct the suitable preventive measures. Although the veterinary practitioner is on the front line of stewardship, the evolving idea of stewardship involves many other fundamentals and actors. For instance, it is essential to plan epidemiological studies aimed at buffalo species to better know the antimicrobial resistance monitoring. And, it is also important to point out the potential role of *Bubalus bubalis* as vector of antimicrobial resistant bacteria dissemination in our Region. Thus, a continued surveillance of the emerging antimicrobial resistance in rural environment is recommended.

[1] WHO, 2012; [2,3] WHO, 2000; [4] WHO 2013; [5] Nizza et al. 2010; [6] Lamagna et al. 2015. [7] Scott Weese et al. in Veterinary Medicine, Fifth Edition (eds S. Giguère, J. F. Prescott and P. M. Dowling), John Wiley & Sons, Inc, Hoboken, NJ, 2013.

DRUG USAGE IN MEDITERRANEAN BUFFALO FOOD CHAIN: FROM FARM TO CONSUMER

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The general principles for responsible use of veterinary drugs may be focused on proper disease diagnosis, right drug choosing, right drug dose, right treatment duration, results verification. Compliance with these principles and a rapid treatment are a guarantee of animal welfare and safe food to the table. Obviously an incorrect drug use can lead resistance, decrease effectiveness and so delete all the efforts to obtain suitable molecules . The drug use should be limited to necessary cases to ensure that the product will be effective and will protect the animal health. In fact, in order to protect human and animal health, the veterinary drugs whole chain is governed by legislative decree no. 193/2006 [1] and 158/2006 [2] and subsequent amendments and additions. In accordance with Art. 88 of the mentioned decree and with the ministerial guidelines [3] issued as part of the "Integrated National Plan 2015/18 on food safety, welfare and animal health, plant health", control of in-farm drug use and prescriptions, takes place through the drug surveillance plan. Within this plan, controls are based on the veterinary drugs loading and unloading register, the treatment records in facilities where animals are raised for food production, the veterinary recipes, the amount and types of various in-farm drugs use. The drug surveillance plan falls in regional planning document in which the drug surveillance in buffalo herds falls into the bovine plan. In fact, the European Commission, based on precise information provided by EMEA, establishes that for MRLs determination, the buffalo is considered to be included in bovine species as laid down in Regulation 2377/90 now replaced by the Regulations EC no. 470/2009 and no. 37/2010 and subsequent amendments and additions. Therefore, all veterinary drugs intended for bovine are also destined to buffalo with the same withdrawal periods. The clarification sent to all the experts by Health Ministry [4] marks a turning point in the buffalo veterinary drugs management. Thus to provide a major drug fair use and a guarantee for food safety.

[1] Decree Law, 158/2006 – IT; [2]Decree Law, 193/2006 – IT; [3]DGSAF, 1466-26/01/2012 –IT ; [4]DGSA, 21474-01/12/2009–IT

DRUG USE IN SMALL RUMINANTS

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In Italy small ruminants are mainly managed under intensive and extensive systems. Sheep is usual breeded for meat and milk purpose, while goat is predominantly used for milking. Generally in extensive systems the management of these small ruminants includes two reproduction seasons: spring/summer and summer/autumn. In goat intensive systems, reproductive season runs from mid-August up to the end of December. This seasonality allows us to manage and plan the majority of pharmacological interventions in periods when the animals are dried-off. The treatments may be prophylactic such as vaccination or parasites control; the use of antibiotic is generally limited to specific clinical cases or when bacterial pathology occurs in young animals during the first three months of breeding. In Italy, drug resistance phenomena (antibiotic and/or antiparasitic) is not been extensively studied, while several research studies are reported in other countries [1,2,3]. For this reason, improve the planning and control system on drug resistance and/or residues in goat and sheep need to be better explored, especially when the impact on human health is considered.

[1] Salgato et al 2016; [2] Sutherland, 2015; [3] Scott e Menzies, 2011

USE OF VETERINARY MEDICINE IN MINOR SPECIES

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All other animal species, which are not considered major, are as a consequence, by default, classed as minor species.
Major food-producing species: cattle (dairy and meat animals); sheep (meat animals); pigs; chickens (including laying hens); salmon.
Major companion animal species: cats; dogs. [1]
The current MUMS guidelines were elaborated in 2004 and 2005 and since that time there have been a number of applicants/companies who have availed of these amended data requirements for products classified as intended for MUMS/limited market. The guidelines are intended to reduce data requirements where possible for products classified as MUMS while still providing assurance of appropriate quality safety and efficacy and complying with the legislation in place and leading to an overall positive benefit-risk balance for the product. The reduction in data requirements has generated considerable debate since the guidelines on MUMS data requirements were introduced. Some stakeholders find this a very valuable component of the policy whereas others consider that in many cases data requirements are only slightly reduced or there are expectations that for any MUMS product all possible data reductions would be applicable. Based on the experience gained to date, after almost 10 years it is considered time to review these guidelines, to ensure that the current guidance is in line with current knowledge and best practice and also provides more predictability and regulatory certainty to applicants in terms of applicability to particular products [2].
The objective of the European Medicine Agency to promote the developement of products for MUMS miserably failed, mainly due to the high level requests for the dossiers that must be submitted. In real terms there are no significant differences between the two dossiers, one for Major species and one for MUMS. This situation does not create the industrial conditions for investing in Minor Species and the result is that no products have been put on the market.
The next review of the current guidelines should come back to the roots, where the goal was to incentivize industries to develop new products for MUMS through more simplified submissions.

[1]http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/12/WC500179577.pdf
[2]http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/11/WC500177461.pdf

WORKSHOP 4

"IL MEDICO VETERINARIO A TUTELA DELLE PRODUZIONI TIPICHE"

THE ROLE OF PUBLIC VETERINARIANS FOR HEALTH PROTECTION AND FOR DEFEND TYPICAL PRODUCTS AND TO SUSTAIN COUNTRY ECONOMY

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On April 20th, 1862 marks a turning point in the history of Western society: Louis Pasteur, performs experiments to demonstrate the effectiveness of the "PASTEURIZATION", but the process was not immediately applied to the milk; it will be only done in 1886 by Franz von Soxhlet. Until 1886, the cheese was made on farm or using milk collected from neighboring farms, and it needed immediate processing. With pasteurization dairies can work very large quantities of product and they can purchased milk in more distant places. Consequently the activity of the dairyman-breeder became marginal. A widespread awareness of discerning consumers that consider raw milk to process cheeses a social and cultural value as well as a productive specificity has been formed in recent years. The traditional cheeses have outstanding prospects of expansion due to their organoleptic characteristics, their delicacy and the resulting increasing of their demand worldwide. Indeed traditional cheeses are the most copied and forged food, worldwide. In that scenario, the veterinarian is the central professional figure and reference in the process control. To accomplish this task the veterinarian needs of knowledge on typical technologies, of the knowledge on health risks related to the raw materials and the processing. The veterinarian must be conscious that most of health guarantees depend not from the heat treatment of food (eg. milk pasteurization), but, rather, by the CCP (Critical Control Points) which should consider the long aging, the biocompetition of useful micro-organisms against pathogens. All those aspects could strongly increases the safety of most traditional products. The veterinarian should evaluate the hazards and then suggest the most appropriate technique to reduce or remove them.

These aspects must be the combined result of expertise and surveys, not necessarily coupled to laboratory assays, able to prove that the steady checks of the processing chain can significantly reduce the hazards. It is unthinkable that European legislation can be invariably applied to all foods, stating processing criteria and microbiological parameters equal for all products, ignoring how ancient food technology are a guarantee of wholesomeness.

Do we want that these products disappear, and with them the whole production chain, as it has already happened to many Italian products? Or do we want to write or rewrite the production disciplinary taking into account of surveys and of field trials in order to ensure an even more precise control of hazards, aiming to increase the health and hygiene guarantees for the consumer?

INTERNATIONAL TRADE AND ITS IMPACT ON THE EU LOCAL PRODUCTION

Dionisi A.

Clear evidence of trading over long distances dates back at least 9,000 years. Today, international trade is at the heart of the global economy. If you walk into a supermarket and are able to buy South American bananas, Indonesian coffee and a bottle of South African wine, you are experiencing the effects of international trade.

The increase in food demand due to a rapid growing population is leading countries to increase their demand for food, therefore the need to safeguard public health while promoting food security and safety. The international trade throughout the entire value chain was never more important.

International trade has its fair share of advantages and disadvantages. Amongst advantages trade has the potential to: maximize a country's capacity to produce and acquire goods improving global efficiency in resource allocation; increase the quality of goods by allowing countries to specialise in the production of those goods and services they do best; create competition both at the international level and local level; increase the local employment rate, and provide monetary gains. Amongst the positive effects on local production: operators will face more competition from abroad, there will be more incentives to increase efficiency; determination to improve the quality of products, to produce innovation through to new ideas, it forces producers to improve their technologies in order to keep up with their competition.

Amongst the disadvantages we can recall the massive mass media campaign which leads to ideological policies, their impact on productions through the destruction of regional and local diversity and difficulties to remain on the market for small entreprises.

Trade is led by respect of standards on food production which are established by international standards setting bodies. Ensuring that requirements for imports and exports are the same the world over, facilitate the movement of goods, services and technologies from country to country.

Many trade partners indicate that international standards would not be binding, but the reality is that the Sanitary and Phytosanitary Agreement of the World Trade Organisation sets out the principles which are considered as reference in case of dispute settlement.

The European Union (EU) is in front line when it comes to approach the global trade. The EU is the world's largest trading block. The EU looks to achieve a stronger position by acting together with one voice on the global stage, rather than with 28 separate trade strategies. It is committed to very high standards of food safety, consumer protection and sanitary risk management based on science and transparency.

His integrated approach is applied to imports and exports through coherent, risk-based control measures throughout the food and feed chain, from farm to table. To meet the challenges of global trade, the EU legislation on animal health, food safety and official controls has been recently updated and enhanced.

The EU is firmly committed to the promotion of open and fair trade with all its trading partners and has specific trade policies in place. Open trade is a recognised engine for growth and job-creation but it requires that fair competition, without distortions, is maintained between domestic and foreign producers.

Trade protectionism is the deliberate attempt to limit imports or promote exports by putting up barriers to trade. Despite the arguments in favor of free trade and increasing trade openness, protectionism is still widely practiced. Standards shall be fulfilled, and EU shall ensure that all the food chain steps are supervised and monitored. EU cannot offer to our trade partner's arguments where the system is considered weak and with different approaches and efficiency, with the risk to make difficult to convince trade partners on opening their market access.

Ensuring that the EU production is safe and the EU legislation is correctly applied at all stages, will help a lot our operators, especially the small and medium and local enterprises to get a more favourable access to international markets. The local production, although it often represents a niche market, cannot escape from that logic without jeopardizing the efforts devoted to increase quality and investments made. Resources cannot be wasted in vain, Italy looks to shine its ability on trade also through the local production.

UPGRADING TRADITIONAL PRODUCTS BY RESEARCH & DEVELOPMENT PROJECTS

Paparella A.

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Inconceivable results were obtained by changes in manufacturing, distribution, and serving methods, such as sliceable eggs, ice creams stable at room temperature, spreadable parmesan, and broth gels.

Moreover, the evolution in packaging generated new opportunities for the food industry, by extending foodshelf-life and creating new markets as automatic vending and on-line sales.

In this respect, the veterinary surgeon can be the reference professional for research&development projects to upgrade the performance and safety of traditional products, without distorting their quality and traditionality.

The seminar aims to describe the evaluation criteria and the technologies for food preservation, considering specific aspects for different food products.

THE VALUE OF ACCREDITED CERTIFICATION FOR THE PROTECTION OF QUALITY PRODUCTS: RULES AND APPLICATIONS

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The quality and variety of agricultural, livestock and aquaculture production of the European Union constitute a strong point and an important competitive advantage for producers and they are an integral part of the cultural and gastronomic heritage. It is for this reason that, since 1992, the EU has carried out a specific protection policy regarding the geographical origins of agricultural and food products. The use of the symbols PGO PGI and TSG has facilitated the identification of quality products on the market, greatly raising their profile and therefore improving customer information.

In 2012 the EU issued a regulation for “quality schemes” of agricultural and food products with two motives: to ensure uniform protection of quality marks as intellectual property and to provide consumer information on the characteristics which provide added value to products.

At the same time, the legislator intended to protect also agri-food products whose methods of production, preservation and maturing have been consolidated over time and are tied to particular geographical areas, though not reaching very large quantities: typical products. These products are recognized as local heritage for their environmental, social and historical characteristics.

The added value of a mark placed on a product is also tied to the trust which consumers place in the checks and controls performed. The product quality control regime relates to the worldwide ISO standardization system and to Regulation 765/2008 concerning accreditation and market surveillance with regard to the marketing of products.

Assessment activities are undertaken in accordance with binding rules for all member states in order to strengthen the principle of mutual recognition of the value of the certificates and test reports which are issued.The presentation report will contain the typologies of quality products, the procedures for obtaining certification and the guarantees offered to the consumer. Some applicable examples will also be presented in the ambit of the production chain of meats, dairy and cheese products, which are useful for understanding how accredited certification against a voluntary standard can help producers to provide added information to consumers so as to give greater value to their products against recognized and shared values. In this sense, certification against the standards of the series ISO 22000 regarding food safety, as well as the effectiveness of a traceability management system, highlight the possibility of extending, on one hand, the guarantees of the protection of healthy foods to all organizations in the production and consumption chain and, on the other hand, extending data recovery and demonstrating compliance with the specific requirements (types of food given to livestock food producers, typologies of breeding, the origin of raw materials, the parameters of applied processes), aimed at satisfying the expectations of clients and consumers.

WORKSHOP 5

" I SARCOMI DEI TESSUTI MOLLI NEI PICCOLI ANIMALI

UN APPROCCIO MULTIDISCIPLINARE PER LA CURA DEL PAZIENTE"

DIAGNOSIS AND PROGNOSIS OF CANINE SOFT TISSUE SARCOMA: THE ROLE OF THE PATHOLOGIST

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Soft tissue sarcomas (STS) are a group of mesenchymal tumors that represents 15% of cutaneous/subcutaneous canine tumors, which rarely metastasize and more commonly recur locally[1,2]. STS classification derives from human medicine and has been inconsistently applied in veterinary literature, causing discrepancies between different studies[3]. Frequently pre-surgical biopsies are performed as first step in the management of STS. On one hand these samples provide useful information (neoplastic nature of the lesion, mesenchymal origin), and on the other there is the chance they are non-diagnostic or non-representative specimens. In addition, the histological grade assessed in pre-surgical biopsies is often incorrect, and therefore the histological exam of a pre-surgical biopsy cannot replace the evaluation of the excised tumor. To reduce these problems, multiple biopsies should be sampled[4]. The evaluation of excised STS starts with the gross evaluation, including the assessment of the size of the tumor, an important prognostic factor, the macroscopic extent of necrosis and sampling of the excision margins. The evaluation of 100% of the excision surface is impractical and the best approach is sampling the areas where neoplastic infiltration is suspected. To allow the correct sampling, the excision surface should not be cut before fixing the tissue, since this can disrupt the architecture of the sample. If a large tumor needs to be incised for a better fixation, this should be done on the cutaneous side. Histological report of STS includes the specific type of sarcoma, and the generic diagnosis of “canine STS” should be avoided if possible. The correct classification is necessary to distinguish benign tumors or even non-neoplastic lesions, such as nodular fasciitis, which may resemble STS and represent a diagnostic problem for the pathologists[1]. After diagnosis and classification, the two most important prognostic factors to be evaluated are surgical margins and histological grade. Completeness of surgical margins is the most reliable parameter to predict local recurrence that occurs in less than 5% of STS with clean margins and in 25% of STS with infiltrated margins[1]. Histological grade is an important predictor of metastases and, in marginally excised STS, also of recurrence[3]. The STS grading, evaluating the subjective parameter of differentiation of the cells, has only moderate inter-observer agreement, and discrepancies between the grade assessed by different pathologists should be taken into account[5]. Therefore, despite some limitations, the pathologist can provide crucial information for the management of canine STS.

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CANINE SOFT TISSUE SARCOMAS (STSS): MULTIMODAL IMAGING APPROACH

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Soft tissue sarcomas (STSs) are common in dogs accounting for up to 15% of all cutaneous/subcutaneous tumours. They represent a true diagnostic challenge for both clinicians and pathologists and a multidisciplinary approach is needed for successful management[1,2]. Radiographs represent the first step: they could reveal soft tissue mineralization, bone erosion, cortical destruction or periosteal reaction. Ultrasonography (US) can be used in the early evaluation of soft tissue lesions, providing information about size, location and consistency (cystic vs solid lesion). B-mode ultrasound has been rarely reported in literature in the study of cutaneous/subcutaneous tumours, due to its low soft tissue detail and margins identification. However, the integrative analysis of these tumours with contrast enhanced ultrasound (CEUS) was recently reported to complete clinical examinations in humans [3]. No specific literature is available about subcutaneous sarcomas, but CEUS and US could be considered as early staging steps to detect neoplastic recurrence or early tumour infiltration. Computed Tomography better assesses skeletal involvement than MRI, showing subtle periosteal reaction, osseous remodelling or cortical destruction. Administration of contrast media allows to evaluate different enhancement patterns, according to tumour perfusion and permeability. CT is also preferred to identify small pulmonary metastases, since non-gated MRI is subjective to cardiac and respiratory motion. US and CT are also important interventional techniques for biopsy procedures. MRI could provide better information in the evaluation of STSs. High field MRI, although more subjective to artefacts, can provide high quality images, allowing accurate evaluation of tumour location and relationship with surrounding tissues. This technique is in fact reported to be excellent in diagnosis and pre-surgical staging of human STSs. STSs usually appear moderately heterogeneous with variable T1 and T2 signal intensities. As for CT, STSs signal intensity features are not specific and grading is only possible through histopathology. Contrast media administration increases MRI potential, providing information about capillary permeability and composition of the interstitial space. MRI spectroscopy could also give information about cellular chemistry, but it's not routinely employed in Veterinary Medicine[4]. Nuclear Medicine can be employed to detect distant metastases and sentinel lymph node involvement, depending on the type of tumour. The use of SPECT or PET techniques with CT fusion can also enhancethe anatomic detail. In humans, metabolic imaging with Nuclear Medicine is very helpful in the evaluation of STSs [5]. Unfortunately, such equipment is not routinely available for veterinary patients.

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CANINE SOFT TISSUE SARCOMAS SURGERY: HOW WIDE THE MARGIN SHOULD BE?

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Canine soft tissue sarcomas (STS) represent a wide range of cutaneous and subcutaneous tumours characterized by similar biologic behavour. They include perivacular wall tumours (PWT), peripheral nerve sheat tumours (PNST) and fibrosarcomas. While the metastatic rates is moderate to low for all of them, therate of local recurrence can vary according to tumour aggressiveness. Recent papers [1,2,3] have demonstrated that the recurrence rate varies according to histologic grade and completeness of surgical excision. Other factors that can influence the outcome are grade of infiltration into deep planes and tumour size (with 5 cm being a cutoff for higher aggressivenss) [4], while the association of a high mitotic index and high histologic grade are prognostic for reduced survival time. In order to achieve a complete exicision of the tumour, an adequate planning of the surgery is important; CT scan of the lesion may help evaluating the real extension of the tumour, and the current guidelines propose a 3 cm lateral and at least 1 fascial plane below the mass. Accurate evaluation of surgical margins is another fundamental step of the procedure. Anyway, wide margins are not always achievable, especially on the extremities. This may imply that adjuvant therapy may be necessary, especially when tumour grade is high. Luckily most of STSs are low grade, and it has been reported that acral PWTs may not require such a wide margin as for more proximal neoplasms of the same origin. In cases where surgical margins are infiltrated, a new excision should be considered with the same 3 cm lateral and 1 fascial plane deep margins, and a new histology performed. According to Bacon et al.'s paper [5], this is preferable to adjuvant radiotherapy or chemotherapy, when a wide margin procedure is foreseen. When this is not possible or the primary masslocated on the limb is too big, an intentional marginal excision follwed by hypofractionated radiotherapy may allow a good clinical outcome [6]. In all cases, a good preoperative planning is fundamental for the good outcome; fine needle aspiration biopsy of the primary mass and any enlarged lymph nodes are easily performed. In cases where knowing the histologic grade may change the surgical approach, a core or incisional biopsy may help achieve better results, keeping in mind that the first surgery provides the best chance for local tumour control. In case of grade I STS excised with incomplete margin (especially acral ones), the tumour can be managed either by active surveillance or by "staging surgery", i.e. the resection of all the scar with a minimal margin (0.5-1 cm) and a subsequent further wide margin re-cut only when histology reports the presence on neoplastic tissue (about 22% of cases) [5]. High grade STSs always require a more aggressive treatment.

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DECISIONAL PROCESS IN THE APPROACH TO CANINE SOFT TISSUE SARCOMAS: WHEN ARE RADIATION THERAPY AND MEDICAL THERAPY WARRANTED?
METASTATIC SOFT TISSUE SARCOMAS: WHEN SURGERY IS NOT AN OPTION ANYMORE

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Canine soft tissue sarcomas are a vast variety of mesenchymal tumors, which most commonly include: hemangiopericytoma (recently reclassified as perivascular wall tumors), fibrosarcoma, and peripheral nerve sheath tumors. Other sarcomas, such as hemangiosarcoma and histiocytic sarcoma, because their biologic behaviour is more aggressive than the other tumors in this group and their cell of origin is hematopoietic, not mesenchymal, they are typically excluded from studies of STS.

The problems associated with post-surgically removed STS are: recurrence of the local disease after inadequate surgery, and the metastatic potential to distant sites. The metastatic potential of STS is poorly described. It is generally considered they have a low to moderate metastatic rate, and more recently, it has been associated with a higher histologic grade and mitotic count. Tumours metastasise most commonly to the lungs, while regional lymph nodes are a relatively rare site of metastases.

In case revision surgery is not feasible for controlling the risk of local recurrence, radiation therapy (RT) may comes into the therapeutical equation and consultation with a board-certified radiation oncologist should be considered. RT can be used as neo-adjuvant treatment before surgery, in the attempt of shrinkage of a large volume primary tumor or in case of difficult locations; however, the use of neo-adjuvant RT is poorly described in veterinary medicine and still it is not clear whether its use could help the surgeon reducing the surgical margins.

Some of the available studies suggest that the use of adjuvant radiotherapy following incomplete excision of STS achieved 1- and 3-year survival rates of 80 to 87% and 61 to 81%, respectively, and median times for development of recurrence of 698 days and greater than 798 days, therefore making RT a recommended treatment option for incompletely resected STS, especially for those with a high mitotic index, which appear to be more at risk of recurrence.

The role of adjuvant RT for low-grade sarcomas has been recently questioned as well as it is not clear which RT protocol leads to the best outcome. Data should be cautiously interpreted because of the lack of comparison groups in all the studies. A board- certified radiation oncologist will suggest the appropriate treatment protocol for an individual patient.

Very few veterinary studies have evaluated the efficacy of chemotherapy for STS, and all of the reports were graded as providing a very low to low level of evidence because of small sample size, frequent retrospective study design, and lack of a comparison group.

The most commonly used molecules typically includes doxorubicin and cyclophosphamide and, although the lack of evidence, the use of chemotherapy is usually reserved to dogs affected by high-grade sarcomas compared to low- to intermediate-grade tumors, that typically have lower proliferation rates and would therefore be expected to be less susceptible to chemotherapy in addition to having lower rates of metastasis.

In the last decade metronomic chemotherapy (daily, low-dose oral chemotherapy) has been suggested as an alternative medical therapy for both local and systemic tumor control. Metronomic chemotherapy works through antiangiogenic and immunomodulatory mechanisms and therefore could potentially control also more chemotherapy resistant cells, such as low-grade STS cells.

A retrospective study of 85 dogs with incompletely resected STS reported that median time to tumor recurrence was nearly doubled in dogs that received low-dose cyclophosphamide daily in combination with a nonsteroidal anti-inflammatory drug compared to those that received no further treatment (410 versus 210 days, respectively). Another study, evaluating daily chlorambucil treatments in dogs with measurable tumors, included nine dogs with STS. One dog experienced complete clinical resolution of the tumor for greater than 17 wk and three more dogs maintained stable disease for at least 7 wk. These encouraging preliminary results warrant further investigation through prospective, randomized trials.

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IMAGING OPTIONS FOR FELINE INJECTION-SITE SARCOMA (ISS)

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Diagnostic imaging techniques are commonly applied for staging and surgical planning of injection-site sarcoma (ISS) in cats. Radiology has low sensitivity in assessing tumour margins and its relationship with the surrounding tissues. Soft tissues mineralization can be occasionally detected on radiographs, while skeletal involvement is rarely observed. Ultrasound (US) is employed for determination of tumour components (solid vs liquid) and margins, for biopsy guidance and assessment of local lymph nodes. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are gold standard modalities, allowing accurate assessment of tumour extension, muscoloskeletal infiltration and metastatic spread. Feline ISS distant metastatic rate is about 10-25%, with thorax, subcutaneous tissues, regional lymph nodes and liver being most frequently involved; local metastatic rate ranges between 14-50% of cases [1]. Pre- and post-contrastographic whole body CT examination is recommended. The patient is positioned in sternal recumbence, with fore limbs extended cranially and the hind limb extended caudally. If the tumour is interscapular, a further post-contrast examination with the fore limbs flexed caudally along the thorax is recommended ("double positioning"). This approach can enhance the relationship between the mass and the surrounding tissues, potentially improving the pre-surgical evaluation of the tumour [2,3]. When CT o MRI exams cannot be performed, staging consists of 3 radiographic projections of the thorax and a full abdominal US. CT, MRI and US features overlap: neoplasms are usually round to irregular in shape, with ill-defined margins, cavitary components and large necrotic centres. Long and thin digitations with associated angiogenesis may be detected and they represent potential soft tissues infiltration. Contrast enhancement is moderate to strong, mostly late and peripheral (ring effect) [2,4,5]. CT and MRI also allow to easily measure tumour volume, usually mildly overestimating it [1]. They should therefore be preferred to detect visceral spread and superficial "skip" metastases, which are subcutaneous nodules not detectable through palpation. Nuclear Medicine techniques complete tumour staging. A nanocolloid-coupled radiopharmaceutical is injected in the subcutaneous tissues around the tumour and absorbed by the lymphatic vessels, accumulating in the sentinel lymph node. Radiopharmaceutical distribution is initially traced by a gamma camera; a specific probe is then employed to exactly identify the sentinel lymph node, which will be excised together with the tumour [6].

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Comunicazioni ORALI

SCIENZE BIOMEDICHE

PROTECTIVE ROLE OF ANTHOCYANINS IN DIABETIC NEPHROPATHY

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Diabetic nephropathy (DN) is a common microvascular complication occurring in approximately 20-40% of patients with type 2 diabetes mellitus (T2DM). It is characterized by the progressive impairment of glomerular function leading to end-stage renal failure (1). Hyperglycaemia, kidney inflammation and oxidative stress are crucial in promoting the development and progression of DN and several factors play a key role in the onset of the disease and its progression (2). Anthocyanins are one class of flavonoid compounds, showing high anti-oxidant and anti-inflammatory activity since their constant intake seems to correlate with a reduction of global oxidative state and with a reduction of inflammation markers (3). A standardized new extract, obtained by properly mixing anthocyanins and other polyphenols, recovered from red orange processing wastes, and eriocitrin and other flavanones, recovered from lemon peel has been developed and used in this study.

We have characterized the pathophysiology of DN in a T2DM/DN animal model and the effect of the anthocyanins in the prevention of DN by evaluating the cause-to-effect relationship between the anthocyanins intake and protection from renal damage. Zucker fa/fa rats and Zucker fa/+ rats (healthy controls) were sacrificed after 6 weeks (T2DM rats without renal damage); 15 weeks (T2DM rats with incipient renal damage) and after 30 weeks (rats with DN). Furthermore, the same groups of Zucker rats were treated by oral intake with specific formulations of anthocyanins to ascertain the protective effect of anthocyanins on the progression of DN.

We have demonstrated in the Zucker fa/fa rat, through the clearance of inulin, that the glomerular filtration rate (GFR) is significantly increased after 15 weeks, while after 30 weeks this value is drastically reduced compared to the control. The analysis of Reactive Oxygen Species (ROS), through the dihydroethidium assay and through the Klotho assessment have shown a significant increase of ROS after 15 and 30 weeks. Treatment with anthocyanins have shown, after 15 weeks, value of GFR similar to the control and a similar reparatory effect we have also found in ROS production. Instead, we have not noticed significant differences between diabetic rats and control rats in inflammatory markers, as such as IL-2, IL-6 and TNFβ before and after treatment.

In conclusion, the use of anthocyanins could reduce the nephrotoxic effect in course of DN through the modulation of ROS productions and open new perspectives in the treatment of patients with T2DM.

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METHIMAZOLE CYTOTOXICITY IN A FELINE IN VITRO MODEL: POSSIBLE ROLE OF THE NATURAL ANTIOXIDANT QUERCETIN

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Feline hyperthyroidism is the most common endocrinopathy in older cats and the antithyroid agent methimazole represents the drug of choice for its medical treatment. Several cases of methimazole-induced tissue injury have been reported and the frequent methimazole-related side-effects including vomiting, diarrhea, hepatotoxicity, and facial pruritus complicate the already difficult management of the hyperthyroid patient [1]. Although the precise mechanisms underlying such effects have not been elucidated so far, oxidative stress and redox unbalance are thought to play a major role in triggering idiosyncratic reactions, suggesting that antioxidants could exert a protective role [2-5]. The aims of the present study were to investigate the methimazole-related cytotoxicity in a feline in vitro model and to evaluate the possible protective role of quercetin, a natural compound known to exhibit antioxidant and anti-inflammatory properties in cultured feline esophageal epithelial cells [4,5]. A feline kidney epithelial cell line (CRFK) was incubated with methimazole alone or in the presence of quercetin at concentrations lacking cytotoxic effects (3 and 6 µM). Methimazole concentrations were selected according to the mean and maximum plasma levels attained in orally treated cats (2 and 4 µM). Cell viability was evaluated after long-term incubation (24-48-72 h) using a colorimetric assay (Neutral Red). One-way ANOVA was performed for statistical analysis with a significance level set at $p<0.05$. According to what already published, methimazole was able to significantly induce cytotoxicity in CRFK cells in a time- and concentration-dependent manner (cell viability after 72 h of incubation: 81±0.07% and 59±0.13%; $p<0.05$). However, such an effect was not reduced by the concomitant incubation with quercetin at any tested concentration and time point. The present study contributes to extend the current knowledge about the methimazole-related side effects frequently seen during feline hyperthyroidism management, by developing a specific in vitro model. Further studies are ongoing in order to elucidate the possible role of oxidative stress and redox unbalance in the methimazole cytotoxicity. Moreover, the possible protective effects of other natural antioxidants are currently investigated.

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EVALUATION OF CYTOTOXIC AND ANTIOXIDANT EFFECTS OF CURCUMIN IN HUMAN AND CANINE MAMMARY CANCER CELLS LINES

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Curcumin is the main component of *Curcuma longa*. It is traditionally used in Ayurvedic medicine and it is also used in Europe, mainly in pharmaceutical preparations, or as food supplement. It is commonly recommended for reducing acute or chronic inflammatory conditions, such as arthritis [1]. In the last decade, Curcumin has been investigated to assess antineoplastic effects, considering a possible correlation between the use of this spice and the decreasing incidence of degenerative and neoplastic diseases [2-3]. The aim of the present study was to evaluate the in vitro cytotoxic effects of Curcumin in cancer cell lines: MCF-7 (derived from metastasis of human mammary adenocarcinoma), CF.41 (primary canine mammary carcinoma), and CHMp (derived from metastasis of canine mammary carcinoma). All cell lines were cultured in Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS - 20%), steptomycin, penicillin and amphotericin B solution (2%) and L-glutamine (2%). Cells were incubated at 37°C and 5% CO^2. At 80% of confluence, cells were detached and seeded in 96-well plates at a concentration of $5*10^3$ cells/well (100 µl) to perform 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay in different conditions: 1) in presence of increasing concentrations of Curcumin (10-4-10-12 M); 2) with hydrogen peroxide (H_2O_2) concentrations ranging from of 2.5 to 200 µM; 3) co-presence of H_2O_2 (2.5-200 µM) and Curcumin (10-4-10-12 M). For each trial, experimental time points were 2, 24, 48 and 72h. The data obtained were statistically analyzed with GraphPad Prism software, using One-way ANOVA and Bonferroni's post- test ($p<0.05$). 1) The Inhibitory Concentration(IC)50 was 10-4.563 M for MCF-7, 10-5.386 M for CF.41 and 10-2.087 M for CHPm cells. At IC50 it was possible to appreciate a significant reduction of proliferation compared to untreated control; 2) all H_2O_2 concentrations induced a statistically significant decrease of the cell amount following a time-dependent inhibitory behavior for MCF-7 and CF.41 cells, while only at 2h CHPm cells showed a concentration-dependent inhibitory behavior; 3) Curcumin seemed not to protect cells against oxidative stimuli, except for CHPm and CF.41 cells that demonstrated a low inhibition rate compared to controls after 72h. These results highlighted different behaviors depending on the type of cell origin (human or canine) and suggested a protective activity of Curcumin agaists oxidative stimuli after a long incubation period.

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THE PROTECTIVE EFFECT OF DELTA-TOCOTRIENOL ON OCHRATOXIN A-INDUCED NEPHROTOXICITY

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Ochratoxin A (OTA) is a natural mycotoxin produced by filamentous mold species belonging to the genera *Aspergillus* and *Penicillium* (1); it is involved in the development of important human and animal diseases. Several studies have shown that OTA is nephrotoxic, hepatotoxic, teratogenic, neurotoxic, immunotoxic, genotoxic and carcinogenetic in several animal species, with the longest half-life in human blood (2); the kidney is the primary target of OTA. The mechanism of OTA toxicity have not yet been clearly elucidated. Several studies have demonstrated that OTA induced nephrotoxicity and hepatotoxicity through oxidative DNA damage in vitro (3,4) and in vivo (5). In this work we have evaluated the protective effect of Delta-tocotrienol (DT3), a member of vitamin E family, on OTA induced nephrotoxicity in rats. In particular we have analyzed trends in body weight, renal damage through the evaluation of glomerular filtration rate (GFR) and oxidative stress through Malondialdehyde (MDA) and Dihydroethidium assay in Sprague Dawley rats. The rats, randomly divided into four groups, were treated for 2 weeks as follows: Group 1 was treated with oral injection of saline solution; Group 2 was treated with oral injection of OTA (0.5 mg/kg); Group 3 was treated with oral injection of DT3 (10 mg/kg) and Group 4 had received both OTA (0.5 mg/kg) and DT3 (10 mg/kg) administered simultaneously. Our data showed that animals treated with OTA presented weight loss and a significant reduction of GFR. We have also showed an increase of the levels of MDA and O_{2^-} in OTA treated animals. The co-treatment with DT3 prevented weight loss and restored the levels of GFR; moreover, we have showed a decrease of MDA and O_{2^-} levels in DT3 treated animals respect to the control. These data show that the nephrotoxic effect induced by OTA is most probably linked with the increase in reactive oxygen species production and indicate that the DT3 is able to prevent renal oxidative stress and the reduction in the GFR secondary to OTA administration.

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MODULATION OF AFLATOXIN B1 TOXICITY IN A BOVINE MAMMARY EPITHELIAL CELL LINE

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Aflatoxin (AF) B1 is a dangerous mycotoxin that contaminates feedstuffs and exhibits several toxic effects upon the bioactivation by drug metabolizing enzymes (DME) [1]. CYP1A and 3A families mediate the synthesis of active metabolites that are highly cytotoxic (i.e. AFB1-epoxide) and can be excreted into milk (i.e. AFM1) contaminating dairy products [2]. Factors modulating DME can influence AFB1 kinetics and toxicity. Dioxin-like (DL) compounds may induce the up-regulation of several genes, including the CYP1A family, via the activation of the AhR pathway. Conversely, a number of natural antioxidants may reduce the generation and/or increase the inactivation of AFB1 metabolites by the inhibition of CYP1A and/or the activation of detoxifying and antioxidant enzymes. The aim of the project was to evaluate in the bovine species the modulation of AFB1 toxicity triggered by Curcumin, an antioxidant that has been reported to counteract AFB1 toxicity in rats and broilers [3-4], and PCB126, a DL-compound mostly involved in dioxin contamination of dairy milk [5]. Such an issue has been addressed by in vitro experiments performed in an immortalized bovine mammary epithelial cell line (BME-UV1), in the light of the active role played by mammary DME in the generation of AFB1 metabolites. Cells were incubated with increasing concentrations of AFB1 (12nM - 3µM) after the pre-incubation for 16 h with Curcumin (5 µM) or PCB126 (10 nM). Cell viability was evaluated by the WST-1 colorimetric assay after 24 and 48 h of AFB1 incubation. Modulation of CYP1A1 enzyme by Curcumin was evaluated incubating cells with increasing concentrations of the antioxidant (0.6 - 5 µM). CYP1A1 expression and activity was assessed by Real-time PCR and EROD activity assay, respectively, at different time-points (from 4 to 48 h). Statistical analysis was performed by 1-way ANOVA followed by Bonferroni's post test. As expected, results indicate that AFB1 cytotoxicity occurs in a time- and dose-dependent manner (EC50 at 24h and 48h equal to 537 and 175nM, respectively), and that PCB 126 is able to enhance such effect of approximately 30% at 24h and 60% at 48h ($P<0.001$). Surprisingly, Curcumin does not increase the viability of cells exposed to AFB1, but it slightly potentiates AFB1 cytotoxicity (around 20% at both 24 and 48h, $P<0.01$). To investigate the possible mechanism of such a phenomenon, we tested if Curcumin could activate the AhR pathway, as suggested by some Authors [6]. However, in BME-UV1 cells Curcumin failed to induce CYP1A1 at both gene and protein level. Further studies are ongoing to unravel the mechanism of the negative effects of Curcumin in our in vitro model and to test the capability of other natural antioxidants to protect BME-UV1 cells from AFB1 toxicity.

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AN INTEGRATED *IN VITRO* MODEL FOR THE EFFICACY ASSESSMENT OF MYCOTOXIN-DETOXIFYING AGENTS IN SEQUESTERING FUMONISIN B_1

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Fumonisins (FBs) are mycotoxins produced by *Fusarium* species, common contaminants in corn samples and their derivative products. Because of the know toxic effect for the animal health and unavoidable presence of the mycotoxins in cereals, research has tended to limit their exposure introducing adsorbents in feed, a procedure permitted by current European legislation [1]. This study was aimed to screen some adsorbent materials (minerals, bio-polymers, agricultural by-products) for their ability in binding FB_1 at different pH values (pH range at 3-7), and to determine the extension of the adsorption using the isotherm adsorption method. Selected adsorbents were subjected to a gastro-intestinal digestion process [2], to measure the bioaccessibility of FB_1. Subsequently, the chyme samples were tested for the antioxidant activity on an intestinal cell line (Caco-2 TC-7 clone), main target of mycotoxin exposure. Three materials (a Na-smectite, a leonardite coal and a bio-polymer) were selected as promising FB_1 adsorbents, sequestering >70% of FB_1 at low adsorbent dosage (0.1% w/v) and 1 µg/mL toxin concentration. Using the isotherm adsorption method, the Na-smectite showed very high binding capacity for FB_1, followed by leonardite coal and the bio-polymer. After digestion process, the organic bio-polymer (particularly rich in polyphenols) showed a good antioxidant activity, while the Na-smectite , the leonardite coal and some agricultural by-products obtained from artichokes did not show anti-oxidant property. Taking into account these preliminary results, a formulation (made by compounds with high toxin binding and antioxidant activity) was subjected to digestion process at two dosages (0.5 and 0.1% w/v) with/without FB_1 . FB_1 bioaccessibility and antioxidant effect were assessed. On an intestinal cell line, a significant reduction in malondialdehyde (MDA) production was observed after exposure of the chyme sample obtained after digestion of the combination FB_1+formulation with respect to the control containing the toxin only. In conclusion, this integrated model allowed the screening of different materials as mycotoxin binders able to protect against the toxic effects of mycotoxins, thus reproducing a physiological condition of exposure to food contaminants. I*n vitro* results will be confirmed by *in vivo* experiments on laboratory animals in order to validate the *in vitro* model.

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STUDYING THE EFFECTS OF ALPHA-ZEARALENOL MYCOTOXIN ON BREAST CANCER CELL LINES

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Endocrine disruptors (ED) are exogenous substances or mixture that interfere with production, release, transport, metabolism or action of natural hormones,1 and which consequently cause adverse health effects in all the organisms. Also some mycotoxins, natural toxins that cause food contamination, are defined as EDs. Zearalenone (ZEN) is a mycotoxin produced by several species of *Fusarium* fungi often found in cereal crops, such as maize, barley, rice, oats, wheat and sorghum. Furthermore, Zeranol, a semi-syntetic derivate of ZEN, is illegally used as anabolic in meat producing animals. ZEN together with its metabolites and derivates have estrogenic effects as infertility and mammary hypertrophy in females and feminization and enlargement of mammary glands in males. It is known that ZEN and its more active metabolite being alpha-zearalenol (alpha-Zol) are able to interact with the estrogen receptors leading to functional and morphological changes in the reproductive system in both animals and humans. It has been already reported that ZEN can stimulate the growth of estrogen receptor-positive human breast carcinoma and a mitogen-activated protein kinase signaling cascade [1]. Moreover, ZEN resulted an anti-apoptotic agent capable to induce the down-regulation of BAX and the up-regulation of BCL-2 expression [2]. In order to understand the toxicodynamic aspects of mycotoxin alpha-Zol, in this study two breast cancer cell lines, MCF-7 and MDA-MB 231, were used to evaluate the cell proliferation and the metabolomics profiling.
In details, two cancer cell lines were plated in cell culture flasks and treated with the increasing concentrations of alpha-Zol from 10E-10 to 10E-6 M. After incubation time of 72h, the cell proliferation and vitality were evaluated. Cell pellets obtained by trypsin digestion were re-suspended in a mixture of H2O, methanol and chloroform, and centrifuged at 10000 rpm for 10 min at 4°C to separate the polar and apolar components. Thereafter, two sub-fractions obtained for each pellet were collected separately and evaporated. 1H-NMR metabolomic analysis was performed on both the cellular polar and apolar fractions using a 600-MHz Bruker spectrometer at 300 K. Some statistical analysis like PCA, OPLS-DA, fold change and T-test were used to identify the differently expressed metabolites between the untreated and treated cells.
Our studies have showed that alpha-Zol was able to increase the cellular proliferation at concentration higher than 10E-8M in MCF7 cells and not in MDA-MB231. This finding is correlated to the different nature of these two breast cancer cell lines. In fact, MCF-7 cells are estrogen-receptor-positive whereas MDA-MB231 cells belong to the triple-negative sub-type. Hence, since it is known that in general EDs act by mining the estrogens, it is reliable that alpha-Zol has effect only on MCF7 cells. Moreover, the metabolomics analysis evidenced that alpha-Zol induces a modulation of the levels of lactate, choline, some amino acids and lipids and, hence, of lipidic and amino acid metabolism. Further studies will regard the validation of these results also on other breast cancer cell lines belonging to estrogen-responsive and triple negative sub-types.

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HPLC DETERMINATION OF OCHRATOXIN A IN PIG TISSUES USING ENZYMATIC DIGESTION COUPLED TO MOLECULARLY IMPRINTED SOLID PHASE PURIFICATION

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Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by various *Aspergillus* and *Penicillium* species with nephrotoxic, carcinogenic, immunotoxic and teratogenic potential effects (1). OTA has been found in several food commodities, including cereals, coffee, beer, wine and spices. OTA can also be present in food of farm animals as a result of carryover from animal contaminated feed (2); consequently, a permitted level of 1 µg/kg OTA in pig meat or pig-derived products was set in Italy, as in other countries. Conventional methods for the determination of OTA in animal tissues are performed by extraction with chloroform and followed by cleaning up with immunoaffinity columns or liquid-liquid partitioning (3). These procedures need a large amount of organic solvents which are environmentally harmful and hazardous to humans. The aim of the present study was to develop a new enzymatic digestion method coupled with molecularly imprinted solid phase purification (MISPE) for quantitative determination of OTA in pig tissues (muscle, liver, or kidney). Five grams of sample aliquot were homogenized with 5 ml of a phosphate buffer using an Ultra Turrax. A 2.5 g aliquot of the homogenate was transferred into a tube, incubated for 1 hour at 37°C with 10 ml solution of 1% pancreatin in a phosphate buffer, ultrasonicated at 75 Hz, purified with MISPE columns (pre-conditioned with 3 ml acetonitrile and 3 ml water). OTA elution was performed with methanol/acetic acid 98:2 (v/v). Final eluate was evaporated to dryness and re-dissolved into 1 ml of HPLC mobile phase and injected into HPLC-FLD. The method was validated according to EU criteria for the confirmatory methods for organic residues and contaminants (4). For all analyzed matrices mean recovery was > 89 %, intra and inter-day repeatability expressed as RSD < 5 % and LOD and LOQ were 0.0018 and 0.0054 µg/kg, respectively. The method can be applied as alternative routine procedure to detect OTA presence in pigs meat products.

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THE ROLE OF ANATOMY WITHIN CLIMAPP: A PROJECT TO IMPROVE SUSTAINABLE DEVELOPMENT IN APENNINE PASTORAL SYSTEMS

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Pastoral systems have to face climate change, since the increasing drought will affect herbage features, carrying capacity and animal welfare, representing a threat to biodiversity conservation and livestock rearing. The downsides of climate change impacts may be reduced by management innovation. Thus, CLIMAPP project is focused on the evaluation of different farming production (milk, wool, meat, etc.) and organization (shepherding, forage chains, flock composition, etc.) scenarios adopting an integrated, multidisciplinary approach which tackles the conservation, socio-economic and cultural components of the decisional context. The aim is achieving a sustainable management of grassland productive ecosystems [1]. Within CLIMAPP project, the anatomical equipe followed groups of animals, reared in pastures with different drought stress intensities; after the maximum pasture flowering (early July) and after the maximum pasture dryness (early September). Some of the animals were slaughtered to collect tissue samples from rumen in order to measure the epithelium keratinization degree [2], and mammary glands to evaluate the immunolocalization and expression of Apelin and its receptor [3]. Obtained data showed modification of the rumen keratinization between the two sampling moments. As mammary gland concerns, apelin was expressed only in samples collected in July, while its receptor was evidenced both in July and in September; data analysis allowed to hypothesize both the endocrine and autocrine action for the apelin at mammary gland level. The anatomical data were integrated with those obtained from the evaluation of variation referred to forage composition, milk production and composition. In additon, the quality and peculiar features of cheese was evaluated by means of a sensory panel. A consumer test associated with an experimental auction was used to evaluate consumer preference and willingness-to-pay [4] [5]. Interesting information emerged by data integration, suggesting a possible strategy to adopt by farmer to differentiate and certificate products obtained by a conservative management of natural grasslands that may also allow to enhance farm economic performance.

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DEVELOPMENT AND VALIDATION OF A SINGLE-SAMPLE IOHEXOL PLASMA CLEARANCE METHOD FOR ESTIMATING GLOMERULAR FILTRATION RATE IN DOGS

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Plasma iohexol clearance (CL_{io}) is a practical method for measuring glomerular filtration rate (GFR) in clinical settings and provides the most useful method for early diagnosing chronic kidney disease (CKD) [1]. Major limitation for a wide use in veterinary practice is the lack of standardized protocols. Furthermore, the standard procedure has the disadvantage to require repeated blood sampling over a period of several hours. In CKD, kidney function decline gradually and a single cut-off value for GFR do not fully discriminate between subjects with and without the disease. To deal with this problem, the construction of a three-zone partition has been proposed in human [2], including a positive and a negative zone and a grey zone of GFR intermediate values between two cut-off levels. A GFR value in the grey zone does not allow the CKD to be scored as either present or absent. Aim of this study was to develop and validate a method for the assessment of CL_{io} in dogs based on the determination of iohexol in a single blood sample (1-BS) and which can be suitable for the assessment of GFR both in daily practice and for research purposes. Furthermore, we also aimed to determine two cut-off levels including a grey zone of borderline intermediate GFR values. Dogs with a wide range of GFR were involved (no. 34), aged 1–16 years and with body weight 4–40 kg. Dogs were classified according to IRIS guidelines for CKD staging. Iohexol (Omnipaque 350) was injected as a bolus at 64.7 mg/kg and blood sampled at 5, 15, 60, 90, 180 min. Plasma iohexol concentrations were measured by HPLC with UV detection [3] and GFR was determined by conventional multisample strategies (5- or 3-blood-samples). Based on substitution of cumulative data from the 3-BS into the Jacobsson formula [3], an equation was derived for back-calculation of GFR with the 1-BS at 60, 90 or 180 min. Pearson test, linear regression analysis, and Bland-Altman plots were used to compare GFR calculated by different methods. ROC analysis was performed to assess diagnostic sensitivity and specificity for CKD. GFR determined by the 1-BS using the CL_{io} at t_{180} found to have the strongest agreement with 3- and 5-BS GFR. 1-BS-t_{180}-GFR values varied from 0.60 to 4.74 ml/min/Kg and without over- or underestimation respect to multisample methods. ROC analysis indicated that the 1-BS-t_{180}-GFR had the best CKD diagnostic power. The grey zone defining the borderline GFR values was identified between 1.98 and 2.28 ml/min/kg. In our population these cut-off correspond respectively to 100% specificity and 91% sensitivity, and 100% sensitivity and 74% specificity. Within this grey zone GFR cannot be classified as normal or reduced, whereas outside certainty of correct CKD diagnosis is assured. In conclusion, we developed a solid protocol that, reducing the dog sampling-associated stress, may enable GFR assessment to become practical in the clinical situation. Furthermore, the identification of a grey zone of borderline GFR values would give the veterinarian a valuable tool for the identification of subclinical/early stages of CKD and, in turn, may appreciably impact the diagnosis and management of pet nephropathies.

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EPIDERMIS AND HAIR FOLLICLE IN BOVINE SKIN EXPRESS THE LEPTIN HORMONE AND ITS RECEPTOR

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Leptin (Ob) is a hormone that acts through the activation of the specific receptor Ob-R. It is mainly secreted by visceral and subcutaneous adipose tissue and represents the first known adipokine [1]. Ob is a pleiotropic molecule and plays an important role in the skin, where it stimulates keratinocytes to proliferate and intervenes in the regulation of wound healing processes. It also participates in the control of hair follicle morphogenesis and its cycles of growth, regression, and rest [2]. Ob may act through endocrine, paracrine and autocrine mechanisms. While it is secreted by skin structures including epidermis and hair follicles, intradermal adipose tissue also seems to have a role in Ob secretion and accordingly in the control of the hair follicle cycle in mice and humans [3]. In this work, the morphological characteristics of the skin in bovine species were evaluated by Hematoxylin-Eosin and Oil Red O staining to investigate the presence and extension of intradermal adipose tissue that may be involved in Ob secretion. Moreover, Ob and Ob-R expressions were analyzed by means of RT-PCR and immunohistochemistry. Through the morphological analysis, a high and thick dermis without adipocytes was observed. Hair follicles and glands were located in the proximal part of the skin, beneath the epidermis, while a thick layer of connective tissue, lacking adipose cells, separated these structures by subcutis. RT-PCR gave a positive outcome, evidencing the transcripts for both molecules in the bovine skin samples examined. By immunohistochemistry, Ob and its receptor were observed in the epidermis and in the outer root sheath of hair follicles during the follicular cycle. The epidermis abundantly expressed Ob; while all layers of cells were involved, the suprabasal layers expressed a stronger signal. Ob-R was observed in the cells of the basal layer. As regards hair follicles, both Ob and Ob-R were expressed by the outer root sheath of hair follicles. Staining mainly extended into the regions of the infundibulum and isthmus while the bulb was negative. Immunostaining persisted in all stages of hair follicles. The expression of Ob-R in the bovine skin proves that Ob acts on this peripheral organ. The identification of Ob in the epidermis and hair follicle epithelium attests that Ob may act through a paracrine and autocrine mechanism on these structures even if an endocrine mechanism cannot be excluded. The absence of adipocytes around hair follicles and, broadly, in all the dermis indicates that the intradermal adipose tissue does not exist in bovine and accordingly cannot exert paracrine control on the hair follicle. The identification of the Ob system in bovine skin provides important information for properly understanding the biological mechanisms that regulate skin structures, and well as for comparing animal species and highlighting their differences.

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INTERSEX OCCURRENCE IN FARMED RAINBOW TROUT (*Oncorhynchus mykiss*): MORPHOLOGICAL AND HISTOLOGICAL CHARACTERIZATION

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Intersex is defined as simultaneous presence of male and female tissue in an individual of a gonochoristic species (Tyler and Jobling 2008). The intersex condition has been documented in both wild, farmed and laboratory animals.

Among the vertebrates, fish retain a remarkable plasticity in gonadal development, since they display different evolutionary adaptations related to sex determination and because they are subject to external influences (exogenous steroids, temperature and pollution) that can alter the normal development (Devlin and Nagahama 2002). This feature has been historically used in aquaculture, where for some species are more cost-effective breeding individuals belonging to one sex: an example is rainbow trout breeding (*Oncorhynchus mykiss*) in which females have a greater commercial yield.

The goal of the present study is to report some cases of intersex occurrence in farmed rainbow trout identified during the routine sampling activities.

During fish health monitoring activities conducted in 2016 by Fish Diseases Laboratory of Istituto Zooprofilattico Sperimentale Piemonte Liguria e Valle d'Aosta (Turin) 30 adult rainbow trout from a fish farm in Veneto Region were examined. All the animals were anaesthetized in a buffered solution of tricaine methanesulfonate (MS-222 100 mg/mL) and subjected to necropsy and microbiological examination. Gonads were grossly observed, photographed, dissected and fixed in 10% neutral buffered formalin. After fixation, tissues were processed by standard paraffin wax techniques. Samples were cut in 4±2 µm sections and stained with hematoxylin and eosin for the evaluation of gonadal morphology. Five of thirty subjects (16.7%) showed the presence of intersex with different distribution pattern, ranging from small ovarian tissue in the context of male gonadal tissue to real intermediate forms with the presence of ovarian tissue well-formed and wide structured in testicular tissue. In one specimen, the presence of intersex was detected only in the right gonad, while the left gonad was composed of a complete testicle, classified as monolateral mixed gonadal tissue (MGT) following Hecker *et al*. 2006. The gonads showed different maturation stages and no pathological changes.

The presence in nature of salmonids with intersex is already known, although this is a very rare phenomenon. These morphological alterations may be induced by the use of hormonal treatment in the fish farm or by the exposure to endocrine disruptor compounds (EDCs). In this case it is very likely that the condition described in some subjects was due to treatment with 17α-methyltestosterone and that these animals were accidentally mixed with trouts for human consumption instead of staying part of the broodstock.

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SOFIVET

EFFECTS OF IN VIVO ELECTROMAGNETIC FIELDS EXPOSURE OF SWINE OVIDUCT ON FERTILIZATION OUTCOME

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All living beings are continuously exposed to different kinds of non ionizing radiations, either of natural origin or from human technological activity, ranging from static magnetic fields to radiofrequency fields. We focused our attention on Extremely low frequency electromagnetic fields (ELF-EMF), originating in electricity transport by power lines, that in Europe have a 50Hz frequency and in USA 60Hz. General population in houses and in shopping centers, schools and hospital, and workers in specific workplaces are always exposed to these fields. ELF-EMFs are classified as possible carcinogens (class 2b) by IARC [1] but data on their possible negative effect on reproduction are still not complete. This led to the need of knowing more about this exposure and of understanding its effects on living beings. Previous studies demonstrated that in vivo exposure of swine spermatozoa to a sinusoidal wave (intensity 1 mT, frequency 50 Hz), negatively affects sperm function, reducing the in vitro fertilization rate. Moreover, direct exposure of oviducts to fields ≥ 0.75 mT without spermatozoa led to negative effects on early embryo development, suggesting a decrease in the embryo cleavage compared to sham exposed [2]. The main aim of the study is the evaluation of in vivo effects of the exposure to ELF-EMF, in particular on the physiology of the oviduct and on fertilization outcome. The experimental protocol has been approved by our Inter-University Ethical Committee for Animal Experimentation (CEISA) prot.43/2011/CEISA/PROG/25. To assess the in vivo effects of the exposure of swine oviduct to ELF-EMF field, a validated protocol was adopted [2]. The estrous cycle was promoted on 3 prepubertal gilts of 91.1±3.1 kg: the follicular growth was stimulated by injection of IM 1250 UI eCG (Folligon, Intervet, MI) and the ovulation was induced 64h later with 750 UI eCG (Corulon, Intervet, MI). Then, we compared oviducts exposed for 1h to the ELF-EMF (intensity of 0.75 mT, frequency of 50 Hz, sinusoidal waveform) and successively surgically inseminated with the contro-lateral non exposed oviduct (sham). Twelve hours after the estimated time of fertilization the fertilized oocytes were collected by flushing the oviducts. Gametes were fixed and observed with Lacmoid staining, to observe oocytes morphology, the percentage of fertilized oocytes, and the polyspermy rate. It was found that oocytes morphology was unaffected; the ELF-EMF exposure determines a significative decrease in the fertilization rate of oocytes (93.3±8.2% sham vs 64.6±10.2% exposed, $p<0.05$) and polispermy rate (60.3±3.8% sham vs 39.2±6.8% exposed, $p<0.01$) , while the number of spermatozoa/polispermic oocytes remained unaffected (5.7±0.6% sham vs 5.3±1.5% exposed, $p>0.05$). So we can confirm that in vivo exposure to an ELF-EMF could negatively affect the fertility outcome, either in terms of oocytes fertilized and on polyspermy rate. Further studies are needed to completely understand the possible interaction between Extremely low frequency electromagnetic fields and living beings, and in particular on reproductive functions.

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ARE THERE DIFFERENCES IN IMPULSIVITY BETWEEN SEXES IN DOMESTIC DOGS?

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Impulsivity is a complex personality trait, which, broadly, relates to a subject tendency to perform choices or actions driven by strong drives and with a lack of proper inhibitory control. Different facets of impulsivity have been shown to differ between sexes in humans and in other animal species [1,2]. However, sex differences in impulsivity have not been described in domestic dogs. The aim of the present study was to address this aspect. Thirty-two pet dogs were enrolled for this experiment (16 males and 16 females) and underwent two assessment procedures: 1) The proximity task, a newly devised procedure for the assessment of impulsive choices, in which dogs are initially trained to learn on which side (right/left) they can find the largest of two amounts of food, which are presented in bowls placed at the same distance (approx. 2.5 m) from the dog. Dogs choices for either of the two bowls are then assessed in a test phase, where the smaller food amount is placed systematically closer to the dog. 2) The cylinder task, a validated procedure that assesses impulsive actions. In this procedure, dogs are first trained to approach the side of an opaque container to get a piece of food. In the following test phase, the front of the container is transparent, and dog's attempts to get the visible food from the front part of the container (failed trials), across 15 consecutive presentations, are indicative of the inability to inhibit an impulsive response upon presentation of an appetitive stimulus. In the proximity task, dogs' choices were significantly affected by the distance of the smaller amount of food ($P<0.001$, Friedman). Dogs chose the smaller amount on only 20% of presentations (median) when the two bowls were at the same distance; choices for the bowl with less food proportionally increased as the latter was placed closer to the dog, reaching 100% (median) when the bowl was at 80 cm from the dog. These results conform to the idea that the dogs' performance in the test is indicative of dogs' inability to resist to an immediate, but smaller, gratification (impulsive choice) and providing a first indication for the validity of the procedure. In the cylinder task, dogs attempted to reach the food from the front of the box on 4 of 15 trials (median; min=1, max=10). There was no correlation between the number of choices for the smaller amount of food (averaged across all distances) in the proximity task and the number failed trials in the cylinder task (Spearman's rho = 0.72, $P=0.64$), suggesting that the two procedures measure different and unrelated facets of impulsivity. There were no differences between sexes in either the number of choices for the smaller food amount in the proximity task ($P=0.76$, Mann-Whitney), or in the number of failed trials in the cylinder task ($P=0.086$), thereby not supporting the existence of sex-related differences in these measures of impulsivity in dogs. However, the large variability and the small p-values found in the comparison of failed trials in the cylinder task warrant the possibility that differences between sexes in impulsive actions may arise if a larger sample was to be tested.

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THE PIGLET AS SPONTANEOUS MODEL OF IRON DEFICIENCY: A FUNCTIONAL TRIAL ON FORTIFIED BREADS

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For the majority of living organisms, iron represents an essential element for a variety of physiological and metabolic processes [1], and its deficiency is the most common nutritional disorder [2]. This situation is highly recurring in pigs as a para-physiological condition induced by zootechnical standards, thus the mandatory need to supplement piglets with exogenous iron within few days of birth [3]. This species is therefore extremely useful for preclinical trials concerning iron deficiency and food supplementation. The aim of this study was to evaluate this piglet model and to investigate the influence of oral iron supplementation, in the form of fortified breads. The study was conducted on 24 hybrid commercial piglets: to begin with, at 5 days of life, animals were divided in 4 groups (n=6) and only 1 group received the mandatory iron dextran supplementation. From day 44, 2 groups, both without iron supplementation, were fed one of two experimental breads, each fortified with ferrous sulphate, either encapsulated or not. The remaining 2, including the supplemented one, were fed standard control bread. After seven days of trial (day 51), animals were sacrificed and samples collected. Weight and hematological parameters were analyzed at 5, 44 and 51 days of life in order to evaluate iron status and overall biological response. Alongside, plasmatic levels of ferritin and gene expression of hepcidin, iron exporter ferroportin, and divalent metal transporter 1 were investigated. All piglets were of similar weight on day 5 and analysis showed that all of the animals enrolled in the study were healthy. The group that received IM iron supplements subsequently gained significantly more weight than those which did not receive the experimental diet ($p<0.05$). Levels of blood variables such as hematocrit, hemoglobin, and red blood cells seemed to be influenced by the kind of bread administered. The iron content in serum varied widely between animals. Despite this large individual variation, it can be noted that the iron levels in orally supplemented groups decreased, while they remained stable in both groups fed with control bread. Our hypothesis is that the increase in nutritional iron availability may stimulate iron storage or tissue-binding mechanisms. Ferritin and gene expression analyses did not show any statistically significant difference among groups. In conclusion, the piglet seems to be a good model for iron deficiency, but still needs some refinement to be more reliable and standardized. Moreover, this study has shown that bread fortified with ferrous sulphate or encapsulated ferrous sulphate can provide effective treatment of iron deficiency statuses in piglets.

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PLASMATIC TRYPTOPHAN/LARGE NEUTRAL AMINO ACIDS RATIO IN DOMESTIC DOGS IS AFFECTED BY MEAL COMPOSITION

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Tryptophan (Trp) is involved in the synthesis of serotonin and melatonin and it competes with other large neutral amino acids (LNAAs) for its uptake into the brain [1]. The aim of this study was to assess the impact of three different diets on plasmatic Trp/LNAAs ratio. This study included five female Labrador Retrievers (2 spayed, 8.6 ± 3.8 years old) from the same bloodline, who were usually fed the same commercial dry food once a day. Each dog received three different diets for one single day each. Isocaloric and isonitrogenous diets, with a carbohydrates content of 47% and proteins content of 28% on dry matter basis, were provided in two meals, one in the morning and one after 12 hours. Dogs received the first diet (D1) and then they returned to their normal diet for 30 days. After that "washout" period, dogs were fed with the second diet (D2), and after 30 more days they received the third diet (D3). D1 was composed of a mix of puffed rice, minced meat and olive oil equally divided into the two meals. D2 was made up of two different meals. The morning meal was composed of puffed rice and olive oil, whereas the evening meal consisted of minced meat and olive oil. D3 consisted of two identical meals of the commercial dry food usually consumed by the sample dogs. Blood was collected right before the first meal (t0) and after 2, 4, 6, 8, 10 and 24 hours. Plasma samples were used for HPLC quantification of Trp and other LNAAs (isoleucine + leucine + phenylalanine + tyrosine + valine) using a method described in literature [2]. Their levels and ratios at t0 and after D1, D2 and D3 were compared using a mixed model for repeated measures ($p<0.05$). Trp concentrations showed no significant difference between D1, D2 and D3 samples at any sampling times. LNAAs levels were similar at t0 in the three experimental days, but they showed different trends depending on the composition of the meal provided. In particular, D2 led to a decrease in LNAAs levels and therefore to higher Trp/LNAAs ratios in the 6 hours period after the provision of carbohydrates. In detail, mean Trp/LNAAs ratio of D2 was statistically higher compared to both D1 and D3 at t2 (D1=0.224; D2=0.306; D3=0.217; $p<0.001$), t4 (D1=0.225; D2=0.327; D3=0.197; $p<0.001$), and t6 (D1=0.244; D2=0.303; D3=0.205; $p<0.015$). In addition, mean Trp/LNAAs ratio after D2 was higher than after D3 also at t8 (D2=0.280; D3=0.206; $p<0.001$) and t10 (D2=0.294; D3=0.224; $p<0.001$). The trend was different at t24, when Trp/LNAAs ratio was found to be significantly lower after being fed D2 compared to D1 (D1=0.210; D2=0.155; $p=0.041$). These results indicate that the diet affects Trp bioavailability. Therefore, it is worthwhile to investigate the effects of diet on Trp bioavailability at the brain level, serotonin and melatonin secretion and the real impact of Trp/LNAAs ratio on dog behaviour.

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CORRELATION BETWEEN OXIDATIVE STRESS MARKERS AND SEMEN PARAMETERS BY SPERM CLASS ANALYZER (SCA) IN DOGS

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Limited amounts of ROS regulate physiological processes necessary for spermatozoa to acquire fertility capability; excessive production of ROS can have a negative effect on the sperm motility and fertilizing power [1]. The aim of this study was to investigate the correlation between oxidative status in dogs and sperm quality assessed by SCA. Ten male dogs aged 1 to 7 years old (4.5±1.2 years) and housed in similar domestic situations were included in this study. Each animal was submitted to clinical examination, including blood and semen sample collections. Seven dogs were fertile; three dogs were hypofertile. Oxidative status was evaluated by measuring hydroperoxide in the blood using d-ROMs test; the antioxidant capacity in serum samples was evaluated by Oxy-Adsorbent test (kits purchased from Diacron International, Grosseto, Italy). The ratio between ROMs and Oxy (×100) (Oxidative Stress index - OSi) is an arbitrary value used as an index of plasma oxidative state; higher values indicate a higher concentration of oxidized molecules than non-enzymatic antioxidants [2]. Semen quality and motility tests were performed by SCA system (Hamilton Thorne Research, Beverly MA, USA). Parameters measured were: percentage of motile (MOT) and progressive motility (PMOT); rapid (RAP), medium (MED), slow (SLOW) and static (STATIC) spermatozoa movement; and sperm concentration (CONC; 10^6/ml). All data were recorded using an Excel spreadsheet (Microsoft® Excel 2011) and then imported into statistical analysis program (JMP® 8.0.2© 2009 SAS Institute Inc.). Data were checked for normalcy of distribution with a Shapiro-Wilk's W test. Multivariate analysis was applied to evaluate the correlation between sperm parameters and OSi. Significance was set at $P \leq 0.05$. The comparison between fertile and hypofertile dogs showed significant difference between the two groups for the following parameters: MOT, PMOT, RAP, MED, SLOW, STATIC, OSi. The correlations between each seminal parameter and OSi were evaluated separately between the fertile and hypofertile groups. In the hypofertile group there was a significant positive correlation between OSi *vs* SLOW (1.000), and STATIC (1.000) and a significant negative correlation between OSi *vs* MOT (-1.000), and RAP (-1.000). In the fertile group there was a significant positive correlation between OSi *vs* SLOW (0.800) and a negative correlation between OSi *vs* PMOT (-0.811). This study reports, for the first time, a direct correlation between oxidative stress and the main parameters of the fertility test in both fertile and hypofertile dogs. These results open new diagnostic, prognostic and therapeutic insights for canine male infertility.

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BEHAVIORAL VISION ASSESSMENT IN PIGS

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The swine species is often the gold standard laboratory animal in ophthalmology research due to the anatomical and physiological similarities of the porcine eye and retina with humans. Despite the importance of the model there is a lack of tools regarding the behavioral vision assessment in the pig.
The aim of the study was to validate a behavioral test to assess vision in the pig model.
The animals used in this study were enrolled in two protocols approved by the Italian Ministry of Health. Two different trials were performed to assess vision during the characterization of an iodoacetic acid IAA induced pig model of photoreceptors degeneration. In the first trial, the visual capacity of pigs was assessed using the sequence of fear tests (Human Approach –HAT-; Novel Object –NOT- and Open Door –ODT-) proposed by van Erp-van der Kooij et al. [1]. Six animals (IAA group=3; C group=3) were individually tested in their home pen before and 2 weeks after IAA administration, the time to complete each test was recorded. The tests were specifically adapted to reduce the possibility of animals to compensate with hearing and smell. For the second trial, 18 pigs (IAA group=11; C group=7) were evaluated before and two weeks after IAA administration with a behavior test proposed for cats in 2013 [2] with some modifications. The testing course was 10 meter long and 2.5 meter wide, with 10 obstacles (one every meter) randomly positioned on the right, the left or the center to obtain 4 configurations. In the morning, before food administration, animals were tested in artificial light and in semidarkness conditions. Time to complete the course and number of collisions with the obstacles were recorded. After a training period of two weeks, the test was performed twice a week for 8 replicates to reduce errors due to smells and noises; to reduce the possibility that animals where learning the obstacles' position, configuration was changed every time the test was performed.
Paired and non-paired analyses were performed for both trials. Our preliminary results show that after IAA administration the average time taken by pigs to carry out all the fear tests increased. However, the variability of the responses observed and the small number of animals tested so far did not allow us to highlight any statistical difference. The data from the second trial instead showed statistically differences between C and IAA groups in light and semidarkness conditions. We suggest that the second trial, named the "obstacle course" is a reliable, sensible and specific test to assess vision in pigs even when a small amount of animals are enrolled in the trial.

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DOSE-EFFECT STUDIES OF *Coridothymus capitatus* AND *Rosmarinus officinalis* ESSENTIAL OILS ON SWINE SPERMATOZOA

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Essential oils (EOs) are products of the secondary metabolism of aromatic plants and are complex mixtures of several compounds. EOs show a wide variety of biological activities widely exploited in both Human and Veterinary Medicine [1;2]. Aim of the study was to evaluate the dose-dependent effects of *Coridothymus capitatus* (Cc) and *Rosmarinus officinalis* (Ro) EOs on the main morpho-functional parameters of swine spermatozoa. Citotoxicity studies on swine spermatozoa have two main outcomes: first, the identification of a cost-effective screening method for EOs evaluation in general, and then a direct application in reproductive medicine (conservation of spermatozoa, reduction of the use of antiobiotics). Beforehand, the exact composition of each EO was tested in Gas Chromatography Flame Ionization Detector (GC-FID). Each experimental dose was prepared by adding a fixed number of spermatozoa in a final volume of 5 ml of medium with 10 different dosages of EO (from 0.2 to 2mg/ml, at intervals of 0.2). After 3 hours of incubation at 16°C (±1°C), the samples were evaluated for the principal morpho-fuctional parameters: viability (Eosine-Nigrosine staining), objective motility (CASA), acrosome status (Comassie blue staining) and pH of the sample. Moreover, for the highest and lowest dosages, the spermatic morphology was evaluated by Scanning Electron Microscopy (SEM). The results showed differences between the 2 EOs: the Cc-treated spermatozoa showed important alterations of motility ($p<0.001$), viability ($p<0.001$) and acrosome status ($p<0.001$) associated with membrane alteration also at low dosages (from 2 mg/ml to 0.4 mg/ml). High dosages of Ro EO determined reduced motility ($p<0.05$ from 2 to 0.4 mg/ml) to but relatively maintained viability on spermatozoa sample if compared to CC-treated samples (Ro Vs Cc: $p<0.001$). The acrosome status was highly alterated when spermatozoa were Cc treated ($p<0.001$), the acrosomes were totally reacted from 2 to 0.4 mg/ml while for Ro, the acrosome status was only slightly alterated ($p<0.05$). Electron microscopy, for both the EOs at 2mg/ml showed strong alterations but with different appearances: Ro seems to alter predominantly the head whilst Cc alters the entire spermatozoa. The results seem to show two different toxicity patterns: either directly on cell membrane or connected to cell fuctionality. In conclusion, studies on spermatozoa might provide new insights regarding the effects of EOs for both toxicology and reproductive medicine.

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DIFFERENCES IN THE DEVELOPMENT OF BEHAVIOURS AND SENSES DURING THE TRANSITION PERIOD IN BEAGLE AND JACK RUSSEL TERRIER PUPPIES

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Developmental changes in a dog's behaviour and physiology begin before birth and extend throughout the dog's life with most changes occurring before sexual and social maturity [1]. While many studies have focused on the socialization period [2, 3], only little attention has been paid to the transition period, during which the puppy develops its senses of vision and of hearing. The objective of this study was to identify and compare the behaviour and onset of senses among a total of 25 purebred puppies representing 2 breeds, namely Beagles (n=12) and Jack Russel terriers (n=13). Litters were all selected from the same breeder. Each litter was videotaped during 1-hour daily sessions beginning 10 days after birth until 21 days of age and coded for the following mutually exclusive behavioural categories: sleeping, suckling and moving. The "moving" category included 6 behaviours: side-to-side head swinging, exploring, rolling, allo-grooming, playing, and vocalising. Additionally, the opening of the eyelids, the onset of the startle response and the ability to stand up with either the front or hind legs were identified. Overall, puppies invested 77.6% of their total observed activity in sleeping, 15% in suckling and 7.4% in moving. When awake, puppies spent the majority of the time staying still while moving the head from side to side (49.1%) and exploring (38.7%). Suckling rate per minute was significantly lower than that of sleeping and moving. Two behavioural categories varied significantly over time. The duration of moving bouts positively correlated to time ($p<0.01$), while the frequency negatively correlated to time ($p<0.01$). The frequency of suckling decreased significantly ($p<0.05$) with pup age, with no significant change in duration. The frequency of moving bouts, particularly exploring, decreased over litter size. By contrast, suckling and moving bout duration was greater ($p<0.01$) in larger litters. Breed differences in puppy mobility and sensory development were observed, with Beagle dogs demonstrating more frequent exploring, grooming, rolling and side-to-side head moving and an earlier acoustic reflex response. Jack Russell terriers showed longer suckling duration and earlier ability of getting up using both front and hind legs. In females we observed a higher frequency of exploring behaviour ($p<0.01$) and an earlier opening of the eyelids ($p<0.05$) than in males. Finally, season of birth showed significant effect on behaviour, with puppies born in the summer displaying shorter but more frequent sleeping, suckling and moving bouts than puppies born in the spring. These findings may be useful to veterinarians, breeders and other professionals to gain a more reliable understanding of behaviour development and predictable differences. This knowledge could be further applied to improving welfare and standards of rearing, training, socialization, and behavioural modification techniques in pet dogs.

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DIFFERENTIAL MODULATION OF IMMEDIATE EARLY GENES IN MAMMARY EPITHELIAL CELLS

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Immediate early genes (IEGs) represent a class of proteins, with different functions, with fast kinetics of up- and down-regulation [1]. In this work we have studied the differential modulation of some IEGs at trascriptional and post-trascriptional levels, in mammary epithelial cells of different origin. We have examined trascription factors of the Egr, Fos and Jun family as well as amphiregulin (AREG), one of the seven ligands for the EGFR tyrosine kinase receptor, which has a fundamental role during pubertal mammary growth [3]. Primary and immortalized mammary epithelial cells (murine, feline, bovine) were used to study how these IEGs are regulated. We have analized the kinetic of expression, the factors and the pathways that affect IEGs modulation both at the mRNA and protein levels. Expression of the IEGs showed different patterns of modulation depending on the gene being analyzed. The Egr family (Egr-1/2/3) and the c-Fos trascrition factor were similarly regulated at the mRNA level, with fast upregulation that reached a significant peak after 45-60 minutes of stimulation with growth medium, followed by rapid downmodulation. Egr-1 protein levels followed exactly mRNA modulation with significant fast up- and down-regulation after growth medium stimulation. On the other hand c-Fos protein expression levels lasted longer being significantly upregulated after one hour but then slowly decreascing after more than 4 hours. Egr-1 and c-Fos depended on the Erk-1/2 pathway activation to sustain their expression both at the mRNA and protein levels. All factors that affected Erk1/2 phosphorylation, like epidermal growth factor or phorbol-mysistate-acetate, were also able to upregulate Egr-1 and c-Fos. mRNA levels of the c-Jun trascription factor were signifantly upregulated by growth medium, but at lower levels the Egr-1 or c-Fos. On the other side c-Jun protein levels were highly incresased by growth medium and remained sustained for 4-6 hours. Inhibiting the Erk1/2, the PI3K or the JUN kinases did not modify c-Jun protein or mRNA levels. AREG, at the mRNA levels, was stimulated but with slower kinetics, reaching a significant peak after 120 minutes of stimulation. A partial reduction of AREG mRNA was observed with Erk1/2, but not PI3K or JUN kinases inhibition. AREG protein levels were superimposable with c-Jun and, similarly, were not altered after Erk1/2, PI3K or JUN kinases inhibition. In conclusion, although IEGs share the common characteristic of fast up- and down-regulation the kinetics, the factors that stimulate them and the pathways that regulate them are different and deserve careful examination in order to unravel mammary gland biology during growth and function [3].

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PHYSIOLOGICAL CHARACTERIZATION OF DRY AND POST-PARTUM PERIOD IN HOLSTEIN AND RENDENA BREEDS

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In the last 60 years genetic selection in Holstein cattle has been mainly focused on the milk yield criterion, and this mono-aptitude selective criterion has led to their high propensity to develop critical physiological conditions in the pregnancy to lactation transition period with repercussions on reproductive efficiency, longevity in farm and resistance to stress and diseases [1][2]. A different scenario appears to happen with autochthonous Italian dairy breeds, which are typically characterized by much better fertility and higher resistance and resilience against disease [3]. Unfortunately, less is known about the biological mechanisms behind this rusticity. In this study, a multidisciplinary approach was applied to compare different physiological parameters related with the metabolism, milk protein profiles and the milk microbiota in Holstein Friesian (HF) and Rendena (REN) breed, reared in the same farm and under the same management conditions. The cows (6 HF and 4 REN) were all among 2 and 4 lactations. The average milk yield was significantly higher in HF compared to REN (HF=5,366 kg vs REN=3,769; p=0.0147). The percentage of milk fat (HF=3.52% vs REN=3.37%) and protein (HF=3.02% vs REN=3.08%) content was comparable in the two breeds. Quarter milk samples and venous blood were collected from each animal at the following time points: dry-off (T1), 1 day after calving (T2), 7-10 days after calving (T3) and 30 days after calving (T4). Blood samples were used for the analysis of plasma metabolites such as: glucose, total cholesterol, urea, inorganic phosphorus, total protein, albumin, total bilirubin, aspartate aminotransferase (GOT), γ-glutamyltransferase (GGT), creatinine, NEFA, β-OH-butyric acid (BHBA), thiol groups (SHp) and ferric reducing antioxidant power (FRAP). Bacteriological analyses, somatic cell counting, protein profiles and characterization of the milk microbiota were perfomed on milk samples. Plasma metabolic parameters were analyzed using a pair wise comparison (least significant difference test). A spatial power covariance structure was used. HF cows showed a more severe fat mobilization (NEFA and BHBA $p<0,05$) and systemic inflammatory response at T2 and T3 in comparison with REN cows. We also observed a lower risk of oxidative stress (FRAP $p<0.05$) and an increased amino acid mobilization (creatinine $p<0.05$) in Rendena cows immediately after calving. Upon bacteriological analysis, contagious bacteria such as Staph. aureus and S. agalactiae were not found, but significant differences were assessed in the general composition of the milk microbiota of the two breeds. Concerning the milk protein abundance profile, several bands associated to immunoglobulin components were present in consistently higher amounts in REN colostrum when compared to HF cows. In addition, the relative abundance of caseins between the two breeds did also vary at all time points, prompting further investigations about its implications on cheesemaking properties. In conclusion, several differences were observed in the two breeds, in spite of the same farming conditions. The physiological observations reported in this work present numerous hints on the factors that may provide autochthonous, more rustic breeds with a better ability to face the critical post-partum period.

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IMPAIRED LYSOSOMAL AUTOPHAGIC PROCESS CONTRIBUTES TO CARDIAC DEFECTS IN MUCOPOLYSACCHARIDOSIS IIIB MOUSE MODEL

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Mucopolysaccharidoses (MPSs) are rare inherited diseases characterized by deficiencies of lysosomal enzymes involved in glycosaminoglycans (GAGs) degradation. The accumulation of GAGs at cellular level results in multiple organ dysfunctions [1]. These diseases have been described both in humans and domestic animals, mainly dogs and cats [2]. To date, there are no specific treatments for MPSs, the prognosis is poor, and commonly the animals are euthanized due to deteriorating quality of life. In affected cats and dogs, cardiovascular abnormalities similar to those seen in children have been reported [3-4]. In the MPS subtype IIIB, the lack or malfunctioning of α-N-acetylglucosaminidase (NAGLU) enzyme required for heparan sulfate (HS) degradation is responsible for clinical manifestations including neurological and cardiovascular disorders. Here, the murine model of the MPS IIIB (NAGLU-/-) [5] was used to explore the molecular mechanisms underlying cardiac involvement in the disease. We investigated cardiac morphology and function in NAGLU-/- mice compared to wild-type (WT) littermates using cardiac ultrasound, histological and biochemical analyses. Echocardiographic and Doppler measurements showed reduced cardiac function and valvular defects in 32-week-old NAGLU-/- mice compared to WT. Enhanced myocardial fiber vacuolization, accumulation of HS in the myocardial vacuoles, fibrosis, recruitment of inflammatory cells and collagen deposition within the myocardium was detected by histological analysis in heart tissues from NAGLU-/- mice compared to WT. Western blotting and RT-PCR analyses demonstrated increased expression levels of hypertrophic and fibrosis markers in heart tissues from NAGLU-/- mice. Finally, cardiac dysfunctions in NAGLU-/- mice resulted to be associated with both lysosomal defects and an impairment of autophagic flux as demonstrated by the increased expression levels of the lysosomal marker LAMP2, the main mediator of the early phase of autophagy Beclin1, and the protein reflective of autophagosome abundance LC3-II. These results indicate that the autophagic process is dysfunctional in the heart of NAGLU-/- mice, thus suggesting that dysregulated autophagic capacity can have a detrimental impact on heart tissues, thus representing an important factor contributing to the physiopathology of cardiac disease in MPS IIIB. Our findings could be relevant in the screening of new therapies for the treatment of MPS IIIB disease.

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INFLAMMATORY RESPONSE IN HOLSTEIN FRIESIAN VERSUS A LOCAL CATTLE BREED (RENDENA) AT DIFFERENT TIME POINTS

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The selective pressure for increased milk production in dairy cows brought about great difficulties in the adaptation of cows to their environment. This normally translates into increased culling rates, reduction of life expectancy and fertility, higher propensity to develop diseases (including mastitis), especially when compared to less selected and lesser producing dairy breeds which are typically characterized by higher resilience. However, not much is known about the biological mechanisms behind the relationship between genetic selection and higher risk of metabolic and infectious diseases (1). It is well known that during the calving period, high-yielding dairy cattle are more susceptible to common environmental stressors. This may have crucial repercussions on disease occurrence and on milk production levels (2).

With the aim of investigating the factors associated to this phenomenon, in this study we compared innate immune response patterns of 6 Holstein Friesian (HF) and 4 Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples and blood were collected from all cows at dry-off (T1), 1 day after calving (T2), 7-10 days after calving (T3) and 30 days after calving (T4). Quarter milk samples were subjected to measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-1β, TNF-α, PTX3, IL-1R8. Blood samples were used for the analysis of plasma metabolites indicators of systemic inflammation such as haptoglobin, ceruloplasmin, total protein, albumin, total bilirubin, and globulin.

HF cows showed a more severe systemic inflammatory response at T2 and T3 in comparison with Rendena cows in terms of haptoglobin, total proteins, globulins and bilirubin. Concerning the milk protein abundance profile, pronounced differences were observed in the colostrum (T2), with significantly higher amounts of protective molecules (immunoglobulins and other immune-related proteins) in Rendena. Moreover, at all time points HF showed higher levels of the inflammation marker cathelicidin in milk. In addition, the expression of innate immune related genes, as well as the CD45/KRT5 expression ratio in milk cells (indicators of leukocyte and epithelial components) were different in HF compared with Rendena. Our results suggest that HF cows develop a systemic and local mammary inflammatory response that could impair the capability of the animal to face the peripartum period and make them more susceptible to disease compared with Rendena cows.

Our findings reveal the importance of the autochthonous breeds in the understanding of the immunity mechanisms and indicate that fundamental effector activities of innate immunity in the mammary gland should be included in the breeding programs of HF cows. This kind of integrated approach can be conducive to a substantial reduction of antibiotic usage in dairy farms as a result of greater disease resistance.

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MILK MICROBIOTA OF HOLSTEIN FRIESIAN COWS PRESENTS HIGHER BIODIVERSITY COMPARED TO RENDENA

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Recently, studies on milk microbiota composition have been intensified, especially concerning the impact that these microorganisms can have on animal health status, as well as on the quality and safety of dairy products (1). Preliminary research performed on the udder microbiota of different ruminant species (cattle, sheep, buffalo) has shown that a complex microbial community is present in raw milk, whose composition is influenced by endogenous and exogenous factors (2).

This study aims to understand the differences in milk microbiota between two different bovine breeds. We compared milk microbial population of 3 Holstein Friesian (HF) and 3 Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples were collected from all cows at dry-off (T1), 1 day after calving (T2), 7-10 days after calving (T3), and 30 days after calving (T4).

In order to evaluate the mammary gland health status, somatic cell count and bacteriological analysis were performed on quarter milk samples. Moreover, a NGS approach based on 16S metagenomics was applied to the milk of HF and REN during the peripartum period. Results were assessed by pooling all lactation time points together.

Upon bacteriological analysis, contagious bacteria such as *Staphylococcus aureus* and *Streptococcus agalactiae* were not found, but significant differences were seen in the general composition of the milk microbiota of the two breeds, with the microbiota biodiversity of Rendena milk being clearly lower than the one of HF milk. At the phylum level, REN milk was dominated by *Firmicutes* (94%, relative abundance), while HF milk contained *Firmicutes* (65%), *Proteobacteria* (15%), *Actinobacteria* (11%) and *Bacterioidetes* (6%). At the genus level, REN milk showed the predominance of *Streptococcus* (71%), followed by *Lactobacillus* (10%) and *Pediococcus* (6%), while HF milk was dominated by *Streptococcus* (29%), followed by *Lactobacillus* (6%), *Corynebacterium* and *Staphylococcus* (4%). Among streptococci, *Streptococcus thermophilus* was the most prevalent (48%) in REN milk, in comparison with only 2% in HF milk.

Streptococcus thermophilus is a lactic acid bacterium used in the production of fermented milks, yogurt, and many cheeses. Therefore, its presence in higher percentages in Rendena milk may be favorable for dairy processing purposes (2).

The advent of high-throughput technologies capable of providing a complete picture of milk microbial composition enables a better understanding and a more efficient comparison of the characteristics of autochthonous breeds (as in the case of Rendena) promoting the breeding and safeguarding of biodiversity.

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WATER BUFFALO MILK MICROBIOTA RELATED TO HEALTH STATUS

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The development of culture-independent techniques by means of high-throughput DNA sequencing has just begun to unravel the impact of the microbiota – the collection of microbial organisms inhabiting a specific environment – on animal health, suggesting the possibility to correlate a determined microbiota to a health status (1). A new concept of pathobiome, which includes pathogenic agents in the microbiota environment, is taking shape (2) and the role of micro-organisms in establishing and modulating diseases is under investigation. The aim of the present study is to provide insights into the microbiota of dairy water buffalo milk related to health status. High-throughput DNA sequencing of the 16S rRNA V1-V2 regions was carried out to determine the bacterial content of milk samples from a total of 137 quarters, divided in three groups: i) healthy quarters (nr 27) ii) quarter with clinical mastitis (nr 27) iii) quarters with sub-clinical mastitis (nr 83). The microbiota diversity of healthy samples was richer as compared to samples with sub-clinical mastitis, whose microbiota diversity were in turn richer as compared to those from clinical mastitis. The core microbiota of water buffalo milk, defined as the asset of micro-organisms shared by all healthy milk samples, includes 15 genera, namely *Micrococcus, Propionibacterium*, 5-7N15, *Solibacillus, Staphylococcus, Aerococcus, Facklamia, Trichococcus, Turicibacter*, 02d06, SMB53, *Clostridium, Acinetobacter, Psychrobacter* and *Pseudomonas*. Only two genera (*Acinetobacter* and *Pseudomonas*) were present in all the samples from sub-clinical mastitis, and no genus was shared across all in clinical mastitis milk samples, where the major genus prevalence was represented by *Bacteroides* and *Pseudomonas*, followed by *Porphyromonas* and *Fusobacterium*. Discriminant analysis shows the evidence that the microbial community of healthy and clinical mastitis could be discriminated on the background of their microbiota profiles. In conclusion, the present study, through a culture-independent metagenome approach, investigated the water buffalo milk microbiota from healthy, clinical and sub-clinical mastitic samples, demonstrating that it is possible to correlate the microbiota to a specific patho-physiological animal status.

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CHARACTERIZATION OF THE BLASTOGENIC RESPONSE TO LPS OF BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS

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Mitogens are diverse compounds of plant and microbial origin, widely employed to test immunocompetence. In healthy, non-immunocompromised hosts, they induce DNA synthesis and division of large leucocyte populations, which can be reasonably associated with the capacity to mount adaptive immune responses. The blastogenic response of bovine peripheral blood mononuclear cells (PBMC) to lypopolysaccharides (LPS) has been investigated for a long time in our laboratories. In particular, a possible correlation between blastogenic response to LPS and disease resistance of periparturient dairy cows had been observed in previous studies (Catalani et al., 2013): low responder cows presented a much higher frequency of disease cases after calving, compared with high responder animals. Owing to the above, different aspects of the blastogenic response to LPS were investigated on PBMC of healthy, dry (2) or lactating (26) Friesian cows, and the extent of the response was evaluated in a 72-hour BrDU incorporation assay, as previously described (Catalani et al., 2013). Unstimulated and BrDU-treated cells were used as negative control. Stimulation with LPS induced little if any increase of cell counts over 72 hours despite consistent, low to moderate BrDU incorporation in all the PBMC samples under study. Poor replication of LPS-stimulated PBMC was confirmed by cell cycle and cell growth flow cytometry analyses. In particular, LPS stimulation gave rise to very low percentages of S phase cells, sometimes lower than in control, unstimulated cells, as opposed to Concanavalin A-stimulated PBMC. Also, LPS-stimulated and BrDU-treated PBMC were submitted to magnetic separation using B and T cell-specific mAb, and Miltenyi anti-mouse IgG MicroBeads. Analysis of BrDU incorporation after stimulation with LPS showed that both B and CD4 T cells are involved in the blastogenic response to LPS, in contrast with current data based on human and murine models. Finally, as opposed to control cells, LPS-stimulated PBMC maintained the expression of IL-1beta and up-regulated both IDO and TDO2 genes (kynurenine pathway, endotoxin tolerance). Both control and LPS-stimulated PBMC down-regulated Ig light chain expression. On the whole, our data indicate that differences in the response to LPS could be accounted for by heterogeneity of responding cells (B and T lymphocytes), that could also cooperate with monocytes in induction and regulation of endotoxin tolerance.

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IMPACT OF CADMIUM EXPOSURE ON SWINE ENTEROCYTES

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Cadmium (Cd) is a toxic and carcinogenic heavy metal widely distributed in the environment. The ingestion of contaminated food and drinking water is the major source of exposure to Cd and gut is the first target of interaction. However, little is known about Cd interaction with the intestinal tract (1). The aim of our study was to investigate the effects of low and high concentrations of Cd on swine enterocytes in terms of gene expression, cytotoxicity, Cd uptake, as well as of host-pathogen interaction. Swine intestinal IPEC-J2 cells were used as model. These were treated with 2 µM or 20 µM Cd solutions and incubated at 37°C in 5% CO2 for 1, 3, 6 or 24 hours. The parameters under study were described in previous studies (2-3-4). Each test was performed twice, untreated wells being used as negative control. The statistical significance of differences among the experimental groups were evaluated by one-way ANOVA or Kruskal-Wallis test. The significance threshold was set at $P< 0.05$. The ability of IPEC-J2 cells to uptake Cd was investigated first. Our data showed a significant ($P<0.001$) increase of intracellular Cd after 3 ($P<0.001$), 6 ($P<0.001$) or 24 hours ($P<0.001$) of exposure with respect to 1 hour. This was confirmed for both 2 µM ($P<0.001$) and 20 µM ($P<0.001$) Cd. The absorption of Cd was related to a significant reduction ($P< 0.0001$) of cell viability after treatment with 20 µM Cd. No effects were shown after treatment with 2 µM Cd. Concerning the modulation of gene expression, cells treated with 2 µM Cd for 1, 3, 6 or 24 hours showed a significant increase ($P<0.05$) of inflammatory gene expression (IL-6, IL-8, MYD88, NFkb1, NFkb-p65, IL-18) at all-time points, respect to untreated wells. These data are in agreement with previous studies (1) and highlight a pro-inflammatory effect of low concentrations of Cd. Treatment with 20 µM Cd caused up-regulation ($P< 0.05$) of IL-8 after 1 hour of exposure followed by a reduction of IL-8, p38, NFkb1, CD14 and STAT3 gene expression after 3 hours of treatment. At the same time, we observed up-regulation of IL-18, TNF-α, MYD88, JNK, IFN-β, BD1, BD2, TLR5 and MD2 gene expression. These effects were followed by up-regulation of Type I IFNs and IL-8 gene expression after 6 hours of exposure. 20 µM Cd caused up-regulation of IL-8, IL-1β, JNK, BD1, BD3 and BD4 and down-regulation of p38, NFkb1, MYD88, NFkb-p65 CD14, and TLR4 after 24 hours of treatment. These data support the ability of Cd to modulate inflammatory responses in swine enterocytes. Moreover, the down-regulation of inflammatory responses observed after 3 h of treatment with 20 µM of Cd, was associated with a significant ($P<0.05$) reduction of *Salmonella typhimurium* penetration into IPEC-J2 cells. In conclusion, our results indicate that exposure to Cd may modify the basal level of cytokine expression, thereby influencing different compartments of the innate immune response.

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EXTRACTS FROM WINE BY-PRODUCTS AFFECT SHEEP CELL PROLIFERATION AND CYTOKINE PRODUCTION

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The Wine sector is one of the most important compartments of the Italian economy; different by-products originate from wine production, among which wine lees. Several studies indicated the possibility of using the different wine by-products in animal feeding as they contain bioactive compounds such as polyphenols [1-2]. Recently, authors demonstrated the ability of these compounds from wine by-products to interfere in the inflammatory cytokines pathways [3], and, therefore, to up-regulate the expression of anti-inflammatory cytokines such as IL-10 [4]. The aim of this study was to evaluate the effects of bioactive compounds extracted from three different wine lees from white (Wh), rosè (Ro) and red wine (Re), on sheep peripheral blood mononuclear cell (PBMC) proliferation and cytokine production. Wine lees extracts were obtained by microwave-assisted extraction with three different solvents: water (W), water/ethanol 1:1 (W-Et) and ethanol (Et), with or without Na_2CO_3 x 10 H_2O as catalyzer (W/k, W-Et/k, Et/k). Subsequently, total phenols, anthocyans and flavonoids content and antioxidant capacity in term of ABTS and FRAP on wine lees extracts were determined using an UV-spectrometer. Sheep PBMC, stimulated with Concanavalin A and LPS, were cultivated for 24 h at 37°C with 5 % of CO_2 and treated with each wine lees extracts at two different concentrations (0.4 ng/mL and 0.8 ng/mL). The free cells supernatant was collected for ELISA interleukin (IL)-6, IL-1β, IL-10 and IFN-γ analysis; on cells, Bromodeoxyuridine proliferation assay was performed. Data were analyzed using ANOVA for mixed models using the MIXED procedure of SAS. PBMC treated with wine lees extracts registered a marked reduction of cells proliferation compared to stimulated cells ($P<0.001$). The levels of IL-10 and IFN-γ were affected by wine lees extracts ($P<0.001$). In particular, the ReW at 0.8 ng/mL led to higher production of both cytokines, probably due to the higher scavenging capacity as demonstrated by ABTS. The proinflammatory cytokines IL-6 and IL-1β were affected by wine lees extracts ($P<0.001$, and $P<0.01$, respectively). Even if any sharp variation among extracts was recorded, the ReW/k at 0.8 ng/mL exerted an increment of IL-6 compared to not stimulated cells. Results from the present experiment demonstrated the ability of wine lee by-products to affect the immune responses of sheep PBMC.

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CIRCULATING REGULATORY T CELLS (TREG) IN OBESE LABRADOR RETRIEVER DOGS: PRELIMINARY RESULTS

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Obesity is the most common nutritional disorder in dogs. This feature has been described as more evident in Labrador Retriever, in which the association between a deletion in the canine pro-opiomelanocortin (POMC) gene and increased body weight has been reported (1,2). Obesity and over-nutrition have been associated with impaired immunity and low-grade inflammation in human and mouse models (3-5). Moreover, a causal role for impaired Treg-dependent mechanisms in the pathogenesis of inflammation and insulin resistance, associated with increased body weight, has been referred in men and mice (6). A reduced number of Treg has been described in visceral adipose tissue and in blood of obese humans (6). However, it is not defined whether canine obesity may affect circulating Treg and whether their number might be associated with systemic inflammation. The aim of this study is to investigate whether in adult obese Labrador Retriever may be present a correlation between Treg and the pro-inflammatory activity. Twenty dogs were enrolled in the study and categorized into two groups based on body condition score (BCS): a control group (CTR: BCS 4–6) and an overweight/obese group (OB: BCS ≥7). Both CTR and OB dogs were considered clinically healthy, basing on review of the medical record at the time of sample collection, evaluation of complete blood count (CBC) and serum biochemistry. No POMC gene evaluation was performed in the enrolled animals. The blood levels of CD3+CD4+, CD3+CD8+ T cells, CD4/CD8 ratio, CD21+ B cells were analyzed. Significant reduction of CD4/CD8 ratio was observed in OB as compared with CTR group ($p<0.05$). Treg number and IFN-γ production, were analyzed by immune-fluorescence and flow cytometry. Intriguingly, preliminary results showed significant reduction of Treg (CD4+CD25+Foxp3+) in the OB ($p<0.05$) and a slight increase of IFN-γ production when comparing OB to CTR dogs. These results may represent new insights into the immunological dysregulation frequently associated to obesity in humans and still undefined in dogs

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CIRCULATING MICRO RNAS AS NOVEL BIOMARKERS OF TRAINING ADAPTATION AND STRESS IN ENDURANCE HORSES

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Endurance exercise induces metabolic adaptation involving musculoskeletal, cardiovascular, respiratory, endocrine, immune systems where muscle remodelling, mitochondrial synthesis and angiogenesis occur. Although these changes have been widely investigated, cellular and molecular mechanisms mediating this adaptation are still not completely elucidated [1-2-3]. Physical exercise has been recently associated to the modulation of a peculiar class of small noncoding RNAs (18–22 nucleotides), micro RNAs (miRNA), that act as post-transcriptionally regulators of gene expression. Released also in the body fluids, therefore named circulating miRNAs (ci-miRNAs), they have been recognized as optimal and accurate biomarkers in respect to classical serum/plasma biomarker [4] and highly stability with high resistance to variations in temperature, pH value and multiple thaw and freezing cycles making samples storage and handling an easier task. The aim of this study was to capture the whole picture of plasma circulating miRNAs through massive parallel sequencing in response to prolonged endurance exercise in samples obtained by four (4) trained and performing Arabian horses. Plasma ci-miRNAs were analyzed before (T0) and two hours after the end of competition (T1), when the majority of the significant changes in ci-miRNAs occur. NGS libraries were built from plasma derived RNA and sequenced producing 50 nucleotide Single-End reads. After cleaning procedures, reads were aligned to the reference genome (equcab 2.0). Differential gene expression analysis, assessed with a count based approach using edgeR package, was applied comparing T1 versus T0 samples. Protein-Protein Interaction (PPI) network and significant enriched pathways of target genes were explored with Cytoscape 3.4.0 suite creating cluster of related targets from which Gene Ontology (GO) enrichment was calculated. Our results reveal the modulation of large set of miRNAs (up regulation of miR-1, 133, 206, 208b, 499-5p, down regulation of miR-486) arising from tissues involved in exercise response such as muscle, heart, liver, and blood and activation of correlated processes like inflammatory response, immunity, angiogenesis and cell proliferation. Ci-miRNAs high throughput sequencing is a promising approach for sport medicine itself beside the value of this specific work in horse athletes. Discovery of putative biomarkers for prediction of disease risks related to prolonged activity (i.e. overtraining syndrome) and metabolic adaptations monitoring to ultimately establish efficient training programs, could be transferred to all “sport species”, including humans.

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CHARACTERIZATION OF CIRCULATING MIRNA SIGNATURE IN WATER BUFFALOES DURING BRUCELLOSIS AND EVALUATION AS POTENTIAL BIOMARKERS IN EARLY AND NON-INVASIVE DIAGNOSIS IN VAGINAL SECRETION

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Brucellosis is one of the most important zoonotic disease in ruminants, caused by *Brucella* species, a Gram negative, facultative, intracellular pathogens. Water buffaloes (*Bubalus bubalis*) are susceptible to both species of *Brucella*, namely *B. abortus* and *B. melitensis*. Both can be transmitted to humans mainly by raw milk consumption, as well as by means of direct contact with infected animals [1]. Serological tests are used for the initial diagnosis of brucellosis, but the results can be negative, especially during the early phases of the disease [2]. A throughout understanding of Brucella biology and the identification of novel biomarkers is essential for diagnosis and prophylaxis protocols. The role of microRNA (miRNA) has been recently highlighted in pathogen-host interactions [3]. At present, their role in brucellosis is virtually unknown. The present study aimed to a) delineate miRNA expression in blood serum of buffaloes; b) evaluate the miRNA expression in vaginal secretion; c) determine whether miRNA can be used as biomarkers to assess brucellosis; d) integrate miRNA to their target genes and to biological processes.

Blood and vaginal secretion samples were collected from 25 *Brucella*-positive (positive to both Rose Bengal and complement fixation test*)* and 20 clinically healthy *Brucella*-negative animals collected from *Brucella*-free farms*.* Buffaloes were multiparous, pregnant and in different lactation phases, including dry period. The profiles of blood circulating miRNA were characterized by NGS and the sequences were mapped against bovine miRNA available in mirBASE. Differentially expressed (DE-)miRNAs were validated by qPCR using TaqMan® probes on both blood serum and vaginal secretion samples. Predicted targets of the significant DE-miRNAs were computationally retrieved from the TargetScan database (http://www.targetscan.org/vert_71/), DAVID (https://david.ncifcrf.gov/) and KEGG (http://www.genome.jp/kegg/) bioinformatics resources. Differentially analysis revealed that 11 known miRNA exhibited significant alterations in expressions, among which 6 were up-regulated, namely miR-let7f, miR-let7i, miR-126-5p, miR-92a, miR-92b, miR215, and 5 were down-regulated, namely miR-30e-5p, miR-320a, miR-339b, miR-127, miR-133a. Five of these miRNAs were selected for further qPCR validation both in blood serum and in vaginal secretion. The comparative analysis in blood serum demonstrated that the level of miR-133a (p=0.013) was significantly lower in *Brucella*-positive compared to negative animals. In vaginal secretion the level of miR-92a, miR-126-5p, miR-320a, miR-let7i and miR-let7f were significantly higher in *Brucella*-positive compared to negative animals. The Areas Under the Curve (AUCs) were good for miR-let7f (0.880) and let7i (0.800) and fair for miR-320a (0.727) and miR-92a (0.760). Computational target prediction and functional genes enrichment identified common biological pathways between different miRNAs, among which metabolic, PI3K-Akt and MAPK pathways and cytokine-cytokine receptor interaction are at the top.

In conclusion, our study investigated, for the first time, the expressions of circulating miRNAs during brucellosis in water buffaloes. Receiver Operating Curve (ROC) analysis suggested that miR-let7f and miR-let7i may be considered promising biomarkers for identification of *Brucella*-positive animals starting from vaginal secretion, paving the way for an early and non-invasive diagnostic procedure.

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A FLOW CYTOMETRY-BASED SYSTEM FOR DETECTION OF BACTERIAL CONTAMINATIONS IN CELL CULTURES

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The control of cell cultures for microbial contaminations is mandatory to set up reliable immunological assays and vaccine production procedures. Microbial contaminations derive from multiple sources and include bacteria, fungi (moulds and yeasts), mycoplasma and viruses. Bacterial contaminations are detected by visual inspection of cell cultures and/or bacteriological procedures. The sensitivity of these is often less than optimal, which may give rise to despicable delays in the detection of contaminated cultures and serious downstream losses. In particular, there is evidence that bacteriological media cannot provide suitable growth conditions for some slow-growing bacterial species and intracellular phases of bacteria such as staphylococci [1]. Moreover, several bacterial pathogens undergo mutations in their environment in order to survive and establish an infection. Many stressors are known to affect their size, growth, division and metabolism [2] and novel control procedures are badly needed. Flow cytometry has been used for a long time to detect bacteria [3], yeasts and fungi [4]. On the basis of these findings, we developed a flow cytometry-based detection procedure of bacterial contaminations of cultured cells and tissue culture media. The protocol is based on two dyes binding to nucleic acids of viable and dead bacterial cells, respectively. Our results showed that it is possible to discriminate the scatter and fluorescence profiles of bacteria from those of nucleoprotein particles released from necrotic and apoptotic cells (non-specific staining). Therefore, a bacterial contamination gate was defined on the basis of both forward scatter and fluorescence, and a threshold number of events in the gate was reckoned following examinations of several uncontaminated cell cultures of different origin, type (fibroblast, epithelial, mesenchymal) and species. Our procedure was shown to detect experimental bacterial contaminations within 4-5 hours of the inoculation. Most important, contaminated cell cultures of our diagnostic laboratory were revealed even before a positive bacteriological test. Each experimental contamination of cell cultures was carried out using one bacterial species at a time. *Mycoplasma* spp. was not investigated. Owing to the above, this novel and rapid test procedure has a considerable potential for routine applications and is conducive to more reliable, robust sterility controls of cell cultures and also immunological products like vaccines.

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MONENSIN AFFECTS THE INNATE IMMUNE RESPONSE IN THE RUMEN

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Monensin is a ionophor with a selecting capacity against Gram- rumen bacteria. In the overall, it increases the production of the rumen propionic acid and fosters liver gluconeogenesis. Previously, we observed that epithelial cells of the bovine forestomachs can recognize and respond differently to changes of rumen milieu and that cows show distinct tendencies to leukocyte infiltration into rumen fluids, in accordance to specific diets and dangerous fermentations (1). Considering the positive metabolic effects Monensin offered as rumen bolus during the transition period, we investigated its effects on rumen fermentations and innate immune responses of the forestomachs. On the whole, 43 cows (13 heifers and 30 multiparous) were allocated to two homogeneous groups: Kexx, which received 32.4 g of monensin by Controlled Release Capsule (Kexxtone®, Elanco Animal Health, UK) 21 days before expected calving, and Ctrl (control). Cows were frequently monitored during the transition period for health status, milk yield and quality, inflammo-metabolic profile (2). Moreover, individual rumen samples were collected with an oro-gastric probe at 30 days in milk (DIM), 6 hours after feed distribution. pH was measured immediately after withdrawal and rumen samples were collected with or without a cryoprotectant (fetal bovine serum and dimethyl sulfoxide). Samples were frozen at –80°C for flow cytometry and molecular assays (CD45= total leukocytes; IL-A24=granulocytes and monocytes; CD3=T lymphocytes; sIgM=B lymphocytes; IGCL=Immunoglobulin light chain) and at -20°C for volatile fatty acids (VFA) and ammonia. Rumen data were analyzed with ANOVA (SAS Inst. Inc., Cary, NC), considering the fixed effect of treatment and parity. As opposed to parity (not significant), treatment led to significant differences. Kexx cows at 30 DIM showed a similar pH (around 6.45), and lower concentration of ammonia (58.7 vs 93.3 mg/L of Ctrl; $P<0.05$), a higher concentration of propionic acid (24.5 vs 22.4 mol/100 mol of Ctrl; $P<0.10$, tendency) and a reduced acetic:propionic ratio (2.5 vs 2.8 of Ctrl; $P<0.10$, tendency). Moreover, Kexx vs Ctrl cows showed a lower number of B lymphocytes (1.66 vs 2.50%; $P<0.01$) and numerically lower amounts of immunoglobulins (IgM and total Ig) and T lymphocytes. The reduced prevalence of T lymphocytes was more marked in heifers receiving monensin ($P<0.10$, tendency, vs Ctrl). On the whole, the activity of the innate immune system was more pronounced in the rumen of Ctrl cows, suggesting that monensin could have contributed to stabilize the rumen milieu and to attenuate the inflammatory responses that commonly occur in forestomachs around calving. These data were supported by clinical inspections (the significant lower incidences of diseases observed in the first two months of lactation) and metabolic data (lower NEFA and BOHB concentrations after calving) observed in the same Kexx cows (2). Our data confirm that nutritional and physiological changes in dairy cows can modify the innate immune response of forestomachs and that the evaluation of the rumen fluid can help evaluate animal health and welfare.

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Scienze Cliniche - CLINICA MEDICA

INTRAVASCULAR HEMOLYSIS ASSOCIATED WITH *Candidatus Mycoplasma hematoparvum* IN A SPLENECTOMIZED DOG IN ITALY

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Mycoplasma haemocanis (Mhc) and *Candidatus* Mycoplasma haematoparvum (CMhp) are hemotropic parasites that infect dogs. Transmission is related to *Rhipicephalus sanguineus* whose main geographical distribution is associated to temperate climates. Illness occurs mainly in immunocompromised and splenectomized dogs, or in non-splenectomized dogs co-infected with other vector diseases. Symptoms range from asymptomatic infection to acute hemolytic anemia accompanied by anorexia, lethargy, dehydration, weight loss and death. The aim of this report is to describe a case of CMhp in a splenectomised dog. A 5-year-old female Beagle dog was presented for anorexia and depression 2 months following splenectomy. Physical examination revealed pale mucous membranes, tachycardia, tachypnea, depression and massive fleas infestation. Abnormalities noted in the complete blood count included regenerative anemia with a marked reticulocytosis. Biochemical profile showed increased renal parameters and serological tests were negative. The dog was treated with fresh whole blood, isotonic fluid therapy, Imidacloprid and Permetrine. Assuming that anemia was caused by severe fleas infestation the dog has been discharged but five days later the animal was presented again with the same symptoms. Second blood transfusion was administered and blood smear examination revealed the presence of 0.5–3 µm basophilic, round, rod, or ring-shaped structures present on erythrocytes individually or in chains. Coomb's test was positive. The partial 16S rDNA and ribonuclease P RNA (RNase P) genes were amplified by *Mycoplasma* spp. specific PCRs. Both sequences were closely related to *Candidatus* Mycoplasma haematoparvum (99% identity with 100% coverage). The subject was treated with 10 mg/kg doxycycline every 24 hours given for 7 months and prednisolone at the tapering dose of 2 mg/kg day. PCR repeated after 30-60-120-200 days was positive while blood smear examination negative . After 200 days the dog showed slight anemia and ciprofloxacine was added (15 mg/kg every 12 hours) for 15 days and anemia solved. After therapy, PCR was still positive so it was decided to discontinue therapy and monitor the dog monthly. This report is the first describing CMhp disease in Italy and provides opportunity for future researches aimed to evaluate the prevalence of haemoplasmas in Sardinian dogs. *Mycoplasma* spp should be considered in differential diagnoses in case of hemolytic anemia in dogs, and PCR analysis for hemoplasma DNA should be considered the gold standard for diagnosis. Therapy needs further investigations, because of the scarce success to eliminate the parasite with standard drugs.

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VALIDATION OF A HUMAN IMMUNOTURBIDIMETRIC ASSAY FOR THE MEASUREMENT OF GLYCATED HEMOGLOBIN (HBA1C) IN DOGS AND COMPARISON OF HBA1C VS SERUM FRUCTOSAMINE FOR ASSESSMENT OF GLYCEMIC CONTROL IN DOGS WITH DIABETES MELLITUS

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Glycated hemoglobin (HbA1c) is used as "gold standard" to monitor long-term effectiveness of treatment in human diabetics. In contrast, measurement of serum fructosamine (SF) is used to assess glycemic control in diabetic dogs[1]. Only a few studies have evaluated HbA1c as an option for therapeutic monitoring of diabetic dogs. The aims of the present study were the validation of a human turbidimetric inhibition immunoassay for HbA1c quantification in dogs and the comparison of blood HbA1c vs SF concentrations for assessment of glycemic control in dogs with diabetes mellitus. Forty healthy dogs and 23 newly diagnosed diabetic dogs treated with insulin BID and re-evaluated for 86 times (in total) were included. All re-evaluations were performed within the first 3 months of insulin treatment. SF (nitrotetrazole blue method, Fructosamine 17350H, Sentinel Diagnostics) and blood HbA1c (HbA1c, OSR6192 Beckman-Coulter) were measured on an automated chemistry analyzer (Beckman-Coulter AU480). To validate the immunoturbidimetric assay for HbA1c the linearity through dilutions was tested; through repeated measurements in whole EDTA blood samples of healthy and diabetic dogs stored at three different temperatures (-80°C, +4°C, +23°C) intra and inter-assay variability (by means of coefficient of variation [CV] %) was evaluated. Using the percentile method on healthy dogs, the reference interval of HbA1c and SF was established. To evaluate the clinical performances of HbA1c and SF in assessing the control of glycemia, the correlation between the two parameters and a clinical score was studied. The clinical score used to classify diabetic dogs in good, moderate or poor glycemic control was set on the basis of body weight, presence of polyuria/polydipsia, median glucose of the blood glucose curves (BGC), blood glucose nadir of the BGC and overall evaluation of the BGC. Data were analyzed using parametric or non-parametric tests on the basis of their distribution. $P<0.05$ was considered significant.

For HbA1c average intra-assay CV was 1.5%, the average inter-assay CV was 10.9%, 15.9% and 19.9% for blood frozen, refrigerated or stored at room temperature, respectively. The assay was linear (r=0.9977). The reference intervals obtained from healthy dogs were 1.6-4.5% for HbA1c and 222-282 µmol for SF. In diabetic dogs HbA1c (median 6.22%, range 2.85-9.23) and SF (median 476 µmol/l, range 270-802) were significantly higher compared to healthy dogs. Both HbA1c and SF were significantly correlated with the clinical score. Even if not significantly, HbA1c identified well-controlled dogs more frequently than SF did (78% vs 61%), while SF identified more frequently dogs with poorly controlled diabetes compared to HbA1c (48% vs 31%). This study is a part of a previous trial and all diabetic dogs were newly diagnosed and were receiving insulin by a maximum of 90 days. This represents a limitation and it is possible that HbA1c was unable to detect poorly controlled diabetic dogs because the hyperglycaemic state in this population study was present only for a short period. In conclusion, the human turbidimetric inhibition immunoassay used in this study is accurate and precise for the measurement of HbA1c in dogs. HbA1c and SF showed similar (but moderate) performances in detecting the glycaemic control of diabetic dogs.

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PREVALENCE OF PROTEINURIA IN DOGS: A MULTICENTRIC STUDY

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Proteinuria, defined as the presence of excess proteins in the urine, is related to different physiological and pathological causes even though when it is persistent and associated with inactive urinary sediment, it is primarily due to kidney disease [1]. Early detection of proteinuria allows to identify several pathological conditions which occur with glomerular disease.

Aim of this study was to evaluate the prevalence of proteinuria in dogs from different veterinary clinics in Italy, and to study possible differences related to the age and geographical distribution.

The study was carried out in seven veterinary clinics, during a period of six weeks. Dogs were enrolled with no restriction of sex or age. Females in estrus as well as dogs with signs of genito-urinary diseases or those previously diagnosed with proteinuric nephropathy, were excluded.

Dipstick test was used to assess proteinuria; samples were collected by ultrasound-guided cystocentesis or by free catch [2][3]. Dogs were either considered "non proteinuric" in case of negative result or "suspected proteinuric", if urine dipstick gave positive results (any colorimetric changes) [4][5]. When possible, proteinuria was confirmed by UPC ratio.

A total of 708 dogs were evaluated: 381 (53.68%) resulted negative, while in 327 samples (46.2%) the urine dipstick showed a positive result. 157 positive urine samples underwent the UPC ratio and proteinuria was confirmed in 72.3% of cases.

Geographical distribution showed statistical differences based on origin of the patients: 32.5% of cases were detected in Turin, 35.2% in Palermo, 55.7% in Milan, 58.2% in Bari, 60% in Naples, 69.2 % in Genoa and 91.2% in Parma.

Proteinuria was assessed both in young/middle aged dogs and older ones, with no differences between dogs under 6 years (48.2%) and over (52.8%).

This study confirms the reliability of dipstick test in detection of proteinuria, also in apparently healthy dogs. Both in human and veterinary medicine, dipstick urinalysis has been used as screening test [5][6] with a high negative predictive value in non proteinuric patients. Results from our study showed a high percentage of proteinuric dogs with no signs of renal diseases or clinicopathological alterations referred to urinary pathologies, undergoing routine clinical examination.

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EMPHYSEMATOUS CYSTITIS: A REVIEW OF 36 DOGS AND 2 CATS

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Emphysematous cystitis (EC) is a rare form of complicated urinary tract infection, with characteristic features being gas within the bladder wall and lumen. The clinical manifestations are various and not always associated with symptoms of lower urinary tract. The literature indicates that it is often associated with diabetes mellitus or other predisposing pathologies [1]. Imaging methods, such the radiology or ultrasonography are pivotal for obtaining a definitive diagnosis of EC [2]. The aim of this study was to describe common traits of dogs and cats with EC and compare them with literature. We retrospectively evaluated subjects with diagnosis of emphysematous cystitis enrolled from 2010 to 2016. Only patients with full clinical records, blood test and urine screening were considered. For any subject, the symptoms, the pre-subsidiary cause and the etiological cause has been established. We evaluated with ultrasonography the stratigraphy of the bladder wall, the appearance of the contents (corpuscular or anechoic), and the localization of air (bladder wall, lumen or both). We found 38 patients (36 dogs and 2 cats), 17 males and 21 females, aged from 5 months to 14 years (mean age: 9 years). The clinical signs were highly variable. Gross hematuria and other symptoms of cystitis (dysuria, pollakiuria) are most common (23/38). Fever, anorexia, vomiting and abdominal pain occurring in 9 subjects, while 6/38 were asymptomatic. Diabetes afflicted only 15.7% of the patients and the most common predisposing factor was recurring urinary tract infection (42%), while 18.4% of patients showed urinary stasis from neurogenic bladdder dysfunction; 24% were other causes already reported in the bibliography. The urinalyses presented glicosuria in three dogs, including 2 non-diabetic. *Escherichia coli* (55%) and *Klebsiella pneumoniae* (13%) were the two major organism isolated in urine cultures. In a few cases we isolated *Proteus mirabilis*, *Enterococcus faecalis* and *Klesbiella Pneumoniae*. Three cultures were negative. The ultrasound showed wall irregularly thickened in 20 subjects with 6/38 with polypoid cystitis; widespread intramural echogenic foci exhibiting dirty acoustic shadowing and a reverberation artifact consistent with gas were seen in 18 patients. Intraluminal gas was seen in 10 patients; the intraluminal foci were mobile and shifted to a nondependent location with postural changes. Ten dogs presented gas both in the bladder wall and in the bladder lumen. Only 5 dogs had radiological study: 4/5 confirmed the EC. In our review there is no difference with veterinary literature for age, sex and clinical symptoms; the predisposing risk factors are different: the number of diabetic cases is considerably less than that other authors (50%). The results of urine cultures in EC patients with no gas producing organism, such as *Enterococcus*, suggest the possibility of mixed infections. With respect to blood analyses there are no specific data suggesting the presence of EC. The use of ultrasound is pivotal for obtaining a definitive diagnosis of EC; ultrasonography seems to be more sensitive than radiography at early stage of the disease and can detect the presence of air in other structures [2]; one dog showed gas in renal pelvis and one in the prostate gland.

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RETROSPECTIVE EVALUATION OF LABORATORY AND CLINICAL FEATURES IN DOGS WITH FATAL PULMONARY HEMORRHAGE DUE TO LEPTOSPIROSIS

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The aim of the study was to retrospectively evaluate clinical signs, CBC, coagulation profile and serum biochemical profile in dogs with leptospirosis, which developed fatal pulmonary hemorrhage. These findings were compared to those of dogs affected by leptospirosis, which did not develop
PH.16 dogs with acute leptospirosis (PCR and/or paired MAT) and referred for hemodialysis were divided into two groups (CG n=11; PH n=5), according to the development of pulmonary hemorrhage during hospitalization. At hospital admission values of CBC, PLT, serum creatinine, urea, total bilirubin, total protein, albumin, total calcium, phosphate, ALT, AST, ALKP, cholesterol, PT, aPTT, fibrinogen and ACT were recorded. Clinical signs of dyspnea, coughing, jaundice, melena, hematemesis, hematuria, hemoptysis and spontaneous mucous membrane bleeding were recorded. Data were tested for normality by Kolmogorov-Smirnov test. Mann-Whitney test was used to compare the laboratory parameters between CG and PH. The prevalence of different clinical signs in CG and PH was compared by Fischer's test. Results were considered statistically significant for $p<0.05$.
At hospital admission, no significant difference in CBC ($p=0.16$), PLT ($p=0.17$), serum creatinine ($p=0.82$), urea ($p=0.15$), total protein ($p=0.69$), albumin ($p=0.64$), total calcium ($p=0.43$), phosphate ($p=0.70$), cholesterol ($p=0.60$), PT ($p=0.19$), aPTT ($p=0.95$), fibrinogen ($p=0.62$) and ACT ($p=0.16$) was found between CG and PH. PH group showed significantly higher values of ALKP ($p=0.07$), AST ($p=0.06$), ALT ($p=0.04$) and bilirubin ($p=0.04$) compared with CG. According to clinical signs, in CG jaundice and melena were present in 7/11 dogs, and hemoptysis and coughing in 1/11. None of the patients of CG showed hematuria, or spontaneous mucous membrane bleeding. In PH group all patients were icteric and 4/5 dogs showed melena. Spontaneous mucous membrane bleeding was present in 2/5 dogs and hematuria in 1/5. None of the patients of PH showed hematemesis or hemoptysis. Dyspnea and hematemesis were absent in both CG and PH. No significant difference in the number of patients showing jaundice ($p=0.24$) or melena ($p=1.0$) was found between CG and PH.
In the dogs of the present study, pulmonary hemorrhage developed as a sudden and dramatic event within hours from hospitalization. In our cohort, clinical signs, coagulation profile and degree of azotemia did not seem to be predictive of the risk for pulmonary hemorrhage. Instead, severely elevated bilirubin and jaundice were constantly found in all patients of HP, and may represent predisposing factors of pulmonary hemorrhage.

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EVALUATION OF CALCIUM-PHOSPHORUS ABNORMALITIES AND FRACTIONAL EXCRETION OF ELECTROLYTES IN DOGS WITH CHRONIC KIDNEY DISEASE (CKD) AT DIFFERENT CKD STAGES

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Chronic kidney disease (CKD) is associated with Calcium (Ca) and Phosphorus (P) metabolism abnormalities such as hyperphosphatemia, hypocalcaemia as documented by reduction of ionized and total Calcium (iCa and tCa, respectively), hypercalcemia and renal secondary hyperparathyroidism (RHPT) that could have a negative effect on kidney function and disease progression. The prevalence of these conditions in CKD dogs is variably documented. In a previous study, high P, decreased tCa and iCa, and RHPT had a frequency of 68.5%, 7.4%, 33.3% and 75.9%, respectively [1]. The aim of the present study was to evaluate clinicopathological variables associated with Ca-P metabolism in CKD dogs focusing on iCa and fractional excretion (FE) of electrolytes. Over a 1-year period, client-owned dogs with naturally occurring CKD were prospectively included and staged following IRIS guidelines [2]. CKD diagnosis was based on history, clinical signs, laboratory and imaging results. CBC, chemistry and blood gas analysis comprehensive of tCa, iCa, P, and intact parathormone (iPTH), urine analysis and chemistry including FE of calcium (FECa) and P (FEP), were evaluated upon admission. CKD dogs were compared to healthy controls (n=67) and dogs affected by acute kidney injury (AKI) (n=113), enrolled in a previous study. Results, presented as median and (range), were compared using non-parametric statistics (significant difference for $p<0.05$). Forty-one CKD dogs, with serum creatinine concentration of 2.64 mg/dL (0.61-14.21), were included and classified in stage 1 (8/41), stage 2 (10/41), stage 3 (15/41), stage 4 (8/41). In CKD dogs, P concentration (mg/dL) and FEP (%) were 5.0 (3.0-15.4) and 24 (0-55), respectively, and increased significantly ($p<0.01$) in stage 3 (P 4.9, 3.5-9.9; FEP 30, 13-55) and 4 (P 9.7, 6.5-15.4; FEP 35, 16-44), if compared to controls (P 4.0, 2.6-5.7; FEP 13, 2-29). No significant differences in FEP were detected between CKD and AKI dogs. Serum tCa concentration (mg/dL) was significantly ($p<0.001$) increased in CKD dogs (10.6, 7.8-13.3) if compared to AKI (9.7, 3.8-12.2) and controls (10.1, 9.0-10.9). Blood iCa (mmol/L) was significantly ($p<0.001$) increased in CKD (1.31, 0.85-1.47) if compared to AKI (1.13, 0.49-1.41), however not significantly different if compared to controls (1.30, 1.07-1.39). FECa (%) increased significantly ($p<0.001$) among the study groups: controls (0.12, 0.05-0.60), CKD (0.4, 0.04-8.20) and AKI (1.0, 0.1-70.0). Serum iPTH concentration (pg/mL) was significantly increased ($p<0.001$) in stage 3 (3.0, 3.0-22.7) and 4 (74.2, 42.7-293.0) if compared to stage 1, stage 2, and controls, in which iPTH was undetectable. In conclusion FEP and FECa could be considered a cost-effective marker of mineral imbalance in the course of CKD. Furthermore, FECa could aid clinicians to discriminate between CKD and AKI, if confirmed in larger studies.

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DIAGNOSTIC AND PROGNOSTIC USEFULNESS OF FRACTIONAL EXCRETION OF ELECTROLYTES IN DOGS WITH SPONTANEOUS ACUTE KIDNEY INJURY (AKI)

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Acute kidney injury (AKI) is a common clinical syndrome associated with reduction of renal function and high mortality in human and veterinary patients [1,2]. AKI diagnosis is still based on clinical and laboratory findings; however, urine electrolyte excretion has been revaluated in AKI patients with promising results [2]. Aim of this study was to investigate the diagnostic and prognostic role of fractional excretion (FE) of electrolytes in dogs with AKI. Canine patients presented to the author's Veterinary Teaching Hospital (February 2014-December 2016) with naturally occurring AKI were prospectively included. AKI diagnosis was based on history, physical exam, and laboratory and imaging findings. Laboratory variables including CBC, serum chemistry, urinalysis, FE of sodium (FENa), potassium (FEK), calcium (FECa), phosphate (FEP), magnesium (FEMg) and urine creatinine to serum creatinine ratio (uCr/sCR) were evaluated upon admission. Dogs were classified as volume responsive-AKI (VR-AKI) and intrinsic-AKI (I-AKI), according to IRIS guidelines [3], and as survivors (S, alive to discharge) and non-survivors (NS, spontaneously died or humanely euthanized), and compared with controls (n=81). Data were reported as median and (min-max), and results were compared using non-parametric statistics or Chi-squared test. P values <.05 were considered significant. A total of 135 dogs fulfilled the inclusion criteria; 52/135 (39%) dogs were classified as VR-AKI, while 69/135 (51%) as I-AKI; 14/135 (10%) dogs were unclassifiable. S and NS were 80/135 (59%) and 55/135 (41%), respectively. Significantly higher frequency of death was detected in I-AKI if compared with VR-AKI dogs (52.2% vs 17.3%; p<.001). If compared to VR-AKI, I-AKI dogs had significantly increased FENa (2.36%, 0.04-75.8 vs 0.24%, 0.01-2.21; p<.001), FECa (4.1%, 0.1-70.0 vs 0.3%, 0.1-3.7; p<.001), FEK (74.9%, 5.3-399.7 vs 23.3%, 1.6-74.1; p<.001), FEMg (10.2%, 0.9-80.9 vs 2.9%, 0.3-13.8; p<.001) and decreased uCr/sCr (14, 1-218 vs 78,10-546; p<.001). Increased FEs were also detected in NS: FeNa (NS: 1.60%; 0.03-75.81 vs S: 0.60%; 0.01-50.45; p=.004), FeCa (NS: 4.0%; 0.1-70.0 vs S: 0.6%; 0.1-56.1; p<.001), FeK (NS: 59.9%; 5.3-399.7 vs S: 34.3%; 1.6-215.9; p<.001), FeMg (NS: 12.0%; 0.3-131.8 vs S: 4.8%; 0.3-70.6; p<.001); and were associated with decreased uCr/sCr (NS: 17.4; 1.2-377.2 vs S: 45.5; 1.9-545.9; p=.006). In conclusion, FE of electrolytes and uCr/sCr may be able to discriminate between VR-AKI and I-AKI, and to predict prognosis in a large population of AKI dogs. Since FE measurement is feasible on a routine basis, its evaluation may be suggested in the daily practice.

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MULTIORGAN DYSFUNCTION IN FELINE SEPSIS: PRELIMINARY STUDY ON 37 CATS

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Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Feline sepsis is associated with substantial morbidity and mortality, however is scarcely documented in veterinary literature. The aim of this prospective observational study was to evaluate the clinical response and the presence of multiorgan dysfunction syndrome (MODS) in relation to outcome in feline sepsis. Cats admitted to the Veterinary University Hospital of Bologna (October 2015 - February 2017) with a diagnosis of sepsis defined by the presence of systemic inflammation associated with cytological or microbiological evidence of infection were included [1]. History, comorbidities, clinical and clinicopathological data including the Feline Acute Patient and Laboratory Evaluation (APPLE) score and serum amyloid A (SAA), origin of infection, treatments and exitus were recorded. Major systems dysfunctions (respiratory, cardiovascular, renal, hepatic and hemostatic) were reported at the time of admission and during hospitalization. Non-parametric statistics with post hoc analysis were used to compare variables between the different groups; $P<0.05$ was considered significant. Thirty-seven cats were included in the study: 20 males (14/20 castrated), 17 females (9/17 spayed). Median age was 6 years (0.2-15). Origin of sepsis was categorized as: thoracic (13/37, 34%), abdominal (8/37, 22%), feline panleukopenia (8/37, 22%) and miscellanea (8/37, 22%). Comorbidities were reported in 26/37 (70%) cats. Clinical presentation was characterized by depressed mental status, hypothermia and hypotension associated with hypovolemic and/or distributive shock in the majority of the subjects. Upon admission, 26/37 (76%) cats had MODS (≥2 organs involved) with an increment up to 86% (32/37) during hospitalization. Mortality rate in the study population was 38% (14/37). Non survivors had significantly lower body temperature, systolic blood pressure, white blood cells count and higher APPLE score and coagulation times at the time of hospital admission, compared with survivors. Frequencies of death were significantly higher in cats with septic shock, acute kidney injury and MODS. By univariate logistic regression analysis, variables independently associated with a poor outcome were: APPLE score, body temperature, septic shock, acute kidney injury and the number of affected organs upon admission. The latter was the only variable retained in the multivariate analysis. No association with outcome was reported for SAA and the presence of comorbidities. The present study contributes to describe the clinical features of sepsis in cats, which is mainly characterized by signs of hypodynamic shock. Septic shock and acute kidney injury are critical sequelae of feline sepsis with prognostic implications. MODS is a common complication of feline sepsis, and seems to significantly increase the odd of death, as reported in dogs [2]. Further studies in a wider population are needed to better characterize MODS in feline sepsis.

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A CORRELATION OF CIBDAI, ENDOSCOPIC AND HISTOPATHOLOGIC EVALUATION IN DOGS WITH INFLAMMATORY BOWEL DISEASE (IBD): PRELIMINARY STUDY

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IBD is a chronic gastrointestinal disease. The pathogenesis seems to be caused by genetic, immunological and environmental factors. To diagnose and to evaluate the severity of IBD, clinical examination, laboratory and instrumental exams are performed; among these, a central role is attributed to Canine IBD activity index (CIBDAI), a method of clinical assessment in patients with IBD. Nevertheless, definitive diagnosis is based on histophatologic evidence of mucosal inflammation, that is critical to choose the proper management and the pharmacological and dietary therapy of the patients.[1] The aim of this study was to investigate correlations between CIBDAI, endoscopic and histopathologic evaluation. In this study 53 dogs with signs of chronic GI disease were reported at the service of flexible endoscopy of Veterinary Teaching Hospital of Perugia University. IBD in these patients was diagnosed through clinical examination, endoscopy and histophatology. Clinical disease activity was assessed by CIBDAI in the following way: 6 gastrointestinal signs scored 0–3 by magnitude: attitude/activity, appetite, vomiting, stool consistency, stool frequency, weight loss. These score were then summed, yielding a total cumulative CIBDAI score (0-3=clinically not significant, 4-5=mild, 6-8=moderate, >9=severe).[1] Performing the endoscopy according to Guilford (2005),[2] macroscopic gastrointestinal lesions considerate were: friability, granularity, erosion, lymphatic dilatation (only in duodenum). These modifications were scored 0–2 by severity. These scores summed provided total cumulative score. The maximum score was 6 in stomach and colon (1-2=mild, 3-4=moderate, 5-6=severe), 8 in duodenum (1-3=mild, 4-6= moderate, 6-8=severe).[1] Multiple mucosal endoscopic samples (5-7) were obtained from stomach, duodenum or colon based on clinical signs. Pathological grading of IBD severity was performed (mild, moderate, severe) according to the classification system proposed by the WSAVA GI standardization group.[1] The correlation between endoscopic and histopathologic data was statistically estimated. The result depends on the gastrointestinal tract considered: the highest correlation level was in colon (66%) but it was not statistically relevant due to low number of cases (6). Duodenum presented a correlation level higher compared with stomach (51% vs 39%) despite the similar number of cases (42 vs 41). This difference could be due to the degree of inflammatory infiltrate. WSAVA GI standardization group estimate the normal GI infiltrate in healthy animals. There is a critical difference between the quality/quantity of infiltrate in duodenum and stomach and this could be more evident in a given IBD patients.[1] Duodenal mucosa, thanks to the presence of villi, have more fragile and “easily to damage” look and this could be the reason of the more evident macroscopical alteration than the gastric one. The statistical inference for endoscopic and CIBDAI scores was studied in linear regression, showing essentially a very low degree of correlation. In conclusion, CIBDAI does not allow diagnose and follow-up of IBD. CIBDAI endoscopic and histopathologic evaluations are not appreciably correlated, therefore all the 3 parameters are needed for a correct diagnosis, prognosis and therapy management of IBD.

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ERYTHROCYTE AND LEUKOCYTE MODIFICATIONS IN CANINE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

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Systemic Inflammatory Response Syndrome (SIRS) is the manifestation of the systemic response to an infectious or non-infectious disease with a massive release of inflammatory mediators. Criteria for SIRS diagnosis in dogs have been previously reported [1]. The aim of this study was to evaluate the association between hematological modifications in canine SIRS and the severity of patient's condition and outcome.
This retrospective study included 3 groups of dogs: 90 with SIRS, 50 healthy, and 50 with chronic disease admitted to the Veterinary Teaching Hospital. A SIRS grading was obtained based on how many criteria were fulfilled. Another clinical score (APPLE FAST)[2] was applied on 50 of 90 dogs of the SIRS group. Survival rate has been assessed after T7 and T15 days from admission. Dogs with a positive cytology or culture were recorded as septic. Dogs hospitalized due to hemolytic or hemorrhagic disorders were excluded. Presence of anemia and nRBCs (nucleated RBC), neutrophil/lymphocyte ratio (NLR), SIRS grading and APPLE FAST were compared to outcome (t-test, chi-square test) and between groups (ANOVA test).
The following dogs with SIRS were sub-grouped using the SIRS score: 47.8% in 2/4, 31.1% in 3/4, and 21.1% in 4/4. Fifty-one dogs (56.5%) died within T15. SIRS group was divided into septic (n=32; 35.5%) and non-septic dogs (n=58; 64.4%). APPLE FAST scores > 25 ($p=0.03$) and SIRS grading >2 ($p=0.001$) were associated with poor outcome. No statistically significant differences were found between hematological alterations and SIRS grading or APPLE FAST score. In the SIRS group, anemia was present in 51 dogs (56.6%) and it was not associated with outcome. Mild (55%), microcytic (55%), and normochromic (92%) anemia was the most represented. Among 51 anemic dogs in the SIRS group, 84% showed non-regenerative anemia, based on the reticulocyte count. Dogs with SIRS showed lower values of RBC, HCT and HGB compared to the other two groups ($p<0.0001$). Twenty-two over 90 dogs (24.4%) showed circulating nRBCs. The nRBC count was significantly higher in SIRS group compared to healthy dogs ($p=0.0007$). In SIRS group, the occurrence of circulating nRBCs was associated with poor outcome ($p=0.005$). NLR was significantly higher in SIRS group compared to control groups ($p=0.0001$) and not associated with outcome. NLR was significantly lower in septic dogs ($p=0.02$).
Dogs with SIRS showed a significant reduction of RBC, HCT and HGB compared to healthy and chronic dogs. A mild non-regenerative anemia was the most frequent type of anemia. This type of anemia is typical of chronic disease, but in SIRS might be a common feature due to concurrent or acute decompensation of a chronic state. To our knowledge, this is the first study showing that dogs with SIRS had a higher NLR, suggesting its usefulness as acute inflammatory marker. APPLE FAST score and SIRS grading may help the clinician as prognostic tools for critically ill dogs. During SIRS, presence of nRBCs in peripheral blood could occur due to the damage of the blood-bone marrow barrier with the release of immature erythroid cells and could be considered a negative prognostic factor for canine SIRS patients.

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PROGNOSTIC FACTORS IN DOGS WITH ACUTE LEPTOSPIROSIS: A PROSPECTIVE STUDY

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Canine leptospirosis is a worldwide zoonotic disease frequently associated with acute kidney injury (AKI), multiple organ damage (MOD) and death [1]. Aims of this study were to asses clinical and clinicopathological features of acute leptospirosis in dogs, and to identify variables with potential prognostic significance. Additionally, the diagnostic accuracy for leptospirosis was compared between a point-of-care test and the microagglutination test (MAT). Dogs hospitalized at a veterinary teaching hospital (January 2014-December 2015) were prospectively selected on the basis of a clinical suspicion of leptospirosis according to history, clinical, laboratory and imaging findings, including evidence of AKI, systemic inflammation and MOD. Leptospiral infection was diagnosed using at least one of the following criteria: a)positive MAT titre ≥800 on serum samples upon admission; b)fourfold increase in MAT titre in paired sera; c)positive qPCR in blood and urine samples. A clinicopathological panel including urine fractional excretion (FE) of electrolytes, and a blood canine leptospira test kit (Witness lepto, ZOETIS) were performed in all dogs. AKI grade and oligo/anuria (urine output <1ml/kg/h over 6h) were classified according to the IRIS guidelines [2]. All dogs were treated with aetiological therapy and supportive care. Dogs were further classified in non-survivors (NS, spontaneously died or humanely euthanized) or survivors (S, alive to discharge). Data were reported as median and min-max. Non-parametric statistics, chi-squared test and logistic regression were used ($P<0.05$ considered significant). Twenty five dogs were included in the study. The median age was 6 years (0.4-11); 15/25 were intact male and 10/25 female (4 spayed); median body weight was 18.3 kg (4.1-49.9). The mortality rate was 52% (13/25). A variable frequency of renal (96%), hepatic (68%), muscular (64%), pulmonary (40%) and haemostatic (32%) involvement was documented, with MOD (>2 organs involved) being present in the 88% of the enrolled dogs. Higher frequency of death was significantly associated with AKI grade severity, presence of oligo/anuria, jaundice and pulmonary involvement. If compared with S, NS had significantly higher FE (%) of sodium (8.2, 1.5-64.3 VS 1.9, 0.2-5.5 ; P=0.02), potassium (163, 73-373 VS 78.9, 24.2-215.9; P=0.01), calcium (6.5, 2.2-70.0 VS 3.4, 0.6-4.9; P=0.03), and magnesium (19.6, 8.4-77.5 VS 7.6, 3.1-28.0; P=0.01). The point-of-care test sensitivity (Se) and specificity (Sp) were, 81.8% and 85.7%, respectively. Se and Sp were not significantly different compared with the MAT performed upon admission. In conclusion, renal dysfunction characterized by a severe urine electrolyte loss had prognostic value in a population of dogs affected by leptospirosis. A rapid test detecting leptospira antibodies in canine blood may be of value in the daily practice, if confirmed in larger studies.

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RENAL EFFECTS ASSOCIATED WITH TETRASTARCH (HYDROXYETHYL STARCH 130/0.4) CONSTANT RATE INFUSION IN DOG

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HES solutions are the most frequently used synthetic colloid in human and veterinary medicine, intravenously administered to provide volume expansion and oncotic support. The majority of research in human medicine has focused on the adverse effects of HES infusion, with emphasis on acute kidney injury (AKI) and coagulation imbalance. The extrapolation of data from human studies should be done aware that there could be species variations and different pharmacokinetics related to different HES solutions. In veterinary medicine the studies are limited, and often differ for the type, amount, interval, and concentration of HES administered. It is also unknown whether there are important differences in the adverse effects of HES solutions when they are administered as constant rate infusion (CRI) rather than as a rapid bolus. For intravascular volume maintenance and long-term colloid osmotic pressure support synthetic colloids are administered as CRI, typically at a rate up to 2 mL/kg/h. These rates correspond to the daily maximal doses in the 20–30 mL/kg/d range, and are largely extrapolated from human literature. The aim of this study was to evaluate the association of the development of acute kidney injury (AKI) when a HES 130/0.5 is used as a CRI (2 mL/kg/h) in a population of canine intensive care unit (ICU) patients. Hypoalbuminemic animals (albumin <2gr/dl), with normal perfusion parameters, requiring intravenous fluid therapy were included. An intravenous catheter was placed into peripheral vein and fluid therapy was set using Lactated Ringer solution (taking into account dehydration, maintenance and on going losses) and HES 130/0.4 (in order to support Colloid Osmotic Pressure, COP). A total of seven dogs were included, and Hydroxyethyl starch 130/0.4 was administered as CRI (2 ml/kg/h) for at least 24 hours. After 12 hours from the start of the infusion and 24 and 48 hours later, samples of blood and urine were collected; serum biochemistry profile (albumin, total protein, blood urea nitrogen, creatinine, glucose, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase), venous blood gas analysis and complete urinalysis (sediment + chemical + renal electrolyte excretion and sodium dodecyl sulphate-agarose gel electrophoresis (SDS-AGE) were performed for each time unit (12-24-48 hours post infusion). Data were collected and analysed with the software Stata 14.2. Normality of data was assessed using the Shapiro-Wilk test. To evaluate the effects of HES 130/0.4 at 2 ml/kg/h in the two times evaluated (T0 vs. T1), a hierarchical linear mixed effects model was used, where the individual subject gives the random effect. Bonferroni correction was applied to detect in which medium the difference was statistically significant. When the data did not meet the assumption of Normality, the comparison was conducted with the Sign test. A value of $P<0.05$ was considered significant. The comparison between serum biochemistry profile and urinalysis at T0, T1 and T2 has not shown statistically significant differences. Even if several randomized controlled trials have shown an association between HES administration and both AKI and mortality in people, further prospective studies are needed to assess both safety and efficacy of HES in dog before recommendations could be made.

ARTERIAL BLOOD GAS IN DOGS DURING HEMODIALYSIS

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Intradialytic hypoxemia is a known phenomenon in human medicine [1]. Extensive epidemiological studies are not yet present and the association of such findings with the patient's prognosis still now requires a studies on a large cohort of patients [2]. Many mechanism of dialytic hypoxemia are hypotesized: a shift in the oxyhemoglobin dissociation curve was hypothesized due to increased of blood serum pH during dialysis; depression of the center of the breath, alveolar hypoventilation secondary to the diffusion of CO_2 in dialysate [3]. To the Author's knowledge respiratory gas exchanges during hemodialysis are not reported in dogs.

The aim of the study was to evaluate arterial blood gas variables, (Ph, PaO_2, PCO_2, SO_2 P/F ratio, P[A-a]O_2 gradient and HCO_3^-) in azotemic dogs during hemodialysis (HD). Arterial blood samples were obtained from the dorsal pedal artery at room air (FiO_2 21%) at hospital admission (T0) and serially from an arterial catheter in the dorsal pedal artery at T1: 3 minutes after starting HD, at T2: 2 hours into treatment, at T3: at the end of HD and at T4: 2 hours after the end of HD. Dogs were then divided in two groups according to outcome: survivors (S) and non-survivors (NS). Normal distribution was assessed using D'Agostino-Pearson test. One-way ANOVA was used to compare pH, $PaCO_2$, PaO_2, P[A−a]O_2 gradient, PaO_2/FiO_2 and HCO_3^- at different times and between (S) and (NS). Twenty-two azotemic dogs referred for HD were enrolled. Fifteen out of 22 (46.8%) did not survive, 7/22 (32%) survived. A statistically significant difference ($p<0.0001$) in increase of pH was found among T0 (7.28±0.085) vs T2 (7.36±0.06) vs T3 (7.38±0.06) vs T4 (7.37±0.06) and for pH at T1 (7.26±0.067) vs T2, T3 and T4. A statistically significant difference in increase of $PaCO_2$ was found between T0 (28.9±4.43 mmHg) and T2 (33.83±3.18 mmHg), T3 (35.5±2.67 mmHg) and T4 (36.5±2.8 mmHg) and between T1 (32±3.18 mmHg) and T4 (36.54±2.8 mmHg). Bicarbonate increase significantly between T0 (13.8±4.7 mEq/L) and T2 (18±3.1 mEq/L), T3 (20±3.62 mEq/L) and T4 (19.21±3.84 mEq/L) and between T1 (14.4±4.2 mEq/L) and T2, T3 and T4. No significant difference in PaO_2, P[A-a]O_2 and $\%SaO_2$ was found at different time samples. No significant difference in arterial gas variables was found between survivors and non-survivors. To the Authors' knowledge this is the first study evaluating respiratory parameters during hemodialysis in dogs. A statistically significant increase in pH, $PaCO_2$ and HCO_3^- as a beneficial effect of hemodialysis in restoring acid-base balance of the patient was found. Ventilation to perfusion (VA/Q) inequalities is the main cause of arterial hypoxemia during HD. Even no significant changes in oxygen tension-based indices were found in this study, further evaluations with a larger number of patients are recommended.

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RESPIRATORY IMPAIRMENT AND OUTCOME IN DOGS WITH ACUTE LEPTOSPIROSIS SUBMITTED TO HEMODIALYSIS

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Canine Leptospirosis is a multiorgan disease, which can cause pulmonary changes in a significant percentage of patients [1,2,3,4]. The aim of the study was to evaluate oxygen tension-based indices, respiratory distress and radiographic patterns in dogs with acute leptospirosis submitted to hemodialysis (HD), and to compare them with survival rate. 19 dogs with acute kidney injury (AKI) secondary to leptospirosis and submitted to HD were enrolled. All the dogs of the present study showed clinical, laboratory and ultrasound findings consistent with AKI. Diagnosis of leptospirosis based on positive serum or urine PCR and/or serum MAT. At hospital admission oxygen tension-based indices (PO2/FiO2, P[A-a]O2 gradient, PaO2, PCO2, SaO2) were obtained by arterial blood sample at room air (FiO2 21%) from the dorsal pedal artery. Chest x-rays (right and left lateral and ventrodorsal) were performed before starting HD. Respiratory distress, (P/F<300 mmHg or P/F<200 mmHg and clinical respiratory effort) and mortality during hospitalization were recorded. Dogs were divided in survivors (S) and non-survivors (NS) according to outcome. Data were tested for normality by Kolmogorov-Smirnov test. Unpaired t-test was used to compare oxygen tension-based indices between S and NS. Prevalence of respiratory distress and radiographic patterns in S and NS were compared by Fisher's, and chi-square test respectively. Survival rate of dogs showing respiratory distress during hospitalization were compared with those that didn't develop any respiratory clinical sign or oxygen impairment, by Kaplan-Meier. Results were considered statistically significant for $p<0.05$.

At hospital admission, no significant difference in oxygen tension-based indices and PaCO2 and SaO2 was found between S (n=11) and NS (n=8). In S group chest x-rays showed normal lungs (1/11), interstitial pattern (5/11), mixed pattern (5/11). In NS group chest x-rays showed alveolar pattern (1/8), interstitial pattern (3/8), mixed pattern (4/8). No significant difference concerning radiographic pattern was found between S and NS. A statistically higher prevalence (p=0.02) of respiratory distress was found in NS (6/8) compared with S (2/11). Dogs with no signs of respiratory distress showed a significantly higher survival rate (p=0.02).

In our cohort of dogs, oxygen tension-based indices and radiographic pattern at hospital admission did not differ significantly between S and NS. The development of respiratory distress during hospitalization seemed to affect significantly outcome of dialyzed dogs with acute leptospirosis.

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THE COMPLETE BLOOD COUNT AND CANINE MAST CELL TUMOR. A RETROSPECTIVE SURVEY IN A VETERINARY TEACHING HOSPITAL IN TUSCANY, ITALY

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Complete blood count (CBC) modifications in canine mast cell tumor (cMCT) are described in dogs showing bone marrow involvement. However, few data are available for dogs affected by MCT in other stages than stage IV MCTs [1-2].
The aims of this study were: 1- to investigate the frequency of cMCT in a Veterinary Teaching Hospital (VTH) of Tuscany, Italy; 2- to assess if breed, gender and sexual status were associated with the frequency of cMCT; 3- to evaluate the association between each CBC parameters and clinical variables in cMCT.
Dogs with cyto/histological diagnosis of cutaneous MCT were enrolled. All dogs were staged with abdominal ultrasound and 3-view chest X-rays. Fine-needle biopsy of the regional lymph node (LN) was performed if LN was palpable or seen at ultrasonography. Fine-needle biopsy of liver and spleen was performed in high-risk MCT (relapsing MCT, inguinal/perineal MCT, large or ulcerated MCT, Patnaik GIII or Kiupel high-grade MCT, presence of nodal metastasis), and if ultrasonography alterations were detected or if requested by clinicians. Bone marrow cytology was performed in IV-stage MCT and if severe peripheral blood cytopenia was found. To investigate frequency of cMCT, dogs with MCT have been selected from our VTH database. To assess breed, gender and sexual status specific frequencies in cMCT, Odds Ratio (OR) with matched dogs without MCT (n. 13,077) in our VTH database has been performed. Dogs were divided in these subgroups: neutered (30)/intact (68), male (47)/female (51), age based on the 25th-, 50th- and 75th-percentile, presence of ulceration (15/89), occurrence of LN (28/88), occurence of visceral metastasis (12/88), and clinical stage (5 stage 0, 26 stage I, 8 stage II, 37 stage III, 12 stage IV). Each single parameter of CBC was used as an independent variable and analyzed statistically for each subgroup of dogs (t-student or if appropriate Mann-Whitney and Kruskal-Wallis tests).
Frequency of cMCT was 0.77% (98/13,175). Among purebreds, Boxer (OR 7.2), Pitt Bull (OR 5.4), French Bulldog (OR 4.4) and Labrador Retriever (OR 2.6) were considered the most predominant. Neutered dogs were also predominant compared to intact dogs (OR 2.1). Up to 67% (66/98) patients had CBC results in the reference ranges. The most common CBC abnormalities were anemia (25%) and leukocytosis/penia (17%). Any hematological abnormalities were not significantly different when analyzed for sex, age, presence of ulceration, occurrence of lymph node or visceral metastasis, and clinical stage.
These findings suggest that some breeds and the neutered status could be at increased frequency for cMCT. As all dogs came from a small area of Italy, a comprehensive examination of inbreeding and gene code associated with cMCT development could suggest a genetic predisposition. As reported in the literature, many dogs with MCT show CBC results in the reference ranges. Any single hematological variable was not associated with specific clinical findings or disease stage, suggesting a minimal role of CBC in the overview of cMCT.

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HEMATOLOGICAL AND BIOCHEMICAL DIFFERENCES IN 5 BREEDS OF HUNTING DOGS BLOOD DONORS

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Hematology and clinical biochemistry represent an important support to evaluate the health status of blood donors in a Veterinary Blood Bank. Numerous studies have shown the importance of breed-related differences between hematological and biochemical results [1-5].
The aim of this study was to compare the hematological and biochemical profiles of 5 hunting-dog breeds from blood donors at the Veterinary Transfusion Unit of the University of Perugia.
The study analyzed blood parameters of 385 clinically healthy dogs: 156 Ariégeois (A), 32 Bleu de Gascogne (B), 44 Bracco/Pointer (C), 103 Segugio (D), 50 Vandeano (E). The age of the dogs ranged from 2 to 8 years, 212 were female and 173 were male and the weight >25 Kg. Analysis of HCT, HGB, MCV, WBC, PLT, Albumin, Total Protein, BUN, Creatinine, AST, ALT, ALP and GGT were performed. Samples (EDTA for CBC and serum for biochemical parameters) were analyzed with Sysmex XT-1800iV (Sysmex) hematology analyzer and Hitachi 904 (Boehringer Mannheim) chemistry analyser. All parameters were within the reference range. Results were statistically evaluated (ANOVA) and significant differences ($P<0.05$) were found among these breeds: HCT (A vs C; C vs D), MCV (A vs C; C vs D,E) , WBC (A vs C), PLT (A vs B,C; C vs D,E), BUN (A vs C,E; B vs C,E; C vs D; D vs E), Creatinine (A vs D,E; B vs E; C vs E), AST (A vs D; B vs D; D vs E), ALT (D vs E), ALP (A vs B,E; C vs E; D vs E) and GGT (A vs C; C vs D). Below the list of the highest and lowest averages of each significant parameter regarding the breeds involved: HCT min (A) 43.7%, max (C) 47.07%; MCV min (A) 63.78 fL, max (C) 67.80 fL; WBC min (C) 9.68 x103/µL, max (A) 11.21 x103/µL; PLT min (A) 318.90 x103/µL, max (C) 391.15 x103/µL; BUN min (B) 23.78 mg/dL, max (C) 36.86 mg/dL; Creatinine min (A) 0.78 mg/dL, max (E) 1.1 mg/dL; AST min (B) 27.71U/L, max (D) 36.25 U7L; ALT min (E) 30.68 U/L, (D) 44.07 U/L; ALP min (E) 39.16 U/L, max (A) 65.99 U/L; GGT min (A) 3.68 U/L, max (C) 7.53U/L.
Significant differences in hematology and clinical biochemistry among the 5 hunting-dog breeds were found in line with earlier studies [1-5]. This study confirms that breed-specific reference intervals for hunting dog blood donors will help avoid misinterpretation of laboratory results in the selection of suitable blood donors.

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EVALUATION OF CANINE RED BLOOD CELL STORAGE LESIONS IN WHOLE BLOOD WITH AND WITHOUT LEUKOREDUCTION

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An increase interest in veterinary transfusion medicine is focused on how to prepare and use stored blood products (1) for extend their storage time, while maintaining the vitality and functionality of the cells (2). During the storage, there are biochemical and morphological modifications, known as Storage Lesions (SLs), which can alter the red blood cells (RBCs) quality and vitality (1; 3). Pre-storage leukoreduction, ie. the removal of white blood cells from the whole blood, would partially reduce the SLs (3). The aim of the study is to evaluate quali-quantitative modifications in dog whole blood leukoreduced (LR) and not (nLR), stored in CPDA1 at 2-6°C for 42 days. Five 350 ml units in 49 ml of CPDA1 were obtained from 5 healthy dog blood donors. Half of each blood unit was filtered, obtaining 5 samples of whole blood LR and 5 nLR. From each of the LR and nLR samples, weekly (from day 0 to 42nd), 15 ml of blood were taken for the following laboratory tests: RBC, Hgb, Hct, WBC, PLT, MCV, erythrocyte morphological index (MI), lactate dehydrogenase (LDH), electrolytes (Na^+,K^+,Cl^-). The erythrocyte fragility at mechanical stresses has been studied as degree of hemolysis induced by ultrasounds in vitro (basal -0" and after 2 and 4 sonication cycles -2" and 4"). Statistical significance was evaluated by Student t-test and ANOVA (R version 3.0.2, 2013). The value of $p < 0.05$ was set as a significance limit. Leukoreduction allowed a reduction in WBC and PLT of 99.58% and 92.8%, respectively and a 99.39% RBCs recovery, evaluated at time 0 in LR versus nLR samples. There was a decrease in Hct from day 0 to 14th and an increase from day 14 to 42nd in both LR and nLR samples, as well as for MCV. The decrease is related to the oxidative damage that results in increased rigidity of the membrane and transformation of the RBCs from discocyte to spherocyte with an increse of hemolysis. The hemolysis was evalueted using the electrolytes and LDH plasma levels as index, and the Na+/K+ pump alterations occurred during storage. Significant increase of LDH was evident at all storage times (already at day 14th) in all units with lower values in LR than in nLR, suggesting a release of LDH by WBCs. A progressive increase of Na+ and K+ were evident in all samples, the latter with statistically significant values already on day 14th. The in vitro ultrasonic resistance test showed that the hemolysis increased significantly by increasing the storage time and the stimulation intensity more in nLR than in LR units, even if the differences between the two groups were not significant, except at 42nd day where nLR units exhibit a basal hemolysis higher than LR. At 14th day there was an increase of MI significantly higher in the nLR samples compared to the LR, supporting the hypothesis that the leukocyte degradation products can contribute to the transformation of the erythrocyte to echinocita.
In conclusion, during the storage period, a progressive impairment of the vitality and resistance of erythrocytes is observed that are no longer suitable for transfusion beyond the 35th day of donation, with effects already apparent on the 14th day. The leukoreduction seems to be able to limit the SLs.

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EARLY ORAL VOLUNTARY NUTRITION IN ANOREXIC CRITICAL ILL DOGS WITH SEVERE PARVOVIRAL INFECTION: A RETROSPECTIVE STUDY IN 49 DOGS (2012-2014)

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Canine Parvovirus (CPV) is the most important cause of canine enteritis. While the clinical signs are typically limited to severe gastrointestinal upset and immunosuppression, sepsis and multiple organ dysfunction syndrome (MODS) may occur in severe cases with marked variations in energy requirement, high risk of malnutrition and its subsequent complications [1] [2]. The aim of this study was to determine retrospectively whether the timing of early oral voluntary nutrition affects mortality and length of hospitalization (LH) in dogs with severe CPV infection, and anorexia. Medical records of anorexic dogs admitted with CPV infection, and clinical criteria of sepsis 3 at OVGVII and OVUD, between January 2012 and August 2014 were reviewed. Clinical data recorded to evaluate illness severity included WBC and platelet count, serum albumin, C-reactive protein, AST, bilirubin, creatinine, and glycaemia. The severity of anorexia was defined by duration of anorexia at time of presentation (mild <24 hours (h), moderate 24-72 h, and severe >72 h). Medical treatment was instituted, and mainly consisted of fluid-therapy support, analgesia, antiemetic therapy, antibiotics, anti-thrombotic treatment, and blood or plasma transfusion when required. Nutritional requirement was calculated by Resting Energy Requirements (RER=70 x BWkg0.75); recovery diet (Royal Canine) or a/d (Hill's) was given via oral voluntary or friendly eating. Mann Whitney test and Spearman's rho test were used for statistical analysis. Forty-nine dogs were included, 21 male, 28 female, with the median age of 3 months and the median body weight of 3 kg. Four dogs (8%) were mildly anorexic, 9 (18%) moderately anorexic and 36 (74%) severely anorexic at time to presentation. Eight dogs (16%) started eating voluntarily within 72 h from admission, 12 dogs (24%) after 72 h from admission, and 29 dogs (60%) never ate food voluntarily. All dogs that never ate food voluntarily died, of them 24 dogs had severe anorexia, and 5 moderate anorexia at presentation. No significant statistical difference was evident for clinical data for comparison illness severity between dogs that never ate, and survivors, except of C-reactive protein (p= 0.04), WBC (p=0.03), and platelet count (p=0.001). In survivor dogs (20) strong correlation was evident between LH, and the time of start eating (r=0.82; p=0.001). Statistically significant difference was observed in LH between dogs that gained voluntary eating within 72 h (median 2 days) and after 72 h (median 4.5 days) from admission (p=0.002). In conclusion, early oral voluntary nutrition in dogs with severe CPV infection, and anorexia is associated with a lower mortality rate and shorter length of hospitalization.

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USE OF INFRARED THERMOGRAPHY TO DETECT INFLAMMATORY FOOT DISEASES IN DAIRY COWS AS A RAPID AND NON-INVASIVE DIAGNOSTIC TOOL

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Foot lesions have a high impact on animal welfare [1] and farm economy and early detection is likely to be valuable in the prevention of further progression and in early effective treatment. The aim of this study was to evaluate the potential usefulness of infrared thermography (IRT) as a non-invasive tool to rapidly screen the most common non-infectious foot lesions in dairy cows. Thirty-eight healthy cows (2-3 years old; mean body weight 625±115 kg) and 38 cows affected by foot diseases (2-3 years old; mean body weight 612±102 kg) were enrolled. The diseased 38 cows showed the following disorders at lateral and medial claw in the hind foot: white line lesion, sole ulcer, sole haemorrhage, horizontal fissure and axial fissure. IRT of hind foot were collected from each animal using a digital infrared camera (ThermaCam P25 Model, Flir Systems, Boston, MA, USA). Foot temperature was measured in four specific regions of interest including central area of the hind foot (A1), interdigital area of the hind foot (A2), lateral (A3) and medial (A4) claw in the hind foot. Ambient temperature at the time of taking the thermal images was 16.7±4.61°C. Two-way repeated measure analysis of variance (ANOVA) showed higher temperature values ($P<0.001$) in the regions A1 and A2 compared to A3 and A4 in both healthy and diseased cows. Cows affected by foot diseases showed higher foot temperature values compared to healthy cows ($P<0.05$) in all considered regions. Inflammation with increased blood flow and tissue metabolism rate occurring in the diseased foot lead to a localised increase in surface temperature detectable by IRTy. This study highlights the potential application of IRT as a reliable, practical tool for detection of hoof lesions in dairy cows. In this aspect, multiple scanning images and comparisons between affected and healthy anatomical structures could be useful in defining the consistency of an abnormality.

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VALIDATION OF A PORTABLE DEVICE FOR THE ASSESSMENT OF HEMOGLOBIN AND PACKED CELL VOLUME IN HORSES

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Hemoglobin (Hb) and packed cell volume (PCV) are important prognostic indicators in many emergency conditions in horses, such as colic, hemolysis and hemorrhage [1,2]. However, they can hardly be assessed in the field. The evaluation of PCV by gravity is possible in the field but time consuming. Contrarily, blood samples must be sent to a laboratory in order to measure their Hb concentration. Having a tool that rapidly measures Hb and PCV could greatly assist the veterinarian in the management of emergency cases in the field. This work aims to assess the reliability and repeatability of a portable human hemoglobinometer for the measurement of Hb and PCV concentration in healthy adult horses. Horses enrolled in the study had blood work performed for other reasons and few drops of the sample were withdrawn for our protocol previous consent of the owner. Subjects were defined as healthy based on their physical examination and complete blood count (CBC) test, when all physiological and hematological values were within normal ranges. All the horses studied were stabled in the same facility where the CBCs were performed in order to reduce to a minimum the time elapsing between sample collection and analysis. Horses underwent jugular venipuncture using a 1ml syringe with a 20G needle. PCV and Hb were immediately assessed in duplicata using the portable human hemoglobinometer (Mission Hb, ACON Laboratories Inc., San Diego, CA, USA). One drop of blood had to be applied on disposable strips and then inserted into the portable device to run the test. Hb detection is based on methemoglobin principle, while PCV is calculated from Hb using an unpublished formula. The remaining blood was transferred into 1ml EDTA blood tube and CBC performed within the next 2 hours using LaserCyte Dx Haemtology analyser (IDEXX Laboratories Inc., Westbrook, ME, USA), defined as the standard method. Linear regression analysis and Bland-Altman test were used to assess the repeatability of the results obtained with the portable device, to describe the relationship between PCV and Hb concentrations obtained with the two methods, and to describe any bias related to the results obtained with the portable device compared to the standard method. A total of 19 horses were studied (average age ± SD: 13 ± 4 years; 14 females and 5 males). Results were obtained in <5 seconds. The repeatability of the results obtained with the portable device was excellent for both values ($r^2=0.97$, $p<0.0001$ for Hb, and $r^2=0.95$, $p<0.0001$ for PCV). A linear relationship between Hb and PCV was found, expressed by the formula $PCV = 2.841*Hb + 1.355$ ($r^2=0.99$, $p<0.0001$). A significant relationship was observed between both Hb and PCV values assessed with both techniques ($r^2=0.90$, $p<0.0001$ for Hb, and $r^2=0.86$, $p<0.0001$ for PCV). Bland-Altman tests revealed a bias of -0.39 ± 0.58 g/dl and 2.6 ± 2% (mean ± SD) for Hb and PCV, respectively, indicating that the portable device slightly underestimates Hb concentration and overestimates PCV. In conclusion, the portable hemoglobinometer we tested provides repeatable and reliable results for the assessment of Hb concentration and PCV in healthy horses.

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ECO-DOPPLER EXAMINATION OF COMMON CAROTID ARTERY IN DONKEYS

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In veterinary practice, donkeys are considered very similar to horses, but several anatomic and physiological differences allow to acknowledge them as a separated species. Doppler parameters of the donkeys' common carotid artery have never been reported. The aim of this study is to identify ultrasonographic specific data for donkeys. Thirteen donkeys differing in weight (average weight: 212.46 ±76.14 Kg), gender (7 ♀and 6 ♂) and age (average age: 9.31 ± 4.99 years) were examined using B-mode real time, colour Doppler and pulsed wave Doppler. Firstly, the donkeys' weight was calculated [1] and a clinical examination was performed in each animal in order to rule out any cardiovascular disease. Then, the caudal third of the right jugular region was sheared at the level of the common carotid artery. The ultrasound evaluation was performed using an echo-Doppler machine (ESAOTE MyLab30 vet Gold) with a high-frequency linear transducer (4-13 MHz). The following parameters were registered for each donkey: vessel diameter (D), heart rate (HR), velocity time integral (VTI), pulsatility index (PI), resistivity index (RI), systolic peak velocity (SPV), average velocity (AV), peak pressure gradient (PPG), mean pressure gradient (MPG), acceleration (A), acceleration time (AT) and carotid output (CO). The following mean values were obtained in the total group of animals: D 0.88 ± 0.03 cm, HR 63 ± 3.79 bpm, VTI 0.30 ± 0.03 m, PI 2.78 ± 0.22, RI 0.95 ± 0.05, SPV 0.91 ± 0.07 m/sec, AV 0.31 ± 0.03 m/s, PPG 3.39 ± 0.49 mmHg, MPG 0.64 ± 0.09 mmHg, A 5.47 ± 0.63 m/s², AT 163.31 ± 12.43 ms and CO 18.98 ± 2.19 ml/sec. Differences among age, sex and weight categories were evaluated. A statistically significant difference between the ♀and ♂was found regarding the CO [2]. This result could be linked to anatomic and functional differences between ♀and ♂. For the horses' vessel diameter no differences between ♀and ♂were described. Conversely, statistically significant differences were found for D and HR in relation with donkeys' weight. In particular, a higher HR in heavier animals was detected. This is unusual and could be explained with a different nature and attitude of the animals. The higher HR evaluated in some donkeys may have been induced by a sympathetic hypertonia in the animals less accustomed to human interaction. Comparing the groups of animals divided in age categories, a statistically significant higher HR was found in older subjects. Moreover, the results could have been influenced by the fact that the animals more accustomed to human interaction were mostly included into the same group. Finally, differently from horses, PSV didn't increase with weight and the A didn't increase in lighter animals.

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COMPARISON OF TWO DIFFERENT METHODS FOR HOLTER ECG MONITORING IN THE HORSE

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Exercise tests are important to evaluate dynamic dysrhythmias and continuous ECG monitoring is the gold standard for the diagnose [1]. The aim of the study was to evaluate two methods [2,3] to record Holter ECG at rest and during exercise.
Ten standardbred female horses (median: 9.5 years) were included in the present prospective study approved by Ethical Committee, Pisa. Mares were divided randomly into 2 groups: in group 1 (n=5) the ECG was recorded using an Holter ECG (Click Holter, Cardioline, Italy) with 7-electrodes [2], while a 4-electrodes method [3] was used for group 2 (n=5). Electrodes were positioned applying ECG gel and glue after hair shaving. Each Holter ECG was recorded for a total of 24h. At 23h, a 15-min aerobic exercise was performed. To check the exercise intensity, the HR was monitored using an HR monitor (Polar, Finland) and lactatemia was measured with a lactatemeter (Accutrend Lactate, Micralab srl, Italy) [4] 5 min before and after the exercise. At 24h the Holter was removed, the record was downloaded and evaluated (Cube Holter Cardioline, Italy) for: 1) number of Total Electrodes (TE) still attached at the end of the 24h-period; 2) number of registered h within the 24h (H); 3) number of Total Artifacts (TA) on the ECG during the 15 min of exercise; 4) percentage of Artifacts (artifact in sec/900 sec x 100) (%TA); 5) percentage of Detached Electrodes in relation with the number of electrodes applied (Detached Electrodes/applied electrodes x 100) (%DE). Average (X) and standard deviation (SD) were calculated. Data were analyzed for distribution, Mann-Whitney and Dunn's test were applied to verify differences between groups ($p<0.05$).
The HR was <180 beat/min during all the 15 min exercise period and no difference in mean plasma lactate pre and post training was found [6]. Statistical differences (group 1 vs 2) were obtained for TA, H and %TA. Our results showed a better performance using the 4-electrodes procedure during the exercise because a lower number of artifacts were present within the 15 minutes exercise ECG. Nerverthless at rest the 7-electrodes method showed a good quality ECG even when several electrodes were detached because of the possibilty to check the other derivations. Based on these results we suggest to use the 4-electrodes method for exercise and switch to the 7-electrodes method if a longer ECG at rest is required.

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URETERAL ENDOSCOPY IN THE HORSE

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Ureteral endoscopy can be a useful tool to evaluate the presence of diseases in this portion of the urinary sistem and to take biopsy or urine samples to confirm diagnosis. It has been described in healthy horses [1], but to the authors' knowledge, no publicaton described the use of this technique in diseased animals. Aim of the study is to present this procedure in 4 horses (3 females and 1 gelding) admitted for various diseases of the urogenital system, that were thought to involved also the ureters. To confirm the diagnosis, ureteral endoscopy was performed using a flexible endoscope (Pentax, 100cm and 6mm in diameter for the three females; Storz, 140cm and 9mm in diameter for the gelding), passed through the urethra and bladder after the animals were sedated (alpha-2 agonist IV, either xylazine 0.4mg/kg or detomidine 0.01mg/kg), and epidural injection of lydocaine was also used. Entrance into the ureter was performed under guidance of a biopsy forceps, except for the gelding, where the dilated organs allowed for an easy entrance without guidance. The endoscopy was easily performed in all animals, as described in the literature [1, 2]. Two horses had urolithiasis (case 1 female, with the stone visible in the ureter, case 2, gelding), one a laceration in the ureter (case 3) and the last one a stricture and renal calcolosis (case 4). The endoscope progressed easily along the organ in all animals, without causing any trauma. The mucosa was normal in three animals (cases 1, 3 and 4), while in case 2, it was hyperemic and with fibrin deposits. The ureters were dilated in two animals: in the gelding (case 2) due to the presence of hydronephrosis and calcolosis of kidneys and bladder, and in the mare with the stenosis (case 4), where there was a pool of hemorrhagic urine and fibrin at 15cm from the bladder, in front of the stricture. Case 3 had a ureteral tear secondary to the removal of a big ovarian tumor, that involved also the ureter: support to the diagnosis came also from laparoscopy performed together with the endoscopy. None of the horses showed signs of discomfort during and after the procedure. The diagnosis was easily made in all animals, and the endoscopy allowed to give the owner a prognosis and select the best treatment plan for the equid: the horse with the laceration (case 3) was euthanized after the procedure, while the gelding (case 2) was euthanized because of renal failure secondary to the urolithiasis, case 4 was discharged, and removal of the urolith of case 1 was performed from the bladder after treatment with non steroidal antinflammatory drugs and antibiotics allowed for it to pass in this organ. In conclusion, ureteral endoscopy appears to be easy to perform and an useful technique to confirm diagnosis of diseases of the urinary system and select the best treatment plan for the patient.

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DESCRIPTION OF A PRODUCTION METHOD FOR PLATELET-RICH PLASMA PREPARATION IN HORSES

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In humans, platelet-rich plasma (PRP) shows different levels of efficacy in inducing regenerative processes, based on different platelet concentrations ([PLT]) and target tissue [1,2]. In equine practice, the most common methods for PRP preparation include centrifugation, using anticoagulated blood collection tubes or commercial systems. However these protocols generally make a small volume of PRP with [PLT] usually lower (from 300,000 to 1,000,000/µL) [3] than those shown to be effective for regeneration of human tissues. The aim of this study was to evaluate the concentrations of PLT, RBC ([RBC]) and WBC ([WBC]) of PRP produced with a double centrifugation technique using quadruple blood bags (BB).
The study was performed on 11 blood donor mares. In each mare, whole blood (WB - 450 ml) was collected using BB (Compoflex, Fresenius Kabi, Germany); a second sampling was made after 3 months for a total of 22 samples. The separation of PRP from WBC and RBC was obtained by BB centrifugation at 1920g for 10' at 22°C (Rotixa 50RS, Hettich, Italia). Based on inclusion or not of buffy coat (0.4 ml), the samples were assigned to group A (n 12) or B (n 10), respectively. PLT concentrates in 15 ml of plasma were provided by a second PRP centrifugation at 3960g for 6'. Automated CBCs were performed both on WB and on final PRP. All data were expressed as mean±SD, and the groups were compared by t-test using a statistical software (MedCalc, 12.6.1). Statistical significance was set at $P<0.05$.
In all 22 WB the [PLT], [RBC] and [WBC] were 153,400±26.000/µL, 6,970,000±1,370,000/µL and 8,490±2,110/µL, respectively; there were no differences between the 2 groups. In group A the [PLT], [RBC] and [WBC] were 3,191,000±698,000/µL, 921,000±245,000/µL and 45,380±27,510/µL, respectively, while in group B were 1,372,000±68,000/µL, 753,000±299,000/µL and 15,130±6,750/µL, respectively. The [PLT] and [WBC] were significantly higher in group A ($P<0.0001$ and $P<0.01$, respectively), whereas no differences were found in [RBC] between the 2 groups.
This study proposes a closed system method for PRP preparation that results in [PLT] and PRP volumes higher than those reported in equine literature. In humans, several studies showed that a lower [PLT] displayed a reduced proliferative response, otherwise higher [PLT] had an inhibitory effect in some tissues whereas in other tissues enhanced the proliferation [1,2]. The WBC role and concentration in PRP have not been clearly established [3]. As expected, both [WBC] and [PLT] obtained by the method we evaluated were very high in group A; diluting the samples, it is therefore possible to modify the [PLT] reducing also the [WBC]. Based on human studies [4], the [RBC] in PRP obtained should not hinder the healing process. In conclusion, the method proposed leads to the production of a high [PLT] and PRP volume, and variable [WBC]. These characteristics make the PRP obtained adjustable to biotherapy based on target tissues.

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INFRARED THERMOGRAPHY AS A RAPID AND NON-INVASIVE DIAGNOSTIC TOOL FOR THE DETECTION OF DIGITAL AND INTERDIGITAL DERMATITIS IN HOLSTEIN FRIESIAN DAIRY COWS

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Digital and interdigital dermatitis are a dynamic and multifactorial infectious foot disease with increasing prevalence in many countries [1,2,3]. Early detection of digital and interdigital dermatitis is the first step towards therapeutic resolution, through rapid treatment and consequent reduction of infection reservoir within the herd [4,5]. Infrared thermography (IRT) has been adopted in animal production studies for different analyses such as metabolic responses to thermal stress and the diagnosis of inflammatory processes. The use of IRT for the identification of lameness in cattle has increased in recent years largely because of its non-invasive properties, ease of automation and continued cost reductions low cost. The aim of this study was to evaluate the potential usefulness of infrared thermography as a non-invasive tool to rapidly screen digital and interdigital dermatitis (DD and ID) in dairy cows. Forty-eight healthy cows and 48 cows affected by DD and ID on central and interdigital regions of hind foot were enrolled in the study. Thermography images of hind foot were collected from each animal using a digital infrared camera. Foot temperature was measured in four regions: central area of the hind foot (R1), interdigital area of the hind foot (R2), lateral (R3) and medial (R4) claw in the hind foot. Two-way analysis of variance (ANOVA) showed higher temperature values in the central (R1) and interdigital area (R2) compared to lateral (R3) and medial (R4) law in both healthy and diseased cows ($P<0.001$). Higher foot temperature values were found in cows affected by DD and ID compared to healthy cows in the regions R1 and R2 ($P<0.001$). The present study showed that IRT is a diagnostic tool in the detection of DD and ID in dairy cows. The obtained result suggest that IRT could contribute in defining the localization area of increased inflammation and/or injury and it would be useful for veterinary podologists to act directly on the lesion detected by thermography rather than on the whole foot.

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OCCURRENCE OF *Prototheca* SPP. IN COW MILK SAMPLES IN ABRUZZO REGION

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Prototheca algae are unicellular organism associated with diseases in humans and animals. *Prototheca* (*P.*) *zopfii* and *P. blaschkeae* have been associated with clinical or subclinical cases of bovine mastitis with *P. zopfii* genotype II being the most prevalent [1,2]. Also, *P. zopfii* is recognized as zoonotic agent in humans. The aim of this study was to investigate the presence of *Prototheca* spp. in cow milk samples from Teramo dairy farms (Italy) by morphologic examination and by PCR [3]. Three hundred and seventeen composite individual samples were collected between September 2015 and June 2016 from 17 dairy herds. Each sample was aseptically collected and underwent to Somatic Cell Count (SCC) and bacteriological culture on blood agar. Furthermore, individual samples were cultured on Sabouraud agar, while bulk tank milk samples were cultured on Prototheca Isolation Medium (PIM). The presence of *Prototheca* spp. was also evaluated in drinking water, environmental and milking equipments swabs in infected dairy farms as well as from stools of each positive cattle. Milk samples were further collected every 2 months for three times, from each cow found to be positive for *Prototheca* infection. Elevated SCC (mean: 1,853 x 103 cell/mL) was detected in 34 out of 317 samples and in 15/34 *Prototheca* spp. was isolated. In particular, algae were identified from 3 bovines with clinical mastitis and in 12 with subclinical mastitis. *Prototheca* spp. was cultured also in two bulk tank milk samples 2/17 (12%), and only from drinking water in both farms. *P. zopfii* genotype II was identified in all positive samples by both morphological examination and PCR. In subsequent checks, despite a persistent increased SCC, in 3 out of 15 cows *Prototheca* spp. was not isolated, although the several attempts. *P. blaschkeae* was not isolated and this data confirm the hypothesis that although *P. blaschkeae* could be cause of mastitis, its prevalence is low [1]. Usually bovine protothecal mastitis is characterized by chronic interstitial mastitis, reduced milk production and increase in SCC [4]. Furthermore, the ability to infect and survive in macrophages and to invade the udder tissue, Prototheca spp. can induce a persistent infection with intermittent shedding as observed in 3 cows here described. Although the occurrence in bovine milk samples was low (5%), consistent with previous studies, bovine protothecal mastitis is increasing worldwide and is currently recognized as endemic. This study represents the first report of bovine protothecosis in the Abruzzo region (Italy). This pathology can lead to significant economic losses and poses a public health problem because of high resistance of *Prototheca* spp. to heat and milk pasteurization [3]. Nowadays no efficacy therapy exists. Drying off the affected quarter and isolation or culling of the infected animals are the only effective counteractions presently applied.

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BOVINE MASTITIS IN DRY PERIOD: MICROBIOLOGICAL AND ANTIBIOTHERAPY EVALUATION

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The transition from lactation to the dry period in dairy cows is a period of high risk for acquiring new intramammary infections (IMI) (1). An important element of many mastitis control programs is the treatment of all cows with antibiotics at the end of lactation, despite of the infection status (2). The aims of this study were to evaluate the microbiological population in milk samples collected from quarters positive to the CMT at the time of drying-off and to assess the use of two different intramammary antibiotic plans as drying-off therapy. Ninety-five Friesian cows and 380 quarters were included. Each quart was evaluated with the CMT at the drying-off time (CMTa). The positive quarters were sampled to perform a complete bacteriological examination. All the 380 quarters were treated with 2 different intramammary antibiotics, based on the mastitis control program of the farm: Cloxacillin (Atb0) and Cephalexin (Atb1). All the 380 quarters were evaluated with the CMT at the beginning of lactation (CTMp). The results were expressed as prevalence. The Fisher's exact test has been performed to compare if one of the two treatments used was more effective on CMTa+ quarters (drying-off time vs beginning of lactation) and/or was more protective on CMTa- quarters (drying-off time vs beginning of lactation).
Sixty-eight/380 quarters (18%) were CMTa+, while 312/380 (82%) were CMTa-. In line with farm' protocol, 136/380 (36%) quarters were treated with Atb0 and 244/380 (64%) with Atb1. Fifty-eight/68 (78%) CMTa+ quarters were negative to the bacteriological evaluation and 15/68 (22%) were positive: 8/15 (53%) *Staphylococcus* sp.; 4/15 (27%) *Streptococcus uberis;* and 3/15 (20%) others. At the time of CMTp, 345/380 (91%) quarters were negative, while 35/380 (9%) were positive. Fifty-four/68 (79%) CMTa+ quarters were negative to the CMTp (44.4% have been treated with Atb0, while 55.6% with Atb1) and 14/68 (21%) were still positive (64.3% have been treated with Atb0, while 35.7% with Atb1). Instead, 291/312 (93.3%) CMTa- quarters remained negative to the CMTp (33% have been treated with Atb0 and 67% with Atb1), while 21/312 (6.7%) became positive (33.3% have been treated with Atb0 and 66.7% with Atb1). There weren't statistically significant differences between the two treatments used both in CMTa+ quarters (p=0.23) (drying-off time *vs* beginning of lactation) and CMTa- quarters (p=1.00) (drying-off time *vs* beginning of lactation). The most commonly isolated bacterial population was the genus *Staphylococcus* that represents the main common pathogen involved in subclinical mastitis in dairy (3). Atb0 and Atb1 showed the same effectiveness in the drying-off therapy (3).

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EVALUATION OF SPEED BIOGRAM TEST FOR DETECTION, IDENTIFICATION AND MICROBIAL SUSCEPTIBILITY TESTING IN CANINE PYODERMA AND EXTERNAL OTITIS

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Point-of-care testing for bacteria and yeast involved in canine pyoderma and external otitis is a possible approach to reduce both turnaround time and costs for detection and microbial susceptibility testing of infections and to reduce emergence of multidrug-resistant (MDR) pathogens that has become a significant health problem (1). The aim of this study was to evaluate a rapid in vitro diagnostic test, Speed Biogram (Bio Veto Test, La Seyne sur Mer, France) for detection, identification and microbial susceptibility testing of pathogens in canine pyoderma and external otitis, diagnosed by citology, comparing the results with those obtained by standard culture (Trypticase soy agar containing 5% of sheep blood, Microbiol, Italy) and antibiogram (Kirby-Bauer susceptibility test) methods. Speed Biogram enables the identification of the genus of the microorganisms and the evaluation of their susceptibilities of commonly used in veterinary practice antibiotics, via a simple colour change in wells 24-48h after inoculation. Sensitivity and specificity for Speed Biogram for identification of the presence of pathogenic microorganisms and for microbial susceptibility testing was calculated by a 2 x 2 table, while the agreement between Speed Biogram and standard culture and antibiogram methods (SM) was evaluated using unweighted k statistic with a 95% confidence interval (95%CI). A total of 34 canine samples were included through cytological evaluation: 11 samples from bacterial otitis, 6 samples from *Malassezia* spp. otitis and 11 sample from pyoderma, while 6 auricular sample were taken in healthy dogs, used as a negative control. The sensitivity and specificity of Speed Biogram for detection of the presence of pathogenic microorganisms were, respectively, 100%, 95%CI 0.88–1.00 and 100 %, 95%CI 0.54–1.00. Standard culture of 22 bacterial positive samples resulted in growth of *Staphylococcus pseudintermedius* (n=15), *Pseudomonas aeruginosa* (n=7), *Proteus mirabili*s (n=8), *Streptococcus β haemoliticus* (n=2), alone or simultaneously present, while *Malassezia* spp. was cultured in 6 auricular samples. Unweighted k statistics on 28 culture positive samples demonstrated a k value of 0.633 (95% CI 0.451 to 0.815) with a good agreement in the assessment of bacterial and *Malassezia* spp. isolation between Speed Biogram and SM. Single or multiple false antimicrobial susceptibilities or resistances with Speed Biogram were observed in 13/22 bacterial standard culture-positive samples, but the sensitivity and specificity of Speed Biogram for antibiogram results were, respectively, 85.71%, 95%CI 0.79-0.91 and 92.81%, 95%CI 0.87-0.96 and unweighted k statistics demonstrated a good or very good agreement between Speed Biogram and SM for all 13 tested antibiotics (average k=0.694) except for enrofloxacin, for which a fair agreement (k=0.54 95%CI 0.184-0.892) has been detected. Speed Biogram is a good and simple to read in house test for detection and identification of bacteria or *Malassezia* spp. in canine pyoderma and external otitis, with statistical good performances in microbial susceptibility testing, but care must be taken, especially regarding enrofloxacin results, as highlighted false antimicrobial susceptibilities may lead to wrong therapeutic choices, increasing the possibility of selection of MDR bacteria.

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SURVEY OF METHICILLIN-RESISTANT COAGULASE POSITIVE *Staphylococcus* SPP. CARRIAGE IN HEALTHY DOGS AND DOGS WITH SKIN DISEASE

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Canine skin diseases (SD) are very common in the veterinary practice and are often complicated by recurrent bacterial infection. Affected dogs usually need multiple cycles of antibiotic treatments (AT) that can lead to development of multidrug resistant strains [1]. Coagulase-positive staphylococci (CPS) are the most often isolated pathogens from canine SD.

The aim of this study is to assess the prevalence of multidrug and methicillin resistant coagulase-positive staphylococci (MRScp) isolated from healthy dogs and dogs with SD, and to evaluate the correlation with clinical scores and previous AT. Forty-nine dogs were enrolled: 25 healthy and 24 with SD. Clinical history and previous AT were recorded. After a complete physical examination, clinical scores (CADESI-3 and pruritus) were calculated. Skin swabs from mouth, ear, genitalia, axilla and skin lesions, when present, were cultured in a nutrient and in a selective medium for MRScp. Suspected *Staphylococcus* colonies were identified by Maldi-Tof MS and specific PCR; methicillin resistance was confirmed by a PCR targeting mecA gene. Susceptibility tests and genetic typing, including spa-typing, SCCmec-typing and MLST were performed on isolates. Normal distribution of data was tested with Shapiro-Wilk test. Data were analyzed using ANOVA and z-test if normally distributed, otherwise with Mann-Whitney Test. Correlations between bacterial resistance and clinical scores or previous AT were assessed by Spearman test. P-value <0.05 was considered significant.

Ninety-five strains of CPS were isolated from 229 samples. A total of 13/95 strains were MRScp and were identified as *Staphylococcus pseudintermedius*. Among them, 10 were multidrug resistant and two were isolated from healthy dogs. The Sequence Type 71, spa-type t02 e SCCmec type II-III, which represents the main clonal strain in Europe [2], was the most frequently identified genetic type (11/13) also in this study. Staphylococci were more commonly isolated from axilla, genitalia and ear conduct of dogs with SD compared with healthy dogs ($p<0.001$). Four out of the 6 MRScp positive dogs had received AT in the previous 6 months. No significant correlations between clinical scores or previous AT and methicillin resistance was found.

Although the low number of dogs included in the study could have affected the results of the investigated correlations, this study confirms the role of *Staphylococcus pseudintermedius* in canine pyoderma and shows that pet dogs may play a significant role as MRScp carriers. Furthermore, close attention should be also paid also to the control of healthy dogs.

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CORNEAL DYSTROPHY IN A COCKER SPANIEL DOG: A CASE REPORT

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In dogs, corneal stromal dystrophy is a primary, hereditary, bilateral opacity of the cornea not associated with eye inflammation or systemic disease [1,2,3]. Because detailed description of corneal dystrophy are available only for few breeds, we present a clinical case in 1.3 year-old female Cocker Spaniel dog referred to the University of Messina VTH for white spots in both corneas and “red eyes”. The spots had appeared several months before. Previous ophthalmic therapy(dexamethasone and cyclosporine) had not been successful. Dog was clinically healthy; blood exams were normal and LeishmaniaPCR was negative. Visual tests were positive (OU). The eye exam showed conjunctival hyperemia, epiphora, and no eyelid anomalies (OU). Slit-lamp examination showed a ring corneal opacities, formed by a myriad of fine, white and small particles, located in axial portion of the cornea in OD. In OS, an arc-shaped opacity of the same density as OD was presented in the subepithelial and posterior stroma of the axial cornea. The epithelium was intact and no neovascularization was observed (OU). STTs were 22mm/min (OU). Fluorescein dye test was negative (OU). Light aqueous flare was present (OU). IOP was 8mmHg (OD) and 6mmHg (OS). Any anomalies were present in lens, vitreous and fundus. Final diagnosis was corneal stromal dystrophy with uveitis-induced. Therapy with both oral Prednisone and topical Dexamethasone resolved the uveitis and relieved eye discomfort. 1%Atropine was topically applied to decrease ciliary spasm. EDTA in ocular solution was applied in both eyes q6h. After a month, eye discomfort and inflammation were resolved. IOP were 13mmHg (OS) and 14mmHg (OD). Cocker Spaniel dog could present two different type of corneal dystrophy: a) epithelial/stromal: a non-inflammatory corneal opacity (white to gray) in one or more of the corneal layers; b) posterior polymorphous dystrophy: multifocal, non-pigmented, vesicular to linear posterior corneal opacities of endothelium. It differs from endothelial dystrophy by the absence of corneal edema. Corneal endothelial cells distant from the corneal opacities are normal [4,5]. In this case report, the lipid accumulation in subepithelial layer may be responsible of the pain and of the subsequent secondary anterior uveitis. No medication will dissolve the opacity but a reduction has been reported with topical cyclosporine and tacrolimus [8]; surgery is not usually recommended [1,6]. Because topical EDTAwas used to remove calciumin corneal degeneration [2,6,7], this therapy was added to the treatment, but no significant efficacy on corneal opacities was recorded. In conclusion, because this lesion is not progressive, treatment is unnecessary unless vision is impaired or the corneal deposits become irritating.

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DETERMINATION OF PLASMA AMINO ACID CONCENTRATIONS DURING A ONE YEAR FOLLOW UP IN A DOG WITH HEPATOCUTANEOUS SYNDROME

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Canine superficial necrolytic dermatitis is an uncommon, progressive, debilitating skin disease that is often associated with a hepatopathy, in this case referred as hepatocutaneous syndrome (HCS); with or without diabetes mellitus (DM) [1]. The prognosis of the HCS is generally poor. Hypoaminoacidemia is a common, but not necessarily a consistent feature of the HCS. An increase in amino acids (AA) catabolism by the liver has been proposed as a mechanism for the low plasma concentrations [2]. The aim of this report was to evaluate the changes in plasma AA in a dog with HCS during a long-term, intermittent treatment protocol.
An 8-year-old male neutered Welsh Terrier presented with a 2-month history of uncontrolled DM and progressive skin lesions. The dog was depressed, thin and anorexic. Physical examination revealed ulcers and crusts that affected the aural pinnae, pressure points and mucocutaneous junctions. Hyperkeratosis and fissures were present on all the footpads. Clinical chemistry findings included an increase in AST, ALT, ALP, glucose, fructosamine and glycosuria. Abdominal ultrasound revealed a honeycomb-like pattern of the liver. Based on histologic examination of multiple skin and liver biopsies, the dog was diagnosed with HCS. Insulin therapy was modified and an AA commercial solution (Dekamin; Monico Spa, Italy) was administered IV through a central catheter (6 mL/kg/h over 8 h) weekly for 3 months, then every 2 weeks for 3 months, followed by every 3 weeks for 2 months. Currently the dog is receiving an AA infusion monthly. Plasma AA concentrations obtained at admission (T0), 1 (T1), 3 (T3) and 12 (T12) months of treatment, were evaluated. The patient showed a general improvement 15 days after treatment and skin lesions completely regressed within 3 months. The DM was also well-controlled.
At T0 the molar ratio of branched-chain AA (BCAA) to aromatic AA (AAA) was 8.2 (normal range 3-4) and half of the AA (including most essential AA) were reduced by 50% or more compared to the reference range means. The concentrations of some of these AA (alanine, glycine, methionine, threonine, tryptophan, tyrosine) administered IV, increased at T1 and T3 and BCAA:AAA ratio decreased to 4.5 and 3.2 respectively. At T12 all of these AA decreased compared with T3 and the BCAA:AAA ratio was 3.2.
An elevated BCAA:AAA ratio at T0 may be explained by starvation and/or concurrent, poorly controlled DM. Given the regression of skin lesions, the normalization of the BCAA:AAA ratio may be related to the interruption of protein catabolism, or they may continue to decline over the long term indicating a progression of the liver disease. The very low concentrations of alanine, glutamine, glycine, proline, and threonine at T0 may be associated with a greater survival time [2].
In the face of a clinical improvement, only a few severely deficient AA increased during therapy. In conclusion, these features could indicate that some AA play a more important role in the pathogenesis of SND. Studies documenting AA patterns and correlating findings with clinical signs over the course of this disease in a larger number of dogs may provide some insight into the underlying mechanisms of HCS.

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EVALUATION OF SYMMETRIC (SDMA) AND ASYMMETRIC (ADMA) DIMETHYLARGININE IN CANINE CHRONIC MITRAL VALVE DISEASE (CMVD): PRELIMINARY DATA

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Serum concentration of symmetric dimethylarginine (SDMA), and its asymmetric isoform (ADMA), have been performed in dogs with chronic mitral valve disease (CMVD) as new potential biomarkers of possible Cardiovascular-Renal Disorders (CvRDs) [1]. Twelve dogs affected by CMVD (Pathological Group; GP) and ten healthy dogs (Control Group; CG) were enrolled at the Cardiology Service of the Teaching Veterinary Hospital of the University of Naples "Federico II". The GP animals were evaluated at inclusion (T0), at 3 months (T1) and at 6 months (T2). All the animals were clinically classified according to the ACVIM guidelines. No dogs showed renal failure according to IRIS criteria at the moment of enrollment. ACE-inhibitors and/or diuretics were administrate to the animals when appropriated at inclusion. ADMA and SDMA concentration were obtained using liquid chromatography-tandem mass spectrometry. The data obtained show a great variability for SDMA and ADMA serum concentration, suggesting a potential influence of diet, similar to what recently reported in humans and in older dog [2,3]. However the trend of SDMA did not change over time as well as between the ACVIM classes enrolled. No difference was also observed between GP and CG. These findings may be due to the presence in our study of several animals at the early stages of CMVD (ACVIM classes B1 and B2), in which probably renal function has not yet been compromised. Regarding ADMA serum concentration, a significant decrease over time was observed. This finding was not related to the clinical setting and/or to the therapy, in disagreement with what reported in humans where ACE-inhibitors showed a protective action on the endothelium against oxidative stress [4]. Any difference in ADMA levels was also found in comparison to the CG and between the ACVIM classes. Under our experimental condition, these biomarkers are not seem to be useful in the early stage of CMVD. Further studies need to better understand the real significance of these parameters in a more large number of dogs at different clinical stages.

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CYTOLOGICAL DIAGNOSIS OF FELINE NASAL HAMARTOMA

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Nasal hamartoma (NH) is a rare benign tumour of the sinonasal tract.[1] Etiology is still unknown. Young age of affected cats suggests hereditary or congenital causes while upper airway infections could represent a trigger factor.[2-3] Histological features are comparable to children's nasal chondromesenchymal hamartoma but cats show a predominant bone component.[1] Aim of the study was to state the diagnostic significance for NH of two cell types we called osteoblast-like (OB-L; polygonal, basophilic cytoplasm, round paracentral nucleus) and osteoclast-like (OC-L; multinucleated giant cells) evaluated in squash preparation (SP) cytology. The 2011-2015 rhinoscopy database of one of the authors (E.B.) was retrospectively reviewed. Feline patients were selected according to the following criteria: presence of a nasal mass, adequate cytological SP sample, definitive histological diagnosis. Within the cases matching the inclusion criteria all NH were included. Among the others, 85 were randomly selected to comply with the database's prevalence of each disease. Therefore 109 cases, with the following histological diagnosis, were included in the study: carcinoma (25), NH (24), lymphoma (22), inflammatory polyp (22), sarcoma (12), nasopharyngeal polyp (4). SP were stained with rapid Romanowsky staining. Slides were blindly evaluated by two cytologists (skilled and unskilled in nasal and SP cytology). The unskilled cytologist counted 500 cells for each smear recording the number of OB-L and OC-L. The skilled cytologist was asked to evaluate 10 fields at 40x magnification with monolayered cells and classify samples into 4 categories for OB-L (0, 1-4, 5-10, >10/ field) and OC-L (0, 1-2, 3-5, >5/ field). The unskilled cytologist evaluation revealed that the absence of one or both of the cell type allows to exclude NH from the differential diagnosis ($p<.001$). The combined presence of OB-L and OC-L provided the highest accuracy, concordance, sensibility and specificity (97.2%, 92.3%, 100%, 96.5% respectively). ROC curves revealed 6/500 (OB-L) and 2/500 (OC-L) as the best cut-off values for NH diagnosis. Skilled cytologist's results showed that more than 10 and less than 5 OB-L/filed were significantly associated with the diagnosis and the exclusion from the differential diagnosis of NH respectively ($p<.001$). All the samples in >5 OC-L category and 5/6 of the 3-5 category were NH. Moreover the simultaneous presence of OB-L (≥5/field) and OC-L (≥3/field) showed 100% concordance with histology and provided 100% accuracy, sensibility, specificity, PPV and NPV for the diagnosis of NH. We demonstrated that OB-L and OC-L allow the diagnosis of NH by means of cytology and described two cytological approaches applicable in a routine diagnostic setting. Diagnostic performances with the detected cut-off values should be confirmed in a prospective study. Effects of different sampling and smear techniques and origin of the two cell types need to be further investigated.

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Scienze Cliniche - SICV

TRAMADOL IN CONTINUOUS INTRAVENOUS INFUSION VS TRAMADOL BOLUS DURING ABDOMINAL SURGERY IN DOG

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This clinical blinded trial study aims to compare the effects of tramadol administered as a constant rate infusion to that of tramadol administered as a single intravenous bolus on physiological parameters and isoflurane requirements during elective ovary-hysterectomy in dog[1] [2]. In this study have been included forty female dogs. Anesthesia was performed by the administration of tiletamine/zolazepam 5mgkg-^{1} intravenously (IV) and acepromazine 0.05 mgkg-^{1} IV, oxygen and isoflurane. Animals were divided in two groups: Group A received 4mgkg^{-1} of tramadol IV as a single bolus, while group B received 1.5 mgkg^{-1} of tramadol IV as a single bolus followed by a tramadol constant rate infusion (CRI) at 2.6 mgkg^{-1}h^{-1}. The following parameters were recorded: heart rate, respiratory rate, blood pressure (systolic, diastolic, mean), body temperature, $EtCO_2$, SpO_2 and the concentration of inspired and expired isoflurane, they dose isoflurane to increase or decrease depending on the variations of: heart rate, respiratory rate, blood pressure, $EtCO_2$. Data were compared with two-way ANOVA for repeated measures followed by Bonferroni test $p < 0.05$.

The heart rate increased in both groups after administration of the tiletamine/zolazepam and acepromazine, however, after intubation and administration of tramadol, it decreased in both groups being the reduction significantly lower in group B p=0.000.The respiratory rate decreased after administration of the injectable anesthetics in both groups and remained constant during the entire surgery. The pressure remained within normal limits throughout, but in group B it is reduced more than basal p=0.000. The SPO_2 remained within physiological limits during the study 98/100%. The isoflurane inspired and expired levels were statistically lower in group B (2.3/2% VS 1.8/1.6%) p=0.000. CRI of tramadol decreases isoflurane requirements compared to tramadol administered as a bolus [1][2]. The presence of transient twitching was observed in individuals that received tramadol as a bolus while no side effects were observed in any of the animals belonging to group B.

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L-BUPIVACINE AND CIS-ATRACURIUM BESILATE WITH PERIBULBAR ANAESTHESIA IN FELINE OPHTHALMIC SURGERY

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The objective of the clinical trial study was to evaluate the effect of L-bupivacaine, local anaesthetic of last generation, in combination with cis-atracurim besilate, administered by peribulbar injection in ophthalmic surgery in cats. [1][2]
The study was approved by the University of Messina Review Board for Animals Care. Experiments were performed following Italian law (D.M. 116192), Europe law (O.J. of E.C. L 358/1 12/18/1986). and USA laws (Animal Welfare Assurance No A5594-01, Department of Health and Human Services, USA). Moreover, the owner of each cat was informed about the study and signed a consent. Forty cats were anesthetized for ophthalmic minor surgery with butorphanol 0.4mg/kg^{-1}, dexmedetomidine 15mcgkg^{-1} and midazolam 0.2 mgkg^{-1} IM and intubated to receive oxygen by mechanical ventilation. Group L received 2 mgkg^{-1} L-bupivacaine, administered by peribulbar injection. Group CL received the same dose of L-bupivacaine, combined with 0 1mg/kg^{-1} of cis-atracurium. Heart rate, *end-tidalCO_2*, oxygen saturation, non invasive blood pressure, intraocular pressure, neuromuscular activity and oculomotor activity: pupil horizontal diameter, eyeball exposure, palpebral akinesia eyeball centralization (for these last three parameters, we used a numerical scoring, ranging from zero to four) were measured. Also, the onset times (eyeball centralization, palpebral akinesia) and duration of the eye muscle motor block were measured. Results were analyzed using Friedman's test, 2 way ANOVA followed by Bonferroni and t-test. Significance was set at *P<0.05.* Physiological variables remained in the physiological range in both groups. Addition of cis-atracurium shortened the onset time of peribulbar anaesthesia (L/LC 4/8 minute-^{1}) *P<0.05*, and achieved a longer duration of complete motor block of the eye muscle (L/LC 60/ 70 minute-^{1}) *P<0.05*. Subjective scores were 3 (3-3) in Group L and 4 (4-4) in Group LC, *P<0.05.* Horizontal pupil diameter was: (L/LC 5±1/8±1mm) *P<0.05.* Intraocular pressure was (L/LC 14±3/12±1mmHg) *P<0.01.* General neuromuscular activity is 95%-100% in both groups No cat in Group LC required supplementary local anesthetic block, while two cats in Group L received an additional peribulbar anesthetic injection 0.5 mgkg^{-1} (the toxic dose of L bupivaciane in cats is 8mgkg $^{-1}$). A combination of cis-atracurium and L-bupivicaine used for peribulbar anesthesia provides effective akinesia of the eye, and shortens the onset of anaesthesia while prolonging the duration of local motor block, thus providing excellent conditions for ophthalmic surgery. [2][3]

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PULSE PRESSURE VARIATIONS AS A DYNAMIC PARAMETER TO PREDICT FLUID RESPONSIVENESS IN HEALTHY DOGS UNDER GENERAL ANESTHESIA

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Various studies have been conducted in human medicine regarding fluid challenge (FC) and its contribution to guide fluid therapy [1]. Traditionally FC is performed using 500 ml of a crystalloid solution and its effectiveness is commonly intended as a cardiac output (CO) improvement ≥15 % respect to baseline. The predicting value of different indexes, static (CVP, PAOP, DO2:VO2) and dynamic (SVP, PPV, SVV) has been studied in order to identify the most accurate. Dynamic parameters allow a real time monitoring thus a continuous flow of information that guides decision making during fluid therapy. A specific cut off value has been obtained in human medicine for pulse pressure variations (PPV) 13% [1]. The scope of this study was to identify a PPV cut off value for predicting fluid responsiveness in dogs under general anesthesia and mechanically ventilated. After the catheterization of a metatarsal artery 15 patients were connected to a dedicated monitor for the measurement of the hemodynamic parameters by means of the pressure recording analytical method (PRAM, MostCare, Vygon Italy). Heart rate (HR), mean arterial pressure (MAP), CO e PPV were recorded before (baseline) and at 5 (t5), 10 (t10) and 15 (t15) minutes after the administration of 5ml/kg ringer lactate solution over 5 minutes. The effects of the fluid challenge on the hemodynamic parameters were assessed using a non parametric Wilcoxon test. Patients were divided into 2 groups according to the percent increase in CO in response to FC. Patients with a CO increase ≥15% were classified as responders (R) whereas all the others as non responders (NR). The comparison of hemodynamic parameters before FC in R and NR patients was assessed using the Mann-Whitney U test. Receiver operating curve (ROC) was generated for PPV, varying the discriminating threshold. A p value less than 0.05 was considered statistically significant. The mean ± sd of HR, MAP, CO and PPV in all the patients at the baseline was 83.71 ± 17.94, 74.28 ± 17.13, 2.53 ± 1.14, 14.48 ± 8.46 respectively. The same parameters post FC were 88.57 ± 15.03, 78.77 ± 16.68, 2.89 ± 1.38, 13.05 ± 7.42 respectively. Out of the 15 patients, 10 were R (66.6%) and 5 NR (33.3%). A PPV cut off value of 6.2% was obtained. The PPV at baseline was significantly higher in R compared to NR. Within R, CO and MAP increased while PPV decreased significantly after FC. These preliminary data indicate PPV as a possible predicting dynamic index to guide fluid therapy in dogs under general anesthesia and mechanically ventilated. The cut off value to discriminate R and NR was 6.2%. Further studies with a larger population are required to confirm these results.

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USE OF PULSE OXYMETRY AT ROOM AIR FOR THE DETERMINATION OF OXYGENATION IN THE POSTOPERATIVE PERIOD IN DOGS

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The gold-standard for the assessment of oxygenation is arterial blood gas analysis.Despite its high validity, repeated arterial samples collection in small animals is more challenging and invasive [1].Pulse oximetry(PO) allows non invasive and continuous estimation of arterial hemoglobin oxygen saturation(SpO2) [2]. Despite the widespread use during general anesthesia, PO is less employed in the postoperative period due to technical problems but also for the lack of adequate prospective clinical studies in small animal patients. The aim of this study was to evaluate the feasibility and efficacy of PO in the postoperative period in dogs as a surrogate of blood gas analysis. For this purpose, 50 healthy dogs recovering from general anesthesia were included in the study. At least 5 minutes after the extubation, with patients breathing room air (FiO2 0.21), SpO2 was recorded (Masimo Rad–87, SET Rainbow) after at least 2 minutes of reading and only if a good quality signal was obtained. In case of poor signal the probe was replaced. At the time of SpO2 recoding an arterial blood sample was collected for the measurement of PaO2 and PaCO2.PaO2 to FiO2 ratio(PF),SpO2 to FiO2 ratio (SF) and the alveolar-arterial oxygen gradient [P(A-a)O2] were calculated. Based on the PaO2 value, patients were divided into three groups: normoxiemic (PaO2>80 mmHg), moderately hypoxemic(PaO2 60-80 mmHg) and severely hypoxemic(PaO2<60 mmHg).Based on the groups of oxygenation the corresponding range of values of SpO2 was computed. The correlation between PaO2 and SpO2 and between PF and SF was evaluated with Pearson test(r).Moreover, for the same values, the linear regression was evaluated and the determination index(r2) was calculated. Receiver operating characteristic (ROC) curve was generated for SpO2 and the discriminating threshold for each category was determined. A p value less than 0.05 was considered statistically significant. 40 patients (80%) were normoxiemic (PaO2>80mmHg;112-82mmHg) corresponding to SpO2 100-90%, SF 476-428 and PF 533-390.The remaining 10 patients were moderately hypoxemic (PaO2 80-60 mmHg;79-62 mmHg) corresponding to SpO2 between 95-85%, SF 404-352 and PF 376-259. A strong(r>0.7) and significative (P<0.05) correlation was obtained between PaO2 and SpO2 and between PF and SF(r^2 0.6). The threshold SpO2 value of 94 allowed discrimination between normoxiemic and moderately hypoxiemic patients with a sensitivity of 65% and a specificity of 90%.The results of this study demonstrated that at room air a cut off value of 94% of SpO2 should be used to discriminate patients with a PaO2 below 80mmHg. Moreover the good correlation between PaO2 and SpO2 indicate that PO could be a useful continuous monitoring for postoperative oxygenation in order to promptly detect and treat hypoxemia.

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DOUBLING TIME, METABOLIC ACTIVITY AND MIGRATING NATURE STUDIED IN DIFFERENT AGES HORSES' FAT-DERIVED MSC CELLS AND THE INFLUENCE OF IFNγ, IL-1 AND TNFα

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In recent years, it has become clear that MSCs can play an important role in tendon regeneration. In this study we used MSCs recovered from three different ages horses and derived from adipose tissue (AMSCs); The aim of the study was to characterize them to find out possible differences linked to the age of the animals subjected to the experimental study. As the first step, we carried out three assays to study their doubling time, metabolic activity and migrating nature. Furthermore, it has been performed a second step, including the expansion of the same cells with the presence of three cytokines: IFNγ, IL-1 and TNFα, in order to investigate their possible influence on the cell's growth. We used the cytokines both one by one and in their triple combination, with different concentrations, by performing the same three assays mentioned before.

AMSCs from the youngest horse displayed the shortest doubling time and the fastest speed of migrating, revealing, in the same time, the best cellularity and morphology too. Concerning the metabolic activity, no clear differences have been demonstrated.

AMSCs with TNFα and IL-1, had the shortest doubling time. Samples with IFNγ clearly displayed the slowest speed in migrating, while the samples with TNFα showed the fastest one. Finally, the metabolic activity of AMSCs with IL-1 and TNFα has been highlighted as the best, as opposed to IFNγ that seemed to negatively influence it.

These findings disclosed that fat-derived MSCs from younger horses could have better performances in tendon injuries treatment pathway and the presence of some cytokines as TNFα and IL-1 could influence their doubling time, and migrating nature.

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ALFAXALONE MIDAZOLAM OR TILETAMINE ZOLAZEPAM FOR SHORT TERM ANAESTHESIA IN WILD BOAR. PRELIMINARY RESULTS

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Objective: To compare induction, quality of sedation, recovery after antagonization in wild boar after alfaxalone/midazolam or tiletamina/zolazepam intra muscular anaesthesia for short term procedure and physiologic parameters during anaesthesia.
Study design: Prospective 'blinded' randomized, clinical study.
Animals: Twelve, healthy wild boars.
Methods: Twelve adults Wild boars, anesthetized for sanitary checks and genetic evaluations, were assigned randomly into two groups: Group Alfa, anaesthesia was induced with an intramuscular combination of alfaxalone (2.5 mg $kg^{-}1$) and midazolam (0.4 mg $kg^{-}1$); Group Zol, anaesthesia was induced with tiletamine/zolazepam (2.5 mg $kg^{-}1$). A combination of azaperone (2 mg $kg^{-}1$), detomidine (0.5 mg $kg^{-}1$) and methadone (0.2 mg $kg^{-}1$) mixed in the same syringe with alfaxalone/midazolam or tiletamine/zolazepam was administered as constant in both groups. Physiological data as heart rate (Hr), respiratory rate (Rr), invasive blood pressure (Mean, Diastolic ad Systolic in mmHg), SpO_2 and body temperature (T° in C°) were collected after 10 minutes from reaching the lateral recumbency, five times every 10 minutes (T0, T1, T2, T3, T4). In the same periods, degree of sedation and the quality of sedation were scored and reflexes evaluated. After one hour from lateral recumbency, intravenous atipamezole (0.12 mg kg−1) and flumazenil (0.01 mg $kg^{-}1$) were administered and the quality of antagonization were assessed. Times of induction, from drugs administration to ataxia, sternal recumbency and lateral recumbency and time of recovery, from antagonization to sternal recumbency, standing position and standing without ataxia, were recorded. All variables were tested for normality via Shapiro-Wilk test. Parametric distributed data reported as mean and standard deviation (SD). Student's t test was performed to detect significant differences between groups. p-value <0.05 was considered statistically significant.
Results: No statistically significant differences in physiological variables were observed between groups with the exception of Rr that was statistically significant higher at every time recorded and the IBP that was statistically significant higher at T1, T2, T3, T4 in the Zol group. The sedation score was higher at T0 in the alfa group and higher at T2, T3, T4 in the zol group. The time of induction was statistically significant shorter in the alfa group. Other statistically significant differences were not observed.
Conclusion: and clinical relevance: Induction of anaesthesia with intramuscular administration of alfaxalone/midazolam was faster, provided better quality of sedation in the first phase and better ventilatory status; however, the sedation scores change during the procedure in favour of zol group. No difference were found between protocols after antagonization. Both protocols would appear appropriate for induction of anaesthesia for short procedures in wild boars. Alfaxalone is suitable for anaesthesia in wild boar allowing rapid induction and good quality of sedation.

Trap-effectiveness and response to tiletamine-zolazepam and medetomidine anaesthesia in Eurasian wild boar captured with cage and corral traps. Barasona JA et al BMC Vet Res. 2013 May 23;9:107

AQUAPORIN 1 (AQP1) EXPRESSION IN DOG TEARS: A PRELIMINARY STUDY

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Aquaporins (AQPs), a group of water channel proteins well characterized for their physiological role related to water transport activity in many tissues, have been recently identified in different compartments of the eye in humans, mice, rats, rabbits, cows and dogs [1]. Although studies of the most prevalent ocular AQPs indicate important roles in maintaining water balance in ocular structures, the functions of many AQPs in ocular tissues remain actually unknown [1]. Particularly, only few studies have focused on the role of AQPs in lacrimal gland fluid secretion [2]. To our knowledge, a study on the expression of AQPs in canine tears has not yet been performed. The aim of this study was to investigate the AQP1 expression in healthy dog tears fluid by using two different methods for tear sampling. Eleven healthy dogs were included. All dogs underwent complete clinical and ophthalmic examination including slit lamp biomicroscopy, Shirmer Tear Test (STT), applanation tonometry, break up time (BUT), and fluorescein and lyssamin green stain before their inclusion in this study. Blood was collected for complete blood count and biochemistry. Tear fluid was collected by a standardized Shirmer Tear Strip (STS) (Schirmer-Plus® GECIS) or by ophthalmic sponges (OS) (bvi Merocel® Beayer-Visitec International, Inc). Dogs were randomly allocated to receive tears collection STS in one eye and OS in the other one. Tears collected in eppendorff tubes were centrifuged according the method adopted by Chen et al., (2011) [3] to obtain tear fluid proteins without gross debris and mucus. Tear samples containing equal amount of total proteins were electrophoretically separated (acrylamide gel 13%) and proteins transferred on nitrocellulose filter. AQP1 expression was determined by western blot analysis according to the method previously described [4].
Results showed that AQP1 was expressed in all examined tear samples, collected with the two methods. In particular, its expression was demonstrated by the presence of multiple bands localized either at AQP1 expected molecular weight corresponding to 28 kDa and other bands (35-60 kDa) compatible with AQP1 glycosylated forms. Moreover, Spearman correlation showed a different correlation between tear volume collected and total protein concentration in consideration of different methods used (STS=0.18 - OS R=0.69).
This study represents the first report of AQP1 expression in dog tears. The results suggest specific roles for AQP1 in water transport in lacrimal gland fluid secretion which need more investigations. The methods of sampling and extraction we describe may be useful in clinical studies to assess AQP1 profiles evaluation in ocular surfaces diseases. Further studies are needed to clarify AQPs functions in canine ocular tissues, as well their implication in ophthalmic diseases and to obtain more opportunities for targeting new therapies to AQPs expression.

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BRONCHOALVEOLAR LAVAGE IN DONKEY: DIAGNOSTIC PROCEDURES AND CLINICAL FINDINGS

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Donkeys and horses usually share similar respiratory problems. Nevertheless, donkeys developed some anatomical differences with horses mainly related to upper airways [1] allowing to reduce their susceptibility to respiratory distresses as well as clinical signs severity [2]. For early diagnosis, the bronchoalveolar lavage (BAL) could represent an useful diagnostic procedure although the technique was never described before in these animals.

The goals of the present investigation were to describe for the first time BAL procedures in healthy donkeys and to evaluate the related clinical findings.

Nine, healthy, large size, female donkeys, with means (±SD) body weight of 338±61kg, wither height of 135.8±6.6cm and thoracic girth of 150.7±10.2cm were enrolled. All of them were submitted to complete clinical examinations (with particular focus on the cardio-respiratory system) and video-endoscopy. After sedation, based on acepromazine maleate 1% (0.025mg/kg IV) and Xylazine hydrochloride 20% (0.8 mg/kg IV), BAL was performed using the "blind technique" and an administration of 120 ml of Ringer lactate at 37°C. At the end of the procedure, the fluid collected was submitted for macroscopic and microscopic evaluation and differential cells count [3]. Blood and parasitological analysis were also performed.

BAL was easily performed in all the subjects. Several epithelial cells (ciliated cells of various type and goblet cells, ranged: 11.2 33%; means±SD: 49.55±14.48), some macrophages (21- 46.50%; 71.66±16.31), few neutrophils (0.5-23.5%; 19.33±18.78), rare lymphocytes (12-47%, 55,44±15,54), and very rare eosinophils (0-5.25%, 4±3,9) were detected.

The technique required analogous level of animal handling reported for horses and no obvious difficulties have been observed. Nevertheless, because of the anatomical differences of the upper airways between the two species [1,4], a good extension of the head (until to create a straight line with the spine) has been necessary both to facilitate the procedure and to use catheters usually employed for horses otherwise too long for donkeys.

Blood investigations revealed normal values in all the subjects while coprological examination were positive for gastrointestinal strongyles in all them, although no larvae in the alveolar bronchial fluid were detected.

BAL's samples allowed to perform a differential count of the cells present in the lower airways of the animals. The means cellular values observed in healthy donkey were significant different from those one described for healthy horses [3]. The difference observed may be related to different specie-specific inflammatory response mechanisms between the two species although further investigation are warranted to confirm this hypothesis.

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IMPING IN BIRDS OF PREY

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Within a Wildlife Rescue and Rehabilitation Centre (CRAS) it is of great importance to preserve a good plumage status of hospitalized birds. Feather condition is as critical during the course of release for birds as is the ability to fly. Any type of damage to the feather structure will interfere with bird's ability to fly, waterproof, and thermoregulate. Thus, birds with compromised feather condition have a low survival ratio following release [1].

When injuries (breakage, bending, wear) affect a relatively small number of feathers (maximum 4 for the wings and 4 for the tail), it is advisable to implement the practice of impinging.

Imping is the process of re-attaching donor feathers onto a bird's wings by the use of small splints/pins inserted into the hollow shaft of the bird's main flight feathers (primaries and secondaries) or tail feathers [2]. It restores flight immediately and is also invaluable in preventing broken blood feathers during a clipped bird's moulting period. Imped feathers will of course also be moulted out and replaced eventually, as though they were the bird's normal feathers as the process doesn't involve neither the calamus, the hollow basal portion of the feather, nor the implant site of the receiving feather.

In this report, ten diurnal birds of prey *consisting* of buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*) and peregrine falcon (*Falco peregrinus*) were submitted to the imping technique. Birds were hospitalized at the CRAS for sequestration, anthropic causes, traumatic events, gunshot wounds, and showed plumage injuries.

After practicing the imping, these birds were examined by using a flight test in a rehabilitation aviary within the CRAS. Both the flight performance and the stability of the imped feather were successful and the immediate release in nature was planned. In fact a prolonged detention during this phase could increase the possibility of damaging the plumage condition, because of any repeated traumas against the nets that delineate aviaries.

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REAC TECHNOLOGY AND EXPERIMENTAL CHONDRAL LESIONS. PRELIMINARY RESULTS IN OVINE ANIMAL MODEL

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Radio Electric Asymmetric Conveyer (REAC) Technology for therapeutic use is a recent technology that utilizes radio electric fields asymmetrically conveyed to induce ionic fluxes and bipolarity optimization in biological structures, for specific bio modulation effects. In previous works, we highlighted that REAC technology decreased nitric oxide and metalloproteinase-3 production in normal and OA chondrocytes, while inducing an increase in proteoglycan synthesis. In most cases, after a traumatic event that cause a cartilage damage, the regenerated tissue shows histological characteristics referable to fibrocartilage with lower biomechanical capacity and potential degenerative events source. In this preliminary experimental work were used four Sardinian race adult females sheep. All subjects underwent a mini arthrotomy of the respective left and right knees articulation, creating an experimental lesion at the level of the medial condyle of each subject. The purpose of our research was to test in ovine animal model, the REAC technology, to healing experimental chondral lesions with particular reference to clinical, biomechanical, histological, immunohistochemical and ultrastructural regenerated tissue and its integration with nearby tissues. The results obtained in this preliminary study seem encouraging, both in terms of quantity and quality and discloses the possibility that REAC technology may be a valuable therapeutic tool in the treatment of cartilage damages.

CONVENTIONAL VERSUS INNOVATIVE THERAPIES TO TREAT SKIN WOUNDS IN VETERINARY MEDICINE

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Wound healing is a complex and dynamic process that begins in response to injury in order to restore the functions and the structure of damaged tissues. The loss of skin integrity may induce important dysfunctions or even death. Particularly, in large wounds, like third and fourth degree burns, chronic wound or deep ulcers, the restitutio ad integrum is hardly achieved and fibrosis and/or scar tissue may develop. The aim of our study is to compare the effectiveness of different topical conventional (hyaluronic acid, Manuka honey, acemannan gel) and innovative (ionized gas plasma, allogeneic mesenchymal stem cells isolated from peripheral blood PB-MSCs) treatments in the process of wound healing of skin lesions, experimentally induced in six sheep. The evaluation is based on clinical, molecular, histological and immunohistochemical analyses. Six 4x4 cm wounds were surgically created on the back of six healthy adult sheep and every single wound was randomly destined to one of the five treatments and to placebo (saline solution). Every week, for five weeks, wounds were clinically evaluated considering different parameters and photographed to evalute the lesions through the use of the software ImageJ©. Tissue biopsies were obtained with a surgical punch (0.6 x 0.6 cm) at time T0, T15 and T42 days from the operation to allow histological and immunohistochemical analyses. Histological examinations considered ulceration, re-epithelization, extension of granulation tissue, superficial and deep inflammation, fibrous tissue and adnexa formation. Immunoresponse, vascularization, and cell proliferation were studied by immunohistochemistry using respectively CD3, CD20, MHCII, von Willebrand factor (vWF) and KI67 antibodies. Real time-PCR investigated the genes expression level of Vascular endothelial growth factors (VEGF), Transforming growth factor beta 1 (TGFβ1), Vimentin, Collagen 1α1 and hair Keratin. Clinical, molecular and histologic assay indicated analogous results. For instance, VEGF mRNA and vWF protein expression confirmed angiogenetic events observed by histological analysis; while TGF-β1 mRNA and CD3/CD20 protein expression indicated the similar presence/absence of inflammation in the used treatments compare to histological evaluation. Innovative therapies led to surprising results regarding regeneration of sheep skin. Ionized gas plasma was effictive treatment and caused a faster wound healing associated to physiological and positive clinical parameters of the wounds. PB-MSCs stimulated mature granulation tissue and skin adnexa development. Finally, among the conventional treatments, hyaluronic acid, only promoted a correct wound healing. Further examinations are ongoing in order to elucidate possible mechanisms explaining the differences observed.

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COMPARISON OF THE ANALGESIC EFFECTS OF MELOXICAM AND ROBENACOXIB IN DOGS AFTER LAPAROSCOPIC SURGERY

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Prophylactic gastropexy prevents a first episode of gastric-dilatation volvulus (GDV) in dogs. Prophylactic gastropexy is often combined with elective ovariectomy in large breed dogs [1]. Pain after laparoscopy depends on surgical procedure, number of instrument cannulas, surgical time and type of gas [2]. Robenacoxib and meloxicam are frequently used for the control of post-operative pain in dogs [3]. Meloxicam has a long duration of action while robenacoxib is cleared rapidly from the central body compartment, but it has a high concentration and long persistence in inflammatory sites [4].
The aim of this study is to compare the efficacy of robenacoxib with that of meloxicam in controlling post-operative pain during the first 24 hours after combined laparoscopic ovariectomy and laparoscopic-assisted gastropexy in dogs.
Twenty-six client-owned dogs undergoing combined laparoscopic ovariectomy and laparoscopic-assisted gastropexy [1] were randomly divided into 2 groups: 13 dogs received robenacoxib 2 mg/kg and 13 dogs received meloxicam 0.2 mg/kg subcutaneously before induction of anesthesia. The degree of pain was assessed by Glasgow pain scale (short form) [5] before surgery and 1, 6, 12, 18 and 24 hours after extubation. All pain measurements were performed by the same experienced individual, blinded to treatment. If Glasgow pain score (GPS) was ≥5, dogs received tramadol 3 mg/kg as rescue analgesia. Data were submitted to ANOVA analysis with treatment, class-age and time of surgery as fixed factors.
Robenacoxib group had a significantly higher GPS (2.18±0.29) than meloxicam group (0.68±0.41) at 24 hours after extubation. Two dogs in meloxicam group and 7 dogs in robenacoxib group required rescue analgesia. GPS was significantly lower at 18 and 24 hours after extubation in the dogs in which surgery time was > 40 minutes compared to those with surgery time was ≤ 40 minutes. Data were re-submitted to ANOVA analysis after the exclusion of dogs which received tramadol in order to avoid the influence of tramadol on GPS. Robenacoxib group had a significantly higher GPS than meloxicam group at 12, 18 and 24 hours after extubation. GPS was significantly lower at 24 hours after surgery in the dogs in which surgery time was >40 minutes. There was no significant difference between class-age.
Preoperative administration of meloxicam or robenacoxib ensures an effective postoperative analgesia. Results point out that meloxicam produces a lower GPS compared to robenacoxib 24 hours after extubation and that the analgesic effect of robenacoxib is better when it is combined with tramadol.

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EVIDENCE SUGGEST THAT OSTEOCLASTS IN SUBCHONDRAL BONE DEGRADE OVERLYING CARTILAGE IN NATURALLY OCCURRED EQUINE OSTEOARTHRITIS

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Equine osteoarthritis (OA) is a degenerative joint disease caused by repeated trauma and is characterised by loss of articular cartilage and subchondral bone remodelling, but cross talk between these adjacent tissues remains poorly understood. Our recent investigation of third carpal bone OA in racehorses revealed that increased osteoclasts (OCs) number in the subchondral bone and a reduction in bone mineral density was correlated to the severity of overlying cartilage pathology [1]. OCs contributing to subchondral bone loss may also have a role in overlying cartilage degradation via the local release of cathepsin K (CK), a cysteine protease causing breakdown of the cartilage collagen network, and exposing at the cleavage site the neoepitope C2K. We hypothesize that there is a biological inter-play between the subchondral bone and cartilage in spontaneous equine OA mediated by OCs, similar to that recently described in experimental rheumatoid arthritis [2].

Our objectives were to demonstrate CK mediated cartilage degradation in naturally occurred equine carpal bone OA and to relate overlying cartilage immunohistochemical changes to OCs density in the subchondral bone. Osteochondral cores were harvested from third carpal bones, with varying degrees of intercarpal joint OA, of 15 racehorses from an abattoir. Cores were decalcified, embedded in paraffin and 5 µm sections were stained with HPS, for cellular and morphologic evaluation, and Safranin O-Fast Green for cartilage histology. Immunohistochemical localization of CK and C2K in the tissues was performed to identify OCs and to score theirs expression in the cartilage. Tissues were also processed for receptor activator of nuclear factor kappa-B ligand (RANKL) expression, a member of the TNF cytokine ligand superfamily, essential for OCs differentiation. To complete the analysis, images of the osteochondral sections were captured with a digital slide scanner at 200x view and processed. Each osteochondral section was then divided digitally into 1-mm-width and 3-mm-depth (subchondral bone) regions of interest (ROI). An OCs count was performed in each ROI (n=120) and a modified Mankin scoring system was employed to assess cartilage structural changes. In the calcified cartilage, microcracks number/mm was assessed. CK, C2K, and RANKL expression in hyaline cartilage were assessed semi-quantitatively by expanding a score for human hyaline cartilage. OCs count was associated with histological cartilage scores and immunohistochemical expression of CK, C2K, RANKL employing a linear model with the horse Id as a random effect. The OC counts in ROIs were positively correlated to the cartilage degeneration scores ($p<0.001$). A focal increased of the OC density is co-localized with the highest inter-individual cartilage score ROIs ($p=0.002$). The OC counts were highly correlated to microcrack number/mm ($p<0.001$), cartilage expression of CK, C2K and RANKL expression ($p<0.001$). Taken together these results suggest that OCs are major players in OA progression and also contribute to calcified and hyaline cartilage degeneration in OA.

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OUTCOME OF 42 MINI-INVASIVE ASSISTED GASTROPEXY

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Prophylactic or therapeutic gastropexy is the only demonstrable way to prevent the occurrence of an acute, life threatening condition known as gastric dilation and volvulus (GDV) in large or giant dogs.The objective of this study was to determine the short- and long-term outcome of endoscopically assisted gastropexy, that seems to be a suitable alternative to open incisional and belt loop gastropexies, cheaper than other mini-invasive procedure and useful for early diagnosis of chronic gastropaty and enteropaty because of the use of gastroscopic vision [1,2,3]. Forty-two healthy dogs, 29 of them great dane, weighting 25 to 88 kg (> 52 for the great dane), over 15 months old, underwent an endoscopic-assisted gastropexy procedure. Dogs were evaluated with abdominal ultrasonography to assess integrity of the gastropexy at 2 month after surgery and 6 to 12 months the long-term outcome was determined via telephone conversations conducted with owners. Mean ± SD surgical time was 19±6 minutes and mean ± SD gastropexy length was 3,2±0.4 cm. Three foreign bodies were found in 2 dogs (2 latex gloves and 1 ball, that underwent gastroscopic removal). Ten dogs showed signs of past gastritis of varying severity. From the second surgery, we modified the technique not positioning the tension suture [3], in order to prevent spleen perforation and to optimize the site of gastropexy twisting the stomach with Babcock forceps through paracostal trans-abdominal approach. Short term follow-up ultrasonography confirms the complete adhesion of the stomach to the abdominal wall at the correct site of gastropexy. Long-term telephonic follow-up was available for 39 dogs, with 1 failure of the technique that resulted in GDV (first dog that underwent classic technique with tension suture) and 38 none of which had any episodes of GDV or dilatation.
According to literature [1,2,3,4] endoscopic-assisted gastropexy truly results mini-invasive in many different ways and can be simple, with short anesthesiology duration, with minimal surgical field exposure, with low-cost instrumentation and reliable method for performing prophylactic gastropexy in dogs [4]. It also allow to diagnosis entero or gastropathy even in absence of clinical evidence [1].

[1] Melanie P. et al Mini-invasive endoscopic-assisted gastropexy for dogs at risk of gastric dilatation-volvulus LXVIII Convegno Nazionale SISV, Convegno SICV Pisa Giugno 2014. [2] Tavakoli a. et al The superiority of paracostal endoscopic-assisted gastropexy over open incisional and belt loop gastropexy in dogs: a comparison of three prophylactic techniques.Iran J Vet Res. 2016. [3] Dujowich M. et al, Evaluation of an endoscopically assisted gastropexy technique in dogs. Am J Vet Res. 2008. [4] Dujowich M. et al Evaluation of short- and long-term complications after endoscopically assisted gastropexy in dogs. J Am Vet Med Assoc. 2010.

REGENERATIVE THERAPY IN CATS. TWO CASES

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In literature, we can find articles about regenerative therapy in horses and dogs, less concerning regenerative therapy for cats. What can we do when we have a cat candidate for a possible regenerative therapy? In case of acute or chronic skin wounds in cats, the answer vary depending of the type of wound, localisation, acute or chronic timing, feline viral disease and owner's compliance. The patients for regenerative therapy need to be selected with criteria. The owners must be informed that the regenerative therapy is not a miracle solution, that we need their collaboration and that sometimes the regenerative therapy cannot produce the expected results. The aim of this article is to open a discussion concerning the difference between cats and dogs as patients and the importance to choose the right type of patient and owner to perform this therapy. We are presenting to you two similar cases, 2 cats (cat A and B), both with chronic, infected skin wound. The wound is open and present for one year. The cat A and B were candidates for regenerative therapy because of luck of integumentary tissue for a successful reconstructive surgery. The therapy for the two patients consisted in curettage of the wound, antibiogram and antibiotics therapy, FIV and FeLV tests and application of Platelet-Rich Plasma (PRP) + heterologous Mesenchymal Stem Cells (MSCs). The cat A was an ideal patient, good character and excellent compliance of the owner FIV and FeLV negative. The cat B was difficult to manage, wary and FIV positive and FeLV negative. The complete healing of the wound in cat A was obtained in 30 days, with perfect closure of the wound. The cat B presented many complications and the complete closure of the wound was obtained in 330 days. The two patients underwent to the same therapy and procedures, but the complete closure of the wound was different. Concerning the cat A, the regenerative therapy was the perfect solution. The regenerative therapy for cat B did not worked so well because of the complications: immunosuppression (FIV+), stress, the lack of compliance of the owner and the reaction of the surgical sutures. The healing of the wound depends on regenerative potential of the each patient and it decreases with the complication that can occur. Are we abusing with regenerative therapy? In cats, is it really working? We need some guidelines, inclusion criteria and the perfect owner to deal with. In this way, we can chose the right patients and the regenerative therapy can be the right solution, not the miracle solution that the owners are looking for.

[1] Regenerative therapy for the management of a large skin wound in a dog",Elena Zubin, Virna Conti, Fabio Leonardi, Stefano Zanichelli, Roberto Ramoni, and Stefano Grolli; clinical case report; Whiley publication 2015. [2] Regenerative therapy for the management of a large skin wounds in 3 dogs, Zubin E., Leonardi F., Grolli S., Conti V. and Zanichelli S. Sisvet Perugia 2015. [3] Pavletic, M.M., (3°edition) 2010. Atlas of Small Animal Wound Management and Reconstructive Surgery. Wiley Blackwell. [4] Williams, J.M., Moores, A., (2° edition) 2009. BSAVA Manual of Canine and Feline Wound Management and Reconstruction. Wiley Blackwell

DETERMINATION OF MINIMUM ALVEOLAR CONCENTRATION (MAC) AND CARDIOPULMONARY EFFECTS OF DESFLURANE IN MECHANICALLY VENTILATED SHEEP

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Aim of the study: To determine the minimum alveolar concentration (MAC) and cardiopulmonary effects of desflurane (Des) in mechanically ventilated sheep.
Material & Methods: Prospective study. The study was approved by local ethical committee (CIBASA prot. n. 22859 del 3-07-2012) Ten healthy adult female Sardinian bred sheep were selected. Anaesthesia was induced with desflurane delivered in oxygen (3 l/min) through a mask with the vaporizer set at 18%. An endotracheal tube was inserted, and a probe for continuous measurement of end-tidal and inspired desflurane concentrations was placed in the trachea via the endotracheal tube. After 30 minutes of equilibration at an end-tidal desflurane concentration of 10.5%, an electrical stimulus [5Hz /1 msec (impulse duration) / 50.0 mA] was applied to the skin of the lateral side of the right antebrachium for one minute or until a gross purposeful movement response was obtained. The vaporizer dial setting was changed in order to obtain an increase or decrease by 0.2% in end-tidal desflurane concentration, dependent upon whether a motor response had been elicited by noxious (electrical) stimulation or not. Following a15-minute equilibration period the noxious stimulation was repeated. The MAC was defined as the mean of the lowest end-tidal desflurane concentration that prevented a positive motor response and the highest concentration that allowed a positive motor response and determined always twice. Physiological parameters, heart rate (Hr), respiratory rate (Rr), invasive blood pressure (Mean, Diastolic ad Systolic in mmHg), and body temperature (T° in C°) were collected at baseline before induction, after induction, first MAC determination and at second MAC determination. Once MAC Des was determined, the delivered concentration of Des was then increased to achieve end-tidal concentrations corresponding at 1.3 MAC and 1.6 MAC and after 15-min of equilibration period the same physiologic parameters were also collected. Time to induction of anaesthesia, time apnoea (i.e restart of spontaneous breathing) time to extubation and time for recovery (i.e. time to standing up) were recorded. All data were analysed by the Wilcoxon Sign rank test for distribution of non-parametric data, the Spearman rank test was performed for the correlation between variables, *p-value* <0.05 was considered statistically significant.
Results: The mean± SD of the MAC of desflurane was 9.8 ± 0.8%. Time to intubation was 288±132 seconds, time apnoea was 242± 145 seconds, time to extubation was 846 ±243 seconds and time to recovery was 1952±748 seconds. The Des, statistically significant affect arterial pressure in every time recorded. Significant difference in other parameters were not observed
Conclusions: Desflurane administration in sheep allows for a smooth induction of anaesthesia and fast extubation and smooth recovery from anaesthesia. Desflurane concentrations between MAC and 1.6 MAC reduces arterial pressure and not affect HR or in sheep

Effects of oxymorphone hydrochloride or hydromorphone hydrochloride on minimal alveolar concentration of desflurane in sheep. Sayre RS et aL Am J Vet Res. 2015 Jul;76(7):583-90

COMPUTED TOMOGRAPHIC FEATURES OF SCLEROSIS AND FOCAL LESIONS OF THE EQUINE DISTAL TARSAL BONES: COMPARISON WITH RADIOGRAPHY

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Tarsal pain is a common cause of hindlimb lameness; however, the severity of radiographic changes is not well correlated with the degree of lameness [1]. Computed tomography (CT) is a cross-sectional technique that has been demonstrated to show degenerative changes of the distal tarsal joints in the absence of radiographic abnormalities or inconclusive findings [2]; however, blinded study are missing. The aim of this study was to compare radiography and CT for the identification of sclerosis and bone lysis of the distal tarsal bones. The tarsi of 16 horses, submitted to euthanasia for reason unrelated to this study, were included; the study was performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Perugia. Limbs were cut at the level of the middle aspect of the tibia and imaged within 6 hours from the death. Radiographic examination was performed using a CR system and the 4 standard projections (Dorsoplantar, Lateromedial, Dorso45°lateral-Plantaromedial Oblique, Dorso45°medial-Plantarolateral Oblique). The CT scans were acquired with a helical CT scanner with the limb positioned mimicking the left lateral recumbency to obtain transverse slices with the metatarsus parallel to the table. Acquisition variables were 120 kV, 100 mA, a slice thickness of 1 mm and a 20 cm field of view; images were reformatted as 1 mm thick using a B70f high and B40f medium kernel and displayed in bone and soft tissue windows. Radiographic and CT images were reviewed by two different clinicians, in a blinded fashion, focusing on the distal tarsal bones of the tarsi. Clinicians were asked to determine the presence and location of sclerosis and focal bone lysis. McNemar test was used to compare radiography and CT for both parameters. Significance was set at $p<0.05$. Sensitivity (Se) and specificity (Sp) of radiography were calculated using CT as a gold standard. On the CT studies, 17/32 tarsi had sclerosis of the distal tarsal bones, most commonly the central tarsal bone (CTB) and the third tarsal bone (T3) and 15/32 had focal bone lysis of the CTB and/or T3 and/or fourth tarsal bone (T4). On the radiographic studies, 9/32 tarsi had sclerosis of the tarsal bones (Se:41%; Sp:87%), most commonly the CTB, and 7/32 had focal bone lysis (Se:40%; Sp:94%) of the CTB and/or T3. There was a significant difference between CT and radiography for detection of sclerosis of the distal tarsal bones ($p<0.05$) and of focal bone lysis ($p<0.05$). Radiography has a low sensitivity for detection of bone sclerosis and lysis and has been demonstrated to be unreliable to identify these abnormalities. CT can be considered a valuable technique in horses with positive diagnostic analgesia of the tarsometatarsal or centrotarsal joint without significant radiographic findings.

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NERVE-REGENERATION IN DISRUPTIVE BRACHIAL PLEXUS LESIONS: A NOVEL SURGICAL APPROACH

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Partial or total disruption of brachial plexus (BP) due to an external mechanical agent (fractured bone ends; penetrating wounds; gun-shot wounds) is a severe occurrence. In less disruptive injuries standard techniques can apply, like direct repair; autograft; allograft or nerve-guides (conduits). However, when a large or complete disruption of the BP occurs, the surgeon may abstain from an immediate treatment, then, proposing reconstructive surgery (nerve or tendon transfers, for example). We designed and implanted a device (called NeuroBox) aimed at protecting the whole brachial plexus as it was a single peripheral nerve. The NeuroBox concept was actually tested successfully in the Rat sciatic nerve in the past. After accessing three different animal models, Rat, Rabbit, Sheep, the last was selected as a translational model for Humans. Eight sardinian old adult female ewes (2.5-5 years old, body weight 39.5±7.7 kg) were implanted after a lesion was produced which consisted of the transection of the BP fibers at the level of the cords, leaving only those directed to the ulnar nerve intact. Successive refinements in the design resulted in a double-halved device with a trapezoidal shape. After surgery, all the animals were unable to move their left anterior limb, then a gradual clinical recovery occurred. At two months, an initial functional recovery was observed. At 32 weeks a functional recovery was observed in one animal which was, then, sacrificed. Retrievals at 10 and 32 weeks showed phases of regeneration (blood clot; initial fibrin phase; late fibrin phase). The retrieval at 32 weeks showed absence of scar tissue and presence of nerve fibers between stump. This proposed new engineered surgical approach is aimed at the early treatment of large disruptive lesions of the BP. Its experimental testing in the sheep is giving clues for human translation thanks to the dimensions of the plexus which is similar in both species. The engineered device may offer a way to standardize, simplify and abbreviate surgery in this demanding district.

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OVERGROUND ENDOSCOPY FINDINGS IN RACEHORSES: A RETROSPECTIVE ANALYSIS

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Dynamic upper respiratory tract (URT) obstructions occur commonly in the equine athlete. Veterinary diagnostic capabilities have improved with the ability to perform endoscopy and to assess URT function during exercise [1]. Several URT abnormalities are reported; these are complex (multiple structures involves) in many cases and became evident only during strenuous exercise [2]. The aims of this study were to describe the clinical features of multiple dynamic obstructions in racehorses. Authors tried to establish which was/were the major abnormalities, and in consequence to identify which ones were the secondary ones. Dynamic videoendoscopy records from 2012-2016 were reviewed; Standardbreds and Throughbreds with multiple dynamic obstructions were included. Eleven horses met the criteria: 5 Standardbreads and 6 Thoroughbreds; all horses exhibited respiratory noise and/or poor performance. All overground endoscopies were performed at training centres with a wireless endoscopy system to recreate the everyday tack routine; after warm-up a session of strenuous exercise was performed, or in any case to work in such a way as to evoke the intolerance in the effort. Retrospective evaluation of the abnormal dynamic findings was evaluated re-adapting a recent diagram publication [2]. The abnormalities identified were: 8 medial axial deviation of aryepiglottic folds (ADAF), 4 recurrent laryngeal neuropathy (RLN), 4 dorsal displacement of soft palate (DDSP), 3 palate instability (PI), 3 vocal fold collapse (VFC), 2 epiglottic entrapment (EE) and 1 epiglottic retroversion (ER).

Seven out of 11 horses exhibited double abnormalities and 4 cases had three or more pathologic conditions. The major abnormalities detected at real movie speed were RLN, DDSP, PI and ADAF; these were combined with minor abnormalities, found in the analysis of the individual and/or very short duration frames, such as: ER, intermittent DDSP and EE. In the four horses with RLN: 2/4 showed concurrent VFC; 2/4 cases was associated with ADAF of severe degree, of the latter one horse also showed a single episode of EE; 1/4 presented mild ADAF. In four cases of DDSP, two were intermittent, one of this associated with preceding PI. There was concurrent presence of ADAF in three horses that was severe in two and mild in one. In 1 case, there was a presence of a subepiglottic mass caused a secondary EE. ADAF was considered of moderate/severe degree in 5/8 cases; 6/8 cases are reported above. In 1/8 was associated with PI and in 1/8 with VFC. PI was noted in three cases, two of which reported above. In one case was in association with epiglottic retroversion (ER), a rare form of airways dynamic obstruction. Grade 2 or 3 of pharyngeal lymphoid hyperplasia (PLH) was noted in four horses, in association with multiple dynamic abnormalities. In case of URT obstructions rest endoscopy are usually silent or in any case insufficient to formulate an accurate diagnosis. Wireless endoscopy is a well tolerated technique that allow to evaluate objectively URT dynamic disorders, in order to formulate an accurate diagnosis and to suggest an appropriate therapy, especially if surgical intervention is required.

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THE IMPACT ON STAGING OF NO-PALPABLE LYMPH NODE EXTIRPATION IN SINGLE CUTANEOUS MAST CELL TUMOR IN DOGS: A MULTICENTRIC STUDY

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Metastasis to regional lymph nodes (RLN) in cutaneous mast cell tumor (cMCT) in dogs were correlated with contraction of survival time and high risk of spreading to distant sites. The extirpation of RLN in dogs with cMCT has been suggested in presence of lymphoadenomegaly, suspected or certain cytological lymph nodes metastasis.[1] On the contrary 29% of dogs with metastasizing grade II cMCT had normal size RLN and 18% of cMCT at first presentation had RLN involvement.[2,3] In this study no palpable or normal size regional lymph node extirpation was included in the surgical management of cMCT in dogs. The lymph node status was assesed using a recent histologic classification[4] and possible correlations with tumour variables were analized.

Ninety-three dogs with single MCT without distant metastasis that underwent wide surgical excision and no palpable or normal size RLN extirpation were included. Data collected were: breed, age, weight, sex, site and size of cMCT, ulceration, presentation (first vs recurrence), 3-tier and 2-tier histological grades, histological margin status (clean vs dirty), RLN location, RLN histological status[4] and admission to adjuvant oncological treatment. The association between nodal status and tumor variables was analyzed by a generalized linear model with multinomial error.

Thirty-three (35.5%) lymph nodes were HN0, 14 (15%) were HN1, 26 (28%) were HN2 and 20 (21.5%) were HN3. The presence of positive no palpable RLN was significantly associated with cMCT located in the genital region and with cMCT larger than 3 cm.

Forty-four dogs received adjuvant chemotherapy. Mean and median follow-up time were 695 and 504 days, respectively (range, 10-2429). Loco-regional relapse with a positive lymph node was detected in 5 cases with a time ranged to 52 to 1071 days from surgery. Metastatic spread to spleen and liver was identified in 5 dogs with a time ranged from 52 to 1071 days (3 out of this 5 dogs had also the loco-regional relapse).

Lymphatic metastases play a crucial role in predicting a correct prognostication and recommendations for adjuvant therapies. These results confirmed the high probability of lymph node metastasis even for no palpable or normal size RLN for dogs with cMCT. No strong association were found to predict the lymph node status. The relative low number of loco-regional and distant metastasis led to the hypothesis that lymph node extirpation could also have a terapeutical value, but further prospective studies are needed.

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BILATERAL FRACTURES OF THE HORIZONTAL *MANDIBULAR RAMUS* IN A MEDITERRANEAN BUFFALO: SURGICAL MANAGEMENT

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Fractures of the mandible are the most common fractures of the cranium in cattle usually involving the interdental space and the molar part of the horizontal ramus (1). In literature only a report about the surgical repair of a mandibular fractures in a new-born buffalo calf is reported (2) and no reports describe the management of mandibular fractures in adult Mediterranean Buffaloes (MB).
This report describes clinical presentation, surgical treatment and follow-up of a bilateral fracture of the molar part of the horizontal *mandibular ramus* in a Mediterranean Buffalo (MB), stabilized by external skeletal fixation (ESF).
A 6 months pregnant, primiparous MB (32 months old) was refereed to Clinical Mobile Service of Veterinary Teaching Hospital of the University of Naples Federico II, following its head knocked by the mixer-wagon.
The buffalo showed severe salivation nasal discharge and an opened mouth. Instability and crepitus were elicited on mandibular palpation. X-ray showed complete bilateral mandibular fractures involving, on the left side, the molar part of the horizontal *mandibular ramus* between second (P2) and third (P3) premolar tooth indeed on the right side, just in front of P2.
The MB was premedicated with Xylazine cloridrate (0.03 mg/kg i.v.) (Rompun®-Bayer) and a mandibulo-alveolar nerve block was performed. After the routine aseptic preparation of the surgical field, a first rostral smooth Kirschner pin was placed caudal to the IV incisive, inserted as a full-pin, through *mandible symphysis* involving both mandiboular ramous. One positive threaded pin (4mm ø x 150mm) was placed caudal to the *incisura vasorum* and other two pins were placed rostrally and caudally to the fracture gap. All pins were placed in both side of mandibular ramous and connected to the connecting bars (6mm ø x 500mm) through ESF clamps (4/6 size) and Polymethyl-Methacrylate (PMMA). The reduction was considered good in order to correct anatomical occlusion of the mouth. Five days after surgery a traumatic partial pull out of left side ESF implant occurred and the latter was removed, leaving in place only the rostral full pin. Thus a telescoping unilateral dynamic External Skeletal Fixator (dESF) (Monotube® TriaxTM Stryker®) was applied to stabilize the left side of fracture.
The dESF was placed using the rostral full-pin and 3 positive threaded half-pins (4mm ø x 16cm) placed through the dedicated clamps. Forty days after the second surgery, the good general clinical conditions and the radiological findings set the timing for the implants removal. The MB was able to eat and drink normally and calved according to the estimated delivery time
The main goal of the surgical treatment of mandible fractures is to achieve stability at the fracture site and a sufficient mouth occlusion to allow a comfortable eating until complete bone healing (1). To our knowledge, in MB repair of fractured bones is not commonly reported (2) and no reports about mandibular fracture in adults exist. Economic constraints, lack of ad-hoc implants and difficult follow-up make the use of ESFs in ruminants usually limited. In this case the use of ESF seems to have been adequate to provide strength and stability to the mandibular fracture. Moreover the use of ESFs recycled by human medicine have been a valid and cheap solution for the present case.

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POST-OPERATIVE EVALUATION OF THE LOAD AND THE GAIT ANALYSIS IN DOGS TREATED WITH CIMICOXIB AFTER TTA SURGERY

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Rupture of the cranial cruciate ligament (CrCL) leads to abnormal cranio-caudal motion of the tibia and excessive internal rotation of the stifle joint, which leads to lameness, pain and progressive osteoarthritis. Restoration of function is obtained surgically and Tibial Tuberosity Advancement(TTA) is one of the most common techniques. Non-steroidal anti-inflammatory drugs are an extremely valuable component of perioperative protocols by virtue of their duration of action, safety profile and efficacy as analgesics for both soft tissue and orthopedic procedures. Cimicoxib is a cox-2 selective NSAID (coxib) and has been approved for use in the postoperative period in dogs (1). Liverpool Osteoarthritis in Dogs (LOAD) and gait analysis(GA) are two clinical metrology instrument widely used in the clinical and experimental setting to monitor diseases associated with impairment of locomotion. The aim of this study was to evaluate the post-operative recovery of dogs, which underwent TTA surgery and treated with cimicoxib in the postoperative period, in order to give to the clinicians some landmarks for the early recognition of complications. In the study were included thirteen dogs affected by an acute rupture of the CrCL and treated with TTA technique. All patients underwent a complete physical examination, x-ray and orthopedic examination, LOAD survey and GA at the time of presentation(t0) and at 15(t15) and 30(t30) days after surgery.Surgery was performed always by the same surgeon. All patients after surgery were treated with cimicoxib 2mg/kg/die for 15 days and re-evaluated at 15 and 30 days after surgery for the follow-up. Cases which developed complication in the postoperative period were excluded from the study. Data obtained from the LOAD survey and the GA of the affected limb were considered for the study. The mean and the standard deviation have been calculated. And the scores were compared between the times of the study with the one way ANOVA for repeated measurements. The LOAD score was 24.5 ± 10.1 at t0 and decreased significantly the t15 (18.5 ± 6.7) and t30 (14.7 ± 5.8) compared to t0.The scores at t15 and t30 were not statistically different. The GA showed a mean value of 13.2 ± 9.6 % at t0 which was similar to the value obtained at t15 (11.3 ± 8.4%).The GA improved significantly at t30 (21.7%)compared to t0 and t15.The results of this study demonstrated that dog undergoing TTA surgery for acute rupture of the CrCL and receiving cimicoxib in the postoperative period improve significantly the ability of locomotion at 15 and 30 days and gain almost the physiological gait on the affected limb 30 days after surgery.

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ASSESSING RELATIONSHIP BETWEEN THE GROUND REACTION FORCES AND MORPHOMETRIC MEASURES IN DIFFERENT CANINE GROUPS USING REGRESSION ANALYSIS

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The force plate gait analysis evaluation allow to measure the ground reaction forces (GRF), that shows limb loading during the walk, allowing to quantitatively define temporo-spatial gait features. In order to minimize variability, current guidelines are to normalize GRF to bodyweight (BW), and to use a narrow velocity range. However, important variability persists in the data. In the last ten years, rescaling of the gait parameters to BW or size, or both combined, and the use of relative velocity, according to the theory of dynamic similarity, was evaluated [1]. Even after full normalization to BW and body size (BS), force plate data of dogs of different breeds are not necessarily comparable; therefore group comparisons should only be made when the groups consist of breeds with similar body conformations [2].
The aim of this study was to compare the results obtained from the sample with the literature findings, and to evaluate the influence of the morphometric measures and their combination, on the GRF and ST in two groups of dogs. Fifty 50 dogs were included in this study, and divided in two groups: mesomorphic (n=36) and dolicomorphic (n=14) dogs. Inclusion criteria were absence of lameness from owner history, absence of clinically detectable lameness on orthopedic examination. Breed, sex, age and weight were recorded for each dog and specific morphometric measurements were taken. GFR had been obtained using a force plate (PASPORT Force Platform, PS-2141, PASCO scientific, California, USA with Capstone software 1.6.0, PASCO scientific, California, USA) embedded in a 4.0 m runway. Force plate data were acquired at walk, with a defined subject velocity of 1.0 ± 0.3 m/s; dog velocity was measured with a motion sensor (Motion Sensor II, CI-6742, PASCO scientific, California, USA). Three valid trials were collected for each limb for all dogs. The influence of the morphometric measures on peak vertical force (PVF), vertical impulse (VI) and stance time (ST) was evaluated by using simple linear regression and multiple linear regression were performed. Linear regression results were similar to those of the reference literature. The influence of variable interactions on GRF was more significant that the one of the single variable, in our model. PVF and VI, in the forelimb of both groups, showed positive linear correlation with the interaction body weight x withers height in both groups. In the hindlimb, GRF showed positive linear correlation with the interaction body weight x medium velocity this result underlines the fact that variable should be considered, for data normalization, as interactions. The use of interactions could give a better explanation of the variables which act on GRF: in fact in nature is the correlation of factors that generally influence the phenomena. The variable interactions between body weight, body size and subject velocity on GRF were the one that caused the major variability in the sample, thus new criteria for normalization should be made, starting from a bigger sample and the combination of kinetic and kinematic analysis.

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ULTRASOUND MONITORING OF EMBRYOS AND FETUS DEVELOPMENT IN CHINCHILLA (*Chinchilla lanigera*) DURING PREGNANCY

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The chinchilla is a rodent that bears one of the finest and most valuable pelts in the world. The wild counterpart is, however, almost extinct because of a drastic past and ongoing population decline. Females typically twin after a gestation of ~112 days and give birth to 2–3 litters per year, resulting in relatively few offspring compared to other rodent species. In light of the situation for this species, the need for immediate conservation actions cannot be overstated: without active management, research and conservation, wild chinchilla populations are likely to become extinct in the near future [1]. Indeed, improvement on physiology and breeding is considered a key-point to avoid the species extinction. Aim of our study was to increase the knowledge on the chinchilla pregnancy using Ultrasound (US).Embryos were imaged through the maternal abdominal wall with a US device (MyLab 30, Esaote, Firenze, IT) equipped with a 12 MHz linear probe. Two to four embryos were imaged in each pregnant female (for a total of 20 pregnant female analyzed during the entire gestational period). Repeated images of implantation sites (90 measurements), placental diameter (58 measurements), crown-rump length (CRL)(45 measurements), head diameter (HD) (49 measurements), body diameter (BD)(65 measurements), eyes (45 measurements), femour (21 measurements), and quantitative variables for monitoring fetal growth were obtained in cross-sectional two-dimensional mode (B-mode) during pregnancy. Because it is not possible to measure each variable for the entire duration of pregnancy, the parameters were divided into four groups as a function of the time at which they were measured. Group 1, from E14 to E30: decidual thickness, gestational sac thickness, implantation site length; Group 2, from E31 to E46: gestational sac thickness, placental length, placental thickness, CRL, HD, BD; Group 3, from E47 to E75: placental length, placental thickness, CRL, HD, BD, eye diameter, latero-lateral (LL) lens diameter, and femour length; Group 4 from E75 to E110: placental length, placental thickness, HD, BD latero-lateral (LL) lens diameter, and femour length. Univariate analysis between each measurement and embryonic day was performed using Spearman's rank correlation (Rs). Continuous linear regression was adopted for multivariate analysis of significant parameters. All statistical tests were two-sided, and a p value <0.05 was considered statistically significant (MedCalc 12, Ostend, Belgium). The study describes the application of US to assess changes in phenotypic parameters in chichilla embryo and fetus during pregnancy and to evaluate physiological fetal and placental growth. US is a valuable phenotyping tool for embryonic chinchilla monitoring and can be used to answer important questions in developmental biology. A database of normal structural and functional parameters of chinchilla pregnancy will provide a useful tool for the immediate conservation actions to avoid the extinction of these species in the near future.

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AUTOLOGOUS CONDITIONED SERUM (ORTHOKINE™) IN DOG OSTEOARTHRITIC JOINT: A PRELIMINARY STUDY

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Osteoarthritis (OA) is one of the most important cause of locomotor disability in dogs and horses. It is a progressive, chronic condition leading to pain and loss of function that dramatically reduces patients' quality of life and athletic career. Pharmacologic treatment options for OA are very limited. They include analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and the intra-articular injection of steroids or hyaluronan (HA).[1] There is a pressing need for novel, improved, mechanism-based agents for treating OA. Biological therapies has been described in the last ten years as a novel therapeutic approach. Among these unconventional therapeutics, the use of the autologous Conditioned serum (ACS) has been described as an encouraging option for the OA treatment.[2]

Meijer et al.[3] noted that exposure of blood to glass beads elicits a vigorous, rapid increase in the synthesis of several anti-inflammatory cytokines, including IL-1 receptor antagonist (IL-1Ra). It could potentially limit the intra-articular actions of IL-1, the most important pro-inflammatory citokyne. ACS was developed in an attempt to generate an injectable material enriched in endogenous IL-1Ra as a novel therapeutic for OA and thereby control the disease process.

The aim of this study was to asses the effectivness of ACS in the control of OA in the dog.

7 dogs, 6 males and 1 female, aged from 9 months to 9 years, different in breeds, weighed from 13 to 40 kg, have been enrolled in the study. The dogs have been investigated with clinical and x-rays examination and OA has been diagnosed in different joint and OA stage (2 hips, 2 radiocarpal joints, 3 elbows and 1 shoulder.) One dog was affected bilaterally by radiocarpal joint OA. 3 joints was staged has low, 3 mild, and 2 severe.

ACS has been obtained with the commercial kit Orthokine ™. The serum obtained has been stocked in 2.5 ml falcon and frozen at -24°C. Serum aliquotes was injected 2.5 ml for each joint once a week for 4 times.

The dogs have been examined clinically (lamness degree, pain at joint passive movments) , LOAD score, and stance analysis before tratment (T0), and weekly for the first month and then monthly.

Risultati e Conclusioni. Results show that clinical examination, LOAD score and stance analysis improve after the second injection till the last one in all joints. In the next follow-up, amelioration persisist in low OA grades, but worse in mild and severe cases.

We can assume that at the early stages of OA, ACS decrease sensibly pain and inflammation, but in mild and severe OA, when exist more osteoarticular modifications , the effects of ACS are lower. For this reason ACS could be succesflully employed for treatment of early stages, but in cases of worse joint conditions appreciable effects should be furtherly investigate.

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TTA "*CANARIA*" FOR SURGICAL REVISION OF FOUR CASES OF FAILURE OF REPAIR OF CCL LIGAMENT DISEASE IN DOGS

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Tibial Tuberosity Advancement osteotomy is a surgical technique to repair LCCr-R that aims to neutralize cranial tibial thrust achieving a 90° angle between the femur-tibial rotule ligament and tibial plateau when the knee has an extension of 135°. TTA "Canaria", compared to the traditional technique, uses a titanium scaffold, highly biocompatible for bone tissue and able to express osteo-inductive and osteo-conductive properties when sustaining cyclical charge.

Our work focused on four unsuccessful LCCr repair case.

The treated patients were the following:

- Mongrel, male, 30kg , BCS 4, 5 years, treated with *tight rope technique;*
- German Sheperd, male, 40kg, BCS 3,5 , 6 years, treated with *TTA like Tepic;*
- Breton, male, 20kg, BCS 3.5, 7 years, treated with *over the top* and *tight rope technique;*
- Rottweiler, male, 44kg, BCS 4.5, 6 years, treated with *TTA modified Maquet technique.*

Despite complications due to previous surgeries, reviewing has proved to be easy to execute. In all cases treated, in the post-operative was achieved a 90° PTA, a negativization of Tibial Compression Test and a reduction in "drawer test".

There was no post-operative complication. In two-weeks follow up, the animals had light lameness that disappeared in the following weeks. The gait of every patient was evaluated at presentation (t0), 15 days after surgery (t15) and at every following control by orthopedic evaluation, rx and LOAD (Liverpool Osteoarthritis in Dog).

Furthermore, for two of these cases a baropodometric evaluation was also carried out to quantify the percentage of weight bearing on each limb.

LOAD results showed a clear clinical improvement and Stance analyzer® showed an increased percentage of weight bearing on the affected limb.

Radiographic follow ups showed a good stability of the implant and significant osteointegration of the scaffold, and in cases where Maquet's fracture had occurred, its progressive cicatrization.

We can state that TTA "Canaria" is an excellent method of rewiew for LCCr surgical repair failure.

POSTOPERATIVE ANALGESIC EFFECTS EITHER OF METADONE OR A CONSTANT RATE INFUSION OF LIDOCAINE, OR THE COMBINATION LIDOCAINE-METADONE IN DOGS UNDERGOING OVARIECTOMY

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The relief of pain in the perioperative period is essential to improve the recovery and comfort of the surgical patient. Postoperative pain produces undesirable effects and to avoid these the use of analgesics with a prolonged action is essential. Drugs such as lidocaine or metadone have been commonly administered to provide inhalant anesthetic-sparing effects and perioperative analgesia in dogs, however, there is limited information available on the efficacy and early post-operative analgesic effects provided by these agents when administered alone, or in combination, in dogs undergoing surgery. This study aimed to evaluate the postoperative analgesic effects either of metadone (MET) or lidocaine (LIDO), or the combination lidocaine-metadone (LM) after ovariectomy in dogs.

Eighteen healthy (ASA 1) adult dogs, aged 1 to 5 years and weighing 8 to 42 kg, were used. Dogs were randomly assigned into three groups of 6 animals, group MET, group LID and group LM. All dogs were premedicated with 0.02 mg/kg of acepromazine IV. After premedication, group MET received 0.2 mg/kg of metadone IV, group LID 2 mg/kg of lidocaine IV and group LM lidocaine-metadone combination. Anesthesia was induced with IV propofol to effect (2-4 mg/kg). Orotracheal intubation was performed, and anesthesia was maintained with isoflurane in 100% oxygen using a small animal rebreathing circuit. During the surgery, only the LID and LM groups received a constant rate infusion of 1 mg/kg/h of lidocaine, immediately after a loading dose of 2 mg/kg IV. The postoperative pain level was measured at 0.5, 1, 2, 4, 8, 12 hrs after extubation using a multiparametric Colorado Acute Pain Scale (CAPS). At the same time, a blood sample was used to determinate glucose measurement. In addition, pain was assessed using a Visual Analogue Scale (VAS, 0-100 mm) to obtain a more occurate overall impression of pain. Rescue analgesia was provided with IV metadone (0.2 mg/kg) when dogs judged to have moderate to severe pain (VAS≥50 or CAPS≥2.3). The Kruskal-Wallis test was used to compare additional metadone requirement and pain score between groups. Differences were considered significant when $P<0.05$. The pain scores were significantly differ between the treatment groups. Requirement for postoperative rescue analgesia was significantly lower in LM than the LID and MED. In the first 12 hr period following surgery, rescue doses of metadone were required for 5 dogs treated with LID, for 2 dogs treated with MED, whereas only rescue doses were needed for 1 dog treated with LM. The level of blood glucose was significantly higher in LID compared with MED and LM. In conclusion, this study showed that LM group had a significantly lower incidence of rescue medication than the LID and MED groups, suggesting a superior level of analgesia when lidocaine was administered with metadone in dogs after ovariectomy in the postoperative pain treatment.

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USE OF DEXMEDETOMIDINE IN THE CHEMICAL IMMOBILIZATION OF WILD DEER (*Dama dama*)

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This clinical study aims to assess the effect of the association tiletamine/zolazepam/dexmedetomidine in the chemical immobilization of wild deer (*Dama dama*). [2]
Forty fallow deer (*Dama dama*) were divided into two groups (A and B). The animals in Group A were immobilized with 2mg/kg^{-1} of xylazine and 1.5mg/kg^{-1} of tiletamine/zolazepam while animals in Group B were immobilized with 35□g/kg $^{-1}$of dexmedetomidine and 1mg/kg^{-1} of tiletamine/zolazepam by immobilization using a carbon dioxide powered rifle (Dan-Inject, Denmark). Heart rate (HR), respiratory rate (RR), body temperature (BT), haemoglobin oxygen saturation (SpO_2), blood lactate concentration (BLC) quality of immobilization (Q. Im.) were evaluated using subjective numerical scoring system (0-4) were evaluated at 10, 20 and 30 minutes after induction; moreover, induction time (IT) was also recorded. The age of the animals was evaluated by inspectioning teeth after capture, while the weight is estimated at sight. After forty minutes of achieved recumbency, we administer in all subjects atipamezole 0.,2mg/kg^{-1} intravenously. Statistical analysis of data was performed using SPSS 15.0 (IBM Company Italy) [1]
By analyzing the mean ± standard deviation on measured data, and the median range for behavioral response score, Kendell's test of concordance was performed on Q.Im. values. Scores were compared using Friedman tests. Physiological data were compared using two way ANOVA for repeated measure followed by Bonferroni test; (IT) and (DR) were compared using one way ANOVA $p<0.05$.
Mean age was 3.2±1.5 years and mean weight 35.3±4 kg. HR is lower than the first survey (10 minutes), along the time line in group B P=0.00. RR is reduced to 20 minutes in group A, while in group B increases at 20 and 30 minutes (P=0.00). SpO_2 values showed hypoxemia in group A (P=0.00). BT as well as BLC were significantly higher in group A (P=0.00). A Kendell's test of concordance of Q.Im. shown a high level of inter-observer agreement (W=0.87) in group A and (W=1) in group B. Q.Im. was statistically higher in group B (P=0.00). IT was 8.1±1.2 in minutes in group A, wile was 6.5±0.5 minutes group ($p<0.05$). Sternal recumbency and quadrupedal station is more rapid in group B , after administration of atipamezole, 7±2.3 minutes^{-1} (group A) and 4±0.2 minutes^{-1} (group B), 20±2.3minutes^{-1} (group A) and 15±0.5 minutes^{-1}(group B) respectively ($p<0.05$).
In conclusion we can say that the dexmedetominina is a viable alternative to the xylazine in chemical immobilization of wild deer.

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VALIDATION OF A LATEROMEDIAL SURGICAL APPROACH FOR PROXIMAL INTERFALANGEAL ARTICULAR CARTILAGE REMOVAL IN EQUINE PROXIMAL INTERFALANGEAL JOINT ARTHRODESIS

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Arthrodesis of the proximal interphalangeal joint (PIJ) is a well-established procedure in equine surgery and is associated with a good prognosis for return to previous levels of activity [1]. Arthrodesis consists of two steps, the first involves articular cartilage removal, the second consists in the joint stabilization. Open surgical removal, closed drilling techniques chemically facilitated ankylosis with sodium carbonate or alcohol [6] have been proposed to obtain articular cartilage removal. Actually, according to the classical arthrodesis technique, the joint is approached dorsally through an inverted-T skin incision. Jones et al. (2009) and Bras et al. (2011) describe less invasive surgical approaches to remove the amount of cartilage necessary to obtain interosseous fusion. [3,4]. A similar technique for cartilage removal has been previously described in 1993 by Bignozzi et al. The paper reported 4 cases operated with a closed lateral approach to remove the cartilage [5]. Aim of this study is to perform on cadaveric horse limbs, the removal of articular cartilage of the PIJ, comparing the amount of cartilage removed using the Jones and Bras approach with the one described by Bignozzi et al. The percentage of surface removed is calculated with the aid of a software Photoshop CS® considering the excised tissue areas. Twenty cadaveric horse limbs were used for this study, 10 hindlimbs and 10 forelimbs. For all techniques was used a 4.5 mm drill bit: for 10 limbs were used the lateral approach, 6 limbs with the use of fluoroscopic guidance and 4 without the use of the fluoroscope. On 10 limbs the technique described in literature by Jones at al. (2009) and by Bras et al. (2011) was used. Bignozzi's technique consists in two small incisions, cranially to the collateral ligament and caudally to the lateral branch of the suspensory ligament; the second caudally to the collateral ligament, taking care to save the nerve, vein and artery. In each incision, a drill sleeve was inserted, with a 4.5 mm drill bit, putting it in contact with the joint surfaces. The drillings were done in a latero-medial direction. From each engraving, three drill lines were made to create a kind of fan. At the end of the drillings, the joints were opened and photographed with a ruler aboard. The images thus obtained were subsequently processed using Adobe Photoshop CS 6 Extended® software in order to obtain the value of the eroded areas, expressed in cm^2. With the dorsal approach, the percentage of articular cartilage erosion was 27.4±8%, and in only four legs the percentage was higher than 30%. With the lateromedial approach the percentage of erosion is 43.33±12%. In particular without the guidance of the fluoroscopy the percentage is 33.51±3%, and with the use of this device the percentage is 48.25±12%. The average percentage of erosion obtained by the dorsal approach (27.40±8%) compared to the one obtained with a latero-medial approach without the use of fluoroscopy (33.51±3%), showed a difference of 6.11 percentage points in favor of the technique with latero/medial approach. The fluoroscopic guidance has further increased the erosion area (48.25±12%), as a better identification of the joint space and, consequently, of the direction of the drill bit.
In this study the erosion rates obtained by performing this technique are comparable, if not better, as reported in the literature for the technique with a dorsal approach.

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COMPARISON OF PERITONEAL LAVAGE WITH LIDOCAINE AND BUPIVACAINE FOR POSTOPERATIVE PAIN MANAGEMENT IN DOGS

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Local Anaesthetics (LA) are widely used in veterinary medicine in perioperative pain management through different routes of administration. Nonetheless, few trials have assessed the intraperitoneal use of LA to control surgical pain [1]. The study aims to compare the effectiveness of lidocaine and bupivacaine peritoneal lavage in providing postoperative pain relief in dogs undergoing laparotomy. Twenty-four owned dogs of different age, gender and breed scheduled for laparotomy were enrolled; inclusion criteria were ASA status ≤ 3 and absence of cardiovascular disease.

After receiving a common premedication (methadone 0.2 mg/kg and dexmedetomidine 5 µg/kg IM) and titrate-to-effect propofol for induction, patients were maintend under general anaesthesia with isoflurane in 100% oxygen to effect.

Before abdominal wall suture, dogs were randomly assigned to one of three groups, with 8 dogs each: group P, administrated with 500 mL of saline 0.9% (placebo); group L, administrated with 200 mg of lidocaine diluted in 500 mL of saline 0.9% and group B, administrated with 2 mg/kg of bupivacaine diluted in 500 mL of saline 0.9%. For dogs under 10 kg, volumes of saline, lidocaine or bupivacaine solutions were of 50 mL/kg.

To perform peritoneal lavage, each solution was poured into the abdominal cavity, left in situ for three minutes and then aspirated; after abdominal wall closure, patients were transferred to the recovery room, where postoperative pain was assessed in the following 6 hours by a single blind operator through the "Glasgow Composite Measure Pain Scale – Short Form" (GCMPS – SF) [2].

Scale scores between 0 and 24 were considered. Scores ≥ 8 were selected as cut-off for administration of "rescue" analgesia (tramadol 2-4 mg/kg IM), representing treatment failure. Statistical analysis was performed with student t test. Within 45 minutes from the beginning of pain evaluation P group showed a percentage of treatment failure of 100%. In L group, one subject received rescue analgesia at 180 minutes, representing a failure percentage of 12.5% that remained constant until the end of the observation period. In B group none of the dogs reached the treatment failure cut-off for for the whole pain evaluation period. The dogs were discharged without adverse effects. Local Anaesthetics administrated through peritoneal lavage showed strong effectiveness in immediate (6 hours) post-operative pain control in dogs undergoing laparotomy. Further extended clinical trials are necessary to describe LA pharmacokinetic profiles after peritoneal lavage administration, to compare short-term and long-term effectiveness of these drugs.

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RETROSPECTIVE EVALUATION OF SMALL-BORE CHEST DRAIN VERSUS TROCAR-TYPE THORACOSTOMY TUBE FOR THE MANAGEMENT OF PYOTHORAX IN CATS

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Thoracostomy tubes are routinely applied for air and fluid removal from the pleural space in cats. Recently, small-bore wire guided chest drains, inserted with a modified Seldinger technique, were proposed for management of pleural space disease in cats (1,2).
The aim of this retrospective study is to evaluate the performance of a small-bore (14 Ga, 2 mm) wire guided chest drain (MILA® International Inc.; SBCD) compared to a (12 Fr, 4 mm) traditional trocar-type chest drain (Redax® S.p.a., TTCD) for the management of pleural effusion secondary to pyothorax in cats.
Cats with a documented pleural septic effusion, treated at least initially with a thoracostomy drain, between June 2008 and March 2017 were included. Two groups were created according to the type of drain applied: traditional TTCD inserted in the thorax with pressure and a SBCD inserted with a Seldinger technique. Clinical data relative to the application of the drain including complications, length of time in use, need of analgesia, surgical exploration and outcome were recorded.
Twenty-one cats with a confirmed diagnosis of pyothorax were included in the study: SBCD was positioned in 8 cats and TTCD in the remaining 13 cats.
Bilateral pleural septic effusion was prevalent in both groups. In the TTCD group, general anesthesia was required for the procedure and the chest drains were positioned bilaterally in 9/13 cases (69%). The application of the SBCD necessitated a mild sedation and was monolateral in 8/9 cats (89%). Post-operative analgesia was significantly different between the two groups with a more frequent requirement of rescue therapy with methadone in the TTCD one ($p=0.02$). No significant differences in the frequency of complications (pneumothorax, malpositioning) were reported during application, however an episode of cardiac arrest occurred in TTCD group. Obstruciotn occurred in both groups during the use of toracostomy tubes. The number of cats who underwent surgery was not statistically different between the two groups.
No deaths were reported in the SBCD group, while 6/13 cats (46%) died in the TTCD group ($p<0.05$).
SBCD can be positionated easily by a Seldinger technique with only mild sedation and appears to be more confortable. Moreover the possibility to apply the drain monolaterally can further decrease the time needed for the application, without reducing their efficacy. The use of the SBCD was only recently introduced in our hospital. Due to the retrospective nature of the study, we could not exclude that a global improvement in the care of the patients or the presence of a more severe clinical condition in the group treated with TTCD, could have influenced the different outcome between the 2 groups. Further prospective studies are necessary to evaluate the impact of the SBCD on the outcome of the feline patients with pyothorax.
In conclusion the SBCD appears easier to place, more confortable for the patient and efficacious in removing dense matherial form the thorax; thus, its use for the management of pleural effusion secondary to pyothorax in cats is warranted.

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COMPARISON BETWEEN TWO RADIOGRAPHIC PRE-OPERATIVE MEASURING TECHNIQUES TO DETERMINE THE CORRECT DEGREE OF PROGRESS IN THE COURSE OF POROUS TTA

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Tibial Tuberosity Advancement osteotomy is a surgical technique to repair LCCr-R that aims to neutralize cranial tibial thrust achieving a 90° angle between the femur-tibial rotule ligament and tibial plateau when the knee has an extension of 135°. It thus neutralises cranial tibial thrust forces, and obtains joint stabilization notwithstanding the ligament tearing.
The necessary advancement to be obtained during surgery has thus to be attentively measured, through evaluation of the PTA (patellar tendon angle). Many studies have been directed to determine the most convenient measuration technique. Currently, two methods are mainly used: the “common tangent” (PTA^{ct}) method, and the more traditional “tibial plateau angle” (PTA^{TPA}) method(1).Literature reports some contrasting data(2)about the preferred, most reliable technique. The aim of this study was to cross-check data obtained from both methods of calculation to evaluate the reliability of obtained PTAs and the existence of any statistically significant difference between the two methods.
For this study, 42 dogs with a definitive diagnosis of CrCL rupture and enlisted for TTA surgery were selected. On the 42 subjects, of different breed, sex, and age, a pre-operatory radiographic evaluation was conducted with both methods, and data was subsequently statistically analyzed with the Bland-Altman Analysis test.Every patient has then undergone surgery and the good result of the performed TTA was radiographically and clinically assessed in the usual way.
The obtained data shows no statistical difference between the two techniques in predicting the correct PTAs, thus confirming the substantial interchangeability of the two.
Also, we can still confirm that the “tibial plateau angle” method is slightly more reliable and should be preferred. Indeed, it leads to post-operatory values that are closer or equal to 90° and it only needs basic instruments (e.g. a dedicated goniometer). The “common tangent” method should be preferred instead in such cases where there is, for example, severe osteophytosis or any other condition impairing the correct visualization of anatomical landmarks, and when it was not possible to obtain a perfect 135° positioning of the stifle joint for the X-ray exam.
In conclusion, we can state that between the “common tangent” method and the “tibial plateau angle” method, both are valid. Since it is easier to perform and less user-dependent, the “tibial plateau angle” should be preferred in most cases.

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ULTRASOUND-GUIDED CERVICAL PLEXUS BLOCK IN DOG: A CADAVERIC PRELIMINARY STUDY

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Ultrasound-guided cervical plexus block is currently part of multimodal anaesthetic management of both elective [1,2] and emergency [3] surgeries involving the region of the neck in human medicine. As this approach has never been described in veterinary medicine, the aim of this study is to preliminarily assess its feasibility in dog cadavers. Three dogs (one mixed-breed female weighing 8 kg, two mixed-breed males of 15 and 37 kg) that underwent euthanasia for reasons not related to the study were enrolled. The dogs were placed in lateral recumbence, the area of the neck was clipped and surgically prepared with alcohol from the transverse process of C1 to the cranial edge of the scapula. A linear probe set at 12 MHz was placed on C1, so as to obtain a transverse scanning plane, and moved in a cranio-caudal direction until the transverse process of C4 was identified. A 22G, 70 mm spinal needle was inserted with an in-plane approach, oriented in a dorso-ventral and slightly latero-medial direction. Methylene blue 15% (at 5, 10 and 20 ml for the small-, medium- and large-breed cadaver respectively) was bilaterally injected in two times: half of the volume over the prevertebral cervical fascia and, after reorienting the needle, half of the volume under the cervical fascia. The anatomical dissection allowed to expose the internal jugular vein and the carotid artery after the sternocephalic and platysma muscles were removed, as well as the transverse processes of C2, C3 and C4. During block performance, in all cadavers the anatomical landmarks were easily identified under US-guidance and it was possible to have a direct and real time visualisation of dye deposition. The spread of methylene blue was homogeneous and the nerve roots of C2, C3 and C4 were stained. In the small-breed subject on the right side, dye infiltration was noted up to the atlas: the dosage might be reduced in small size animals in future studies. In conclusion, the ultrasound-guided approach seems to be effective to preform cervical plexus block in dogs and further studies are warranted to assess its clinical effectiveness.

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OCULAR MELANOMA ASSOCIATED WITH *PHTHISIS BULBI* IN A CAT

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Eyes presenting *phthisis bulbi* are blind, small, opaque and not painful, and they are not usually monitored. Until now, two cases of feline intraocular sarcoma have been reported in stray cats associated to blindness and hypotonia of the affected eye[1]. Previously, a case of an osteogenic sarcoma developed in a phthisical eye of a cat, after an injury, had been reported[2]. The authors showed that monitoring and early enucleation of eyes of cats with *phthisis bulbi* should be considered. Here we describe a case of intraocular poorly pigmented melanoma in a 13-year-old, female spayed, Domestic Shorthaired cat, which had been adopted when it was 3 months old and with a history of a right eye *phthisis bulbi*. The cat was referred for evaluation of buphthalmia of the right eye which, according to the owner, had developed in the previous month. On physical examination there were buphthalmia, moderate clear discharge and a large pink/greyish mass involving the entire ocular surface. The mass precluded visualization of the intraocular structures and measurement of IOP. The left eye examination was unremarkable, and the complete physical examination was normal.

Ultrasonography demonstrated a severe distortion of the right eye that showed a “hourglass” shape with an anterior larger portion, irregularly oval shaped, echoic and highly vascularized, and a rounded smaller, mixed echoic posterior portion. The posterior portion was attributable to the vitreous cavity of the phthisical right eye, and showed a hyperechoic line, compatible with the retinal layer and an anechoic content compatible with the vitreous body, partially invaded by the anterior mass; no structures attributable to the lens were detectable.

A tentative diagnosis of an intraocular tumour, developed on a blind and hypotonic eye, was made and an orbital exenteration of the affected eye was performed. Histopathology confirmed the diagnosis of primary poorly pigmented iris melanoma. Six months later the cat was clinically healthy with no sign of recurrence or metastasis.

In our case sonography allowed the tentative diagnosis of an intraocular tumour with high accuracy without requiring anaesthesia of the patient. Immunohistochemistry showed positivity of the neoplastic cells for S100 and Melan A confirmed the diagnosis of amelocitic melanoma of the iris. In conclusion, development of intraocular melanoma in association with *phthisis bulbi* represents a rare finding in cats and, to our knowledge, this is the first report of an iris melanoma associated with *phthisis bulbi* in a cat. Therefore, this tumour has to be considered when a blind and hypotonic eye for many years becomes suddenly buphthalmic.

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Scienze Cliniche – SIRA

Riproduzione nei piccoli animali

PSEUDO-PLACENTATIONAL ENDOMETRIAL HYPERPLASIA IN THE BITCH: ASSESSMENT OF MAST CELLS

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Cystic lesions of the canine uterus have been recently reviewed [1]. Pseudo-placentational endometrial hyperplasia (PEH) is a rare lesion in which the endometrium grows in an ordinate architecture that strictly resembles the maternal placenta of the bitch. Pathogenesis is not totally understood, to date, even if a specific reactivity of portions of the endometrium to physical or infectious agents has been suggested by experimental studies of the University of Osaka [1]. Uterine-derived histamine produced by resident mast cells (MCs) has long been suspected as a key regulator in implantation and decidualization [2]. The aim of this study was to report two new cases of PEH and to assess the number of MCs in the lesion, comparing it with pregnant and non pregnant uterus at the same stage of the oestrous cycle. The genital tracts of two bitches were collected after routine ovariohysterectomy. They were in oestrus two months before and were not mated. Grossly, ovoid (2-3 cm in diameter) swellings were found in the uterine horn compatible with early pregnancies. On section, the uterine mucosa was pale red and thickened; at swelling level no foetal structures were found but a soft greyish tissue adhered to the mucosa with a cloudy fluid in the lumen. Histologically, ovaries and uterus were in a late dioestrous phase. The mucosal swellings showed an organized architecture, resembling the normal maternal decidua. The glandular, connective, spongy layers were recognized and even the compact layer (labyrinth) in a case. There were no inflammatory infiltrates with the exception of a large number of mast cells (MCs), identified for the pyramidal shape, the round nucleus and typical metachromasia when stained with Toluidine Blue. Additional 5 cases of unpregnant canine uteri (UU) at about 60 days of the oestrous cycle and 5 cases of placental sites (PS) of pregnant uteri at term were stained with Toluidine Blue to assess and compare the number and distribution of MCs. Shapiro-Wilks test was used to assess normality of data, paired t-test to compare the means of MCs in the different layers and between PEH, PS and UU. MCs were detected mainly ($p<0.01$) in myometrium of PEH and UU and in the connective layer of decidua of PEH and PS. There was a significant ($p<0.01$) increase of MCs in PEH versus PS and UU, and in PS versus UU. PEH is an enigmatic pathology of the canine uterus and especially in the complete form is extremely rare [1]. PEH seems also a good model to study placentation in this species. Presence and distribution of MCs suggest that they may promote the myometrial contraction, the formation of the decidua or pseudo-decidua and its vascularization [3] without the exclusion of their role in modulating the inflammatory status of uterus and placenta.

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CRYPTORCHIDISM AND CYST OF THE VAS DEFERENS ACQUIRED BY THE DOG

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Cryptorchidism is a congenital disease consisting in failure of the descent of one or both testicles in the scrotum. Undescended testicles in dogs are predisposed to develop seminoma and sertolioma [1]. Genito-urinary cysts are not common to find [2] and, according to the etiopathogenesis, they can be classified as acquired, like prostatic or paraprotatic cyst, urethra and bladder diverticula, or congenital, residues coming from the mesonephric or paramesophrenic duct and uracali which may evolve into cystic structure because of hormonal stimulation by sex hormones [3, 4, 5]. Our work was developed on Spike, a 10 years old male beagle. The clinical history of the dog has been reported by the owner: when he was 4 years old he was affected by cryptorchidism. The abdominal testicle was surgically removed while the other one was left in the scrotum. The work began when the patient was subjected to an ultrasound exam to search for a prostate disease. A non-vascularised round formation with corpuscular contents was discovered during the ultrasound. It was suspected to be an infected paraprostatic cyst. Moreover, hypoechoic formation with anechoic cavities and hyperechoic septa of about 4 cm was found behind the caudal pole of the left kidney and it was suspected to be a neoformation. After that, a CT scan showed 2 round formations with sharp borders with mitigation of the liquid in the right caudal abdomen and to the left of the bladder. These structures were connected by a thin tubular structure which ended nearby the prostate. A masculine cystic uterus was suspected (differential diagnosis with undescended testicle and cystic disease of the deferent). It also showed a moderate stretching of the vas deferens with fluids to the right under the skin in the inguinal area. Surgery was suggested to shed light on the clinical picture. The histological report described the lesion on the left of the bladder as a nodular fibrotic and necrotic structure due to an undescended testicle while the injury to the right of the bladder was described as a cystic structure of the vas deferens containing cellular debris, macrophages and neutrophils. Even the inguinal injury revealed to be a cyst of the vas deferens with necrotic and inflammatory material. From the clinical examination of the case, it was shown that the undescended testicle has not been removed and probably the derived hormonal imbalance led to the formation of the two genitourinary cysts.

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BIOCHEMICAL COMPOSITION OF AMNIOTIC FLUID IN NORMAL PUPPIES AT TERM OF PREGNANCY: PRELIMINARY DATA

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Similarly to other species, the full knowledge of the normal fetal fluids composition could be useful also in the dog for the better management of newborns, especially for reducing the perinatal mortality. The aim of the present study was hence to define the biochemical composition of amniotic fluid collected from the amniotic sac of puppies born by elective Caesarean section (CS) at term of a physiologic pregnancy. The study was done on 24 purebred bitches classified on the basis of the FCI standards into small size (<10 kg) and large size breeds (>20kg). All the bitches were healthy and clinically monitored from mating, throughout pregnancy, until parturition. An elective CS at term of pregnancy was performed due to an identified high risk of dystocia[1]. For each puppy, the amniotic fluid was collected and immediately centrifuged and frozen at –20°C until analysis for Albumin, Amylase (AMY), Total Bilirubin, Cholesterol (CHOL), Creatine Kinase (CK), Alkaline Phosphatase, Gamma Glutamil Transferase, Transaminases, Lactate Dehydrogenase (LDH), Triglycerides, Urea, Glucose (Glc), Total Proteins, Creatinine , Lipase, Mg, Ca, K, Na, Cl, and Globulins. Data were analyzed by ANCOVA to verify the possible effects of parity, the breed body size and newborn gender on amniotic biochemical composition. From 24 bitches, 1.5-9 years old, 1-4 parity, a total of 69 amniotic fluid samples were collected from the amniotic sacs of healthy, normal puppies. The amniotic mean ± SD and min-max values for each parameter were defined. LDH ($p<0.01$) and CK activity ($p<0.05$), as well as Glc concentrations ($p<0.0001$) were negatively influenced by the parity. AMY activity was significantly ($p<0.05$) higher in large sized (44.2±20.87 U/L) respect to small sized dogs (30.3±19.89 U/L), while lower ($p<0.05$) CHOL amniotic concentrations were found in small sized (3.0±2.71 mg/dl) as compared to large sized (3.9±2.93 mg/dl) dogs. Gender of the newborn did not influence the amniotic biochemical composition. The preliminary results of this study showed some similarities as well as some differences concerning the biochemical composition of the amniotic fluid in dogs at term of pregnancy if compared to data reported for the cat [2]. Furthermore, the results suggested that, in dogs, some amniotic parameters could be influenced by breed body size and by parity, especially some parameters related to the energetic metabolism and the liver, pancreas and muscular enzymes activity. These last findings could contribute to a better understanding of the influence of body size and parity on the definite composition of the amniotic fluid, which is recognized to play many important roles in fetal growth and development, as well as for fetal well being.

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DEHYDROEPIANDROSTERONE SULFATE CLAWS CONCENTRATIONS IN DOGS FROM BIRTH TO 30 DAYS OF AGE: PRELIMINARY RESULTS

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Similarly to all other species, also in the dog improvements in the knowledge on perinatology are mandatory for a better management of newborns, mainly aimed to reduce the impact of perinatal mortality. However, until recently, the study of canine perinatology was limited mainly because of the invasiveness of many investigation procedures, such as repeated blood sampling. In recent times, the claws/nails were proved to be a useful, non invasive, matrix for long time-frame retrospective hormone concentrations analysis also in babies and puppies [1,2], providing a suitable matrix for perinatal long-term hormonal changes studies. The last intrauterine foetal stage of development and the neonatal period represent the most challenging phases for the mammals offspring. It was demonstrated that the activation of the fetal hypothalamic-pituitary-adrenal (HPA) axis leads to dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) secretion, the major fetal steroids. Thus, DHEA-S measured at birth in newborns could be considered as a marker of offspring HPA axis activity, under the maternal influence. This study was aimed to assess the DHEA-S concentrations in newborn puppies claws, collected at birth and at 30 days of age, and to evaluate the possible influence of age, gender and type of birth on DHEA-S claws accumulation. The study was performed on 58 large purebred, normal, healthy, viable (Apgar≥7) puppies, 31 males and 27 females, born by vaginal spontaneous (N=22) or caesarean (N=36) parturition. DHEA-S was analysed by RIA. The mean ± SD DHEA-S claws concentration significantly ($p<0.01$) decreased from birth (210±152.00 pg/mg) to 30 days (91±72.63 pg/mg), evidencing the higher fetal DHEA-S secretion in the last fetal stage of pregnancy in comparison to the first postnatal month of age. According to the type of parturition, higher ($p<0.001$) DHEA-S claws concentrations were found at birth in puppies born by spontaneous than caesarean parturition (300±167.05 vs 154±112.23 pg/mg, respectively); this finding deserves further investigations. No influence of newborn gender was found. Claws DHEA-S values at birth were a bit higher, but with a lower SD, in comparison to data reported for babies 1-3 weeks old [1]. The trend of decrease is in agreement with data reported for cortisol [2] in dead puppies, and suggests, beside the role of cortisol, the important effects of DHEA-S around the time of birth, also in puppies, as reported for babies [1].

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Scienze Cliniche – SIRA

Biotecnologie riproduttive

RELATIONSHIP BETWEEN OVARIAN FOLLICLE CHARACTERISTICS AND OXIDATIVE STRESS IN BOVINE GRANULOSA CELLS

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Oxidative stress is the result of a delicate balance between the activity of endogenous or environmental oxidants and the defense mechanisms acting to counteract their effects inside and outside the cell. Mural granulosa cells (GC), living within a confined environment such as the ovarian follicle, are partially preserved from environmental oxidants. However, their high metabolic activity, which is mainly directed to the production of sex hormones, involves the generation of reactive oxygen species (ROS). Moreover, during follicle selection, specific metabolic signals activate apoptosis mechanisms leading to degeneration/death of GC. The aim of this study was to evaluate, in bovine GC, the oxidative stress dynamics related to the size and the quality of the follicle. A total number of 33 ovarian follicles of sexually mature heifers were dissected and morphologically analyzed [1]. The follicle diameter ranged from 9 to 15 mm. GC were collected and the corresponding cumulus-oocyte complexes (COC) were morphologically classified [2]. GC were incubated with specific fluorochromes to evaluate: lipid peroxidation (5 µM C11-BODIPY581,591), the production of peroxides and superoxide radicals by using 10 µM 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) and 30 µM dihydroethidium (DHE), respectively. A number of 400*103 GC were incubated with C11-BODIPY581,591 and H2DCFDA for 30 min, then, washed and incubated in PBS for additional 30 min before read. Since C11-BODIPY581,591 is an effective tracer of lipid trafficking and can be used to measure antioxidant activity, C11-loaded GC were stressed by exposure to menadione, CuSO4 and H2O2 [3] for 30 min before read. DHE was incubated with a number of 2*106 GC for 20 min. All samples were read with a spectrofluorometer. The excitation wavelengths were set at 490, 350 and 488 nm and the emission spectra were recorded in the range of 500–650, 550-670 and 500-560 nm for C11-BODIPY581,591, DHE and H2DCFDA, respectively. Lipid peroxidation estimation was obtained by comparing the two fluorescence intensity peaks ((Fo~520/ (Fo~520+Fo~594))*100) of C11-BODIPY581,591 whereas the ROS generation was evaluated on DHE and H2DCFDA fluorescence intensity peaks at ~595 and ~520 nm, respectively. The follicle size did not significantly affect the GC ability to counteract lipid peroxidation; however, this ability was higher (P< 0.01) in healthy follicles and decreased in atretic follicles. Both H2DCFDA- and DHE-detected ROS decreased together with increasing follicle atresia. Overall data, COC morphology better discriminated follicle atresia than follicle morphology. In conclusion, bovine GC from healthy follicles generate ROS that are counteracted by a high antioxidant activity. Both ROS generation and antioxidant defense systems are impaired by atresia occurrence and are not affected by follicle size.

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CORRELATION BETWEEN MORPHOMETRIC PARAMETERS AND CHROMATIN FRAGMENTATION IN SPERMATOZOA OF *Canis familiaris*

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Sperm morphometric analysis plays, until now, a secondary role in evaluation of sperm quality because it's difficult to identify a sperm morphometric class with excellent fertilization capacity. Many techniques have been developed to evaluate sperm quality and fertility, as morphology, motility and viability [1]. Moreover sperm DNA integrity has been considered as an important index of fertility [2]. Aim of this study is to verify the existence of a correlation between morphometric parameters and chromatin fragmentation of sperm in dogs so that morphometric evaluations could be used as an objective method for sperm quality assessment and the prediction of the potential fertility. Semen was collected from 15 healthy dogs bred by kennels or individuals in the provinces of Naples, Salerno and Caserta, aged from 1 to 14 years. Sperm DNA fragmentation was assessed using Sperm-Halomax Kit (Halotech®dna for canine semen) following the manufacturer's instructions. For morphometric analysis fresh semen was diluted in physiological solution and two drops of solution were used to prepare slides. Hematoxylin-eosin technique was used for staining the slides that were observed with a Nikon Eclipse 80i microscope at 100x magnification. Major axis, minor axis, perimeter, area, shape factor and roughness of 200 spermatozoa/animal were measured with the software Nis Elements (Nikon) for morphometric analysis. Three-hundred spermatozoa/animal were analysed for Halomax test. All parameters were statistically analysed by IBM SPSS Statistics Version 20 with Anova and correlation test. Dogs have been divided in three groups based on age (G1 from 1 to 2 years; G2 from 3 to 5 years; G3 from 8 to 14 years). No statistical differences in total ejaculate volume and concentration have been found among groups. All morphometric parameters are different with a statistic significance ($P<0.05$) among groups. Percentage of spermatozoa with DNA fragmentation progressively increases with the age of the dog but without exceeding fertility range. According to our data there is a correlation between spermatozoa area and DNA fragmentation so that a high area value correspond to minor degree of DNA fragmentation. These preliminary results indicate that the sperms area can be a predictive morphometrical parameters for fertility.

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BIOENERGETIC/OXIDATIVE BALANCE IN FELINE MORULAE AND BLASTOCYSTS PRODUCED IN VITRO AFTER TEMPORARY OOCYTE HOLDING AT ROOM TEMPERATURE VERSUS COLD OVARY STORAGE

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A major problem in in vitro embryo production (IVEP) is the availability of methods for ovary or oocyte preservation and transport. The suitability of a given method can be assessed by analizing the bioenergetic/oxidative balance of obtained embryos, indicating oocyte/embryo health status during the whole IVEP chain. Unlike other species, cat oocytes have the unique ability to mature in vitro after cold ovary storage (COS; 1), which provides opportunities to rescue oocytes after ovariohysterectomy or sudden death. An alternative strategy is oocyte holding at room temperature (r.t.) in a medium without meiotic inhibitors, namely EH medium, as established in the mare (EH, 2). The aim of this study was to evaluate the effects of temporary (6h) oocyte holding at r.t. in EH medium versus COS on the development and bioenergetic/oxidative balance of feline late stage embryos, morulae (M) and blastocysts (Bl), obtained after in vitro maturation (IVM), fertilization (IVF) and embryo culture (IVEC). The study was conducted in autumn 2016 in 7 replicates. Ovaries were collected from 17 anestrous queens by routine ovariohysterectomy at the Veterinary Hospital of University of Bari. For each queen, one ovary underwent immediate slicing and retrieved cumulus-oocyte complexes (COCs) were placed at r.t. (22-27°C) in the dark for 6h in EH medium (40% Earle's, 40% Hank's salts-buffered M199 and 20% FCS; 2), and the other ovary was placed in PBS solution and stored for 6h at 4°C (1) until slicing. Only COCs with uniform, darkly pigmented ooplasm and intact cumulus (1) underwent 24h IVM in a TCM199-based medium at 38.5°C under 5% CO2 in air (3). Fresh sperm cells were collected by flushing cauda epididymis of male cats, following routine orchiectomy. COCs and sperm cells ($2x10^6$/ml) were coincubated for 18-22h for IVF under 5% CO2 in air. IVEC lasted for 7 days under 5% CO2, 5% O2, 90% N2. IVF and IVEC were performed in SOF media (3). Embryo development was followed up to M and Bl stage and confirmed by nuclear chromatin evaluation. The bioenergetic/oxidative status of M and Bl was assessed by confocal microscopy after mitochondria and reactive oxygen species (ROS) labeling (4). Data were analysed by Chi-square and Student's t Test (Significance when $P \leq 0.05$). A total of 359 oocytes were used, 185 after EH and 174 after COS. No differences were observed between the two groups for embryo cleavage (22/185, 12% vs 21/174, 12%; $P>0.05$), M/cleaved (4/22, 18% vs 8/21, 38%; $P>0.05$), Bl/cleaved (2/22, 9% vs 5/21, 24%; $P>0.05$) and number of nuclei (M: 39.5±30.4 vs 40±0; $P>0.05$; Bl: 100±14.1 vs 107.6±21.9; $P>0.05$). Compared with the EH group, COS-embryos showed mitochondria over-activity (arbitrary densitometry units, ADU: 38.2±15.0 vs 19.9±12.2; $P<0.0001$), increased ROS levels (ADU: 17.8±7.9 vs 10.9±4.1; $P<0.0001$) and significantly lower mitochondria/ROS colocalization (Pearson coefficient: 0.25±0.1 vs 0.34±0.2; $P<0.001$), overall indicating worse health conditions. We conclude that EH could be a valid alternative to COS for feline oocyte short-term preservation, as it allowed to produce similar rates of M+Bl and these embryos showed improved bioenergetic/oxidative balance.

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CLEAVAGE IRREGULARITIES IN EQUINE EMBRYOS OBTAINED WITH OOCYTES EXPOSED TO DEHP DURING IVM, FERTILIZED BY PIEZO-ICSI AND MONITORED BY LONG-TERM TIME-LAPSE IMAGING

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Phthalates are a class of organic chemicals, widely used as plasticizers in industrial applications. The di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used. It has been shown to affect oocyte maturation (1) and embryo development (2,3), even though the events underlying reduced embryo developmental competence need to be investigated more extensively. The time-lapse microscopy allows to monitor early embryo development in vitro and to observe cleavage abnormalities, reported as quite frequent and related to many different factors, including environmental pollutants (4). The aim of the present study was to monitor, by long-term time-lapse imaging, the in vitro development of equine embryos obtained after oocyte exposure to DEHP during IVM, piezo intracytoplasmic sperm injection (piezo-ICSI) and in vitro culture to the blastocyst stage. Cumulus-oocyte complexes (COCs) were recovered from the ovaries of slaughtered mares, held overnight (5) and cultured for IVM (1). DEHP was added at 0.5 µM, selected on the basis of previous studies (1). COCs cultured in absence of DEHP were used as controls. Matured oocytes were fertilized by piezo-ICSI with fresh sperm. Sperm preparation via swim-up, piezo-ICSI, and subsequent 2h oocyte holding were conducted in CZB media (6). Injected oocytes were cultured in DMEM/F-12 with 10% FCS at 38.2 C° under 5% O2, 5% CO2, 90% N2 up to 9 days. A group of embryos was evaluated by standard visual assessment, whereas a second group was monitored by the PrimoVision™ time-lapse imaging system and images of each embryo were recorded every 10 min and retrospectively annotated to record the cleavage rates and the morula and blastocyst (Bl) formation rates. At day 9, all embryos underwent Hoechst 33258 staining and total cell number evaluation. Data were analysed by the Chi-square test and presented for DEHP-exposed and controls, respectively. Differences were considered to be significant when $P<0.05$. A total of 98 oocytes were cultured for IVM. Four replicates were performed. No significant differences were observed for nuclear maturation rates (24/49, 49%; vs 26/49, 53%; $P>0.05$), overall Bl formation rates (3/23, 13% vs 4/25, 16%, $P>0.05$), Bl developmental stage (Early: 13% vs 16%, Expanded: 13% vs 16%; Hatched: 0% vs 4%, $P>0.05$) and Bl total cell number (139±36 vs 177±75; $P>0.05$). In time-lapse monitored embryos, a higher rate of abnormally cleaved embryos, with direct cleavage of a cell in more than two blastomeres and extensive fragmentation during division in two or more cells (5/11, 45.5% vs 1/12, 8.3%; $P<0.05$) and a reduced rate of normally cleaved embryos (6/11, 54.5% vs 11/12, 91.7%; $P<0.05$) was found in DEHP-exposed oocytes compared with controls. In both groups, the abnormally cleaved embryos did not develop beyond the 16-cell stage. In conclusion 0.5 µM DEHP, added during IVM, induces abnormal cleavage which is associated with lower Bl formation rate. Whether this embryo abnormality corresponds to altered spindle integrity in mitotic events and mis-segregation of genetic material, remains to be investigated.

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OVIDUCTAL MICROVESICLES AND THEIR EFFECT ON *IN VITRO* MATURATION OF CANINE OOCYTES

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Efficiency of *in vitro* maturation (IVM) of canine oocytes is limited due to difficulties in reproducing the oviductal microenvironment, where the *in vivo* maturation occurs. Canine oocytes are usually cultured in the presence of oviductal epithelial cells to mimic the intratubal conditions *in vitro* (Hewitt and England, 1998). However, these cells can de-differentiate if cultured in monolayer (Gualtieri et al., 2012). Multicellular spheroids of oviductal cells (MSOCs), obtained after squeezing of canine oviducts, could better mimic what occur *in vivo,* probably thank to the cell-to-cell interactions between epithelial cells and between epithelial and mesenchymal cells, which are present within these aggregates. Moreover, since cells are able to communicate with each other by paracrine mechanisms, MSOCs can be *in vitro* cultured to obtain the conditioned *medium* (CM) consisting of soluble factors and microvesicles (MVs) that represent a carrier for non-soluble molecules, including micro-RNAs (miRNAs). Aim of the present work was to assess the effect of the addition of CM or MVs, secreted by MSOCs, to the canine IVM *medium*. To generate CM, MSOCs of three animals in late oestrus were cultured for 5 days at 38.5°C and 5% CO_2. One aliquot of culture *medium* was collected, centrifuged at 2500g and stored at –80°C. MVs were obtained by ultracentrifugation of the remaining CM at 100,000g for 1h at 4°C. MVs concentration and size were assessed by Nanosight technology. Canine ovaries were obtained from 64 healthy domestic bitches (1–4 years old) that underwent ovariectomy regardless of the estrous cycle. Cumulus–oocyte complexes (COCs) were released by slicing the ovarian cortex and only COCs with 110-120 µm in diameter were selected. COCs were cultured at 38.5°C, 5% CO_2 and 5% of O_2 in a bi-phasic systems: 24h in sinthetic oviductal fluid (SOF) supplemented with 5 µg/ml LH followed by 48h in SOF supplemented with 10% of oestrous bitch serum and 10% CM or 50-75-100-150 x 10^6 MVs/ml labelled with PKH-26, which was used to track MVs under fluorescence microscopy. Control (CTR) was the same medium without CM or MVs. Oocytes were observed under a fluorescent microscope to detect metaphase II (MII) by Hoechst staining and incorporation of MVs. Statistical analysis was performed by chi-squared test. Results showed that canine oviductal cells secreted MVs of 234±23 nm in size underling their categorization to the shedding vesicles group. The incorporation of labeled MVs occurred at first in cumulus cells, at 48h of maturation, and then, at 72h in oocyte cytoplasm. Supplementation of IVM medium with MVs had a positive effect on MII rate at the concentration of 75 and 100 x 10^6 MVs/ml compared to CM and CTR (12/59=20.34% and 12/55=21.82% vs 8/88=9.09% and 7/81=8.64%, respectively). The concentration of 150 x 10^6 MVs/ml provided only 5/54=9.26% of MII. To understand the role of MVs, the expression of three miRNA (miRNA-30b, miR-375 e miR-503) that are involved in some key pathways regulating follicular development and meiotic resumption, was assessed. The lower rate of MII with the higher concentration of MVs was possibly due to the high level of miR-375 that recent literature shows to suppress the TGF-β pathway leading to impaired oocyte maturation (da Silveira et al., 2012.) In conclusion, oviductal MVs could be involved in cellular trafficking during oocyte maturation and their possible use *in vitro* could facilitate the canine reproductive biotechnologies.

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OOCYTES FROM AGED MARES SHOW A HIGHER INCIDENCE OF CHROMOSOME MISALIGNMENT AND SPINDLE ABNORMALITIES, AND A REDUCED ABILITY TO RECOVER FROM SPINDLE DEPOLYMERIZATION

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Maternal aging is associated with an increase in embryonic aneuploidy and early miscarriage, as a result of errors in chromosome segregation [1]. Failure to achieve correct alignment of the chromosomes on the spindle is an important factor contributing to mis-segregation errors in oocytes [2,3]. Since little data is available in horses, the aim of this study was to evaluate the effect of maternal aging on spindle morphology and the incidence of chromosomes misalignment. Cumulus oocyte complexes (COCs) were recovered from slaughtered mares, divided into groups depending on mare age (young, < 15 years; old, ≥15 years) and matured in vitro for 26h. After maturation and denudation, only the oocytes that had reached MII were used and these were further subdivided into Control and Nocodazole groups (total = 4 groups; n=20 per group). Oocytes in the Nocodazole groups were incubated for 10 min in medium containing 20 µM Nocodazole, to induce tubulin depolymerization, washed and then incubated for 2 hrs in maturation medium. In order to destabilize any tubulin fibers not properly attached to the kinetochores samples were subjected to cold shock, fixed and stored at 4˚C prior to immunofluorescent staining for DNA and alpha-tubulin. Spindle morphology and the incidence of chromosome misalignment were evaluated by confocal microscopy and 3D image analysis (Imaris 8.3). Spindle morphology was scored as normal (fusiform, bipolar) or abnormal (tri- or tetra-polar, severely misshapen), chromosome misalignment was scored as absent, mild (1-5 misaligned chromosomes) or severe (>5 misaligned chromosomes). Oocytes from aged mares showed higher rates of mild and severe chromosome misalignments when compared to those from young mares, both in normal condition (mild 37 vs 5%; severe 11 vs 0%) and after Nocodazole treatment (mild 42 vs 15%; severe 21 vs 0%). Moreover, oocytes from old mares were more likely to show abnormal spindle morphology both under control conditions (5 vs 0%) and after Nocodazole treatment (10 vs 0%). Although nocodazole treatment did not result in a significant increase in chromosome misalignment and spindle abnormalities, the incidence of chromosomal misalignments increased numerically in both aged and young groups (total % of misalignment without treatment 47.4 and 4.5% vs 63.2 and 15% after nocodazole treatment). We suggest that the compromised ability to form a normal meiotic spindle and correctly align the chromosomes observed in MII oocytes from aged mares might reflect an impaired function of the spindle assembly check point components, and explain the age-related reduction in oocyte developmental competence.

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Scienze Cliniche – SIRA

Riproduzione in grossi animali e in specie non convenzionali

INTRA MAMMARY EFFECT OF CONDITIONED *MEDIUM* IN THE TREATMENT OF BOVINE MASTITIS: A PILOT STUDY

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Bovine mastitis is considered one of the most economically important diseases for the dairy industry in developed countries. Although antibiotics are very useful to treat infections, they do not directly protect the gland from being damaged. Mastitis is an animal welfare issue, but it is also a food safety problem because dairy cows produce milk for human consumption. For this reason, many countries have highlighted the need for alternatives to the use of antibiotics. Since mastitis induces disruption of the normal secretory functions of the mammary gland by reducing the number of epithelial cells and their activity, a different approach for the treatment of mastitis might be to stimulate the regeneration of the glandular tissue, providing a therapeutic agent composed of soluble factors and no-soluble factors with regenerative and antimicrobial effect. This substance might be the conditioned *medium* (CM) that represent the secretion of cells during *in vitro* culture and that it has already been used in animal models for limb ischemia (Mirabella et al., 2011), healing of wounds (Lee et al., 2011) and liver diseases (Chen et al., 2015). For the first time in veterinary medicine, the CM was used in equine spontaneous tendon lesions with results overlapping those obtained by the use of their origin amniotic stem cells (Lange-Consiglio et al., 2013). Since, infection of bovine udders induces a variety of changes in gene expression, including a decrease in α-LA and casein mRNA and, on the contrary, an increase in mRNA of several growth factors (Sheffield, 1997), a possible role of peptide growth factors in tissue protection or repair processes is conceivable.

In this context, the aim of our study was to evaluate the effect of heterologous conditioned medium (CM) administration for the control of clinical acute and chronic mastitis. The CM was prepared culturing bovine amniotic-derived cells at passage three for four nights. Then, the supernatants were centrifuged at 2000g for 20 minutes and stored at -80°C.

Twelve cows affected by mastitis (3 acute and 9 chronic) were treated twice daily with 3 ml of CM after milking for three consecutive days. Other twelve cows (8 acute and 4 chronic) were used as controls and treated with antibiotics/anti-inflammatory (Ketoprofene+Marbofloxacina+Cefalexina). The experimental groups were heterogeneous due to the decision of some farmers to use quarters affected by chronic mastitis.

Diagnosis of acute and chronic mastitis was based on somatic cell count and bacteriological analyses of milk from affected quarters, together with the observation of the general conditions of the animals. The statistical analyzes were performed by Shapiro-Wilk, Fisher and Student tests with significance value <0.05.

Our data show that, for acute mastitis, the treatment with only CM did not show statistically significant differences regarding the decreases of somatic cells compared to antibiotic alone (50% vs 44% of quarters presenting decrease of somatic cells). The CM was better than the use of only antibiotic for chronic mastitis (25% vs 0% of somatic cells decrease). In addition, no relapses were registered in the treatment of acute mastitis with CM compared to antibiotic (0% vs 25%). On these bases, it is possible to assert that CM performed significantly better than antibiotic alone, either for the recovery of the affected mammary quarters or for somatic cell count reduction. Our results show that CM alone may be useful for a quick resolution of the inflammatory response, playing a role in limiting the tissue damage to the mammary gland parenchyma and reducing the recurrence rates.

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COMPARISON OF RECIPIENTS' PREGNANCY RATES AFTER TRANSFER OF FRESH OR COOLED EMBRYOS IN MARES

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At the Veterinary Teaching Hospital (VTH) of the Pisa University, an Embryo-Transfer (ET) clinical program in horses is offered since 1998. In the last two years, 2015 and 2016, there have been changes in Veterinarian customers needs, with an increased request of transferring cooled transported embryos in VTH's recipient mares. The aim of this work was to compare pregnancy rates in recipient mares after ET of fresh or cooled/shipped embryos. Embryos were collected by uterine flushing with Lactate Ringer (Galenica Senese, Industria farmaceutica Galenica Senese Srl, SI) 8 days after the detection of ovulation and transcervically transferred in recipient mares between the 5^{th} and the 8^{th} day of diestrus. Embryos collected at VTH where transferred within 30 minutes from collection (fresh embryos), embryos collected elsewhere where cooled at around 15°C, shipped in a polystyrene box and transferred in recipients between 6 and 12 hours from collection (cooled embryos). For both fresh and cooled embryos, zwitterionic holding media (EMCARE™ Holding Solution EMCARE™ ICP, Auckland, New Zealand or Embryo holding medium, IMV Technologies, Piacenza) were employed. Pregnancies were diagnosed by transrectal ultrasound at day 14 and checked again at day 40 after the donors' ovulation. Pregnancy rates after ET of fresh and cooled/shipped embryos were 27/38 (71.1%) and 26/34 (76.5%) at 14 days, 24/38 (63.2%) and 23/34 (67.6%) at 40 days, respectively. There were no differences between diameters and quality of fresh and cooled/shipped embryos. Also factors strictly related to the recipient mare (such as age, previous pregnancies and management) seemed not to affect pregnancy rates [1, 2]. This work confirms that recipients' pregnancy rates after transfer of fresh or cooled/shipped (for 6-12 hours) embryos are similar [3, 4]. Cooling and shipping equine embryos may allow practitioners to be able to collect the embryo where the donor is stabled and just send it to a specialized centre for the transfer. The implementation of this protocol might increase collaboration between private Veterinarians and University Veterinary Teaching Hospitals or large embryo recipient centres.

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EFFECT OF THE ADMINISTRATION OF ALFAPROSTOL AT DAY 6 AFTER OVULATION ON CORPUS LUTEUM MAXIMUM CROSS-SECTIONAL AND VASCULARIZED AREA AND PROGESTERONE CONCENTRATION IN AMIATA JENNIES

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The effect of PGF2alpha on corpus luteum (CL) ultrasonographic characteristics and serum progesterone concentration ([P4]) in the jenny has been reported in just a few studies [1-3]. The aim of this work was to evaluate the CL ultrasonographic characteristics (diameter, area and vascularized area) and serum [P4] of the donkey jennies after PGF2α administration. During two reproductive cycles of 6 Amiata jennies vascularized area of the CLs and [P4] were daily monitored from ovulation to the hastening of the next oestrus by B-Mode, Doppler ultrasound and RIA [4], respectively. In one cycle a PGF2alpha analogue (3 mg/im of Alfaprostol; Gabbrostim, CEVA, Agrate, MB, Italy) was administered at day 6 (PG6) after ovulation, in a second cycle jennies were left untreated (CTRL). In this study, we confirmed a positive correlation between the analysed properties of corpus luteum and [P4] ($P<0.0001$; Area: $r2=0.21$, vascularized area: $r2=0.54$). Moreover, we observed how the PG6 group interovulatory period (15±1.8 days) was significantly reduced by Alfaprostol treatment compared to CTRL group (24.5±2.9 days) ($P<0.05$). [P4] in PG6 group dropped under 1ng/mL in 6/6 jennies 2 days after administration, remaining under this concentration until estrus and subsequent ovulation. These results confirm what previously observed by Carluccio [1] and Mirò [3] on induced luteolysis in the donkey jenny.

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COMBINED USE OF SUPEROXIDE DISMUTASE, CATALASE AND GLUTATHIONE PEROXIDASE IMPROVES LONGEVITY OF STALLION SEMEN DURING CHILLED STORAGE

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Artificial insemination with fresh or cooled-shipped semen is the predominant reproductive technique used in many horse breeds. Slow-cooling and controlled storage at 5°C is considered the best way to preserve sperm motility, membrane integrity and DNA integrity for up to 40h [1]. However, the fertilizing capacity of stallion sperm decreases with storage time, probably due to an increase in Reactive Oxygen Species (ROS) concentrations [2]. Low intracellular ROS concentrations appear to be a prerequisite for normal sperm function. By contrast, an excess of extracellular or intracellular ROS damages spermatozoa by inducing apoptosis-like changes. Maintaining the ROS concentration in stored semen at a low level may be a key to optimal preservation of the fertilizing capacity of spermatozoa during cooling and storage. Spermatozoa, seminal plasma, the epididymes and testes are endowed with antioxidant systems to protect against ROS, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). We hypothesized that supplementing the semen extender with naturally occurring antioxidants would improve the quality of stored semen. The aim of this study was to investigate the protective effect of combined SOD, CAT, and GPX addition to a semen extender on stallion sperm DNA integrity and motility parameters. Two ejaculates from each of seven stallions were divided into two equal aliquots and diluted with INRA96 without addition of antioxidants (control) or after addition of 15 IU each of SOD, CAT and GPX (treated). Both aliquots were stored at 5°C for 72h and evaluated every 24h for sperm velocity and progression characteristics by Sperm Class Analyzer (SCA) and DNA integrity (APO-BrdUTUNEL assay). Chilled storage resulted in a progressive reduction in total and progressive motility and DNA integrity, independent of treatment (two-factorial ANOVA for repeated measurements; $P<0.05$). However, the decline in semen quality parameters was less pronounced in semen extender containing antioxidants, with significant differences between groups (ANOVA; $P<0.005$) at 48 and 72h: e.g. % of sperm with fragmented DNA was 36.3±9.2 at 48h and 47.4±7.9 at 72h in control samples compared to 22.5±6.8 at 48h and 31.3±5.8 at 72h in treated extender. Semen from one stallion showed a slower decline in quality in the control extender than in extender with antioxidants ($P<0.01$). The combined addition of antioxidants naturally present in the male genital tract improved longevity of chilled stored stallion semen. This indirectly suggests that ROS production during storage adversely affects semen quality. Furthermore, endogenous levels of antioxidants in stallion spermatozoa and/or seminal plasma seem to be suboptimal for prolonged in vitro preservation, which may be enhanced by exogenous supplementation. The adverse effects of antioxidants observed for semen from one stallion may reflect an individual incompatibility with the tested concentration or combination of antioxidants. In conclusion, the combined addition of superoxide dismutase, catalase, and glutathione peroxidase to a commercial semen extender attenuated the decline in motility and DNA integrity of stallion sperm during storage at 5°C.

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EVALUATION OF TWO DIFFERENT PIGLET FOSTERING APPROACHES

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Being a polytocous species, the sow may ovulate till 30 follicles during each oestrus cycle, so the swine prolificity has been strongly pursue to improve the litter size in pig herds [1]. Nowadays, these more prolific sows are able to deliver till 20 piglets born alive per farrowing [2]. The difference of the birthweight and growth of the suckling piglets within a litter could be two to three fold different; this variation is associated with a high level of mortality and the litter size has the largest influence [3]. Weaning weight is also a key piece of information on farrowing room performance. Unfortunately, many causes could impair the sow to nurse and wean all the piglets born alive (number of teats, disgalactiae syndrome, gastric health). For these reasons, the "supernumerary" and "underprivileged" piglets need fostering to survive: foster mothers, associated to the bump or split-weaning management, and the artificial milk feeder [3]. The aim of this study was to evaluate the efficacy of the foster mother and the use of the artificial milk feeder in the modern suckling piglets management. The farrowing and weaning data of one pig farm, located in the north of Italy, adopting both the foster mothers and the artificial milk feeder for suckling piglets management were recorded within 75 weeks (2013-2014). The farm vets organized the grouping of the newborns by piglets and litter size. 4,410 farrowing, with a mean number of piglets born alive/sow of 15.64±0.7, and 55,201 piglets weaned were considered. Suckling piglets with any health disease were not included in the study. Piglet body weights at weaning, grouped by feeding method were analysed by ANOVA through the univariate procedure of the general linear model, using the feeding methods (mother, foster mother, artificial milk feeder) as fixed factor, the week of weaning as random variable and lactation length as covariate. 49,147 piglets were weaned by their own mother (group M), 3470 by the foster mothers (group FM) using the bump weaning and 2584 by artificial milk feeders (group MF). The mean age at weaning was 26.5±1.64 days; while LS-means of weaning body weights were 6.38, 6.43 and 5.84 kg for M, FM and MF respectively, with 0.053 SE value. The statistical comparison between groups M and MS was not different, while group MF weight resulted different in comparison with M and FM ($P \leq 0.001$). In this study 6,054 piglets were weaned by fostering methods, the foster mother performing equal to the mother; even if the MF group was the worst, the mean body weights of 5.84 kg is acceptable and allowed piglet weaning without craving of additional sows, clearly reducing the weaning herd costs and efforts. In this study the artificial milk feeder allowed to save surplus piglets and reduces losses that might occur as a result of post-partum sow problems. In conclusion, the author would like to introduce a new parameter farrowing room: the total efficiency of the farrowing room/sow unit, in the modern pig production, corresponds to the efficiency of the sow (no. piglets weaned per farrowing) added technical efficiency (no. piglets weaned per farrowing) that comes from nursing or fostering closely related to the farrowing room staff work.

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PROGESTERONE ROLE IN THE REGULATION OF THE FEMALE REPRODUCTIVE CYCLE IN CAPTIVE ROYAL PYTHON (*P. regius*): PRELIMINARY STUDY

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Current studies support an important role for progesterone in the reproduction activity of reptiles [1]. Although detection of hormone levels in serum or plasma is the most direct method, blood sampling is quite invasive and therefore not easily repeatable on healthy animal for monitoring purposes, mostly in non-conventional and wild animals, particularly susceptible to stress. Noninvasive monitoring using feces has been successfully applied to many species and correlations between serum hormone levels and fecal metabolites have been demonstrated. So far very few studies regarding the monitoring of fecal steroid hormones metabolites in reptiles are reported in literature [2]. Royal python (*P. regius*) is one of the snake species most bred in captivity. However, studies regarding the evaluation of sex steroid hormones in the *P. regius* female reproductive cycle are not currently reported. The aim of the present study was to evaluate the variations of progesterone levels during the female reproductive cycle of captive bred Royal python, through faecal progesterone metabolites measurement. A total of 86 adult female were studied between January 2016 and January 2017. All animals were captive born, fed a diet of commercially raised rats and individually housed in rack at 28°C under a 12:12 h L/D cycle. Faecal samples were monthly collected and classified according to reproductive stages, identified by ultrasound scans of ovaries, follicles and eggs [3]. The samples were then processed by enzyme-linked immunosorbent assay using a species-independent kit (Progesterone Enzyme Immunoassay Kit K025-H5, Arbor Assays DetectX®, Ann Arbor, Michigan, USA) and mean fecal progesterone level (MFP) for each phase of the reproductive cycle was calculated. Progesterone faecal metabolites reveal an increasing trend during the reproductive cycle. Metabolites concentrations corresponding to the reproductive quiescence (anovulatory phase - MFP: 9.5±6 ng/g) and the next stage in which restarts the ovarian activity (transition - MFP: 88.2±18 ng/g) are lower than those detected during the later stages of follicular and eggs development (folliculogenesis and embryogenesis - MFP, respectively: 112.6±7 ng/g and 120.5±9 ng/g) until deposition, with statistically significant difference ($P<0.01$). The high levels of progesterone highlighted during gestation, with a sharp decrease in correspondence to egg laying, recall the plasma progesterone trend evidenced during the reproductive cycle in some species of viviparous snakes [1]. From the results of this study, it can be supposed that progesterone plays a role in in the maintenance of pregnancy in captive Royal python.

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Oncology I

CEREBRAL INTRAVASCULAR LYMPHOMA IN DOGS

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Intravascular lymphoma (IVL) is a rare angiotropic large-cell lymphoma in which neoplastic lymphocytes proliferate within the lumina of small blood vessels in the absence of a primary extravascular mass or leukemia. IVL is described in humans, dogs, one cat and one horse. The clinical symptoms of the disease are dependent on the specific organ involvement, which most often includes the central nervous system (CNS) and skin. The aim of our study was to characterize the clinical and neuropathological features of 10 cases of canine IVL restricted to the CNS. The study included 6 females and 4 males with an average age of 8 years (range 2.5 to 13 years). Immunohistochemistry (IHC) using anti-CD3 and anti-CD20 antibodies was performed to typify the neoplastic lymphocytes. Anti-CD44 and anti-CD29 antibodies were used to investigate the pathogenetic mechanism leading to the intravascular aggregation of the neoplastic lymphocytes, since CD44 and CD29 are molecules known to be involved in lymphocyte and endothelial adhesion phenomena. The same IHC panel was also applied on 8 cases of primary and metastatic canine CNS lymphoma in order to compare IVL immunoreactivity. The main clinical signs shown by dogs with cerebral IVL were depression, seizures and gait deficits. Magnetic resonance imaging showed several areas of hyperintensity distributed mainly in the forebrain with almost no significant enhancement post intravenous gadolinium administration. Grossly, lesions were found in 6 cases and included focal extensive or multiple hemorrhagic areas. Microscopic examination revealed numerous veins and capillaries filled with neoplastic lymphoid cells, involving both neuroparenchymal and meningeal vessels, and accompanied by various degrees of edema, hemorrhage and thrombosis. Three IVLs were typified as T-cell ($CD3^+$), 3 as B-cell ($CD20^+$) and 4 as non-B non-T ($CD3^-$, $CD20^-$). Regarding primary and metastatic canine CNS lymphomas, 4 were classified as T-cell, 3 as B-cell, and one as non-B non-T. In IVLs, neoplastic lymphocytes showed marked expression of CD44, whereas in primary and metastatic lymphomas CD44 positive cells were detected only in 2 cases. CD29 immunolabeled cells were observed in 4 IVLs and in one primary CNS lymphoma. In human IVL, CD44 is invariably expressed on the cytoplasmic membrane of neoplastic cells, presumably predisposing to the formation of lymphocytes aggregates. Moreover, the transvascular lymphocyte migration could be impaired because of lack of CD29 expression on neoplastic cells, limiting their proliferation within the intravascular compartment. CD44 immunoreactivity in canine IVL was consistent with the findings reported in human IVL, whereas CD29 was inconsistently immunonegative, confirming only partially the pathogenetic mechanism suggested for the human counterpart.

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A COMPARATIVE ASSESSMENT OF HISTOLOGY, IMMUNOHISTOCHEMISTRY AND CLONALITY IN THE DIFFERENTIAL DIAGNOSIS OF SPLENIC LYMPHOID NODULES IN DOGS

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Canine splenic lymphoid nodules are histologically classified as indolent lymphomas (marginal zone lymphoma – MZL; mantle cell lymphoma - MCL) or lymphoid nodular hyperplasia, simple (SNH) or complex (CNH) type. Nevertheless, their differentiation may be difficult on a plain morphological basis, because of similar histologic appearance and poorly defined diagnostic criteria. In order to evaluate the possible contribution of image analysis, immunohistochemistry (IHC) and clonality, we reviewed 30 surgical samples of splenic lymphoid nodules histologically diagnosed as 10 MZL, 3 MCL, 11 SNH and 6 CNH. Proliferative activity was evaluated together with immunophenotype with a double staining technique for Ki67-index and CD3 or CD79a. Image analysis was then performed to assess in each nodule the percentage of T/B-cell areas and the corresponding proliferative activity. Three cases formerly diagnosed as MZL were reclassified as lymphoid hyperplasia upon evaluation of CD3 and CD79a patterns. The percentage of CD79a-positive areas was significantly higher in lymphomas (mean, 3.5 ± 11.3%) compared with nodular hyperplasia (mean, 24.2 ± 10.9%; P = 0.001). The proliferative activity of B-cells was lower in hyperplastic lesions than in lymphomas (median Ki67-index, 2.2% and 5.5%, respectively; P=0.014). Regardless of the histological diagnosis, Ki67-index was higher in non-B cells than in B cells in all cases (median, 18.7%; P < 0.001). The best cut off value discriminating between lesions diagnosed as hyperplasia or lymphoma was a B cell area of at least 27% with a Ki67 index above 3%. None of the dogs except one received adjuvant treatment besides splenectomy. Dogs were monitored for a median follow-up time of 947 days (range, 133-2261) and in no case a relapse was documented. Overall median survival time was 1237 days, with no significant difference between lymphomas and hyperplasia. Surprisingly, clonality results showed a monoclonal or biclonal rearrangement also in the vast majority of cases diagnosed as hyperplasia, suggesting a pathogenetic continuum with lymphoma. In conclusion, the combination of histology and IHC may help to improve the diagnostic accuracy of canine splenic lymphoid nodules, even if the long-term behavior of these lesions appears similar

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EXPRESSION ANALYSIS OF MICRORNAS IN FFPE SAMPLES OF CANINE CUTANEOUS AND ORAL MELANOMA BY RT-QPCR

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MicroRNA (miRNA), a class of small, non-coding RNA - regulating post-transcriptionally protein expression - are emerging as clinical biomarkers in many pathologies, including cancer [1]. Since miRNA are supposed to represent fundamental key regulators, better understanding of melanoma tumour biology is essential to improve both disease grading and staging and, consequently, therapy options and prognosis. The aim of the study was to investigate whether miRNA expression can vary in canine melanoma samples derived from formalin-fixed-paraffin-embedded (FFPE) tissues. Experimental design of the study included three groups, each one composed of 7 animals: i) control healthy skin group ii) oral melanoma group iii) skin melanoma group. Two tissue slides were used for miRNA extraction. The expression levels of seven miRNA - miR-145, miR-146a, miR-425-5p, miR-223, miR-365, miR-155 and miR-134 - were detected and assessed by qPCR using TaqMan® probes [2-7]. Five miRNA were significantly up-(n=3) or down-(n=2) regulated. In details, miR-146a and miR-155 abundance was increased as compared with control in both oral and skin melanoma (p = 0.004 and 0.014 and p = 0.043 and 0.035 respectively), while the levels of miR-145 and miR-365 were lower (p = 0.018 and 0.008 and p = 0.01 and 0.028, respectively). MiR-425-5p was upregulated (p = 0.039) only in skin melanoma Furthermore, functional analysis, carried out using miRNet web-based tool [8], showed that 76 genes related to cancer-associated pathways were possible target of these five microRNA (p = 6.95E-9); in particular, 21 target genes were associated with melanoma (p = 1.47E-5), including BRAF, KRAS, AKT1 and CDK, E2F,FGF, EGFR and PIK3 families. In conclusion, miR-145, miR-146a, miR-425-5p, miR-365 and miR-155 are differentially expressed in melanoma and healthy FFPE samples, suggesting that they may play a role in canine melanoma pathogenesis and/or progression.

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PATTERNS OF MIRNA EXPRESSION IN CANINE MENINGIOMA

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miRNAs are a class of non-coding small RNAs highly conserved between human and dog [1]. They regulate gene expression by binding to complementary sequences in their target mRNAs, promoting their degradation or repressing their translation. Increasing evidences support that miRNAs play an important role in tumor development as oncogenes or tumor suppressors. In recent years miRNAs have become a major focus of research also in veterinary and comparative oncology.

The aim of this study is to investigate the biological role of miRNAs in the meningioma of the dog, trying to contribute to understanding the molecular bases of the most common primary cerebral tumor in this species. The expression of 12 miRNAs (miR-335, miR-200a, miR-98, miR-96, miR-190a, miR-29c, miR-219-5p, miR-155, miR-146a, miR-145, miR-136, miR-451) associated with human meningioma has been analyzed by qRT-PCR in 41 FFPE canine tumors and compared to normal arachnoid tissue [2, 3, 4]. The correlation between miRNA expression and tumor grade/histotype has been evaluated.

Total RNA was extracted with FFPE-RNA Purification Kit (Norgen). Reverse-transcription, pre-amplification, and PCR reactions were performed using TaqMan Advanced miRNA Assays (Applied Biosystems). One way ANOVA followed by Tukey's post-hoc test and t-test were used to compare miRNA expression among groups (Rcmdr, R Commander).

Our analyses revealed miR-29c and miR-200a upregulation in grade III meningiomas. Furthermore, miR-200a was upregulated in anaplastic meningiomas whereas miR-98 and miR-136 were downregulated in papillary and meningothelial meningiomas, respectively. Finally, miR-145 was downregulated in meningiomas compared to normal arachnoid tissue.

In contrast to our findings, in human meningioma the expression of miR-29c decreases with tumor grade, in association with high recurrence rate [3]. Similarly, miR-200a upregulation in canine grade III and anaplastic meningiomas is an unexpected result considered the proved tumor suppressor activity of this miRNA in human meningioma. In fact, miR-200a inhibits growth and migration of neoplastic meningeal cells increasing the expression of E-cadherin, blocking the Wnt/β-catenin signaling pathway and targeting NMHCIIb [2]. As for histotypes, the different miRNA expression observed suggests a possible role of miR-200a, miR-98 and miR-136 in guiding histological pattern expression.

Our study represents the first attempt to investigate miRNAs potentially involved in the biological behavior of canine meningioma. Based on data reported for human meningioma, a different biological role of the examined miRNAs should be taken into consideration in the canine neoplasia.

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THE DIAGNOSTIC ROLE OF IMMUNOHISTOCHEMISTRY IN FISH CANCER IDENTIFICATION

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Tumors are nowadays more and more found in aquatic animals and the study of carcinogenesis is becoming very important in the field of research. Particularly in teleosts almost all tumour types affecting all tissues and organs have been reported. The diagnosis of tumors in fish is not always easy to achieve and the use of antibodies is today essential in order to make a definitive and unambiguous identification. In this order, different tumor types, such as skin papilloma, dermal fibroma, dermal schwannoma, skin melanoma, sampled from 7 fishes were studied and their gross, histological, histochemical and immunohistochemical patterns are reported. The following antibodies were used: pancytokeratins, vimentin, S-100, melan A, calretinin, GFAP, actin, and Ki-67. The data obtained underline the importance and the value of immunohistochemistry as diagnostic tool in fish tumor diagnosis and especially for the identification of the origin of the tumor cells. Moreover, the first reports of papilloma in a bream, of fibroma in a Mediterranean steenbras, of schwannoma in a crucian carp are here provided.

EXPRESSION OF CARNITINE PALMITOYLTRANSFERASE 1 A IN CANINE MAMMARY TUMOURS. PRELIMINARY INVESTIGATIONS

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Neoplastic cells gain a survival and growth advantage by adapting their metabolism to respond to environmental stress, a process known as metabolic transformation. The best known aspect of this process is the Warburg effect, whereby cancer cells up-regulate glycolysis under aerobic conditions. However, neoplastic cells can undergo metabolic transformation utilizing other nutrients such as fatty acids, drawn from the microenvironment, to produce ATP. Fatty acid oxidation (FAO) is regulated by carnitine-palmitoyl-transferase system which is responsible for transporting long-chain Acyl-CoA from cytoplasm into mitochondria for β oxidation. The first component of this system is carnitine palmitoyltransferase 1 A (CPT1A) which catalyzes the FAO rate limiting step. A FAO involvement has been demonstrated in different human tumors including breast cancer, and, pharmacological inhibition of CPT1A can reduce cancer cell survival (1). Spontaneously occurring canine mammary tumors are known as suitable model for human breast cancer. Thus, the aim of the present study was to investigate FAO in 5 samples of normal mammary gland tissue, in 5 benign and 10 malignant spontaneous canine mammary tumors evaluating CPT1A expression by immunohistochemistry and Western blot analysis. Neoplastic samples were classified according to WHO criteria and divided into grades I to III, (G1 to G3) applying Elston Ellis parameters. Immunoreactivity was scored by two independent observers under blinded condiction, selecting 20 fields at 400X magnification and counting immunostained cells. Results were expressed as percentage. In normal mammary glands, 98.2% out of epithelial ductal cells showed strong expression of CPT1A characterized by cytoplasmic small granules. Myoepithelial cells were negative. In G1 and G2 tumors, the intensity of immunostaining was weaker than in normal mammary gland, and the number of immunostained cells was decreased (68.2% and 57% respectively.). However, in these tumors clusters of neoplastic cells showed strong immunostaining for CPT1A, characterized by cytoplasmic large and widespread granules. In grade III tumors, the intensity of immunostaining was very weak and the number of positive cells was further decreased (14.6%). Western blot analysis confirmed the cross-reactivity of the anti-human CPT1A antibodies in canine mammary gland. The mechanisms mediating metabolic transformation in cancer remain still quite undefined. Our results show for the first time, the expression of CPT1A in canine normal mammary gland, in benign and malignant tumors. The upregulation of this enzyme observed only in some clusters of neoplastic cells in G1 and G2 carcinomas, suggests that only few cells can adapt to this alternative metabolic pathway. We hypothesize that the loss of CPT1A immunostaining in G3 carcinomas, could be the consequence of mitochondrial damage which would render FAO impossible.

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EXPRESSION OF CD79A AND IMMUNOGLOBULINS IN THE EPITHELIUM OF CANINE MAMMARY GLAND TUMORS

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Immunoglobulins (Ig) are traditionally considered as an exclusive product of B cell lineage and as molecules that play a crucial role in the regulation of the immune system mechanisms. Noteworthy, in the last decades, has been highlighted that many physiological and pathological “non B” cells, including epithelial cells and neurons may also express immunoglobulins (i.e. IgA, IgG and IgM). Igs, and their receptor CD79a, have been found in human cancerous cells and their role in cancerogenesis, as well as the therapeutic usefulness, has been raising interest [1,2]. On the basis of similarities among the epidemiology, biological behaviors, histopathological presentation and risk factors between the human and dog, our aim was to evaluate the epithelial expression of immunoglobulins in mammary gland tumors of the female dog. By the mean of immunohistochemistry, epithelial expression for CD79a and IgG, IgA ed IgM on 43 cases of mammary lesions (10 hyperplasia, 10 simple carcinoma, 5 solid carcinoma, 8 micropapillary carcinoma and 10 cases of mixed carcinoma) was evaluated. At the same time, an evaluation of number and distribution of CD79a+ immune cells was performed. In the mammary gland the number of CD79a+ immune cells decreased in hyperplastic area; in the malignant lesions, above all in peritumoral stroma, a high increase in the number of CD79a+ immune cells was observed. Rare cells were detectable in the intratumoral stroma. Moreover, the positivity for the antibody CD79a was also detectable in the basal cells of the solid tumors and in glandular cells of simple and micropapillary carcinomas. Both in preneoplastic and neoplastic lesions even with differences in intensity and cell localization, epithelial cells showed a positivity to IgA and IgG while the positivity for IgM was weak or absent. On the basis of our preliminary results and literature data, we suggest that such as immune cells and molecules could be directly involved in the progression or regression of mammary gland tumors as underlined by recent scientific evidences [3,4,5,6]. The canine mammary gland tumor, already recognized as a model in comparative oncology, represents once again an important support for the advancement of the veterinary oncology.

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AIPVET

Forensic pathology I

EVALUATION OF MUSCULAR PROTEINS DEGRADATION TO DEFINE POST MORTEM INTERVAL (PMI) IN DOGS

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The delimitation of the postmortem interval (PMI) is a very important aspect in Veterinary Forensic Pathology (1). The time of death and survival period may be used to determine criminal charges in animal cruelty cases (1). Many post-mortem modifications have been studied in human, less in animals, but, to our knowledge, no one considered the modifications of muscles in dogs. The skeletal muscle could be a viable target tissue for PMI analysis because it has a much greater delay in postmortem change compared to other organs (4). The aim of the present study was to evaluate the immunoreactivity modifications of two cytoskeletal proteins, desmin and dystrophin in muscles of dead dogs over the time to establish if there were statistical correlation with increasing of the PMI. For this study, muscles of twenty-five adult dogs (age range 6-12 years) were evaluated for 6 days after death in standard condition of temperature (23°C). Muscle samples were obtained from the Vastus lateralis and Triceps brachi at different time of death (respectively 0, 3, 24, 48, 72, 96, 120 and 148 hours after death). All samples were immediately frozen in isopentane pre-cooled in liquid nitrogen, and stored at −80°C until further processed. Tissue sections of 10 µm were cut in a transverse plane with a cryostat and processed for immunohistochemistry and haematoxylin and eosin staining. Immunohistochemical analysis was performed using anti-desmin and anti-dystrophin antibody diluited 1:200 for 2 hours. The degree of the immunoreactivity was scored as follows: 4 (>80% positively stained fibrers in the section); 3 (50-80% positively stained fibres in the section); 2 (30-50% positively stained fibres in the section); 1 (1-30% positively stained fibres in the section) and 0 (negative staining observed in the fibers of the section). The one way analysis of variance was used to compare degree of immunoreactivity among the different times of death. The histological examination showed foci of muscle disintegration characterized by ruptured fibres and a loss of cell borders after 4 days post mortem (dpm). Immunohistochemical examination showed a more rapid dystrophin degradation with complete disappearance of the immunoreactivity after 4 dpm. In contrast, desmin was detected in dog muscle for all 6 days of observation with progressive reduction of immunoreactivity cells during the time ($P<0.001$). This study demonstrates that the muscle proteins have a time dependent degradation. Moreover, our immunohistochemical findings indicate that there is a difference in degradation among the various proteins of the muscle during storage. These results were in line with previous studies performed in fish muscle and suggest a different resistance of this protein to the process of autolysis (2,3). These studies will be useful to better evaluate the PMI in Forensic Veterinary Pathology.

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ANIMAL ABUSE AND INTERPERSONAL VIOLENCE: THE ROLE OF THE VETERINARIAN IN FORENSIC MEDICINE

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In recent years, investigations on animal abuse and interpersonal violence have demonstrated a strong relationship between these two forms of aggressive behaviours. Animal welfare, professionals, and social services now acknowledge that episodes of cruelty to animals, as well as maltreatment, domestic violence, and abuse of the elderly, are closely related. This phenomenon is commonly referred to as "THE LINK".
It is imperative that changes in social policies are introduced in order to raise awareness about this subject, and also that professional figures, who are involved in various ways in the fight against violence, develop collaborative approaches to limit animal abuse and other forms of domestic violence. We know that no professionalism, alone, can successfully deal with this alarming situation. Breaking the cycle of violence has become a top priority for today's society.
Understanding and tackling the connection between animal violence and violence inflicted on people represents an important tool for veterinary surgeons to protect animals' welfare.
The current problem related to the relationship between acts of cruelty to animals and violence against human beings is largely represented by the role of veterinarian in the recognition of abuse on domestic and wild animals and the ability to produce clinical and pathological evidence.
It is therefore necessary to undertake a university education programme to recognise and document external/internal body injuries suggesting abuse.
Veterinarians have many opportunities in civil and professional life to play their part in preventing crimes in the "One Health" perspective.
In the US as well as in some European countries (The Netherlands, the UK, Spain and Sweden) (National Link Coalition - June 28, 2016 - Minneapolis), strategies for the detection of animal abuse have been adopted in order to prevent violence towards the weaker categories.
This study aims to raise academic awareness about cruelty to animals, to encourage veterinarians to include non-accidental injuries (NAIs) in differential diagnosis and to report animal abuse cases, guaranteeing, at the same time, their anonymity. We will also present a selection of case studies.

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BIOMARKERS OF PATHOLOGICAL CHANGES IN VETERINARY FORENSIC SCIENCE. A CASE STUDY ON FATAL HYPOTHERMIA IN A DOG

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Primary hypothermia occurs from prolonged exposure to cold, due to the failure of thermoregulation biochemical mechanism. The postmortem diagnosis of primary hypothermia as cause of death is often difficult in forensic sciences because of negative or inconstant macroscopic and microscopic findings [1]. In human legal medicine there have recently been promising results focused on the investigation of hypothermia-induced biomarkers. Particularly, the beta-hydroxybutyric acid (B-HB) as metabolite derived from fat catabolism via Acetyl-CoA pathway occurring in stress reactions following the insulin depletion, has been reputed a valid, measurable intra vitam indicator of fatal hypothermia [2].
We have retrospectively revised a post mortem diagnosis concerning a 4 year old Pincher, female, housed outdoor in a little enclosure, died during a night with external temperature under zero degrees. The macroscopic and histopathological findings as well as the screening for parasitological and microbiological infectious agents were inconclusive. Thereafter, the post-mortem diagnosis was newly reconsidered through a biochemistry approach.
The thawed liver and humor vitreous of animal were sampled and inocula from the liver homogenate and surnatant of centrifuged humor vitreous were analysed for the B-HB using a commercial enzyme-colorimetric probe (Beta-HB assay kit, ABCAM, UK). In the same test, two inocula from liver homogenates and one from vitreous of fresh carcasses of suddenly died dogs, previously tested negative for the presence of urinary ketones, were also analysed as in house negative controls.
The analyte concentrations in liver and vitreous of the test-animal were 38.47 and 11.18 ng/microL respectively and their percent variations face to negative controls were 32.5% (average of two livers) and 136.8% (humor vitreous).
Data from this few trial do not allow a conclusive speculation. However, the B-HB concentration in the vitreous, although of lesser level, would seem more discriminating. Further investigations are needed using, in absence of blood sample, instantly preserved inocula from cadaveric liver and fluids such as the humor vitreous, pericardial and cerebrospinal liquids.
The ketogenesis in dog is slower than in the man [3], thus the B-HB concentration reaches a significant level in a longer time. Hence, the proposed biochemistry approach could find a better application in the forensic cases when they make us suspect that intra vitam pathological changes affect the onset of the ketogenesis pathway such as in the prolonged exposure to cold injury or the prolonged fasting.

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AIPVET

Oncology II

DISCOVERY OF *Ovis aries* PAPILLOMAVIRUS 3-RELATED PROTEINS IN OVINE CUTANEOUS SQUAMOUS CELL CARCINOMA

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Cutaneous squamous cell carcinoma (SCC) is widely described in animals and is the most common form of skin cancer in sheep [1]. The cause of this neoplasm appears to be multifactorial, although prolonged exposure to ultraviolet radiation and poor skin pigmentation are considered primary risk factors for tumor development [2]. However, oncogenic viruses, such as papillomaviruses (PVs) are often associated with benign and malignant tumors of the skin and mucous membranes in both humans and animals [3]. Recently, *Ovis aries* papillomavirus 3 (OaPV3) has been reported in ovine SCCs suggesting a role in the etiology of this neoplasm [4]. Nevertheless, the molecular pathways involved in viral-host interaction and skin cancer development has not been investigated. The aim of this study was to discover and validate differentially expressed proteins as candidate biomarkers of the OaPV3 infection in ovine cutaneous SCC, by proteomic and immunohistochemical (IHC) analysis. Fresh frozen non-SCC samples (N=3), OaPV3 negative SCCs (N=3) and OaPV3 positive SCCs (N=3) obtained from the udders of Sardinian ewes, were subjected to protein extraction and trypsin digestion with the Filter-Aided Sample Preparation procedure. Peptide mixtures were separated by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis and proteins identified by the Proteome Discoverer Software. Spectral counts and normalized spectral abundance factor were calculated in order to estimate the protein fold changes between the different histological samples and their relative abundance. IHC expression of deregulated proteins was analyzed in 10% formalin fixed and paraffin embedded non-SCC samples (N=10), OaPV3 negative SCCs (N=10) and OaPV3 positive SCCs (N=10). A list of 70 proteins, mainly involved in epithelial cell differentiation, extracellular matrix organization and apoptotic signaling pathway were differentially expressed in OaPV3 positive SCCs compared to non-SCC and OaPV3 negative SCCs samples ($P<0.05$). Among the deregulated proteins, IHC results revealed an increased expression of cytokeratin 13 in the intermediate and superficial layers of keratin pearls in 10/10 OaPV3 positive SCCs, suggesting a putative role of this protein as OaPV3 biomarker in SCC. To the best of our knowledge this is the first proteomic approach investigating the deregulated biological processes related to the PV viral infection in ovine SCC, and it opens new insights on the pathogenesis of the virus-host interaction.

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EQUINE SARCOID: ROLE OF ANGIOGENESIS IN EXTRACELLULAR MATRIX (ECM) REMODELING

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Equine sarcoids are benign fibroblastic skin tumors, locally invasive and rarely regressive [1]. They are notoriously difficult to treat, as they are associated with a high recurrence rate following surgical intervention [2]. It is accepted that they can develop subsequently to injury and scarring in genetically predisposed equines and that BPV-1 and less commonly BPV-2 are widely recognized as the causative agents of the disease [3]. Even though, the viral etiology, biology, morphology and epidemiology of equine sarcoids are known, the pathogenic events leading to the development of tumor are poorly understood. The aim of this study was to further understand the pathogenesis of sarcoids, investigating the immunohistochemical expression of Ki67, Bcl2, Factor VIII and VEGF in 15 sarcoid samples positive to BPV-1 and BPV-2, and 3 normal skin samples, previously processed by routine histological methods. Twenty fields of each section (at least 1,000 cells), at X400 magnification, were randomly chosen in order to quantify the immunohistochemical labeling and the results were expressed as percentage. In 100% of sarcoids samples, Ki67 immunoreactivity was strong in the majority of epidermal basal cells (> 90%) and weack to strong in dermal cells, in which the percentage ranged from 5% to 10%. Bcl2 immunoreactivity was detected in 8 of 15 sarcoids (53%). The percentage of Bcl2 positive fibroblasts,located immediately under the epidermis, ranged from 20 to 50% and the immunostaining was moderate. These results seem to suggest a central role of keratinocytes in the regulation of fibroblast proliferation and survival. Furthermore, in all sarcoids samples ,VEGF showed a strong and finely granular cytoplasmic staining pattern in the majority (>90%) of keratinocytes, fibroblasts and endothelial cells. Numerous small vessels, often irregular in shape and without a distinct lumen, were immunostained with Factor VIII. These data seem to support the important role of VEGF in microvascular regeneration and that the partial or total occlusion of vascular lumina could be responsible to maintain a deficient oxygen gradient within the tissue and, paradoxically, exacerbate angiogenesis [4]. In conclusion, mild hypoxia could increase production of VEGF, which in turn could increase collagen production by fibroblasts and promoting their survival with concomitant intensification of ECM deposition and reduction of its degradation, due to an alterate expression of MMPs and TIMPs [5]. These results strongly support the hypothesis that sarcoid formation is due to an imbalance between production and degradation of collagen and demonstrate that VEGF and hypoxia play a crucial role in its pathogenesis.

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Felis catus PAPILLOMAVIRUS TYPE-2 E6 ONCOGENE IMPAIRS P53 PROTEIN STABILITY IN AN IN VITRO FELINE MODEL OF VIRAL PATHOGENESIS

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Felis catus PV type-2 (FcaPV-2) is the causative agent of feline squamous cell carcinomas (SCCs); its oncogenes E6 and E7 are transcribed in tumour samples and display transforming properties in vitro [1]. The p53 tumour suppressor protein is the main target of PVs E6 [2]. High risk human PVs (HR-HPVs) E6 promote ubiquitination of p53 by forming a ternary complex with the ubiquitin ligase E6AP, thus leading p53 to accelerated proteasomal degradation [2]. We have recently demonstrated that FcaPV-2 E6 binds to and impairs p53 protein level in feline epithelial cells [1]. The aim of this study was to gain new insights into the molecular events underlying p53 downregulation by FcaPV-2 E6 and check for E6AP expression in order to hypothesize its possible involvement as in human counterpart. CRFK cells stably expressing cloned FcaPV-2 E6 or the empty pCEFL vector were analysed by western blotting (WB) and double immunofluorescence (IF) for p53 and E6AP. To investigate p53 half-life, cells were treated with protein synthesis inhibitor cycloheximide 20 µg/mL for 0, 0.5, 1, 2.5, 5 hours (h) and subjected to WB for p53. Concomitantly, p53 and E6AP protein rescue was evaluated by WB after incubation of cells with the proteasome inhibitor MG132 30 µM for 4 h. Three feline oral SCCs cell lines (SCCF1, SCCF2, SCCF3), provided by Prof. T.J. Rosol from Ohio State University, were further included in the study as in vitro model of SCC. SCCF1, SCCF2 and SCCF3 cells were checked for p53 and E6AP expression by WB. All the WB experiments were normalized for β-actin as loading control. Expression of p53 was confirmed to be lower in CRFKE6 compared to CRFKpCEFL. Interestingly, E6AP protein levels showed the same trend. By IF staining E6AP co-localized with p53 in the cytoplasm of CRFKE6 but not in control cells. The p53 half-life was shorter in CRFKE6 and, importantly, the percentage of protein reduction with respect to time 0 was higher at each time point compared to control cells. Consistently, proteasome inhibition induced higher accumulation of p53 in cells expressing E6 and the same was observed for E6AP. Taken together, these results suggest that FcaPV-2 E6 may promote E6AP-p53 physical interaction and downregulate p53 protein by accelerating its degradation through the proteasome pathway, similarly to HR-HPVs E6 [2]. Moreover, the data indicate that FcaPV-2 E6 may promote E6AP proteasomal degradation as well, thus disrupting ubiquitination pathway and protein homeostasis within cells as already known for HPV16 [3]. In addition, consistent preliminary data were obtained in SCCF cells. Interestingly, p53 was undetectable and E6AP expressed at lower levels in SCCF3 cells, in which expression of FcaPV-2 E6 had been exclusively detected, suggesting a similar scenario in both the experimental cell models.

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PLACENTAL PAPILLOMATOSIS IN WATER BUFFALO ASSOCIATED WITH BOVINE DELTAPAPILLOMAVIRUS

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Bovine papillomaviruses (BPVs) are small DNA oncogenic viruses that can infect both epithelial and mesenchymal tissues of many animals causing both benign and malignant lesions. BPV comprise twenty-one types, which have been classified in to four genera: Deltapapillomavirus (BPV-1, -2, -13, 14), Xipapillomavirus (BPV-3, -4, -6, -9, -11, -12, -17 and -20), Epsilonpapillomavirus (BPV-5, -8). Dyoxipapillomavirus (BPV-7, -16 and -18). BPV-19 and BPV-21 are currently unclassified. The only papillomaviruses showing clear evidence of transmission across species barriers are Deltapapillomavirus. There are numerous reports of BPV infection in other animals including horses, cape mountain zebras, giraffe and antelope. In water buffalo, Deltapapillomavirus infection has been described in urinary bladder tumours [1], and in fibropapilloma of the skin and vulva [2]. To our knowledge, study of BPV infection associated with neoplastic lesions in placenta and other reproductive disorders have been poorly investigated in large animals. Recently BPV-2 infection has been shown to occur in placental trophoblast cells of pregnant cows [3]. In this study we describe the presence of Deltapapillomavirus in papillomas of the placenta in water buffalo (bubalus bubalis). Six buffalo placentas with evidence of papillomas were macroscopically examined. Samples from the lesions were collected for routine histology, immunohistochemistry and for subsequent molecular procedures. Macroscopically, the lesions were distributed in groups and ranged from 1 to 20 mm in diameter. The larger tumours appeared pedunculate, brown-white in color, with finger like projections. The smaller tumours were sessile, flattened, yellow with a pitted surface. Histologically, the lesions consist in a marked proliferation of epithelial cells with large, mildly eosinophilic and vacuolated cytoplasm, and nuclei with dense chromatin. DNA Deltapapillomavirus was amplified in papilloma samples. Western blot analysis revealed a marked E5 protein expression in placenta papilloma. A cytoplasmic E5 immunoreactivity was clearly shown in 50% of neoplastic epithelial cells, valuated selecting 10 fields at 20x magnification for each section. This study shows an association between Deltapapillomavirus infection and papilloma of the placenta; furthermore, papillomavirus may be involved in buffalo reproductive disorders. It is worthwhile noting that the incidence of reproductive disorders in large animals, like in fertility and abortions, caused by infectious agents is continuously increasing thus leading to substantial economic losses. This is even more so as the major causes of miscarriage are only rarely identified. Further investigation is needed to better understand the role of BPVs in placental pathology that may result in adverse pregnancy outcomes.

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AIPVET

Infectious Diseases

FATAL *Leucocytozoon* INFECTION IN A CAPTIVE GREY-HEADED PARROT (*Poicephalus robustus suahelicus* Reichenow, 1898)

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Haemoparasites infect all species of birds and are transmitted by arthropod vectors. Asymptomatic infections are common, but protozoal parasites can also cause the death of infected birds. A fatal infection by *Leucocytozoon* in a 1 year old captive female Grey-Headed Parrot (*Poicephalus robustus suahelicus*) is described. Necropsy was performed and tissues were collected for light and transmission electron microscopy, biomolecular examination and for evaluating iron accumulation in tissues. At necropsy severe spleen and liver enlargement were observed. Viscera appeared pale. Pulmonary oedema and some multifocal areas of necrosis in the liver and myocardium were also observed. Histologically, the presence of few schizonts were observed in hepatocytes and endothelial cells. A very large number of macrophages filled by characteristically shaped and basophilic merozoites suggestive of Leucocytozoon spp. was observed in the liver, spleen, and the lung parenchyma. Perls'stain showed that iron was present at a very highconcentration in liver, both in macrophages and in hepatocytes. Spleen and kidney contained also scattered deposits of stainable iron. Ultrastructurally, numerous early or mature schizonts containing 1.5 to 3 µm in size, round to elongate merozoites were present within hepatocytes. All organs tested by nested-PCR resulted positive for *Leucocytozoon* spp. An iron level of 74.40 mg/kg was recorded in liver. *Leucocytozoon* frequently shows a higher pathogenicity compared to *Haemoproteus*, as the gametocyte phase can occur within either erythrocytes or leucocytes depending on the host and the species, frequently leading to megaloschizonts occurring in many organs and muscle tissues, and causing severe damage and necrosis. Fatal *Leucocytozoon* infections are rarely reported in parrots in Europe [1, 6] and haemoprotozoa are rarely reported in African parrots. To the authors' knowledge there is only a single report of *Plasmodium* infection in *Poicephalus meyeri* [3]. Nevertheless, *Haemoproteus* has been recorded in *Poicephalus robustus* [4,5], in captive birds in London Zoo. The clinical history and post mortem examination of the individual bird suggest that death caused by *Leucocytozoon* in this aviary may be due to a range of predisposing factors influencing susceptibility to infection. Blood parasite infections and concurrent diseases can be associated with breakdown of tissues and blood cells resulting in excess iron accumulation in the liver and spleen [2]. Anamnesis associated with gross, histological, transmission electron microscopy, molecular and chemical findings, clearly indicates a fatal haemoprotozoal infection in a captive Grey-Headed parrot. This case is the first description of fatal *Leucocytozoon* infection in an African Parrot and the first report of *Leucocytozoon* in psittacine birds in which morphological identification is confirmed by PCR analysis.

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PATHOLOGICAL AND MICROBIOLOGICAL INVESTIGATIONS ON GREAT CORMORANT (*Phalacrocorax carbo*) POPULATIONS IN THE PROVINCE OF CUNEO (ITALY)

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The Great Cormorant is a cosmopolitan and ichtyophagous aquatic bird belonging to the *Suliformes* order [1]. It is a migratory species, although it has recently become nesting in some regions (e.g. Piedmont). Due to its demographic increase and to the damage caused to fish farms and rivers, this species is subjected to population control programmes.

Aims of the work were to evaluate the presence of diseases by anatomo-histopathological and microbiological investigations on cormorant, to hypothesise any public health risk and to evaluate environmental contaminants (cadmium and lead) to assess water pollution.

Fifty-seven animals were subjected to a necroscopic examination and sampled for microbiological, parasitological and toxicological investigations. Liver, lung, heart, spleen, kidney and skeletal muscle were fixed in 10% buffered formalin for histological examination. For virological (West Nile Virus, Newcastle Disease and Influenza A), bacteriological (*Borrelia burgdorferi, Chlamydia* spp., *Rickettsia* and *Coxiella burnetii*) and parasitological (*Toxoplasma gondii, Neospora caninum, Haemoproteus* spp., *Plasmodium* spp., *Leucocytozoon* spp. and gastrointestinal parasites) investigations, and for the quantification of cadmium and lead, samples were frozen at -20°C.

Histologically the most affected organ was the liver, with 54.7% animals showing lymphocytic infiltration. Virological and bacteriological examinations resulted negative, with the exception for *Chlamydia* spp. (6/57 animals were positive and in 5/6 cases *C. psittaci* was identified). Positivities for *Haemoproteus* spp., *Plasmodium* spp. and *Leucocytozoon* spp. were also detected. Helminths were found in 92.6%, 100% and 33.3% of animals examined for *Contracaecum rudolphii, Paryphostomum radiatum* and flatworms respectively. The quantification of heavy metals revealed normal values in liver and feathers for cadmium, whereas lead resulted increased in 6 subjects (1 liver and 5 feathers). Such deviations may indicate accumulation due to biomagnifications, or to direct environmental contact between the matrix and the pollutant [2].

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AIPVET

Miscellaneous

AN UNCOMMON CARDIOMYOPATHY IN A CAPTIVE CHIMPANZEE (*Pan troglodytes*)

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Heart disease is the main cause of death in captive chimpanzees (*Pan troglodytes*) reaching a prevalence of 68% in adult animals [1]. The present case report describes the findings of an uncommon cardiomyopathy in a captive chimpanzee.

A 41 year old male chimpanzee was submitted for post-mortem examination. The animal was hosted in a zoological garden. During late 2015, poor body conditions, depression, tachipnea and abdominal breathing were observed. A moderate pleural effusion was found, drained, and classified as transudate at cytology. Clinical and instrumental findings suggested the diagnosis of atrioventricular valve regurgitation and dilated cardiomyopathy. Even though a therapeutic protocol was promptly started, clinical conditions gradually deteriorated through the following months and eventually the animal was found dead.

A complete necropsy was performed. A subcutaneous abscess was observed on the left hemithorax, and a serous/hemorrhagic transudate was revealed at the opening of the thorax. The heart was markedly enlarged and lungs were compressed. The epicardium presented small fibrin depositions on the surface and the right ventricle was dilated. At the heart dissection, both atrioventricular valves were atrophic. Prominent nodular thickening of free margins of both valves was observed. Hepatomegaly and perihepatitis were also noticed.

Histologically, both atrioventricular valves were repleted with blood and foci of chronic mononuclear inflammation were observed. Myocardial fibres were extensively replaced with connective and adipose tissue and the remaining ones showed a marked variation in size. Interstitial fibrosis was diffusively detected and a considerable amount of oedema was evident between atrophied fibres. The lungs showed mild perivascular mononuclear cell infiltrates and alveolar oedema.

Several samples were submitted to bacteriological investigations. *Staphylococcus aureus* was isolated from lungs and exudate. Routine parasitological exam and molecular analyses (carried out for the detection of *Herpesviridae*, Hepatitis A, Hepatitis B, Hepatitis C, and *Toxoplasma gondii*) tested negative.

Fibrosing cardiomyopathy complicated by chronic endocarditis and toxic myocarditis was diagnosed.

Interstitial fibrosis is widely reported in aged chimpanzees and is considered a significant cause of cardiac disease in this species [1]. In the present case report, a secondary pathological chronic process has been found. The chronic trend and the growth of *S. aureus* in two specimens lead to presume that the cardiac lesions, other than interstitial fibrosis, could be caused by a slow dissemination of the bacterium and its toxins from the subcutaneous abscess. In humans *S. aureus* is considered the predominant cause of infective endocarditis [2]. Moreover *S. aureus* toxins have been shown to induce localized cytotoxicity and persistent inflammation, preventing the healing of the damaged site [3]. The findings described in this case report may help to understand cardiovascular disease in great apes.

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IMPRESSION CYTOLOGY OF HEALTHY OCULAR SURFACE IN HORSES: COMPARISON WITH CYTOBRUSH TECHNIQUE

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Cytobrushing is the most common technique used to sample the equine ocular surface. Impression cytology (IC) is an innovative, non-invasive method, which allows to collect the superficial layers of ocular epithelium. It's routinely used in human medicine while in veterinary medicine it has not been commonly applied yet. IC has been experimented first in dogs and cats [1,2], but few studies in large animals have been done [3]. The aims of this study are: 1) to compare cytobrush technique and impression cytology of the cornea and bulbar conjunctiva of healthy equine eyes; 2) to assess the agreement between two observers with different level of expertise; and 3) to evaluate the normal pattern of ocular impression cytology in the horse.

Twenty-four horses were sampled few minutes after slaughtering. All samples were obtained from superior limbus using IC on the left eye and cytobrush on the right eye. Specimens were stained with Wright-Giemsa stain and evaluated by two observers with different expertise, a board-certified clinical pathologist and a post-doc researcher with 3 years of experience in cytology. Cellularity, preservations of morphology, different cellular types' morphology, presence of goblet and inflammatory cells were evaluated using a four-grades scoring system.

In IC samples, corneal and conjunctival cells were clearly recognized and separated. IC samples showed good preservation and cellularity for both cell population in 16 out of 24 samples (66%) and 6/24 (25%), respectively. Good preservation and cellularity were limited to only one cell population in 8 samples (33%) and 7 (29%), respectively. In 6 IC samples (25%) inflammatory cells were noticed and in only 5 samples (24%) goblet cells were present. Cytobrush specimens appeared well preserved in 15/24 (63%) cases and with good cellularity in 7/24 (29%) for both cells types. Both corneal and conjunctival cells were present, but without a clear separation. Cellularity, preservation and the enumeration of goblet cells were higher in IC compared to brush technique (P= 0.013; P=0.004; P=0.031). There was moderate to fair agreement about cellular morphology in IC between observers.

IC of ocular surface in horses gives samples with good cellularity and well-preserved, better than cytobrush samples. Moreover, goblet cells were found easier with impression cytology. However, cytobrushing, that allows bigger magnification, is recommended to evaluate more specific details of cytoplasm and nucleus.

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A DEGENERATIVE LEUKOMYELOENCEPHALOPATHY OF UNKNOWN ORIGIN IN AN AZAWAKH DOG

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The term ”leukodystrophy” is generally used to describe inherited and progressive disorders primarily and directly affecting the myelin of the Central Nervous System (CNS) [1]. In veterinary neuropathology leukodystrophies are more strictly considered as disorders of myelin synthesis and maintenance distinguished from insufficient or retarded production of myelin (hypomyelination), affecting bilaterally symmetrical selective areas of the white matter, with destruction of myelin and eventually axons [2]. Diseases displaying a comparable confluent primary progressive loss of myelin, but lacking a frank evidence of genetic origin are referred to as “leukoencephalopathies” [1]. A number of leukodystrophies and degenerative myelopathies have been described in selective canine breeds, not infrequently of difficult classification. In Azawakh dog, degenerative diseases have been reported neither in the CNS, nor in the Peripheral Nervous System.

The aim of this study is to describe neuropathological findings of a degenerative disorder primarily affecting the spinal cord in a 6-year-old male Azawakh dog showing a history of slowly progressive ataxic syndrome of seven months duration associated with sensory disorders.

Gross and histological examination by Luxol fast blue-PAS and Bielschowsky stains were performed on CNS. Additional IHC was performed on selected FFPE brain and spinal cord sections using avidin-biotin peroxidase complex staining for glial fibrillary acidic protein (GFAP, Dako, Carpenteria, California, USA) and neurofilaments (NF, Biomol, Plymouth Meeting, PA, USA). Additional semithin and ultrathin sections were also obtained from the cervical spinal cord and selected peripheral nerves.

A diffuse bilaterally symmetrical leukomyelopathy was observed consistent with severe bilaterally symmetrical demyelination and vacuolisation confined to the dorsal columns along all the spinal cord with a minor axonal degeneration. The main changes of myelin sheath consisted in splitting and intramyelin edema. Myelin sheath free axons were scattered in a network of astrocytic processes and isolated collagen fibres. Few reactive macrophages exhibiting a foamy pattern were observed adjacent to the small vessels. In the brain, a spongy change was observed in the raphe nuclei, spinal tract and nuclei of trigeminal nerve, and rostral cerebellar peduncles. Lesions were no found in peripheral nerves, nor in the spinal ganglia.

To our knowledge, this is the first neurodegenerative disease described in Azawakh dog. Considering the late age of onset and the lack of a similar neurological disorder in relative dogs, an inherited origin remains doubtful. A nutritional or toxic-metabolic disorder cannot be excluded in the pathogenesis of the lesions.

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UNRAVELLING A MYSTERY: CHARACTERIZATION OF INFLAMMATORY INFILTRATE OF ULCERATIVE DERMATITIS IN MICE

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Ulcerative dermatitis (UD) is an idiopathic, spontaneous and progressive disease typically affecting aged mice on a C57BL/6 background [1]. This condition is clinically characterized by intense pruritus and scratching leading to severe skin lesions that are resilient to treatment [1]. Although several etiologic factors have been implicated in the development and progression of UD, the etiopathogenesis of this disease is still largely unknown [1]. Hence, we have evaluated the phenotypes of inflammatory infiltrate in UD skin lesions in order to provide some new insights on UD pathogenesis. For this study, we analyzed moderate to severe cases of ulcerative dermatitis observed in 20 out of 60 transgenic mice with a C57BL/6 background. Immediately after euthanasia, skin samples were fixed in 10% neutral buffered formalin for histopathology or cryopreserved for molecular analysis. 10 unaffected skin biopsies from mice with a C57BL/6 background were used as negative controls. Four-µm paraffin sections were stained with haematoxylin and eosin (HE) and toluidine blue (TB). Moreover, immunohistochemical analysis for CD3, CD45, CD4, CD8, IL-17 and MHC II was performed to characterize and quantify lymphocytic infiltrate. Macroscopically, lesions varied from coalescing crusts to irregularly shaped areas of ulceration extending mostly to the dorsal cervical region. Histologically, affected skin revealed extensive areas of ulceration and a diffuse, severe and mixed inflammatory infiltrate in the dermis, often reaching deep underlying structures, consisting mostly of lymphocytes, neutrophils and macrophages. No relevant changes were observed in control's skin. We also observed an increase of mast cells in the affected skin compared to controls, a predominant CD3 and CD4 positive lymphocytes with a fewer number of CD45 positive cells and IL-17 positive lymphocytes and mast cells. No immunoreaction was observed for CD8 antibody. Moreover, a MHC class II immunoreaction of dermal and subcutaneous endothelial cells as well as inflammatory cells was also detected. Gene expression array of affected mice showed an upregulation of ST2 gene. ST2 gene have been demonstrated to play an essential role in allergic Th2 response [2], while little is known about the interaction between ST2 gene products and Th17 cells, but some authors recently suggested that also Th17 cells, together with Th2 cells, may be involved in the pathogenesis of allergic airway disease in mice [3]. Although the causative trigger of ulcerative dermatitis is still not completely understood, our preliminary data lead to further investigate the hypothesis that UD in mice have an allergic etiopathogenesis.

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ALOPECIA X, CYCLICAL FLANK ALOPECIA AND FOLLICULAR DYSPLASIA: EXPRESSION OF P63 IN CANINE HAIR FOLLICLE

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Skin diseases characterized by alopecia are generally associated with a deregulation of the Hair Cycle (HC) or an abnormal development of the Hair Follicle (HF), although their pathogenesis is not completely defined. Adult skin contains several reservoirs of stem cells (SCs) located in the epidermis, HFs and probably sebaceous glands. P63, a member of the p53 family of proto-oncogenes, is a transcription factor that is likely to play a key role in regulating self-renewal and long-term proliferative capacity of SCs [1]. P63 is also important in skin development and homeostasis, by promoting cell proliferation in basal and suprabasal epidermal cells, as well as in the outer root sheat (ORS) and matrix of HFs [2]. The immunohistochemical evaluation of p63 expression in the scalp of affected (frontal) and unaffected (occipital) skin of human patients with androgenetic alopecia showed that the root sheaths from occipital skin had significantly higher expression of p63 in comparison to frontal areas [3]. The aim of our study was to evaluate the immunohistochemical expression of p63 in 15 necropsy samples of canine normal skin and 16 skin biopsy samples from dogs affected by Alopecia X (n=10), Cyclical flank alopecia (n=4) and Canine follicular dysplasia (n=2), in order to determine if this molecule may be involved in the pathological mechanisms of HC arrest and if there are differences in p63 expression between the various types of canine alopecia. The number of p63-positive nuclei in HF cells for each sample was calculated in 10 randomly selected high-power (×400) fields, counting at least 1000 positive nuclei, and expressed as a percentage. P63 expression was only detectable in the ORS cells and bulbs of HFs, with a significantly higher nuclear score in canine normal skin (89.9%; +/- 2%) when compared to affected skin (76.3%; +/- 4,7) ($p<0.001$). On the other hand, HF papilla and inner root sheath (IRS) were negative for p63 in both normal and pathological samples. Although p63 immunoexpression appeared to be higher in Alopecia X (77.2%) when compared to the other pathological groups, especially Canine follicular dysplasia (71%), differences did not reach statistical significance due to the limited number of samples available for dysplastic diseases. In addition, a higher variability of expression was detected between the different pathological samples in comparison to normal samples. These findings indicate a partial loss of p63 expression in canine HF diseases, similarly to the changes observed in human androgenic alopecia [2]. On the basis of these results, p63 may be supposed to play a role in the pathogenesis of impaired follicular regeneration and HC arrest occurring in the investigated canine HF diseases.

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EXPRESSION AND BIOCHEMICAL PROPERTIES OF CELLULAR PRION PROTEIN IN SKELETAL MUSCLE OF COWS ARE NOT AGE DEPENDENT

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The cellular prion protein (PrP^C) is a membrane-bound sialoglycoprotein highly expressed in the nervous tissue while present at a lower level in skeletal muscle and other organs. PrP^C is essential for prion propagation in human and animal prion diseases, characterized by accumulation of an aberrant, proteinase resistant, isoform of PrP (called PrP^{sc}) predominantly in the brain. PrP^C overexpression has been described in many different human neuromuscular disorders (1). Little is known about the expression of PrP^C in health and disease in bovine's muscle tissue. The aim of this study was to evaluate the presence, the molecular and biochemical properties of PrP^C in skeletal muscle of cows as well as their possible changes in muscle aging. Skeletal muscle and brain samples of 12 aged (7-22 years old) and 8 young (1 year old) cross-breed Podolica cows were collected at the slaughterhouse and snap frozen in liquid nitrogen. All the brains were tested BSE negative. Muscle cryosections were examined by a standard panel of histological and histoenzymatic stains as well as immunohistochemistry for PrP and desmin. Immunoblot was performed on muscle and brain homogenates in order to analyze the amount of PrP^C, its glycosylation profile, proteinase resistance, solubility levels after high speed centrifugation and tendency to aggregate by sucrose gradient assay (2). Histologically, muscle biopsies of aged cows showed myopathic features such as vacuolated fibers (67%), angular atrophy (58%), degenerated fibers (66%) and lymphocytic inflammation (40%) as already described in bovine sarcopenia (3). Vacuolated muscle fibers, degenerated fibers and less severe angular atrophy were found in a lower number of young animals (25 and 12%, respectively). Immunohistochemistry for PrP revealed a distinct granular positivity in intramuscular nerve branches and muscle spindles in all cases. A faint membrane positivity was evident in about 50% of cases, occasionally associated with angular and atrophic fibers. A focal subsarcolemmal granular positivity was found in degenerated fibers (desmin depleted) of both young (30%) and old (66%) animals with an increased immunolabeling of rimmed vacuoles. Occasionally, mononuclear endomysial infiltrate was PrP positive as well. No differences between muscles and brains of young and old animals were detected by quantitative immunoblot and Proteinase K titration. The glycoform profile and the molecular mass of PrP^C in muscle samples appeared slightly different compared to the brain. Sucrose gradient velocity sedimentation gave variable results, with a generally lower amount of PrP in high density sucrose fractions in the muscle compared to the brain. After high speed centrifugation the majority of muscle PrP^C was found in the soluble fraction, without significant differences between young and old animals. Our preliminary data suggest that: 1) PrP^C is overexpressed in degenerated, vacuolated and atrophic muscle fibers; 2) The glycoform profile of PrP^C differs between brain and muscle tissue; 3) Detergent solubility, PK resistance and PrP^C aggregation in skeletal muscle do not increase with age.

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HER2 PROTEIN OVEREXPRESSION AND GENE AMPLIFICATION IN FELINE PULMONARY CARCINOMA

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Pulmonary carcinomas are aggressive and clinically silent neoplasm of old cats [1]. The molecular carcinogenesis in human lung cancer is orchestrated by several oncogenes such as *EGFR, RAS, HER2, ALK* [2]. *HER2* gene encodes for the homonym tirosin kinase receptor that undergoes ligand independent dimerization, phosphorylation and linking with downstream proliferation pathways such as PI3K and MAPK [3]. The aim of this study was to investigate the HER2 protein overexpression and gene amplification status in feline pulmonary carcinoma.

Thirteen feline primary pulmonary carcinomas were retrospectively selected and analyzed on HE stained sections. The diagnoses were revised based on the current WHO classification system [4, 1]. Serial sections were immunohistochemically processed using antibodies against TTF1 and HER2. TTF1 was semiquantitatively evaluated, either positive or negative. HER2 protein was scored based on the ASCO CAP guidelines and grouped in tumors with positive (3+), equivocal (2+) and negative expression (1+, 0) [5]. All the HER2 positive and equivocal tumors and a subset of negative tumors were subjected to fluorescence in situ hybridization (FISH) with an ERBB2 probe in a dual core tissue microarray assay. Based on the "Agenzia Italiana del Farmaco" recommendation, *HER2* was considered amplified with > 4 gene copy numbers.

Ten adenocarcinomas (6 solid, 2 lepidic and 2 papillary patterns), 2 squamous cell carcinomas and 1 adenosquamous carcinoma were diagnosed. TTF1 was positive in 5 cases (38.5%). HER2 was overexpressed (3+) in 2 cases (15%), equivocal (2+) in 5 cases (38%) and negative (1+, 0) in 6 cases (46%). Eight cases were evaluated by FISH analysis, but 2 cases (25%) were *HER2* indeterminate, because technically inadequate. *HER2* gene status was amplified in 3 cases (38%), gain of function but not amplified in 2 cases (25%) and not amplified with a diploid copy number in 1 case (13%). *HER2* gene amplification was detected both in tumor with protein overexpression and equivocal and negative expression.

This is the first study that describes the presence of HER2 protein overexpression and gene amplification in feline pulmonary carcinoma and provides data for targeted therapeutic perspectives in this species.

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Produzioni Animali e Sicurezza Alimentare

COLOUR CHANGES IN THAWED TUNA TRETATED WITH VEGETABLE EXTRACTS VACUUM PACKAGED AND STORED AT REFRIGERATION TEMPERATURE

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Tuna fish is one of the largest selling fishery products. Naturally fresh tuna has a red pigmentation but over time pigmentation turns brown depending to oxidation process. Nitrates and nitrites are used in foods as antimicrobials but also because their property to enhance color [1]. Use of these additives is not authorised in unprocessed fishery products; however food operators use vegetable extracts containing nitrates and nitrites to enhance tuna's colour. This treatment is not allowed because masks the real freshness of the fish; so public health issues have to be considered regarding potential high level of histamine and formation of carcinogenic nitrosamines during cooking. Aim of the work was to evaluate the colour changes in thawed tuna fillets treated with a commercial blend containing vegetable extracts. For the trial were used no. 4 fillets of thawed tuna (*Thunnus albacares*) each of 2.5 kg belonging from the same lot, originated from tuna caught 2 months earlier, prepared on board and quickly frozen. The commercial mixture was prepared according to the manufacturer's specifications and injected by the means of a multi-needle automatic machine in n. 3 fillets. No. 1 fillet was used as control. After treatment, each fillets was packed in nylon-polyethylene pouches with a Multivac model A 300 vacuum packaging machine (Bury, Lancs., UK) and stored at refrigeration temperature. Before treatment (T0) and after 7 days (T1) a slice (200 g) of each fillets was subjected to colorimetric assessment (Konica Minolta CR300, Minolta, Osaka, Japan), histamine [2], nitrates [2], TVB-N [3] and TBA [4] analysis. After 7 days treated fillets showed a bright red colour with no significant differences with respect to colorimeter values of T0. Differently control samples colour turns brown and significant differences were highlighted compared to colorimeter values of T0. Nitrates were found only in treated samples, reflecting that commercial blend contains these compounds. Histamine at T0 was <5 ppm. After 7 days storage levels detected in treated samples were 580±110 ppm, higher than those set by the Reg. EC 2073/05. The average values of TVB-N at T0 were 9.35 mg/100g. At the end of trial concentrations of 17.33 mg/100g were detected, indicating a good tuna quality. Malonaldehyde values were lower than those reported in literature for lipid oxidation. Reg. EU 1129/2011 in unprocessed fishery products allows only ascorbates, citrates, erythorbates and polyphosphates. If in the next future UE legislation will allow the use of vegetable extracts, in our opinion is mandatory analysis on histamine residues on fillets treated. In fact histamine levels detected in our study confirm that this treatment should mask potential high level of histamine.

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STUDY ON MICROPLASTICS DETECTION IN BIVALVE MOLLUSCS REGULARLY COMMERCIALIZED

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Microplastics in food could represent a risk for human health due to their potential of adsorbing chemical pollutants [1]. Plastic debris can easily reach the top of the food chain, since they are gathered in the digestive tract of several seafood [2]. Moreover, molluscs are mostly used as bio-indicators and can be used for the control of the level of sea-contamination [3]. For all these reasons, the EFSA Panel for Contaminants in the Food Chain was asked to deliver a statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood [1]. The aim of the present study was to evaluate and quantify the presence of plastic extraneous particles (PEP) in 2 species of bivalves regularly commercialized. A total of 51 fresh samples (33 mussels, *Mytilus galloprovincialis* and 18 clams, *Tapes decussatus*), from different breeding in Mediterranean Sea (FAO 37.2.2 and 37.2.1), were analyzed for the presence of PEP. A total amount of 10 g of pulp and intervalve liquid from each sample was collected for the soft tissue digestion using hydrogen peroxide 30% Vol, and then processed for flotation, with an over-saturated salt solution (NaCl 1-2g/mL). This process allows the separation of the plastic debris from sediment, based on the specific weight [4]. The supernatant was subsequently filtered by filters with pores diameter of 5 µm and then observed under a stereo-microscope. Plastic debris were identified in 82.4% (42) of the total samples, in particular: 100% (33) of mussels and 50% (9) of clams. The total abundance of plastic fragments (n=120) was 0.3±0.3 PEP/g. Plastic fibers represented the 84.2% (n 101) of PEP, while the 15.8% (n=19) was identified as microplastics. Among different species, *M. galloprovincialis* revealed a level of contamination (0.36±0.30 PEP/g) significant higher ($p<0.05$) than *Tapes decussatus* (0.12±0.22 PEP/g). The study confirmed the wide diffusion of plastic debris in bivalves from Mediterranean Sea, with important differences between mussels and clams. The potential unsafe impact on human health, after consumption of contaminated seafood, needs to be further investigated.

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Pagellus erythrinus: THE COMPLETE MITOCHONDRIAL GENOME

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The study of mitochondrial DNA (mtDNA) has become a very common approach in population genetics and evolutionary studies. MtDNA is used as marker to detect fraudulent substitutions in prepared and transformed fish products [1]; the target nucleotide sequences are fragments belonging to the genes cytochrome b, ribosomal 16S and 12S subunits, and cytochrome c oxidase subunit 1 [2]. However, the use of short segments of the mtDNA may give ambiguous results, because fragments are too short to contain sufficient genetic information. The analysis of the complete mitochondrial genome (mitogenome) of fishery products allows to tackle more thoroughly fish species identities. The *Sparidae* family comprises about 41 species, some of which are of considerable economic importance [3]. However, yet there is a paucity of genetic information regarding these species. The common pandora (*Pagellus erythrinus*) is one of the most commercially-caught *Sparidae* species in the Mediterranean Sea and is often fraudulently replaced with less expensive species of the same genus. The aim of this research was to obtain the complete nucleotide sequence of the *P. erythrinus* mitogenome in order to obtain more information for unambiguous species identification. A whole specimen was obtained from fish market and identified based on morphological and molecular features. Total DNA was extracted from dorsal fin using the DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's instructions. The mitogenome of *P. erythrinus* was determined by using a combination of long and short PCR, followed by Sanger and Illumina MiSeq sequencing methods. The total lenght of the common pandora complete mitogenome was determined to be 16,694 bp. It contains 13 protein-coding genes, 2 ribosomal RNA genes (12S rRNA and 16S rRNA), 22 transfer RNA genes (tRNA) and 1 control region. Most of the genes were encoded on the heavy strand, with only the NADH dehydrogenase subunit 6 (ND6) and eight tRNA genes [Gln, Ala, Asn, Cys, Tyr, Ser (UCN), Glu, Pro] encoded on the light strand. The nucleotide composition is 27.4% A, 28.2% C, 27.5% T and 16.9% G, which is similar to other *Sparidae* mitogenomes [4]. All the protein-coding genes began with an ATG start codon, except for COX1 and NAD4, which started with GTG. Five types of stop codons revealed are TAA (ND1, ND2, ATP8, ATP6, COX3, ND4L, ND6), AGG (COX1), and T (COX2, ND3, ND4, CYTB). The 12S and 16S rRNA genes were located between the tRNA-Phe (GAA) and tRNA-Leu (TAA) genes, and were separated by the tRNA-Val gene. The 22 tRNA genes vary from 66 to 74 bp in length. The 994 bp-long control region was located between tRNA-Pro (TGG) and tRNA-Phe (GAA). In agreement with Regulation (EU) 1379/2013, the complete mitogenome characterization will allow a complete comparison and analysis of fish species. Other *Sparidae* mitogenomes are currently under sequencing for comparison studies.

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PREVALENCE AND MEAN INTENSITY OF *Anisakis* SPP. IN TWO ANGLERFISH SPECIES (*Lophius piscatorius, Lophius budegassa*) CAUGHT IN MEDITERRANEAN SEA

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Anisakiasis is the human infection with third larval stage of nematodes belonging to the families *Anisakidae* or *Raphidascaridae*. *Anisakis* is the genera most associated with anisakiasis. Marine mammals are definitive hosts, while aquatic invertebrates and fish are intermediate or paratenic hosts. In intermediate fish the larvae penetrate the intestine and invade the body cavity or the muscles, where they become encapsulated as the third stage. In wild Anglerfish (*Lophius budegassa* and *Lophius piscatorius*) a high prevalence of *Anisakis* and *Pseudoterranova* larvae in muscles was demonstrated [1], for this reason an epidemiological survey of *Anisakidae* larvae was carried out. A total of 58 viscera and muscle of Anglerfish (*Lophius budegassa* and *Lophius piscatorius*) from Tyrrenian sea and Adriatic sea (F.A.O. zone 37.1.3 and 37.2.1 respectively) were examined for *Anisakidae* larvae detection by digestion method. Extracted parasites were counted and mean intensity was calculated. Parasites were identified by traditional and molecular techniques and the most frequent species isolated was *Anisakis pegreffi*. In viscera, the main localizations of the larvae were under the gastric serosa and intestinal serosa, where several parasites, alive and dead, were found. The visceral prevalence was 75% and the mean intensity was 4.48 for fish caught in Tyrrenian sea while no larvae were found in edibile parts. For the fish coming from Adriatic sea a visceral prevalence was 30%, mean intensity was 13.44 and only in one fish were isolated seven larvae in belly flaps. A comparison among different fisheries area is important for risk assessment of anisakiasis: in an analogous study performed in Scotland waters, Petrie et al. [1] reported, a prevalence and a mean intensity of *Anisakis* spp., in edible parts, in anglerfish (*Lophius budegassa* and *Lophius piscatorius*) equal to 26.96% and 1.70 respectively. As stated in the present study, *Anisakis* spp. in Mediterranean anglerfish proved a lower prevalence if compared to the same fish species examined previously by Petrie et al. [1]. These are the first data reported on the prevalence and mean intensity of *A. pegreffi* in Anglerfish caught in Mediterranean sea. These findings have an important consequence on epidemiology of anisakidosis and public health risk assessment.

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OCCURRENCE AND GENETIC DIVERSITY OF POTENTIALLY PATHOGENIC ARCOBACTERS IN SHELLFISH AND LETTUCE

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Even though campylobacteriosis is the most commonly reported zoonosis in the European Union (EU)[1], in some cases *Campylobacter* spp. can be confused with *Arcobacter* species, an emerging foodborne pathogen of particular interest for food safety and public health [2,3]. Although the infectious dose has not yet been established, point-source *Arcobacter* spp. outbreaks have been associated with exposure to faecally-contaminated drinking-water wells, as well with the manipulation or consumption of contaminated raw or poorly-cooked food products, or direct contact with animals [4]. Currently, the true incidence of arcobacters in food is underestimated, and a direct link between a specific source and human infection is missing [2]. Therefore, the aim of this study was to assess the occurrence of *Arcobacter* spp. in shellfish and lettuce. The presence and the identity of the *Arcobacter* isolates was determined using a multiplex-PCR [5]. Subsequently, in order to confirm the m-PCR results [6], the 16S rDNA-RFLP was performed [7]. A further aim of this study was to evaluate the heterogeneity between *Arcobacter* spp. isolates using MLST. A total of 80 samples, made up of 40 ready-to-eat lettuce, and 40 shellfish (including 25 *Mytilus galloprovincialis* and 15 *Tapes decussatus*), were purchased from different markets in the Apulia region (SE Italy). *Arcobacter* spp. were detected in 17.5%, and in 22.5% shellfish and lettuce samples, respectively. Subsequently, biomolecular assays revealed *A. butzleri* in 75% and *A. cryaerophilus* in 25% of the isolates. The *Arcobacter* population was diverse, as revealed by the MLST data that identified a large number of alleles and sequence types (STs). Overall, 19 previously unreported alleles and 12 STs were found in this study. These results confirm that food products are an important potential transmission route of *Arcobacter* and that the isolates in this study constitute a more diverse population than those previously observed [8,9,10]. Our research provides more information regarding the health risks associated with the traditional consumption of raw shellfish and of RTE lettuce. In addition, our findings emphasize the need to enhance a synergic One-Health approach between veterinarians and local hospital research epidemiologists in order to improve attribution of arcobacter-related cases to food vehicles and to preserve human and animal health.

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LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR THE DETECTION OF *Anisakis simplex*

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Rapid identification of *Anisakis* spp. larvae is very interesting for fish food industry. Nevertheless, biomolecular techniques are labour-intensive and time-consuming, and they require technical know-how and expensive equipment. Loop-mediated isothermal amplification (LAMP) is a relatively new technique and it proposes significant advantages in terms of ease and speed; moreover, it has already been developed and extensively used as a diagnostic tool for some pathogenic bacteria sush as Listeria monocytogenes and parasites like *Toxoplasma gondii* [1, 2]. In this study, a specific primer was designed and a fluorescence-based real-time LAMP assay was studied, providing an alternative and primarily screening approach for the identification of the genus *Anisakis*. The primer developed was tested directly against *Anisakis* spp. and other non-pathogenic *Anisakidae* larvae (*Hysterothylacium fabri*). Subsequently, the kit was tested against homogenized fish muscle voluntarily contaminated with a decreasing number of larvae, previously isolated and identified to genus level. Finally, baby food samples (80g) contaminated with one, two and three larvae were also tested. The specificity of the kit developed to amplify DNA sequences belonging to *Anisakis* spp. was confirmed as larvae belonging to *Anisakis simplex* sensu strictu and *A. pegreffii* were amplified and recognized by the kit, while *Hysterothylacium fabri*, a non zoonotic parasite morphologically similar to *Anisakis* spp. and belonging to the same family, was not recognized. The tests performed using mixed fish muscle in order to evaluate the sensitivity of the kit resulted to be in agreement with the biomolecular tecniques actually used [3, 4] with a sensitivity equal to 20 larvae/kg of product. Comparable sensitivity was also confirmed with the test perfomed using baby food [5]. LAMP tecnique applied could have promising practical outcomes as it is able to produce good identification results in short times and with low costs. Important advantages could be especially produced if applied to the periodical control of fish-based products (like baby food) or to quality control of extensive productions before commercialization.

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EFFECTS OF INCLUDING LINSEED AND VITAMIN E IN A CONCENTRATE FED TO CHAROLAISE X PODOLICA YOUNG BULLS ON LIPID QUALITY AND BEEF COLOR

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Consumer awareness on relationships between diet and health has increased the interest in the nutritional value of the animal source foods. Aim of this research was to evaluate the effects of linseed or linseed plus high vit. E levels diet inclusion on beef quality traits such as lipid composition, color and fat stability. Eighteen Charolaise x Podolica young bulls (385±10 kg, age 8-9 mo.) were divided into 3 groups (N=6), each receiving concentrate without linseed (C), with linseed (80 g/kg, i.e. 440 g/head/day)(L), and with linseed (80 g/kg, i.e. 440 g/head/day) plus vit. E (2,500 IU/head/day)(L+E). Wheat straw was offered *ad libitum*. After 8 days of adaptation, animals were treated for 91±18 days and then slaughtered in 6 batches of 3 animals per week, one animal/group/batch. Samples of *longissimus thoracis* and subcutaneous fat were taken (1) and frozen at -20°C for lipid analysis or stored at +4°C for color measurement. Fats were extracted (2) and FAME separated by GC. Cholesterol amounts were obtained by HPLC. Meat color was measured daily for 6 days on steaks stored at 4°C, recording the CIEL*a*b* parameters by a Minolta CM-700d spectrophotometer (10° obs., illuminant A, aperture 8 mm) (3). Lipid stability was assessed measuring the hexanal content of subcutaneous fat by Panseri's method (4).

Data on composition and stability of lipids were analysed by ANOVA GLM GEN proc with the diet as fixed effects, while color data were analysed by GLM REP proc including the diet as between factor and the day as within factor (IBM SPSS 20.0). The interaction diet x day was evaluated. Comparison among means was performed with Bonferroni test. Differences with $P<0.05$ were considered statistically significant. L+E diet ameliorated lipid profile, with a strong decrease ($P<0.05$) of cholesterol content (mg/100g of meat) in L+E (28.1±10.2) vs L (49.8±17.1) and C (45.9±3.5) groups and with a high increase ($P<0.01$) of PUFA% in L+E (20.1±6.9) vs L (8.7±2.1), while in C (13.6±5.5) was not statistically different from the other groups. Respect C group (17.8±2.5), in L+E (6.4±0.4) and L (6.3±1.8) we recorded a remarkable reduction ($P<0.001$) of the n-6/n-3 ratio, that reached values close to those recommended (5). Meat color resulted badly affected by L diet: a* was reduced ($P<0.01$) in L (19.3±2.1) vs C (22.5±1.9), b* was lower ($P<0.05$) in L (15.5±2.0) than in C (17.2±1.6), and also Chroma was reduced ($P<0.01$) in L (24.8±2.6) vs C (28.3±2.4) group. In L+E all color parameters cited were not statistically different from C and L groups, while Hue value was slightly increased ($P<0.01$) both in L (38.6±2.9) and L+E (38.6±2.6) vs C (37.3±1.7). Moreover, significant interaction diet × day was found for Hue. Hexanal (ng/g of fat) was higher ($P<0.001$) in L (245.8±69.3) than in L+E (62.1±9.8) and C (67.1±9.1) groups, suggesting a positive effect of vit. E in protecting lipids from oxidation. In conclusion, adding 80 g/kg of extruded linseed in young bulls concentrate, can unfavorably affect color and lipid stability with only a mild improvement on lipid profile. Conversely, linseed plus high levels of vit. E supplementation can be a useful strategy to ameliorate beef nutritional value without impairing color and lipid stability.

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CURRENT SITUATION OF ANTIMICROBIAL USE IN VEAL CALVES PRODUCTION IN ITALY

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The use of antimicrobial compounds in food animal production provides demonstrated benefits, including improved animal health, higher production and, in some cases, reduction in foodborne pathogens [1]. However, the treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial (AM) agents [2]. The interest in this topic has been increased tremendously in the last twenty years eather in humans and animals science. The main concern is related to the AM use in livestock productions for human consumption, and the possibility for resistent bacteria to be transferred to humans through animal food consumption. Several studies have been published on poultry and pig production because of the shorter cycle and the higher level of AM use mainly as a preventive strategy in the whole group, compare to dairy and beef industry. In Italy the veal calves represent a relevant meat production (560000 hd/year), and it has been involved in the European surveillance program (Decision EC 652/2013). The aim of this study was to summaryze the data on AM use in veal calves farms. Data were collected in 2012, 2013 and 2014 for 146,854, 150,598 and 171,925 animals, respectively. Each AM was grouped in classes: Aminoglycosides, Betalactamine, Fluorochinolons, Lincosamides, Macrolides, Polymyxins, Sulfonamides, Tetracyclines, Trimetoprim, divided per way of administration (oral vs. parenteral), and expressed as g or ml of active ingredient per head during the productive cycle. The data were summaryzed per year, and the mean, min and max of AM used were calculated. Then the farms were categorized based on the quantity of AM used (<60, 61/100, 101-140, >140 g,ml/hd/cycle). The results showed a total use of AM of 123.8, 130.4 and 119.6 g,ml/hd/cycle in 2012, 2013 and 2014, respectively, with a higher use of AM *per OS* (116.9, 122.7 and 110.3, in the 2012, 2013 and 2014, respectively). The AM per OS mainly used were the Tetracyclines (67.9,68.3,58.0 g,ml/hd/cycle in the 2012, 2013 and 2014) and Sulfonamides (21.3, 11.8, 13.9 g,ml/hd/cycle in the 2012, 2013 and 2014). The min e max values of AM use showed a high variability between farms analyzed (i.e. tetracyclins ranged from 9.9 to 374.1; 11.7 to 286.0; and 0.3 to 242.9 g,ml/hd/cycle in the 2012, 2013 and 2014, respectively). We can conclude that currently the AM in veal calves production is mainly used for group treatments with a metaphylactic approach, as confirmed by the higher use of AM *per OS*. Considering the variability of AM use among farms we can hypothesize the possibility to reduce the AM use, improving the farm enviroment condition and management. More information on the herd management good practices and biosecurity are needed as a strategy to reduce the AMR risk in veal calves production.

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NUTRITIONAL MANAGEMENT TO PREVENT STRUVITE UROLITHIASIS IN CATS

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The formation of urolithes along the urinary tract is typical of several anatomo-physiological conditions and pathological processes. Urolitjiasis is universally recognized as one of the most common nutritional diseases. The most frequently isolated compounds are struvite crystals, but other uroliths, such as oxalate, urate and cystine or mixed, may also occur [1]. Usually, the uroliths are amenable to dietary or medical dissolution, but surgery is necessary in some cases and, in both cases, calculosis often recurs. Aim of the present study was to test an innovative cat diet able to reduce the recurrence risk. The experimental diet (CP 33.0, EE 16.5, CF 1.20, Ca 0.70, P 0.65, Mg 0.06 % a.f.; ME 3842 kcal/kg a.f.) supplemented with specific urine acidifiers (ammonium chloride 3.5 g/kg a.f. and DL-methionine 2.5 g/kg a.f.) was prepared. The diet was administered to 45 adult cats (in ratio of 100 kcal of ME/kg $BW^{0.67}$) that successfully finished (at least from 60 days) a dietary therapy for the dissolution of struvite crystals. The experimental period lasted 10 months. Clinical examination, blood chemistry and urinalysis were performed every two months in order to assess the possible recurrence of urolithiasis. All the data were subjected to the analysis of variance using JMP software of SAS (SAS Institute, NC, USA). Before starting the experimental diet administration (time 0) the serum levels of urea and creatinine were high in all subjects: more than 50% of cats showed urea values higher than the physiological range (mean value 74.45; physiological range 20-50 mg/dl) and the creatinine ones corresponded to the upper limit in all cats (mean value 1.94; physiological range 0.5-2 mg/dl). At the first urinary sampling 24 cats showed crystalluria of different nature (18 struvite and 6 other minerals). During the trial, the crystals presence, the urinary pH and density, as well as urea and creatinine levels progressively decreased. After 10 months of nutritional treatment only 3 cats showed the presence of urinary sediment (2 struvite and 1 other minerals), but in all cases the urine density resulted higher than the physiological range (mean value 10.59; physiological value 10.40 g/dl), confirming the high susceptibility to develop urolithiasis, regardless of urinary pH. The low protein content of the experimental diet since the third blod sampling resulted in a recovery within the physiological ranges of both indices of renal function (44.5 and 1.77 mg/dl for urea and creatinine, respectively). In 40 cats the urinary sediment disappeared. The dissolution of struvite crystals was due to the urinary pH acidifier [2], while the low protein, calcium, phosphorus, magnesium and vitamin D3 levels may be responsible for the reduction of the crystals composed by other minerals. [3]. These data confirm that the composition of the tested diet is useful to reduce and prevent the formation of crystals along the urinary tract in cats. In any event, the high susceptibility of cats to develop urolithiasis strongly suggests that the nutritional management and a periodic clinical examination play a critical role in the prevention of urolithiasis recurrence.

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FKBP51 GENE EXPRESSION IN SKELETAL MUSCLE AS A POTENTIAL BIOMARKER FOR GLUCOCORTICOID ILLEGAL TREATMENT IN VEAL CALVES

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The use of glucocorticoids (GCs) as growth promoters is banned in EU. The chemical analysis of drug residues is the exclusive accepted method to identify illicitly treated animals, but GCs and their metabolites are no longer detectable by LC/MS-MS methods in biological fluids [1]. This study aimed to elucidate the effect on the expression of genes involved in the GC signalling pathway in the skeletal muscle of veal calves following a prolonged treatment with dexamethasone (DEX) or prednisolone (PRD). A gene expression change may indicate new potential molecular biomarkers of GC treatment.
Twenty-two Friesian veal calves, 6 months old, were divided as follows: gr. A (n=6) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.4 mg/day of DEX for 31 days; gr. B (n=8) treated with 15 mg/day of PRD for 31 days; gr. K (n=8) was the control. The calves were slaughtered at 3 days after the last treatment. Samples of the biceps brachii (BB), longissimus dorsi (LD) and vastus lateralis (VL) muscle were collected from each animal and subjected to quantitative PCR for the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), FK506 binding protein 51 (FKBP51) and FKBP52. Statistical differences were determined by ANOVA, followed by Dunnett's post test.
MR gene expression increased in the BB muscle of gr. A ($P<0.01$), while decreased in the LD muscle of gr. B ($P<0.05$). 11β-HSD1 gene expression increased only in the BB muscle of gr. B ($P<0.05$). FKBP51 gene expression decreased in all considered muscles of gr. A ($P<0.01$), whereas increased in the LD ($P<0.05$) and VL muscles ($P<0.05$) of gr. B. No change of GR and FKBP52 expression was observed.
Despite the skeletal muscle is one of the main target organs of GCs, the expression of the genes involved in the GC signalling pathway seems only in part affected by DEX or PRD treatment, as previously reported in beef cattle [2]. However, the GC signalling is stochastic and differs greatly among tissues. The evaluation of FKBP51 gene expression in the skeletal muscle could play an important role in the indirect identification of GC-treated calves, as reported in beef cattle [3]. Particularly, the increase of FKBP51 gene expression following PRD administartion seems to be very promising, although the increase of FKBP51 expression observed in BB muscle was not significant. Probably, a greater number of animals have to be considered and further investigations are needed. However, so far an effective biomarker of PRD treatment is not been yet and it is remarkable that the analysis of the expression of a unique gene in a tissue may distinguish the dispensed molecules. The skeletal muscle can be easily sampled at the slaughterhouse, representing a good target tissue for the development of a screening test.

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MICROBIOLOGICAL QUALITY OF ETHNIC READY-TO-EAT FOODS SOLD IN TUSCANY: PRELIMINARY RESULTS

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In recent years, consumers who enjoy ethnic foods have increased in number and ethnic restaurants have become popular in Europe and also in Italy. From 2011 to 2014, this catering sector grew by 1.6%. It counts about 190,000 throughout the country and this number is expected to grow [1]. The aim of this study was to evaluate the microbiological quality of some ethnic ready-to-eat foods retailed in Tuscany. From September 2016 to March 2017, 185 food samples were collected from take-away shops in 4 Tuscany provinces (LI, LU, PI, PO). The samples included various types of ready-to-eat foods that were stored precooked for reheating on demand: 101 Chinese foods (47 pig/beef meat, 34 fish, 17 poultry meat and 3 eggs), 70 kebabs (35 sandwiches and 35 meat alone) and 14 Turkish or Indian foodstuff (8 poultry meat and 6 mixed meat). The food samples were analyzed within 4 hours after they were purchased. The analyses were focused on the main safety and hygiene indicator microorganisms. *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci (CPS), *Salmonella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* were investigated using the methods prescribed by the relevant ISO standards. All samples were negative for *Salmonella* spp. and *L. monocytogenes*. *Y. enterocolitica* was isolated from one Chinese sample, a fish soup. As concern CPS, 82/185 samples (44.32%) presented bacterial counts under detectable limit, 84/185 (45.40%) between 10^2 and 10^5 CFU/g and 19/185 (10.27%) above 10^5 CFU/g. Regarding *Enterobacteriaceae* 102/185 samples (55.13%) showed bacterial counts under detectable limit, 63/185 (34.05%) between 10^2 and 10^5 CFU/g and 20/185 (10.81%) exceeding 10^5 CFU/g. For *E. coli*, 168/185 samples (90.81%) presented bacterial counts under detectable limit and 17/185 (9.19%) between 10^2 and 10^5 CFU/g. A percentage of 4.86% samples showed bacterial counts above 10^5 CFU/g for both CPS and *Enterobacteriaceae*, specifically 6 Chinese foods (2 pig/beef meat, 2 poultry meat, 2 fish) and 3 kebab sandwiches. The results of this investigation show that all samples except one resulted negative for major bacterial pathogens, in accordance with studies of other authors [2;3]; besides many samples presented low bacterial contamination. However, few samples showed high bacterial contamination, especially for *Enterobacteriaceae* and CPS. Although final heating reduces the levels of microorganisms present in foods, it can't inactivate any toxins if present. Hence, preliminary results suggest the need to improve good hygiene practices in take-away restaurants, in particular handling in ready-to-eat products.

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Yersinia enterocolitica ISOLATE FROM WILD BOARS PRESENCE OF GENES OF PATHOGENICITY

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Yersinia enterocolitica represent zoonotic bacteria able to infect humans and animals, and are recognized as the third cause of foodborne disease in Europe [1]. The epidemiology of the infection and the distribution of serotypes need to be further understood [1]. *Y. enterocolitica* comprises six biotypes: 1A, 1B, 2, 3, 4 and 5. The virulence of the strains belonging to biotypes 1B and 2–5 depends on the presence of both chromosomal and plasmid-borne genes [2]. The aim of our study was to evaluate *Y. enterocolitica* pathogenicity in strains isolated from wild boar hunted in Liguria Region (Italy) between 2013 and 2017. In this period, we collected a total of 4,282 liver samples from Liguria provinces: Imperia, Savona, Genoa and La Spezia. All samples were analysed according to procedure ISO10273-2003, i.e. the European reference method for *Yersinia enterocolitica* detection in food. All *Y. enterocolitica* strains isolated were biotyped and serotyped according to procedure ISO10273-2003 and tested by RT-PCR to detect the following virulence genes: ail (attachment and invasion locus), ystA (*Yersinia* stable toxin), ystB (*Yersinia* stable toxin), inv (invasin), myfA (mucoid *Yersinia* factor) and ymoA (*Yersinia* modulator) [3]. DNA was extracted from pure colonies using QIAmp DNA mini kit® and amplified as previously described [3]. Out of 4282 liver samples, 126 (2.9%) resulted positive, *Y. enterocolitica* strains being isolated in samples collected from both Genoa (85%) and La Spezia (15%) provinces. No positive samples were found in the other areas. Regarding biotyping, in agreement with previous studies [4], we predominantly found *Y. enterocolitica* 1A (91.8%) and a small percentage of *Y. enterocolitica* 1B (7.2 %) and 2 (1 %). Most isolated strain resulted O-non typeable (44.3%); in particular, *Y. enterocolitica* 1A strains belonged to serotypes O:1.2 (4.1%), O:3 (7.2%), O:5 (11.3%), O:8 (30.9%), O:9 (3%), and *Y. enterocolitica* 1B to serotypes O:1.2, O:5, O:8. Most strains were included in serotypes O:5 and O:8, the most relevant serotypes causing gastroenteritis in humans in Europe [4]. Concerning the genes of pathogenicity, we observed the presence of ystB in 59.3% of the strains under study followed by ymoA (42.2%), ail (35.2%), ystA (16.4%), myfA (10.2%) and inv (7.8%). The biotype 1A strains have been considered to be non-pathogenic, since they do not have pYV plasmid and some chromosomal virulence genes, e.g. ystA and myfA [5]. Although inv is present, it seems to be non-functional in most 1A strains [6]. However, in our study we demonstrated that 1A strains carry other virulence genes, like ystA, ail, ystB, ymoA and myfA; moreover, some biotype 1A strains have been isolated from humans with gastrointestinal infections. These results indicate that *Y. enterocolitica* 1A may infect humans and animals; however other studies are needed to demonstrate our hypothesis and the ability of some strains to invade and modulate immune responses in gut.

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EVALUATION OF THE ON-CHAIN REFRIGERATION OF BROILER CARCASSES ON *Campylobacter* CONTAMINATION

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Thermophilic Campylobacters are the first cause of foodborne illness in the European Union (about 230,000 cases in 2015) [1]; it is estimated that about 20-30% of human Campylobacteriosis cases are linked to the consumption of broiler meat [2]. The prevalence of Campylobacters on broiler carcasses after slaughtering is very high [3], due to their wide distribution in the chicken population and to the cross contamination during slaughtering. *Campylobacter* is not able to grow on the meat surfaces and is gradually inactivated by refrigeration [4], especially when low temperatures are combined with a drying of meat surface, as can be achieved with air-chilling.This study aimed to evaluate the effect of on-chain air chilling on *Campylobacter* contamination of broiler carcasses in an industrial slaughterhouse. During three sampling sessions (form March to July), a total of 14 slaughtered broiler batches were analyzed as follows:
- Detection of *Campylobacter* spp. in the caecal content (one pooled sample from 5 caeca for each batch);
- Detection and count of *Campylobacter* spp. in neck/breast skin samples (3 pools from 10 broiler carcasses for each batch), taken before and after the air refrigeration (4°C for 45 min).
For the analyses, ISO methods were applied, and isolates were identified by PCR. The temperature of the carcasses during the refrigeration (on the surface and under the skin) was also registered. The refrigeration process led to a mean temperature decay of the carcasses of 14.7°C (surface) and 9.1°C (under the skin). All the broiler batches showed the presence of Campylobacters both in caecal content and on carcass surfaces, with about half of the pooled carcass samples having counts >3 Log CFU/g. The refrigeration of the carcasses resulted in a mean decrease of 0.27 Log CFU/g in *Campylobacter* counts, a slight but statistically significant effect. The main frequency class of counts was higher for pre-refrigeration samples (3-4 Log CFU/g vs 2-3 Log CFU/g). A high correlation was shown between counts pre and post refrigeration, stressing the importance of the previous phases to achieve a low contamination level. Due to the extremely high diffusion of Campylobacters, no differences were detected considering the slaughtering order of the batches or the sampling season. All the isolates belonged to the species *C. jejuni* or *C. coli*, with the prevalence of *C. jejuni* (71%) and of *C. coli* (86%) in pre and post refrigeration samples, respectively. The application of this chilling step could be useful, in combination with further refrigeration phases (during marketing and storage), in order to achieve constant low counts on broiler meats.

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USING PROTECTIVE CULTURES TO CONTROL *Pseudomonas* SPP CONTAMINATION IN *RICOTTA FRESCA* CHEESE

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Ricotta fresca cheese in Sardinia (Italy) is traditionally obtained from the whey remaining after the production of sheep's milk hard cheeses. At industrial level, the manufacturing of *Ricotta fresca* is very similar to the artisanal batch production, which exposes the product to post-process contamination originating from handling and processing environment [1]. As result of the high temperatures applied during production, *Ricotta fresca* is poor in natural competitive microflora. Therefore, during refrigerated storage grow of pathogens or spoilage psychotropic microorganism can occur, the latter being represented mainly by *Pseudomonas* spp [2]. Providing this product with a competitive microflora could be an option to inhibit the growth of unwanted bacteria [3]. With this objective, the present study was designed to assess the efficacy of two different protective cultures against the growth of *Pseudomonas* spp in naturally contaminated *Ricotta fresca*. Lyofast FPR 2 (including *Enterococcus faecium*, *Lactobacillus plantarum* e *Lactobacillus rhamnosus*) and Lyofast CNBAL (*Carnobacterium* spp) were inoculated on the surface of *Ricotta fresca* samples, which were MAP packed and stored at 4°C. Triplicate samples were analyzed the day of inoculation (T_0) and after 7, 14 and 21 days (T_7, T_{14} and T_{21}) of storage. The determination of headspace gas composition (O_2 and CO_2), physico-chemical characteristics (pH, a_W, moisture, fat, protein), aerobic mesophilic bacteria, mesophilic lactic acid bacteria, *Enterobacteriaceae, Pseudomonas* spp*, L. monocytogenes,* yeast and molds was conducted. Overall were analyzed 108 *Ricotta fresca* cheeses, including 36 samples for each of the following experimental units: control samples, samples inoculated with Lyofast FPR2 and samples inoculated with Lyofast CNBAL. Intrinsic properties and composition of *Ricotta fresca* showed no significant differences among test units. The O_2 and CO_2 content ranged from 0.72±0.45% and 14.59±2.56% at T_0 and from 0.21±0.61% and 8.48±3.81% at T_{21}, respectively. The pH ranged between 6.69±0.1 at T_0 and 6.47±0.1 at T_{21}, a_W between 0.991±0.004 at T_0 and 0.992±0.006 at T_{21}. Moisture, fat and proteins ranged from 72.98±4.11%, 16.33±5.51%, and 9.76±0.61% at T_0, to 72.86±3.17%, 15.06±2.31% and 10.08±1.59% at T_{21}, respectively. *L. monocytogenes* was never detected, while yeast and molds were occasionally recovered. In control samples *Enterobacteriaceae* and *Pseudomonas* spp during storage increased from 2.20±1.02 to 4.58±1.68 and from 2.64±0.59 to 6.83±0.91 $\log_{10}$ cfu g^{-1}, respectively. Lyofast FPR2 showed no control against *Enterobacteriaceae* and *Pseudomonas* spp. Conversely, in samples inoculated with CNBAL, *Carnobacterium* spp increased from 6.28±0.35 at T_0 to 8.59±0.47 at T_{21} $\log_{10}$ cfu g^{-1} showing a significant (P<0.05) reduction in *Pseudomonas* spp and *Enterobacteriaceae* respectively of 1.93 and 2.66 $\log_{10}$ cfu g^{-1}. *Carnobacterium* demonstrated a good adaptation to growth on refrigerated *Ricotta fresca* and to control the growth of *Pseudomonas* spp.

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PREVALENCE, BIO/SEROTYPING AND ANTIBIOTIC RESISTANCE OF *Yersinia enterocolitica* DETECTED IN FINISHING PIGS AT SLAUGHTER IN SARDINIA

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In 2015, yersiniosis was the third most common reported zoonoses in the European Union and *Y. enterocolitica* was isolated from 99.5% of all human cases [1]. Pigs are a common source of human infection by *Y. enterocolitica* through consumption of raw or undercooked meat or by direct contact with contaminated carcasses. Aims of the present work were to evaluate *Y. enterocolitica* prevalence in pig slaughterhouses, determine the biotype/serotype and evaluate the antimicrobial susceptibility of the isolates. The study was conducted in 9 pig slaughterhouses in Sardinia. Samples of tonsils, mesenteric lymph nodes, colon content and carcass surface were collected from 126 finishing pigs and 35 piglets. Detection of *Y. enterocolitica* was carried out following the ISO 10273-2003 method, with some modifications. Confirmed *Y. enterocolitica* isolates (47) were biotyped [2] and serotyped by agglutination tests. Isolates were screened for antimicrobial susceptibility of ampicillin, amoxicillin and clavulanic acid 2:1, cefotaxime, ciprofloxacin, cefalothin, ceftazidime, colistine, chloramphenicol, enrofloxacin, gentamicin, kanamycin, nalidixic acid, neomycin, streptomycin, sulphonamide, tetracycline and trimethoprim-sulphametoxazole by the disc-diffusion method [3, 4]. *Y.enterocolitica* was detected from 30/126 (23,8%) *adult pigs*. Prevalence of *Y. enterocolitica* in adult pigs was 15.8% (20/126) in carcass surface, 11% (14/126) in colon content, 2.4% (3/126) in tonsils and 1.5 % (2/126) in lymph nodes. *Y.enterocolitica* was never detected from samples collected from piglets. The following bio/serotypes were identified: 4/O:3 in 33/47 (70.2%) isolates, 2/O:5 in 4/47 (8.5%) isolates, 2/O-untypeable in 3/47 (6.4%) isolates, 1A/O-untypeable in 7/47 (14.9%) isolates. All 47 isolates were susceptible to cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, sulphonamide, tetracycline and trimethoprim-sulphametoxazole. Resistance to ampicillin and cefalothin was the most common (100%), including in 4/O:3, 2/O:5, 2/O-untypeable isolates, followed by amoxicillin and clavulanic acid (39/47) and streptomycin (2/47). Resistance to amoxicillin and clavulanic acid 2:1 was reported among bio/serotype 2/O:5 and 2/O-untypeable (both 100%), 4/O:3 (81.8%, 27/33) and 1A (71.4%, 5/7). Resistance to streptomycin was observed in 6.1% (2/33) of 4/O:3 isolates only. Our results reflect a low infection prevalence in the pig population slaughtered in Sardinia, as compared to similar studies [5]. Results on antimicrobial resistance are in accordance with similar studies (2). The high prevalence of resistance against amoxicillin and clavulanic acid 2:1 in bio/serotype pathogenic to humans (4/O:3 and 2/O) is of particular concern since pig meat could play a role in the spreading of antimicrobial resistance of *Y. enterocolitica*.

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INFLUENCE OF DAIRY PRODUCTS ENVIRONMENT ON THE GROWTH OF *Bacillus cereus*

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Bacillus cereus is a spore forming foodborne pathogen associated with two types of diseases, the emetic and the diarrheal syndromes [1]. Raw milk could be contaminated at farm level but also post-pasteurization recontamination of milk and dairy products by vegetative cells or endospores could occur [2, 3]. It is well known that pH is one of the most important parameters used to control bacterial replication in foodstuffs and nowadays low-pH foods are widely produced and consumed as they guarantee bacterial stability. Dairy products are characterized by different substrate acidity levels that can significantly influence bacterial growth. In the present study the ability of two Bacillus cereus strains, one clinical human isolate (GPe2) and one isolated from a dairy product (D43), were investigated to evaluate the in vitro growth at different pH values (from 3.5 to 7.5) at two different temperatures (15 and 37°C). Moreover, the spores germination and growth of these two strains in different typologies of dairy products (unflavoured yogurt, taleggio cheese, mascarpone cheese, raw and pasteurized milk) was investigated at 15°C. The spores were inoculated (inoculum concentration around 2-3 Log CFU/g) and microbiological analyses were performed at settled times during the storage of the products at 15°C, minimum temperature of growth of the two microroganisms. *B. cereus* GPe2 and D43 showed their ability to grow at 37°C from 5.5 to 7.5 and from 5.0 to 7.5, respectively. The growth ability of the two strains was moderately affected by the temperature of 15°C, as growth was detected in a pH range between 5.5 and 7.5 and between 6.0 and 7.5 for GPe2 and D43 strains, respectively. Considering the behaviour in dairy substrates, no growth was observed in yogurt, likely due to the combined effect of low pH (<5) and the presence of high Lactic Acid Bacteria counts (>7 Log CFU/g). An inhibitory effect of the natural microflora on *B. cereus* growth may also be assumed for taleggio cheese and raw milk, as in these matrices high natural Lactic Acid Bacteria (LAB) population and permissive pH values (>5.8 in taleggio cheese, >7 in raw milk) were detected. Finally, in pasteurized milk and mascarpone cheese, where pH was not preventive for the growth of B. cereus and where LAB loads were not significant, growth occurred quickly up to loads nearly of 7 Log CFU/g. Especially for the last two products, where pH and natural microflora do not exert an inhibition, correct hygienic procedures should be regularly applied during the production with the goal to reduce the risk of contamination by pathogenic *B. cereus* strains.

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MOLECULAR CHARACTERIZATION AND BIOFILM FORMATION OF *Staphylococcus aureus* ISOLATED FROM DAIRY PRODUCTS

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Staphylococcus aureus (*S. aureus*) is a foodborne pathogen considered to be the world's third most important causative agent of foodborne illnesses [1]. Besides the production of enterotoxins, the formation of biofilm is increasingly being recognized as an important virulence factor in *S. aureus* [4]. The present study focused on the molecular characterization of *S. aureus* strains isolated from dairy products analyzing the biofilm-forming ability of the isolates. Forty strains were characterized by *spa* typing [2] and screened by PCR for the presence of enterotoxin encoding genes, and some genetic markers associated to the biofilm production [3]. Furthermore, the strains were tested for the biofilm production on polystyrene at 37°C [4]. A total of 19 spa types were found, the most frequent were t524 (7/40) and t2953 (5/40), which have been frequently reported also in other European countries. Majority of isolates (83%) showed similar distribution of adhesion genes (*ica*A*, ica*D*, cna, fnb*A and *fnb*B), toxin genes (*hla* and *hlb*), and staphylococcal regulators (*sar*A). Biofilm formation was observed in the 48% (19/40) of the isolates, of which 11% (2/19) strains formed biofilms strongly, 42% (8/19) moderately, and 47% (8/19) weakly. Interestingly, all strains carrying agr type III (5/40) were found to be biofilm producer, including the two strong biofilm producers. Furthermore, 62.5% of the isolates (25/40) were found to be potentially able to produce enterotoxins, carrying at least one gene encoding for staphylococcal enterotoxins. In conclusion, this study underlines the ability of *S. aureus* strains from dairy products to form biofilm. The biofilm formed by *S. aureus* on milk equipment surfaces can lead to significant food safety issues. The majority of strains (13/19) classified as biofilm producers, in fact, were found to be potentially able to produce enterotoxins. Currently, a regular cleaning and disinfecting is the first and most important step to get rid of raw milk residues and prevent biofilm formation by *S. aureus* in the dairy industry.

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DETERMINATION OF RADIOSTRONTIUM FOR IDENTIFYING A MECHANICALLY SEPARATED MEAT: PRELIMINARY STUDY

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According to Regulations (EC) No 853/2004 and No 999/2001, mechanically separated meat (MSM) is defined "the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure". The identification of MSM is important for quality and safety evaluation of meat products. MSM identity technique consists in calcium content determination. This value shall not exceed 1000 ppm of fresh product in not MSM products. However, EFSA recommended studies on differentiation of MSM from other meat products, based on the analysis of combination of different parameters, both chemical and physical, since the determination of calcium content alone does not allow differentiation between low pressure MSM (P< 104 kPa) and other meat products [1]. Strontium-90 (Sr-90) is considered an important radioactive isotope, and after nuclear accidents, nuclear weapons tests and not correct waste management, this may be released in the environment. As demostrated in recent studies [2], if Sr-90 is present in the environment, it may contaminate the raw materials used in animal fed. Consequently, this radionuclide may be accumulated also in animal bones, since it is chemically similar to calcium. Aim of study was to determinate ultra-low-levels of Sr-90 in meat products using an ultra-sensible radiochemical method, and to evaluate if this may be used as marker in the MSM identification. The Italian National Reference Centre for the Detection of Radioactivity in Feed and Foodstuff uses an ultra-sensible radiochemical method for the determination of Sr-90 in meat products, at ultra-low-levels. So, this technique was tested in order to evalute if the determination of Sr-90 levels may be used as tool in the MSM identification. Ten samples (n. 5 wurstel with MSM, and n. 5 fresh meats not composed of MSM) were analysed, and the Sr-90 levels were statistically compared by t-test. The results showed significant differences ($p<0.05$) between MSM and not MSM products, confirming that this parameter is very interesting. Moreover, in this preliminary evaluation, a "cut-off" value corresponding to 40 mBq/kg may be suggested for discriminating between MSM and not MSM.

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DEVELOPMENT AND VALIDATION OF A GC-MS/MS METHOD FOR CONTAMINANTS DETECTION IN SHELLFISH

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Environmental contamination is an issue in food safety programs. In the last decades, various essential elements and toxic contaminants were investigated in seafood in order to limit exposure of consumers [1]. Fish, and to a lesser extent the molluscs, bioaccumulate contaminants, such as toxic metals and Persistent Organic Pollutants (POPs), which can represent a risk for human. The aim of this study was to develop and validate a QuEChERS extraction and clean up procedure, followed by GC-MS/MS analysis for the detection of 33 contaminants (organochlorines pesticides (OCPs); polychlorobiphenyls (PCBs); polybromodiphenylethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs)) in mussels and clams. Uncontaminated mussel and clam samples were used to validate the method.The validation parameters were good for all analytes. The linearity, expressed as R2, was higher than 0.985, the recoveries were in the range 70–120% and the repeatability, expressed as coefficient of variation (CV %) was always lower than 20%, therefore this method meets the validation criteria required by EU guidelines [2]. Finally, the procedure was applied to 10 samples from the wholesale fish market of Milan. DDT metabolites, Benzopyrene, PCBs were often found, especially in mussels. Our results show that the method is effective for the analysis of POPs in shellfish

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DETERMINATION OF CARMINIC ACID (E120) IN FRESH SAUSAGES USING A SIMPLE EXTRACTION METHOD FOLLOWED BY LC-HRMS ANALYSIS

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Carminic acid (E120) is a natural, water soluble red dye, secreted by the cochineal insect as a deterrent to predators. It is stable under conditions of indoor scattered rays, large temperature range, pH variation and oxidation-resistant [1]. This colorant is used for several uses from ink to cosmetics and foods. Regards food, it is usually added to preserve and maintain the red color of meat products during their shelf-life as well [2]. The quality of fresh meat products so may be compromised by this not admitted procedure. On this purpose, Regulation 1129/11/EC [3] prohibits the use of E120 in the fresh meat preparations, which also includes sausages . The aim of this study was to develop a method for carminic acid determination and to detect it in fresh sausages in order to guarantee to consumers a product free of illegal dye addition. In literature, almost all works detect carminic acid by liquid chromatography with UV Detector (LC-UV) system or use elaborated extraction procedures for different foods, especially for drinks [2]. In this work we developed a simple liquid extraction method with water, followed by a defatting step with hexane before analysis by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). The LOD and LOQ of the method were 3 and 10 µg/ kg, respectively. The recovery was 99%. The other performances of the analytical method, effectiveness and robustness particularly, were evaluated following the Decision 657/2002/EC [4]. The protocol was then applied on 95 fresh sausage samples collected from different supermarkets and food suppliers, showing 23% of non-compliance with concentrations ranging from 45 to 1218 µg/ kg. The E120 evidence, not indicated on the product label of the sausages, collected directly from the different stores, suggests the importance of monitoring fresh products, safeguarding consumer health.

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APPLICATIONS OF CONE-BEAM COMPUTED TOMOGRAPHY (CBCT) IN THE FOOD SECTOR

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Cone beam computed tomography (CBCT) is an imaging modality that is commonly applied for medical applications mainly in orthodontic assessment. CBCT provides immediate and accurate two- and three-dimensional radiographic images of a solid structure. , Recently CBCT systems are relatively inexpensive and small due to: the development of compact, relatively low cost, high quality, large, flat-panel detector arrays, the availability of low cost computers with processing power sufficient for cone-beam image reconstruction and, finally, the fabrication of highly efficient X-ray tubes capable of multiple exposures. [1]. The cost reduction, toghether with the previously mentioned ability of CBCT to provide three-dimensional images and the lower emissions make its use possible in the food sector. Moreover the mechanical complexity of the cone beam tomographers is much lower than fan-beam tomographers making it possible to integrate such an apparatus in food production lines. These features contribute to the cost reduction and give the possibility to place the CBCT apparatus in convenient lead-shielded boxes that breack down X-ray emissions to negligible levels making it compatible with worker's safety.The aim of this study was to explore the potential of CBCT in food production,it was, then, decided to try CBCT for several applications in different matrices.In hard cheeses such as Pecorino and Parmigiano Reggiano it was possible to demonstrate defects of the texture, splits and the presence of air bubbles . In softer cheese CBCT was used to assess the number and volume of the bubbles inside the matrix which extremely important for cheeses characterized by propionic fermentation (Emmenthaler). In mortadella and cooked ham samples, it was possible to calculate the lean/fat ratio and regions of different density in the matrix. Some cured (whole) hams have been scanned, but the possibility to detect defects in the muscles or nearby the bone still have to be investigated.The technique is able to detect foreign bodies in all the tested matrices. Results show that the technique is very sensitive in detecting metal and high density bodies but it can also detect non metal, low density foreign bodies. (parts of plastic gloves). In conclusion, CBCT looks extremely promising for the food sector, representing a valid tool for the detection of foreign bodies undetected by conventional systems and a powerful help for product calssification.

ANIV

LIVESTOCK

ANTIMICROBIAL SUSCEPTIBILITY AND GENOTYPING OF *Staphylococcus aureus* ISOLATES COLLECTED IN SARDINIA FROM OVINE MASTITIS

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Staphylococcus aureus mastitis is the one of the most common health problems affecting dairy sheep. Sardinia, an island located in the middle of the Mediterranean, has approximately 3.5 million milking *sarda* sheep, corresponding to half of the total Italian stock. In the control of ovine staphylococcal mastitis, antimicrobial therapy continues to play an important role. In several studies, the antibiotic resistance of different *S. aureus* isolates from cases of ovine mastitis have been described [1,2]. Genotyping of *S. aureus* is an important tool in epidemiological studies of mastitis and contributes to our understanding of the pathogen's dissemination. Several molecular methods have been developed for typing *S. aureus* isolates, such as MLST, ribotyping, AFLP, PFGE and staphylococcal protein A (*spa*) typing [3,4]. The aim of the present study was to characterise the clonal diversity of 330 *S. aureus* isolates collected in Sardinia from clinical ovine mastitis and used for the preparation of inactivated autogenous vaccines. We investigated their antimicrobial susceptibility as well as the possible use of *spa* typing and MLST, in combination with PFGE, for tracking MRSA transmission among ovine population. Susceptibility to 12 antimicrobial agents was tested according to CLSI recommendations. Resistance genes were detected by PCR assays. The most of isolates (85.5%) were susceptible to all antimicrobials tested, suggesting that did not exist change of resistance over time. Ninety-four percent of the tetracycline-resistant isolates (n=16) harboured the *tet*(K) gene, indicating that resistance to tetracycline is mainly by efflux pumps. Two isolates were multidrug-resistant (MDR), one of them showed resistance to seven antibiotics including oxacillin and erythromycin. This MRSA 1496 harboured SCC*mec* type IV and the *erm*(C) gene. Isolates were characterized by *spa* typing, and macro-restriction pulsed-field gel electrophoresis (PFGE) analysis. For selected isolates, multi-locus sequence typing (MLST) was performed. Isolates belonged to 29 *spa* types: t1773 (n=186), t2678 (n=53), t7754 (n=14), t1532 (n=5), t524 (n=5) and t6060 (n=4) were the most frequent *spa* types found in Sardinia. The majority of ovine isolates (t1773, t7754 and t1532) was grouped in MLST CC130 (n=205) followed by CC133 (n=57). Into the main CC130, *S. aureus* isolates showed a PFGE profile type A with 12 closely related subtypes (A1-A12) whereas within to CC130, isolates displayed a different profile type B. MRSA 1496 was classified as t3896, ST1 and CC1, a clonal complex common in human and also reported in cattle and pig. This study suggests that the CC130/ST700/t1773 is the prevalent *S. aureus* lineage associated with ovine mastitis in Sardinia.

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SMALL RUMINANT LENTIVIRUS A8 IN VALDOSTANA GOATS

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Small Ruminant lentiviruses are heterogeneous group of virus able to infect goat and sheep. Four different genotypes were classified, and several subtypes were recognized in each group. This heterogeneity can influence both the host interaction and the diagnostic test results. In vivo and in vitro properties were investigated for different SRLV genotypes and recent studies demonstrated how small changes in the genetic sequences can interfere with the cell tropism during the viral replicative cycle. In the same way, given the antigenic diversity, diagnostic tests can fail in the identification of seropositive animals when they are based on heterologous antigens. In 2012 we conducted serological screening on 581 goats in Aosta Valley, finding a very high prevalence (49.57%). The 75% of the flocks showed a high reactivity against SRLV-A antigens, associated to ovine hosts. Molecular characterization confirmed that the SRLV-A8 subgroup were specifically associated to the Valdostana breed.
In this report a viral strain was isolated and further characterised.
A three years old Valdostana goat was selected based on specific immunoresponse and genetic sequence characterization (SRLV-A8). During regular slaughter, target tissues were collected and tissue explants were obtained from the mammary gland and lymph nodes, lung, mediastinal lymph nodes, synovial membrane, choroid plexus, and spleen. Cultures were maintained over five passages and weekly examined (CPE and RT activity). RT activity was detected only in spleen culture with a decreasing signal over time, suggesting a scarce susceptibility of the spleen fibroblasts selected during tissue explantation. A foetal caprine lung cell line, known to be permissive to SRLV, was also unsuccessful when used in co-culture. No CPE was observed in any culture. We then infect blood derived macrophage (BDM) obtained from SRLV free herd using the supernatant of the first passage spleen explant, resulting in the highest RT activity after 6 dpi. Supernatant was collected, centrifuged for eliminating the cells debris and concentrated using Amicon-15 100 kDa centrifugal filter tubes. Total RNA was extracted from the concentrated supernatant and reverse transcribed as double stranded cDNA. Nextera XT protocol was applied and MiSeq platform was used to fully sequence the virus on a 2x250 V2 Nano flowcell. A total of 165,156 reads were obtained and assembled with Ray. The analysis of the Gag sequence confirmed the subtype definition as well as the Pol gene sequence. Interestingly, the assembly procedure resulted in two different consensus sequences, showing differences along Tat and Env codifying sequences. Env protein is known to play a role in cell receptor and co-receptor binding. In particular, the hyper variable region HV2 was recognized as an important receptor binding site as well as a linear neutralizing epitope: in one of the two consensus sequence the HV2 region was absent, and the deletion was confirmed by the high coverage (1600X against 700X) and by Sanger sequencing. As previously demonstrated, mutation of the HV2 region may lead to a restricted tissue tropims and a marker of low pathogenic potential of A8 subtype in goat. The second env variant, harbouring a complete HV2 motif was not selected during in vitro propagation suggesting no advantages in terms of tissue tropism. Further studies are planned to verify this hypothesis including entry assays using viral pseudotypes using both env variant.

ONCOLYTIC POTENTIALS OF CAPRINE HERPESVIRUS 1 (CPHV-1) ON HUMAN CANCER CELL LINES

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Oncolytic virus immunotherapy is a therapeutic approach to cancer treatment that utilizes native or genetically modified viruses that selectively replicate in cancer cells while displaying minimal adverse effects in normal healthy cells. To date, a wide variety of viruses have been evaluated for their oncolytic potential, including DNA viruses as well as RNA viruses [1]. CpHV-1 is a species-specific herpesvirus closely related to Bovine herpesvirus type 1 (BHV-1) [2]. Our group previously demonstrated that CpHV 1 is able to induce apoptosis in goat peripheral blood mononuclear cells, moreover we have investigated on the pro apoptotic potential of CpHV 1 in Madin Darby bovine kidney cells, evaluating apoptotic profiles like chromatin condensation and DNA laddering. Recently, we have characterized in more detail the intracellular pathway by which CpHV 1 is able to induce apoptosis by analyzing the gene expression response during the apoptotic phase of CpHV 1 infection in a murine neuroblastoma cell line (Neuro 2a) [3]. Thus, the aim of the present research was to investigate the ability of CpHV-1 to replicate, cause cell death and affect cellular viability in a panel of human cancer cells lines. Human breast adenocarcinoma (MDA-MB-468), Human cervical adenocarcinoma (HeLa), Human osteosarcoma (U2OS), Human prostatic adenocarcinoma (PC3), Human lung carcinoma (A549), Human malignant melanoma (A375) and Chronic Myelogenous Leukemia (K562) cell lines were used. Human Embryonic kidney (293T) and Madin Darby bovine kidney (MDBK) cell lines were used as control. In a first series of experiment we have analyzed the effect of CpHV-1 infection on cell viability by means of the MTT assay at different time post infection (p.i.) and several multiplicity of infection (MOI). All cell lines, except K562 cells, showed a marked cytopatic effect (CPE), demonstrating an oncolytic potential of CpHV-1 in tested human cancer cells. The reduction of cells viability was associated with significant levels of viral production as assayed by TCID50 after 24 h p.i. for MDBK, 293T, MDA-MD468, A549, U2OS, A374 and after 48 h p.i. for PC3, HeLa and K562 cell lines. Then, we investigated virus induced cytotoxicity, viability, and apoptosis within a single assay well, by using the ApoTox-Glo Triplex assay. Analysis of virus-infected cells revealed activation of caspase-3, a marker of apoptosis at 24 h post-infection. Analysis of virus-infected, cells by western blot assay, revealed activation of caspase-3 and cleaved caspase 3 at 24 h p. i. in MDBK, PC3, MDA-MD-468, A375 and U2OS cell lines. Our findings are significant because this is the first published study showing the effect of CpHV-1 infection in neoplastic cell lines in terms of caspase activation and apoptosis modulation.

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INDUCTION OF ANTI-HUMAN CCR5 ANTIBODIES BY A BOVINE HERPESVIRUS TYPE-4 BASED VECTOR

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Bovine herpesvirus 4 (BoHV-4) is a promising vector for the delivery and intracellular expression of recombinant antigens and can thus be considered as a new prototype vaccine formulation system. [1] An interesting, and actively pursued, antigen in the context of HIV infection prophylaxis (and therapy) is the CCR5 co-receptor, whose blockage by specific antibodies has been shown to inhibit both viral entry and cell-to-cell transmission of the virus. [2] Building on our previous work on the BoHV-4 vector system, we have engineered and tested a replication-competent derivative of BoHV4 (BoHV-4-CMV-hCCR5ΔTK) bearing a human CCR5 expression cassette. We show here that CCR5 is indeed expressed at high levels in multiple types of BoHV-4-CMV-hCCR5ΔTK-infected cells. More importantly, two intravenous inoculations of CCR5-expressing BoHV-4 virions into rabbits led to the production of anti-CCR5 antibodies capable of reacting with the CCR5 receptor exposed on the surface of HEK293T cells through specific recognition of the amino-terminal region (aa. 14-34) of the protein. Given the growing interest for anti-CCR5 immunization as an HIV control strategy and the many advantages of virus-based immunogen formulations (especially for poorly immunogenic or self antigens), the results reported in this study provide preliminary validation of BoHV-4 as a safe viral vector suitable for CCR5 vaccination.

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IDENTIFICATION AND GENETIC CHARACTERIZATION OF EQUINE HEPACIVIRUSES IN ITALY

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Recently, several new hepaciviruses (HVs) have been discovered in different animal species. Among those, equine HV (EqHV), represents the closest known relative of hepatitis C virus (HCV). Although detected worldwide, information on EqHV epidemiology, genetic diversity and pathogenicity is still limited. In this study we investigated the prevalence and genetic diversity of EqHV in Italian equids. A collection of 2066 serum samples, including equine and donkey sera, was screened for the presence of EqHV RNA by using a TaqMan-based molecular assay [1]. Out of 1932 equine sera, 91 (4.7%) tested positive in real time RT-PCR, whilst EqHV was not detected in donkey sera (134). Eighteen samples with high RNA copy number (> 103 copies/ml) in Real time RT-PCR were selected for sequencing and phylogenetic analysis of 5'UTR, NS3 and NS5B genomic portions. Sequence editing, multiple codon-based (translation) alignments and phylogenetic analysis of detected viruses were performed by Geneious software version 9.1.6. Phylogenetic trees of 5'UTR, NS3 and NS5B genomic regions were inferred by using the neighbor joining method, with the Maximum Composite Likelihood algorithm of distance correction and with bootstrapping over 1,000 replicates. Identity between the Italian strains and strains isolated in other countries ranged from 89.69 to 100% in the 5'UTR, 79.01 to 100% in the NS3 and 77.21 to 100% in the NS5B. The moderate genetic diversity of the various EqHV strains, more marked in the NS5B region (up to 22.69%) may account for a rather short evolutionary history of EqHVs with a recent divergence after a bottleneck event. Upon sequence comparison and phylogenetic analysis of the Italian EqHV sequences segregated into two main clades and were distributed into various sub-lineages and intermingled with strain of various geographical origin. This chaotic strain diversity would be consistent with multiple/repeated introduction of EqHV strains and could be related to trading of meat horses, or, more likely, to international movement of competition horses that nowadays interest, basically, all the continents. Multi-target analysis spanning nearly the complete genome sequence of EqHV did not reveal inconsistencies in the inferred phylogenies, that would be suggestive of potential recombination events. These findings may indicate that recombination is not common among EqHV strains. However, multi-target results of the Italian EqHV strains revealed apparently inconsistent, with some strains matching two viruses in different genomic regions, a pattern that could be interpreted with the presence of recombination.

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COMPARATIVE CYTOTOXICITY AND EFFICACY AGAINST *Rhodococcus equi* OF AZITHROMYCIN AND RIFAMPICIN VERSUS THEIR BINARY COMBINATIONS

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Rhodococcus equi is the main infectious agent of pneumonia and, in some countries, the principal cause of morbidity and mortality in foals [1]. Currently, the oral combined therapy with azithromycin and rifampicin is recommended, being the antimicrobial combinatorial treatment one of the leading and powerful mechanisms to contrast the antibiotic resistance and to enhance the therapeutic dose [2]. Indeed, a positive antibiotic interaction potentially allows to reduce dosage and, consequently, adverse effects. However, the efficacy of this combination compared with the single pharmaceutical molecules was not evaluated in depth and data concerning the most effective ratio between azithromycin and rifampicin are missing as well as studies regard to the combination safety. This study is designed to evaluate the potential synergism between azithromycin and rifampicin combined as a micronized dry powder. The collateral purpose is to provide an assessment on the safety of this antibiotic combo. Three binary combinations of azithromycin and rifampicin as dry powders were developed according to the ratios 1:1, 2:1 and 1:2 by using a Mini Spray Dryer model B-290. Spray-dried azithromycin and rifampicin were prepared independently for comparison. Firstly, a broth microdilution test was performed in triplicate for the purpose of determining the Minimal Inhibitory Concentration (MIC) of the combinations and single drugs against *R. equi* ATCC 33701 at 24 and 48 hours, considered the slow growth of this organism, subsequently, the Minimal Bactericidal Concentration (MBC) was established. The calculation of the Fractional Inhibitory Concentration (FIC) index was used as predictor of synergy. Secondly, the cytotoxicity of azithromycin and rifampicin individually and in combination was determined on BEAS-2B cells as epithelial model for investigating cellular viability using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay. Except for azithromycin plus rifampicin ratio 1:2, the combinations MIC kept lower than the single antibiotics over time and FIC index confirmed the favourable additive effect, whereas MBC assay demonstrated the more powerful killing activity of all three combinations than the single compounds. Besides, the BEAS-2B treatment with the drugs combination was well tolerated, exhibiting the 50% cytotoxic concentration at 100 µg/ml. The cellular viability was found below 90% at concentrations >20 µg/ml, >75 µg/ml and >40 µg/ml for azithromycin, rifampicin and their combination respectively, at least 40 times MIC value of the compound showing the most significant cellular effect. Obtained the preliminary data in regard to the safety and the greater efficacy of the azithromycin and rifampicin combinations compared to single drugs, further studies are desirable in order to evaluate the intracellular antimicrobial activity and, consequently, to identify the most effective ratio. Taking into account the importance of rhodococcosis in equine medicine, the advantages related to antimicrobial interaction and the proprieties of the binary combinations examined, the hypothesis of a future realization of a commercial antibiotic combo cannot be completely off the table.

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WHOLE GENOME SEQUENCING AND GENETIC CHARACTERIZATION OF *Brucella abortus* ISOLATED FROM WATER BUFFALO HERDS OF THE CAMPANIA REGION

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Brucellosis is a worldwide disease caused by intracellular bacteria of the genus *Brucella*. The bacterial species are classified according to their preferred animal host, each comprising several biovars: *abortus, melitensis, suis, canis, ovis, neotomae* and others less widespread (1). Brucellosis in water buffalo (*Bubalus bubalis*) is generally caused by *B. abortus* and may cause reproductive disorders. Despite the huge efforts invested in the control of animal brucellosis, the disease is still highly present in Campania (2). This study characterized the genome of 18 *B. abortus* strains isolated from water buffalo aborted fetuses collected in Campania region and analyzed by the Istituto Zooprofilattico Sperimentale del Mezzogiorno. The analysis was carried out through NGS sequencing for genetic characterization of the strains.

Bacteria were grown onto Brucella agar and identified by automated bio-chemical tests (VITEK, biomerieux). Species and biovar identification were performed by Italian National Centre for brucellosis. Genomic DNA was isolated by QIAamp DNA Mini Kit (Qiagen) according to manufacturer's protocol. DNA concentration was determined by Qubit® 2.0 (Invitrogen). Libraries were prepared with Ion Xpress™ Plus Fragment Library kit (Thermo Fisher Scientific) using 1 ng of DNA. Finally, whole genome sequencing was performed on Ion Torrent PGM instrument (Thermofisher). The sequenced paired-end reads were assembled with SPAdes v3.9.(3). Resulting scaffolds were ordered using Abacas and the B abortus (NCBI:A13334) as a reference genome. Gaps were manually checked. Annotation was performed using Prodigal and Uniprot database to confirm predictions. Orthologs were determinded by ProteinOrtho. Our results indicated the presence of a core genome, common to all strains, including 1764 genes, and an accessory genome, with 2093 genes. Among all these genes, 1259 allelic variants were identified and classified according to their function. Most loci (73.9%) were involved in the physiology of the bacterium, others were responsible for bacterial virulence (1.6%), pathogenicity (1.7%) or defense mechanisms (4.2%), while the remaining genes (18.6%) coded for uncharacterized proteins. Phylogenetic analysis showed clusterization of strains according to their geographical origin.

These results highlight the complexity of *Brucella abortus* and might allow a better comprehension of the pathogenicity of this bacterium in water buffalo.

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ENHANCED ONCOLYTIC ACTIVITY OF BOHV-4-BASED VECTOR DELIVERING A MIRNA SEQUENCE AGAINST ENOLASE 2 TRANSCRIPT

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Cancer diseases are the second globally most frequent cause of mortality. To help to prevent and cure these diseases new innovative anticancer therapies and treatments are needed. Oncolytic viruses, because of their ability to infect and replicate selectively into tumor cells, could represent an interesting considerable anticancer strategy. [1] Experimental studies/data have shown how these viruses have marked tropism for brain tumor cells such as glioblastoma cells. Glioblastoma Multiforme (GBM) is a very aggressive brain tumor originating into the glia, characterized by high replication rate, which nowadays still has no cure. Genetic analysis revealed that progressive oncogenes and tumor suppressor genes mutations are involved in the development of GBM and one of the most frequent mutation observed in these tumor cells is enolase 1 (ENO1) homozygous deletion. ENO1 is an isoenzyme expressed in a variety of tissues, including brain, involved into glycolysis and gluconeogenesis cell energetic processes. The intense energetic demands of neuronal ENO1(-/-)tumor cells is modestly compensated by ENO2 gene expression, making ENO2 an ideal target for GBM anticancer therapy. [2] The aim of this work was to generate a recombinant Bovine Herpesvirus 4 (BoHV-4) based vector expressing a microRNA for ENO2 (miR-ENO2) to improve the BoHV-4 oncolytic activity in ENO1-deleted GBM cells. An expression cassette, caring miR-ENO2 downstream of Turbo RFP and under the transcriptional control of Cytomegalovirus Immediate Early promoter, was designed to subsequently generate the recombinant virus. [3] The oncolytic efficiency of recombinant BoHV-4-TurboRFP-ENO2 to selectively limit GMB cells growth and survival was tested on Gli56 and D423, ENO1(-/-)/ENO2(+/+),cell lines and evaluated by Cristal Violet, MTT assay and Western Immunoblotting. In vitro results showed how BoHV-4 is able to infect and replicate into ENO1-deleted GMB cells, inducing a diffuse cytopathic effect (CPE) on cells monolayer, and how ENO2 inhibition through miR-ENO2 expression has significantly increased its oncolytic activity. This increased activity could be, in fact, attributable to ENO2 post-transcriptional downregulation, thus causing the loss of energy source in GBM cells. These in vitro data could suggest a possible BoHV-4-TurboRFP-ENO2 use as a GBM therapeutic tool. However, in vivo studies on animal models will be necessary to consider BoHV-4 based vector as a valid alternative for GBM anticancer treatment.

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HIGHLIGHTING PRIORITY AREAS FOR BOVINE VIRAL DIARRHEA CONTROL IN CATTLE IN ITALY: A PHYLOGEOGRAPHIC APPROACH

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The bovine viral diarrhea virus (BVDV) prevalence and genetic diversity in a geographic area are largely influenced by live animal trade and management practices [1]. Despite control and eradication programs are underway in several European countries, the risk of BVDV spread within and among countries is still present [2]. Two BVDV recognized species have been identified in cattle, BVDV-1 and BVDV-2, and a putative third one, HoBi-like pestivirus, recently associated with sporadic BVDV-like clinical forms [3]. BVDV-1 is the predominant genotype circulating in European cattle population. In this study,a phylogeographic analysis was applied to BVDV-1 most prevalent subtypes in Italy [4] to reconstruct the origin and spatio-temporal distribution and to trace the main viral flows between different locations to highlight priority areas for BVDV control. With this aim a comprehensive dataset of BVDV-1b (n=173) and 1e (n=172) 5'UTR sequences has been analyzed, including both novel and published sequences from Italy and European countries bordering and/or with cattle commercial flows with Italy. A common phylogeographic pattern was observed for BVDV-1b and 1e subtypes: interspersion from multiple areas was widespread until the end of the last century, whereas significant local clusters were observed starting from 2000. These findings support a continuous viral flow among different areas over long time scales with no evidence of significant geographical structure, while a local system of viral evolution is limited to more recent years. Northern Italy has been highlighted as the area of origin of the main clades of both BVDV subtypes at national level, acting both as a crucial area for introduction and a maintenance source for other areas. Other Italian regions mainly of central and southern areas contributed to limited geographical distribution and local BVDV persistence. Interestingly a similar pattern has been recently observed also for BVDV-1f. On the whole, priority control measures should to be focused on: i) breaking the gravity-like dynamic of the infection, originating in larger animal populations from Northern regions and diffusing to smaller populations; ii) stopping the dynamic of infections in regions with self-maintenance of BVDV as demonstrated by significant spatial clusters observe in recent years.

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CONGENITAL TREMOR AND HYPOMYELINATION ASSOCIATED WITH TRANSPLACENTAL INFECTION WITH BOVINE VIRAL DIARRHEA VIRUS (BVDV) IN AN ITALIAN CATTLE HERD

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BVDV belongs to the genus Pestivirus within the family *Flaviviridae* and it is responsible of severe losses in cattle farms all over the world. The infection of naïve pregnant cattle in the first 120 days of gestation can lead to the generation of persistently infected (PI) calves which are the main source of infection within and between herds. In particular, the fetal infection between 100 and 150 days' gestation causes teratogenic consequences which typically affect the brain, the development of the retina, hair-coat and bones. Hypomyelination as predominant CNS pathology is considered an uncommon outcome as it was rarely reported in calves congenitally infected with BVDV.

We describe an outbreak of BVD in a Holstein naïve dairy herd, resulting in a high prevalence of calves with neurologic signs. The calves were affected by congenital generalized tremors more or less disability to assume and maintain quadrupedal stance and showed generalized ataxia.

The brain and the spinal cord of three neurologic calves were examined. Tissue sample sections were stained with Luxol fast blue method. BVDV RNA was extracted, with a commercial kit, from the brain of 5 calves with neurologic symptoms and from the whole blood of an age-matched PI calf without neurologic signs. Reverse transcription and PCR assays targeting different regions of the genome were performed and the amplicons were cloned and sequenced.

Histologically, a mild to moderate, multifocal gliosis was evidenced through the brain and the spinal cord where few swollen axons were identified, hypomyelination was the predominant lesion observed, while the most classic neuropathological findings, as cerebellar hypoplasia and dysgenesis, were not present.

Luxol fast blue-stain permitted a histologic diagnosis of multifocal neuraxial hypomyelination. The 5'-UTR and Npro regions showed a 99.7-100% nucleotide identity between the 6 samples and the Phylogenetic analysis performed classified the BVDV as type 1 subtype b. The sequence analyses of E2 and NS2 regions demonstrated 99.4-100% identity between the clones obtained from the brain samples and the main part of the clones obtained from the blood of the healthy PI animal. In the latter, the presence of a mixed infection was shown in NS2-3 clones suggesting the presence of quasispecies.

Hypomyelination is a rare manifestation of in utero BVDV infection in cattle, possible reasons for these unusual manifestations include the gestational age of infection, virus neurovirulence or infectious dose and other factors such as breed, age, and immune status.

The pathogenesis of hypomyelination is poorly understood and previously described cases were not linked to a specific viral genotype but only the most conserved genomic regions 5'UTR were investigated [1, 2]. Our results may suggest a possible neurotrophism of the predominant variant identified in diseased animals and in the blood of the PI healthy calf, an indeep genomic analysis will be necessary to confirm this hypothesis.

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IDENTIFICATION OF THE GENES CONTROLLED BY THE FAS AND MGA TRANSCRIPTIONAL REGULATORS OF *Streptococcus equi* USING A TRANSCRIPTOMICS APPROACH

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Strepococcus equi subspecies equi (*S. equi*) is the causative agent of strangles, but despite its economic impact the only available vaccine, Equilis StrepE, is rarely used because it causes adverse reactions [1]. Recently, a technique known as transposon directed insertion-site sequencing (TraDIS) [2] was used to identify which genes in *S. equi* were required for the formation of adverse reactions following vaccination with three transposon mutant pools of a new live attenuated strangles vaccine, Se4592. Interestingly, rather than identifying genes lost from the population that were essential for survival at the injection site, 43% of surviving mutants contained disrupted fas or mga genes. These data suggest that the loss of function of these genes promotes the formation of adverse reactions. The genes fas and mga encode the putative transcriptional regulators: fibronectin/fibrinogen binding/haemolytic activity/streptokinase regulator and multiple virulence gene regulator of Group A *Streptococcus* (GAS), respectively. The aim of the present study was to use a transcriptomics approach to identify genes controlled by Fas and Mga, which may influence the ability of live *S. equi* vaccines to cause adverse reactions.
Transposon mutants of fas and mga were identified from individual *S. equi* colonies recovered from an adverse reaction by PCR using a transposon-specific forward primer and a set of fas or mga reverse primers. The PCR products were sequenced to confirm the insertion sites. RNA was isolated from the two mutants and the wild-type bacteria (Se4592) for transcriptomic analysis. The indexed sequencing reads for each isolate were mapped against the reference genome of Se4592 using Bowtie2, and transcriptomes were reconstructed using Cufflinks. The transcriptomes of the mutant strains were compared to the transcriptome of Se4592 using Cuffdiff [3]. Genes controlled by the regulators were identified by virtue of decreased or increased copies of mRNA sequence in the fas and mga mutant strains relative to Se4592. Nine genes were differentially transcribed in the mga mutant and seven genes in the fas mutant ($P<0.001$), compared to the vaccine strain. These results are very interesting because show that a mutation of fas and mga alters the transcription of a series of genes that deserve further investigation. Our data shed light on the processes that underlie the formation of adverse vaccine reactions towards improving the safety of live attenuated strangles vaccines.

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ANIV

PETS

EVALUATION OF ANTIBACTERIAL PROPERTIES OF NON TRANSFUSIONAL HEMO-COMPONENTS: AN IN VITRO STUDY IN VETERINARY MEDICINE

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The decline in the antibiotic pipeline and the continuous increase in resistance have become a serious global threat in human and veterinary medicine [1]. In recent years, more and more interest was addressed in discovering new natural antibacterial molecules. Activated non transfusional hemo-components (NTHC) have been demonstrated to promote tissue regeneration, to enhance the action of the natural physiological responses, and to inhibit bacterial growth [2]. The aims of the study were to evaluate: -the antibacterial activity of NTHC obtained from canine blood; -the role of leucocytes and platelets in antibacterial effect; -the properties of NTHC in relation to the different Gram's stain affinity to antibiotic susceptibility profile. Platelet lysate, fibrin glue, thrombin, pure- and leucocyte-platelet-poor plasma (P-PPP, L-PPP), and pure- and leucocyte-platelet-rich plasma (P-PRP, L-PRP) with and without calcium gluconate or thrombin, pure- and leucocyte-platelet gel (P-PG, L-PG) at different quantities (10, 20, 40, 180µl) were tested. Different strains of *Staphylococcus aureus*, *Staphylococcus cohnii*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, previously isolated from canine skin infections and classified as sensitive, multi-resistant and resistant to a whole panel of ten commercial antibiotics, were used. The assessment was carried out at 4, 18, 24 hours both by Kirby Bauer method and inhibition in broth into microtiter plate with a spectrophotometer reading (OD_{540nm}). In comparison to each bacterial control growth (OD_{540nm}), platelet lysate, fibrin glue, thrombin, PPP and PG, with and without leucocytes, showed greater inhibitory effect against Gram negative bacteria ($P<0.05$). Antibacterial activity of P-PG and L-PG was documented already after 4 hours of incubation and confirmed after 18 and 24 hours (Kirby-Bauer). The maximum bacteriostatic effect was highlighted at 18 hours (microdilution in broth). The antibacterial action was directly proportional to the amount of PG, when tested against Gram negative (L-PG 180µl: mean OD=0.90 vs. control OD=1.60, $P=0.001$; P-PG 180µl: mean OD=0.70 vs. control OD=1.60, $P<10^{-4}$), multi-resistant (L-PG 180µl P=0.031; P-PG 180µl P=0.019) and resistant to all panel strains (P-PG 180µl P=0.021). The similar bacteriostatic action of PPP and PRP ($P>0.05$) demonstrated to be linked to plasma components rather than to platelets. The presence of leucocytes in NTHC did not result in a significant reduction of bacterial growth ($P>0.05$). The study allowed demonstrating the antibacterial efficacy of NTHC against different microorganisms, in particular resistant strains. For the first time, the activity of platelet lysate was evaluated in veterinary medicine. The results contributed to the enrichment of knowledge on this field, in order to provide answers to relevant questions recently raised by different Authors in human medicine. Further studies are in progress to evaluate the mechanism of action underlying the antimicrobial activity of NTHC.

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SEROLOGICAL TESTING TO MONITOR IMMUNITY TO CANINE VACCINES: AN USEFUL TOOL IN VETERINARY PRACTICE

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Today there are well-validated rapid and simple in-practice serological tests that can detect the presence of protective antibodies specific for core vaccine antigens: canine adenovirus (CAV, responsible for canine infectious hepatitis, ICH), canine parvovirus type 2 (CPV) and canine distemper virus (CDV) for dogs; feline herpesvirus (FHV), feline calicivirus (FCV) and feline panleucopenia virus (FPV) for cats. These kits offer a good alternative to routine core revaccination in adult dogs, but they can be used also to evaluate interference of maternal-derived antibodies in puppies and kittens, decide whether to vaccinate an old pet or an animal without history of vaccination, manage vaccination in allergic patients, and evaluate the situation in shelters. The 2015 WSAVA guidelines for the vaccination of dogs and cats support their use [1, 2]. The purpose of our study was to test the applicability of one of these tests in a wide canine population, different for sex, breed/size, age, and vaccination history. For this purpose, 106 owner dogs (57 females and 49 males) were tested with Vaccicheck Canine (Agrolabo), an in-practice kit based on a solid-phase ELISA test. Animals were divided by: (1) breed/size: 38 small size (<10 kg), 28 medium size (10-25 kg) and 40 large size (>25 kg); (2) age: 8 puppies (<1.5 yrs, that began or completed their primary core vaccinations), 36 young adults (1.5-3 yrs), 35 adults (3-6 yrs), 14 seniors (6-9 yrs), and 13 geriatrics (>9 yrs). The results of the test were interpreted according to the indications of the kit: a dog was considered protected with an antibody titre ≥1:16 for ICH, ≥1:80 for CPV, and ≥1:32 for CDV. The mean antibody titre for the whole canine population was always highly protective (ICH ≥1:170, CPV ≥1:430, CDV ≥1:230). Females always had a mean titre higher than males (ICH ≥1:190 vs ≥1:150 - CPV ≥1:460 vs ≥1:400 - CDV ≥1:260 vs ≥1:200), while no big differences between dogs of different size/breed were noted (small size ICH ≥1:180, CPV ≥1:450, CDV ≥1:220 - medium size ICH ≥1:150, CPV ≥1:420, CDV ≥1:240 - large size ICH ≥1:180, CPV ≥1:430, CDV ≥1:240). At increasing age, the titre tends to decrease, but remains always protective (puppies ICH ≥1:200, CPV ≥1:490, CDV ≥1:340 - young adults ICH ≥1:200, CPV ≥1:480, CDV ≥1:240 - adults ICH ≥1:170, CPV ≥1:420, CDV ≥1:250 - seniors ICH ≥1:120, CPV ≥1:380, CDV ≥1:210 - geriatrics ICH ≥1:130, CPV ≥1:360, CDV ≥1:120). Dogs vaccinated less than 1 year before had mean titres slightly higher than dogs vaccinated more than 1 year before, but titres were always highly protective in both groups (ICH ≥1:190 vs ≥1:140; CPV ≥1:450 vs ≥1:400; CDV ≥1:240 vs 1:200). Two geriatric dogs (two sisters Irish Setter-Belgian Shepherd crossbreed, 13 yrs old), vaccinated 4 years before for the last time, had high titres for the 3 vaccines (ICH ≥1:160, CPV >1:640, CDV >1:80), and even another geriatric dog (small size crossbreed female, 15 yrs old), vaccinated only when she was a puppy, had protective titres (≥1:160) for all vaccines. Our results support the use of Vaccicheck Canine as in-practice serological kit in order to test dogs before vaccination and confirm the long duration of a protective immunity in dogs vaccinated much more than one year before.

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THE ROLE OF DOG CIRCOVIRUS IN THE DEVELOPMENT OF CANINE ACUTE GASTROENTERITIS

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Dog circovirus (DogCV) is a canine virus whose pathogenetic role is still uncertain. Based on recent data suggesting its role as entheropathogen, a case-control study was conducted between 2013 and 2016 to investigate the association of DogCV with the occurrence of acute gastroenteritis in dogs, alone or in co-infections with canine parvovirus (CPV), canine coronavirus (CCoV) and canine distemper virus (CDV).

A total of 219 dogs suffering from acute gastroenteritis and 67 controls randomly recruited among animals without clinical signs of gastroenteritis were screened by real-time PCR assays for detection of DogCV [1], CPV [2], CCoV [3] and CDV [4]. The age of the selected animals, their clinical data and vaccination records were collected for further analyses.

A high prevalence of viral infections was detected in dogs with acute gastroenteritis (77.16%), whereas only 31.35% of the controls were found to shed any virus. Not surprisingly, CPV was the most common enteropathogen (57.99% of cases), followed by DogCV (32.42%) and CCoV (24.65%). Occurrence of pathogens was more likely to be reported in young dogs than in adults ($p<0.0001$) and in kennelled than in client-owned dogs ($p=0.001466$).

Co-infections were detected in 34.25% of the clinical cases. DogCV was found in a significant proportion (77.33%), mostly associated with CPV (70.68%). Detection of DogCV in control dogs (28.35%) occurred with a similar frequency to the clinical cases, so that the correlation of single DogCV infections with gastrointestinal disease was not statistically supported ($p=0.3925$). However, all positive samples from the control group were single infections, showing a significant correlation of DogCV co-infections with acute gastroenteritis ($p<0.00001$).

Recently, DogCV was associated with different clinical forms, including acute gastroenteritis [1]. However, subsequent studies have suggested that the pathogenic potential of this emerging virus may be restricted to co-infections with other viruses, mainly CPV [5, 6]. The results of the present study support the role of DogCV as a co-pathogen in the occurrence and, probably, severity of gastrointestinal disease, thus acting in synergism with other, more pathogenic viruses.

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CANINE PARVOVIRUS (CPV) 2C INFECTION IN A CAT

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Canine parvovirus 2 (CPV-2) emerged as dog pathogen in the late 1970s and originated as a host range variant from Feline panleukopenia virus (FPLV) [1]. CPV-2 has been rapidly and totally replaced by three variants (CPV-2a/2b/2c). Unlike CPV-2, variants regained the ability to infect feline tissues and were also isolated from healthy cats or from cats with mild or subclinical disease. CPV-2c was rarely reported in cats, alone or in co-infection with FPLV or other CPV types [2,3,4,5]. This study reports the evidence of CPV-2c in a cat. A 3 months old kitten was submitted to IZS of Sicily for necropsy. Oedema of thoracic and visceral serous membranes, intense enteritis with bland necrosis of mucous membrane and diarrhoea during necropsy were observed. The organs were submitted to bacteriological, virological and toxicological assays. DNA extracted from intestine, spleen and lungs was screened by conventional PCR assay for FPLV and CPV [6]. PCR products were digested using the enzyme MboII [6]. Whole parvovirus genome from intestine was sequenced using a primer panel [7]. Sequence analysis and alignments with sequences available in GenBank were performed. All the samples tested positive for parvovirus, RFLP generated two fragments typical of 426Glu mutants. Sequence analysis confirmed CPV-2c type, showing a nucleotide identity of 99.82% (GU362935) and 99.60% (HQ025913) with CPV-2c strains previously collected from cats in Italy [2,4]. The NS1 and VP2 sequences showed a nucleotide identity of 99.85 and 100% respectively with those of CPV-2c dog strains from the same region. The VP2 sequence presented one non synonimous (g415a → Val139Ile) and two synonimous mutations (a1716t, a1746g) that we observed in CPV strains collected from dogs. Toxicological and bacteriological assays tested negative. This study is the first evidence of a CPV-2c strain with uncommon amino acid and nucleotide changes in a domestic cat. The amino acid change Val139Ile, firstly described in Europe in 2011 in other CPV types [8], has never been reported in european CPV-2c strains. We observed the same mutation in dog samples too (unpublished data). Role of CPV-2c type in cat remains unclear but this finding highlights the circulation of CPV-2c strain with specific changes also in cats. Further and large scale investigation are suggested to better evaluate prevalence and role of CPV strains in cats.

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CLINICAL AND BACTERIOLOGICAL EVALUATION OF SURGICAL SKIN WOUNDS TREATED WITH OZONIZED OLIVE OIL: A PRELIMINARY STUDY

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Since in the last years the overuse of antibiotics and the appearance of "multi-drug resistant" bacteria constantly increased, scientific research was driven towards the study of new antimicrobial, especially of natural origin. The aim of the study was to evaluate the bacteriological and healing activity of an ozonized olive oil (OOL) used to treat abdominal surgical skin wounds in dogs and cats. A randomized blind controlled clinical trial was performed at the School of Biosciences and Veterinary Medicine, University of Camerino. For assessing the clinical efficacy, the ASEPSIS Scale, the Wound Evaluation Scale (WES) [1] and the Total Bacterial Count (TBC) analysis were performed for each patient [2]. The degree of owner satisfaction and the possible highlight of complications or adverse effects, were also considered. Animals were randomly divided into two groups: treated group (GA), that received topical application of OOL twice a day for three consecutive days, beginning the day of surgery, and control group (GC) that did not receive any kind of treatment. Comparing the TBC at T_0, T_1 and T_2 between GC and GA, no significant differences were revealed. Within GC, it was observed a not significant increase in the TBC comparing T0 vs T1, T1 vs T2 and T0 vs T2. In the GA, between T_0-T_1 and T_0-T_2 the TBC value increased, while the TBC between T_1-T_2 (t=0.59, P=0.56) decreased, but not significantly. Comparing the frequency of optimal healing of both groups at the same time, a significant difference was observed at T_2 (P=0.034) in favor of GA. Regarding the WES, a significant increase of patients of GA with optimal healing process from T_0 to T_2 was observed, while in the GC the percentage of optimal healing decreased from T_0 to T_1 and from T_0 to T_2 and increased from T_1 to T_2, but not significantly. Considering ASEPSIS score, the difference between GA and GC was not significant at T_0 (T=0.05; P=0.96), at T_1 (T=0.29; P=0.77), and at T_2 (T=1.17; P=0.24). At T_2, GA showed a high number of patients with satisfactory healing, but without any significant differences between the times of observation. This study allows to conclude that the OOL could be a medical device applicable to skin wounds without any collateral effect, it facilitates the healing process with a relative short period of treatment (3 days) and the owners, also reported an excellent grade of applicability and compliance. The absence of significant results related to the decrease of TBC mean values could find its explanation on a correct surgery preparation with iodophor agents. The results obtained with this study shows slight different results from the conclusions of a similar trial conducted by Kim et al. [3]. The explanations could be related to the shorter period of treatment and to the wound location, always in the abdominal area, with the consequent biological and physiological factors. No adverse events were reported. Infection related to licking was the only complication affecting 10% of patients in GC and 12.5% in GA

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Malattie parassitarie

LIVESTOCK

SEQUENCE VARIATION IN THE B1 GENE AMONG *Toxoplasma gondii* ISOLATES FROM SWINE AND CATS IN ITALY

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Among parasitic zoonoses *Toxoplasma gondii* infection has the highest incidence worldwide. The risk derived from cat ownership or meat consumption in terms of acquiring human toxoplasmosis is still controversial. The present work evaluates the sequence variation of the B1 gene of *T. gondii* among isolates from domestic swine, wild boars and domestic cats of Umbria region (central Italy). For this tool between September 2014 and December 2015, 77 faecal samples were individually collected from pet cats enrolled on the basis of the presence of *T. gondii*-associated risk factors deduced from a questionnaire completed by the owners (e.g. outdoor access, hunting activities, feeding with raw meat). At the same time, samples of diaphragm pillars were collected from 498 slaughtered fattening pigs managed in intensive farms that were found to be positive for *T. gondii* in previous epidemiological surveys, and from 97 wild boars hunted in the hunting season 2014. Meat juice samples obtained from the tissues through consecutive cycles of freezing and thawing, were screened by Immunofluorescent Antibody Test (IFAT) using a commercial kit (MegaScreen® FLUO TOXOPLASMA *gondii* Test) and the tissue samples tested positive by immunoassay were subjected to genomic DNA extraction using the QIAamp® DNA Mini Kit (Qiagen). Genomic DNA was isolated from the cat faeces using the QIAamp® Fast DNA Stool Mini Kit (Qiagen). DNA extracted was tested by a nested-PCR (n-PCR) protocol [1], amplifying a fragment of ~130 bp of the B1 gene of *T. gondii,* a 35-fold-repetitive gene which is routinely and widely used for biomolecular detection of *Toxoplasma,* especially from human specimens. The amplicons obtained were purified and sequenced. The sequences were compared with those of other isolates available in the GenBank™ by the Nucleotide-Nucleotide "Basic Local Alignment Search Tool" (BLAST), and analysed using Data Analysis in Molecular Biology and Evolution version 4.5.55 (DAMBE) and Mega Evolutionary Genetic Analysis version 7.0.20 (MEGA7) software.

Among the swine, 36 pigs and 20 wild boars tested positive to IFAT. Among the 133 specimens examined by the B1 n-PCR screening, overall 36 specimens (i.e. 10 pigs, 13 wild boars and 13 cats) produced products of the expected size. BLAST analysis showed for all the 36 amplicons an identity score from 99 to 100% with *T. gondii* sequences of sheep origin reported in GenBank™ (a.n. KX270388.1; KX270387.1), however a scant coverage (<50%) was observed with sequences of cat and swine origin, due to the fact that the fragment of the B1 gene selected for the amplification in the present study has not been used in previous molecular investigations in such species. Sequence analysis did not show intraspecific variation for either species but revealed a single base pair polymorphism (C/T) at position 35 in sequences from swine (both pig and wild boars) and cats, respectively. Moreover, comparing the sequences obtained from swine with representative sequences of other species (i.e. sheep, human, birds etc.) the SNP seems to be stable present. The alignment of all the sequences resulted in a total of 91 analyzed characters including 81 conserved and 10 variable sites, of which 1 were parsimony-informative and 9 were singletons, with an overall pairwise distance of 1.240. The nucleotide frequencies were 30.9% (A), 21.3% (T), 27.0% (C), 20.8% (G) with an overall transitions/transversion bias of 1.52.

The results obtained in the present study seem to highlight a genetic variation between isolates from swine and cats. This sequences detected here should be compared with those recovered from human isolates of the same geographical areas, to establish a relationship between human toxoplasmosis and different sources of infection.

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NATURAL VEGETABLE MIXTURE USED FOR THE TREATMENT OF GASTROINTESTINAL STRONGYLES IN SHEEP: EFFECTIVENESS AND BENEFITS FOR ANIMALS

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Gastrointestinal (GI) strongyles are a major factor for reduced productivity in sheep farms. Nowadays the control of parasitic diseases is based mainly on the use of synthetic drugs. The medical herbs and natural mixtures had been used for many years by farmers in the local tradition for the treatment of parasitic diseases in sheep and to improve the productions. These blends are now used on organic farms. However, the scientific evidence on the efficacy of anthelmintic medicinal herbs is very limited. For these reasons the aim of our study was to evaluate the effectiveness of some vegetable extracts (E) used for the treatment of GI strongyles in sheep.

To evaluate their effectiveness tests have been performed on a complementary feed (E_1) made from herbal extracts and essential oils of *Compositae, Cesalpinacae, Liliacae, Bromeliaceae*, (registered for the treatment of nematode infections in sheep) and on two natural extracts in water, used in the local traditions. The first (E_2), based on tannin present in the pomegranate peel *(Punica granatum),* and the second (E_3) based on extracts (bark and leaves) of willow *(Salix alba).*

The study was done between Sept 2015/Sept 2016 in three Calabrian farms. In these farms we have taken individual samples of feces from 60 sheep, for laboratory exams, 180 in total (T-7). From this parasitological screening was possible to select the sheep for the test groups (10 for group): TG ($E_{1, 2, 3}$), Treated Group treated with natural mixtures and CG Control Group untreated.

The timing was: T_0 the formation of groups, sampling feces and treatment with the dosages recommended by the manufacturer; T_7, T_{14} and T_{21} sampling feces and calculated the fecal eggs count reduction (FECR) for evaluation anthelmintic effectiveness.

All individual fecal samples were examined by Flotac *basic technique,* with a satured solution of NaCl (s.g.1200).

The formulas used to evaluate the anthelmintic efficacy (based on the arithmetic mean of the control and treated group), $FECR=100x(1-[epg_T/epg_C])$, are those of the World Association for the Advancement of Veterinary Parasitology (WAAVP).

The results of tests (E_1, E_2 and E_3), with the dosages used, the epg (mean) of the groups and FECR (%) are:

- E_1 (10 ml/sheep/OS) T_0: CG 240 epg, TG 246 epg; T_7: CG 262 epg, TG 314 epg (FECR -20%); T_{14}: CG 362 epg, TG 284 epg (FECR 22%); T_{21}: CG 492 epg, TG 659 epg (FECR -37%).
- E_2 (50 ml/sheep/OS) T_0: CG 142 epg, TG 141 epg; T_7: CG 262 epg, TG 146 epg (FECR 44.3%); T_{14}: CG 168 epg, TG 121 epg (FECR 27.98%); T_{21}: CG 301 epg, TG 181 epg (FECR 39.9%).
- E_3 (50 ml/sheep/OS) T_0: CG 442 epg, TG 440 epg; T_7: CG 223 epg, TG 328 epg (FECR -46.8%); T_{14}: CG 192 epg, TG 266 epg (FECR -38.4%); T_{21}: CG 186 epg, TG 238 epg (FECR -27.8%).

The present study reports that these formulations were ineffective against GI strongyles in sheep, without any benefit for animals and for the farmer.

However, further research is required to verify the validity of the medical plants and of the traditional mixtures used for the treatment of intestinal parasites. It is indeed clear that the scientific validation is essential to confirm the potential anthelmintic of the natural mixtures and to assess its safety.

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Toxoplasma gondii IN BREEDING PIGS IN ESTONIA – A SEROEPIDEMIOLOGICAL STUDY

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Toxoplasma gondii is among the most relevant foodborne parasites [1], and pork is considered a major source of human *T. gondii* infection. A recent study estimated that *T. gondii* seroprevalence in humans was almost 60% in Estonia [2], where the seroprevalence is high also in domestic cats [3], cattle [4], and free-ranging wild boars [5]. Aims of this study were to estimate *T. gondii* seroprevalence in breeding pigs raised in Estonia, and to evaluate a selection of plausible risk factors for seropositivity. Altogether 382 domestic pigs from 14 breeding pig herds were included in this study; 374 of the pigs had also been included in a study estimating *Trichinella* spp. seroprevalence [6]. The sera were analysed with a commercial direct agglutination test for anti-*T. gondii* immunoglobulin G antibodies. Cut-off titer for seropositivity was 40. Data available for the risk factor analyses included age, gender, and breed of each pig, and herd size and location of each farm. Twenty-two (5.8%) of the 382 pigs tested seropositive, and at least one seropositive pig was found in six (42.9%) of the 14 herds. Sows had more than five times higher odds to test seropositive than boars. Age, breed, herd size, and location of the farm did not appear as significant factors in the statistical analyses. This was the first cross-sectional study on *T. gondii* seroprevalence in domestic pigs in Estonia. The *T. gondii* seroprevalence in these domestic pigs was significantly lower than that in free-ranging wild boars [5]. Moreover, *Trichinella* spp. are highly prevalent in the sylvatic cycles in Estonia [6]. The domestic pigs that tested positive for anti-*T. gondii* antibodies in this study were negative for anti-*Trichinella* spp. antibodies [6], which illustrates that the industry was able to prevent exposure to *Trichinella* spp. but not to *T. gondii*, in a country where both *T. gondii* infection pressure and *Trichinella* spp. infection pressure are substantial.

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PREVALENCE OF METACESTODES IN SHEEP IN THE BASILICATA REGION OF SOUTHERN ITALY

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The Basilicata region of southern Italy has a long tradition for sheep farming (242,360 ovine heads; Banca Dati Nazionale, 2017) thus representing an important reality for the regional economy. Parasitic diseases have a great impact on health and production of small rumimants, in fact they are among the main causes of morbidity, mortality and financial losses [1, 2]. In particular, metacestodes - i.e. the larval stages of Taenidae of carnivores infecting small ruminants as intermediate hosts - are responsible of severe tissue damage in different organs, and also cause reduction in milk and meat production and a considerable economic loss due to condemnation of the infected organs of slaughtered animals [3]. The present abattoir survey was performed to assess the prevalence of metacestodes in sheep in Basilicata, a region of southern Italy. A Geographical Information System was constructed in order to uniformly sample the farms throughout the entire region, using as datalayers the administrative boundaries at the provincial and municipal level. Specifically, the region was divided into 100 quadrants, by overlaying a grid of 10 x 10 km. In each quadrant 6 sheep were trasported to the regional slaughterhouse for parasitological investigations; organs from 600 sheep on 393 farms in the Basilicata region were investigated. The abattoir survey was aimed at detecting the following metacestodes in ovine organs and tissues (liver, brain, lungs, heart, abdominal serous membrane): hydatids, larval form of *Echinococcus granulosus; Cysticercus tenuicollis*, larval form of *Taenia hydatigena; Cysticercus ovis*, larval form of *Taenia ovis* and *Coenurus cerebralis*, larval form of *Taenia multiceps*. During the observation period at the slaughterhouse, 407/600 sheep (67.5%) resulted infected by *E. granulosus* and 61/600 (10.1%) by *C. tenuicollis*. The farm prevalence was 75.6% (297/393) for *E. granulosus* and 13.5% (53/393) for *C. tenuicollis*. Mixed infections by the two metacestodes were also common. These results indicate that metacestode infections were widespread in sheep from Basilicata and considering the zoonotic potential of some of them (e.g. *E. granulosus*), it is a great concern for both public and veterinary health. Therefore it is necessary to monitor the spread of metacetodes through specific programs of prevention and control.

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SPATIAL DISTRIBUTION COMPARISON OF BOVINE ANAPLASMOSIS AND BABESIOSIS IN PALERMO PROVINCE (SOUTHERN ITALY)

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Some tick-borne diseases are considered endemic in the Mediterranean basin and they are responsible of huge losses in zootechnical production. Sicily region is ecologically favourable to vector-borne pathogens diffusion, due to its position among the 35th and the 40th parallel, and to its climatic characteristics. Moreover, time of exposition to infected vectors is enhanced by prevalent extensive cattle breeding management, rising the risk to develop the disease. In endemic areas, cattle becomes infected at a young age and develops a long-term immunity. However, outbreaks can occur in these endemic areas if exposure to ticks by young animals is interrupted or immuno-naïve cattle are introduced.

Aims of this survey were to analyze the prevalence of Anaplasmosis and Babesiosis in cattle herds of Palermo province and to correlate the results to pedoclimatic characteristics of the investigated sites.

Palermo province (Sicily, Italy) covers a territory of 4,992 km² and for this study, surface has been subdivided in 14 geographic quadrants (each of 10 km² x 5 km²) where 1 to 2 herds were selected. During spring and summer of 2016, serum samples, whole blood and ticks were collected from a representative number of animals on each selected herd of the territory of Palermo. Geographical coordinates were recorded and morphological identification was performed on ticks. Specimens such as serum and blood were analysed by serological (IFAT and c-ELISA) and molecular (PCR) methods as previously described for the detection of *Babesia bovis, Babesia bigemina* and *Anaplasma marginale* [1]. Confidence intervals of 95% (CI) were calculated for the total prevalence of the infections.

A total of 14 herds distributed in different zones of Palermo province were included in the study and the number of animals tested ranged from 3 to 34. Out of 128 samples, 90.6% (CI 90.47%- 90.73%, median 100%), 71% (CI 63.2%-78.8%, median 65.5%) and 38% (CI 29.6%-46.4%, median 36%) were serologically positive to *A. marginale, B. bigemina* and *B. bovis* respectively. The DNA of *A. marginale* was the only one found in 45.7% specimens (CI 35.9%-55.5%, median 45%) by PCR. Geographically, altitudes ranged from 239 and 823 meters above sea level and all the herds were at least positive to one pathogen investigated (Serology and/or PCR). In herds where highest prevalence of tick-borne pathogens was observed, the competent vectors were present, indeed *Rhipicephalus annulatus* was the mainly tick found in positive *B. bovis* herds.

Data confirm that antibodies against *A. marginale* are the most frequently found in sicilian territory, followed by *B. bigemina* and then *B. bovis* [2-4]. However it is well know that the distribution of tick borne pathogens is not homogeneous in endemic areas. *A. marginale* and *B. bigemina* show the same distribution pattern while B. bovis has a spotty spreading. These data underline the tight relation between ecology and interactions among host-vector-pathogen.

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FLUCONAZOLE SUSCEPTIBILITY IN ASPERGILLUS FUMIGATUS STRAIN ISOLATED FROM ANIMALS

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In the last years, the increased attention to mycoses has led to a more accessible sharing of information about fungal behavior, pathogenic and/or zoonotic role and ability to respond to drugs. Nevertheless, fungal infections are still difficult to diagnose and to treat.
Mycoses are important both in human and veterinary medicine especially for zoonotic features of some fungi; moreover, the therapy could be problematic since the resistance of some strains to common antifungals. Resistance can be related to widespread use of these drugs in mycotic infections and to agricultural practices. Within filamentous fungi, in human medicine the intrinsic resistance of *Aspergillus fumigatus* to fluconazole is well known but few data are available in veterinary practice.
It is known that *A. fumigatus* is intrinsically resistant to fluconazole despite other triazoles are active in vitro and in vivo.
The main mechanism for triazole resistance in *A. fumigatus* is a point mutation in the cyp51A gene encoding a 14-α sterol demethylase; furthermore, some isolates are characterized by a genetic alteration consisting of a 34bp tandem repeat in the promotor.
This study describes an anomalous behavior of an *A. fumigatus* strain from avian origin towards fluconazole and evaluates the origin of resistance through molecular analysis.
The antifungal susceptibility of the strain has been investigated with E-test and minimum inhibitory concentration (MIC), both performed by three different operators. For MIC SensititreY10® plates were inoculated and then incubated at 25°C for 24-72 hours. For E-test a fungal suspension was performed in a sterile tube; spores were counted at light microscope and then 200µl of the suspension were put in a sterile plate and uniformly distributed; after a drying period, fluconazole strip was laid and incubated at 37°C for 24-48 hours. *A. fumigatus* ATCC 204305 was used as control.
The strains have been subjected to PCR and sequencing for cyp51A and promotor using specific primers and amplification conditions. The final sequences were aligned with the GenBank-European nucleotide archive database.
E-test demonstrated the resistance to fluconazole (>256µg/ml) in all *A. fumigatus* strains while MIC SensititreY10® showed increased susceptibility to fluconazole (8µg/ml) in the strain from avian origin. These results were obtained by all the three operators. The control was resistant to fluconazole in both methods (MIC SensititreY10®=128µg/ml; E-test=>256µg/ml). Molecular analysis showed that all *A. fumigatus* tested lack any known cyp51A mutation or tandem repeat in the promotor.
This work shows a discrepancy between MIC SensititreY10 and E-test. PCR and sequencing demonstrate that the differences were not due to azole target mutations. Further investigation will be performed to verify the possible same behavior in other *A. fumigatus* strains isolated from different animals. In case this phenomenon is frequently found more efforts may be focused on looking at other possible molecular mechanisms.

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Malattie parassitarie

VECTOR-BORNE DISEASES

MOLECULAR SURVEY ON *Babesia* SPP. *Borrelia burgdorferi* SENSU LATO, *Anaplasma phagocytophilum* AND *Rickettsia* SPP. IN *Ixodes ricinus* TICKS INFESTING DOGS IN CENTRAL ITALY

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Dogs represent a common feeding host for *Ixodes ricinus,* a vector of several infectious agents. They may act as reservoir hosts for zoonotic tick-borne pathogens (TBPs) and as bringing-host of infected ticks in human settings. Since to date limited information on the pathogens detectable from *I. ricinus* specimens feeding on dogs are available in Italy, the present study aimed to investigate the presence of TBPs of public health concern (*Anaplasma phagocytophilum*, *Rickettsia* spp*., Borrelia burgdorferi* sensu lato (s.l.)*,* and *Babesia* spp.) in tick specimens collected from dogs living in urban and sub-urban settings of a TBP-endemic area of central Italy. A total of 212 ticks, morphologically identified as *I. ricinus,* were collected from the coat of owned dogs living in urban and peri-urban settings of Umbria region and attending the Veterinary Teaching Hospital of Perugia. Genomic DNA was extracted individually from each specimen with the QIAmp Blood and Tissue Extraction Kit (Qiagen). The DNA was then screened for *A. phagocytophilum* and *B. burgdorferi* s.l. by real-time PCR protocols targeting a 77 pb region of msp2 and a 75 pb fragment of 23S rRNA genome region, respectively [1,2]. DNA extracts, positive in real-time PCR, were additionally investigated with a nested PCR targeting the 16S rRNA gene to differentiate variants of *A. phagocytophilum* and with a conventional PCR targeting a conserved region of the *fla* gene to identification of *B. burgdorferi* s.l. genospecies [3,4]. For the detection of *Babesia* spp. and *Rickettsia* spp. DNA conventional PCRs targeting a fragment of 18S rRNA gene and part of citrate synthase-encoding gene ***(gltA)*** were implemented, respectively [5,6]. The amplicons were bidirectionally sequenced, assembled and edited with Bioedit software 7.2.5 (Ibis Biosciences). The sequences obtained were compared with representative sequences available in GenBank using Basic Local Alignment Search Tool (BLAST). Sixty-one out of 212 *I. ricinus* specimens (28.8%) were positive for TBPs. *Rickettsia* spp. were the most frequently identified pathogens (n=39; 18.4%). Among these 32 (15.1%) were positive for *Rickettsia monacensis* and 7 (3.3%) for *R. helvetica*. The other TBPs detected were *A. phagocytophilum* (n. 22; 10.4%), *Borrelia burgdorferi* s.l. (n. 3; 1.4%), *Babesia venatorum* (formely *Babesia* spp. EU1) (n.1; 0.5%). The present findings show a significant exposure of dogs to TBPs of public health concern and provide data about the role of dogs in the circulation of *I. ricinus*-borne pathogens in central Italy.

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TRANSMISSION OF *Rickettsia raoultii* AND *Rickettsia massiliae* BY IN VITRO FEEDING *Dermacentor reticulatus* AND *Rhipicephalus sanguineus* TICKS

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In Europe, *Rickettsia raoultii*, was recently identified as a Spotted Fever Group Rickettsiae (SFGR) causing Scalp eschar neck lymphadenopathy in human. *Dermacentor reticulatus* is consider one of the main vector ticks of *R. raoultii*. *Rickettsia massiliae*, a SFGR, is a worldwide rickettsia of which human pathogenicity was recently confirmed and *Rhipicephalus sanguineus* is recognized as potential vector [2]. The primary aim of this study was to demonstrate the transmission of SFG rickettsiae by *in vitro* feeding *D. reticulatus* and *R. sanguineus* ticks. The secondary aim was to study the maintenance of SFGR between generations of *D. reticulatus*.

D. reticulatus and *R. sanguineus* used in the *in vitro* feeding were obtained from colonies maintained at the Utrecht Centre for Tick-borne Diseases. Ticks were distributed in the feeding units, composed of a Plexiglas tubing in which were fixed the silicone membranes. The units were placed into pre-warmed blood, previously collected from a cattle and distributed over a six-well cell culture plates. Plates were left in the incubator. At intervals, the feeding units were checked for attachment, blood was collected and changed. The hemolymph of *D. reticulatus* from the laboratory colony of the 5th generation was collected. For detection of Rickettsia species the PCR/Reverse line blotting hybridization was performed on the blood samples collected in the *in vitro* feeding and the related ticks used, the hemolymph samples and *D. reticulatus* selected from the colony.

D. reticulatus and *R. sanguineus* ticks fed on blood through the silicone membranes. The presence of the *R. raoultii* and *R. massiliae* in the blood samples was demonstrated. DNA of *R. massiliae* was already detected 8h post application The 71.1% of *D. reticulatus* used in the *in vitro* feeding experiments, was positive for *R. raoultii*. Overall 48.6% *D. reticulatus* randomly selected from the colony were positive for *R. raoultii*. R sanguineus ticks from *in vitro* feeding were all positive for *R. massiliae*. The 80% of hemolymph samples resulted positive for *R. raoultii.*

This study evidence for the first time *in vitro* the ability of *D. reticulatus* and *R. sanguineus* ticks to transmit *R. raoultii* and *R. massiliae*, respectively. A high rate of *R. raoultii* in the hemolymph suggests that the infection is systemic. The prevalence of *R. raoultii* among ticks resulted high. *R. raoultii* was preserved for five generations, proving that *D. reticulatus* could sustain *R. raoultii* infection over generations. Our results suggest that *R. raoultii* could be considered a symbiont. Noteworthy, the speed of transmission of *R. massiliae*. In a recent study was demonstrated the ability of *R. sanguineus* to transmit *Ehrlichia canis* within 8 hours after the attachment to the membrane [3]. In conclusion the detection of *R. raoultii* and *R. massiliae* in the blood samples definitely proved the vectorial capacity of the ticks. The simultaneous transmission of *R. massiliae* and *E. canis* by *R. sanguineus* confirms the need of acaricides with a rapid effect. The *in vitro* feeding methodology results as an alternative artificial method for researches.

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FELINE AND CANINE LEISHMANIOSIS AND OTHER VECTOR-BORNE DISEASES IN THE AEOLIAN ISLANDS: PATHOGEN AND VECTOR CIRCULATION IN A CONFINED ENVIRONMENT

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Leishmaniosis and Vector-Borne Diseases (VBDs) are prevalent in dog populations worldwide, and their epidemiology in dogs is widely investigated and studied. Conversely, epidemiology of feline VBDs is less investigated, and scant data are available; even though, feline leishmaniosis (FeL) is increasingly reported in cats in endemic areas. Comprehensive investigations on the distribution of VBDs in populations of cats and dogs living in relatively small geographical areas, such as islands, are scare [1]. The aim of this study was to investigate the prevalence of VBD causing pathogens in cohorts of cats and dogs living in the Aeolian Islands.

The study was conducted from January 2015 to June 2016 on owned dogs and cats living in Lipari and Vulcano, two main islands of the Aeolian archipelago (Sicily, Italy). For all the animals, data on age, sex, breed, and antiparasitic treatments were collected. Systemic signs, as well as skin and ocular disorders suggestive of VBDs, were recorded. Dogs and cats were also examined for the presence of ticks and fleas. Conjunctival swabs and blood samples for serological, cytological and molecular testing were collected. Leishmania infantum infection was assessed by IFAT and quantitative PCR (qPCR) on blood and conjunctival swabs, while the presence of other pathogens was diagnosed by cytology and PCR on blood samples.

A total of 330 cats (151 males and 179 females) and 263 dogs (147 males and 116 females) were tested. Eighty-five cats (25.8%) were positive for *L. infantum* (85 to IFAT, 7 IFAT+ qPCR on blood, 6 IFAT+ qPCR on conjunctival swabs), 13 (3.9%) for *Bartonella* spp. (qPCR), and 1 (0.3%) for *Hepatozoon felis* (qPCR). One-hundred and ten dogs (41.8%) were positive for *L. infantum*, and three (1.1%) for *Hepatozoon canis*. *Leishmania infantum* prevalence (p=0.0001) and year incidence (p=0.0003) were higher in dogs (41.8%; 27%) than in cats (28.8%; 14.7%). Thirty-four cats (10.3%) scored positive for ticks, identified as *Ixodes ventalloi* and *Rhipicephalus pusillus*. Conversely, *Rhipicephalus sanguineus* was the only species identified in dogs (10.6%). Flea infestation by *Ctenocephalides felis* was larger in cats (n=91; 27.6%) than in dogs (n=33; 12.5%; p=0.0001). Moreover, a female of *Nosopsyllus fasciatus* and a male of *Spilopsyllus cuniculi* were identified in two flea-infected cats.

VBDs are endemic in the Aeolian Islands, *L. infantum* being the most prevalent pathogen which circulates in cats and dogs. The overall seroprevalence of FeL recorded in this study is larger than the one assessed, in populations of cats in Greece and in Spain [1; 2; 3]. Since *L. infantum* and VBDs are commonly associated with dogs, the recognition of cats as hosts of different vector-borne pathogens is a factor of paramount importance in view of a better management of these diseases in both animals and humans.

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SPECIES DISTRIBUTON MODELS: THE CASE STUDY OF *Ixodes ricinus*

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Zoonotic tick-borne diseases represent an increasing health burden in Europe due to environmental anthropogenic alterations affecting the biology of vectors ticks and disease transmission. Data on the tick *Ixodes ricinus* suggest that an extension of its northern and altitudinal ranges have been accompanied by an increased prevalence of tick-borne pathogens [1]. We developed a habitat suitability model (HSM) for *I. ricinus*, as a proactive tool for institutions and policy makes dealing with tick-borne pathogens. Disease predictive models allow to better understand the entity of tick vector presence at regional scale (Piedmont Region, Northwestern Italy), facilitate the diagnosis of tick-borne diseases in humans, as well as optimize screening and preventive actions within the Regional territory. Environmental tick presence and abundance were evaluated by dragging from May to October 2015, with 40 monthly-repeated sampling transects (n=240 transects). Tick presence data were used to train a predictive model for *I. ricinus* presence/abundance which was developed using MaxEnt 3.3.3 (http://www.cs.princeton.edu). Maximum Entropy models use presence only (PO) data (tick presence) and correlates those PO data to environmental factors that can favor or limit vector ecological niches. Biotic and abiotic parameters like temperature, ground elevation, solar exposure, vegetation coverage and land cover were used as covariates to train species occurrence models. A total of 1553 *I. ricinus* ticks were recovered with abundance peaking in August. Model evaluation and "best-model" selection was carried out manually, based on area under receiver-operator curve (AUC) and lower model complexity. Covariates to be retained were selected by backward step-wise model selection excluding at each step the feature with the lowest permutation importance [2]. The best performing model had an AUC value of 0.919 with 5 significant predictive covariates: mean temperature of the coldest quarter of the year, mean summer Normalized Difference Vegetation Index (NDVI), ground slope and solar exposure, and land use. The increasing interaction between wildlife, humans and domestic animals has led to the emergence of several diseases many of which are vector-borne. Active surveillance of sentinel animals and predictive epidemiology have been shown to be effective in mitigating the impact of these diseases. In order to promote a more efficient and cost-effective tool for disease surveillance and management in wildlife, we propose the use predictive models for vector presence and abundance like the one developed for *I. ricinus*.

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VECTOR BORNE PATHOGENS IN DOGS LIVING IN PANTELLERIA ISLAND

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Vector borne diseases are largely distributed in temperate area and Sicily is considered endemic for some of these, which also have a zoonotic interest.
The aim of the present study was to get a picture of the presence, the circulation and the diffusion of some Vector Borne Pathogens (VBPs) in dogs living in a restricted area like Pantelleria Island (Sicily, Italy).
During February 2017, 137 dogs living in Pantelleria Island were included in the study to investigate the presence of antibodies and the DNA of some VBPs including *Leishmania infantum, Rickettsia conorii, Ehrlichia canis, Babesia canis, Anaplasma phagocytophilum* and *Coxiella burnetii*. A clinical form was recorded and specimens such as serum, EDTA blood and lymph node aspirated when reactive were collected from all animals. Serological (IFAT) and molecular analysis (real time PCR for *L. infantum* and PCR for others) were performed as previously described [1].
Antibodies against the VBPs investigated were found with a prevalence of 59.1%, 50.4%, 19.7%, 18.2%, 1.6% and 0.7% for *L. infantum, R. conorii, A. phagocytophilum, E. canis, C. burnetii* and *B. canis* respectively while only DNA of the first pathogen was amplified (21.1%), mainly from lymph node. Only one dog with IFAT titer of 1:5120 for *L. infantum* was positive to both blood and lymph node.
Except for *C. burnetii* and *B. canis*, this is the first report that proves the circulation of VBPs in canine population living in Pantelleria Island, accordingly with previous data from Southern Italy [2-5]. Although only DNA of *L. infantum* was found, due to the rapid bacteremia and parasitemia of VBPs that underlines the difficulty to detect their DNA, the large number of animals seroreacting against the investigated pathogens confirms the important role of dogs as sentinels. However, this data suggests that PCR should not be the only diagnostic method for population screening.

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ANIV

Other Species – Public Health

NOROVIRUS ACCUMULATION AND DEPURATION KINETICS IN SHELLFISH: PRELIMINARY RESULTS

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Fresh shellfish belonging to harvesting areas denominated B and C must be depurated prior to commercialization. The depuration process, intended to reduce the likelihood of transmitting infection agents to consumers, is carried out by placing mollusks in big tanks with clean seawater. It uses the natural shellfish pumping activity and has been demostrated to be effective in reducing fecal bacteria levels in their intestine [1]. With respect to viruses, it is well known that depuration is less effective in virus removal, as demonstrated by the periodic report of viral gastroenteritis outbreaks linked to the consumption of depurated shellfish [2]. Among the virus involved in the episodes of gastroenteritis, human norovirus is the virus most frequently associated to human outbreaks linked to mussels. In this study we evaluated the kinetics by which the norovirus accumulates in the mussels as well as its removal trend, with the aim to improve the purification systems in use. Since human norovirus is not cultivable, a surrogate, murine norovirus, was used in the experiments. Bioaccumulation trials were carried in 4 liter clean seawater tanks containing 300g of mussels. Virus (final concentration 106 Plaque Forming Units/L) was mixed with Nannochloropsis oculata ($4x10^5$ cells/ml) and added to each tank. Three experiments were carried out at different temperatures of water (14, 18, 22°C) to assess the effect of temperature on virus uptake. For each experiment mussels were sampled at various times (1-72h) after virus supplement. Depuration of experimentally contaminated mussels was carried out in 25L tanks provided with filtered (1 µm pore size) and disinfected (irradiation by means of a UV lamp of 55 W) seawater at a flow of 1.4 L/min. Mussels were depurated under controlled temperature (18°C) for 6 days and sampled at regular intervals during the process (1, 2, 3, 6 days). Virus quantification analysis was performed by Real-time reverse transcription quantitative polymerase chain reaction following a protocol from literature [3]. For the scope nucleic acids were extracted from pooled epatopancreas according to the ISO/TS 15216-1:2013 using Qiasymphony automated system (Qiagen). Bioaccumulation experiments demonstrated that the infection rate is positively correlated to the temperature, showing that at 22°C virus is already present in the mussels after 1 hour, reaching the highest concentration after 6 hours. Depuration trials showed a three-phase depuration trend, with a pronunced decrease of the virus (70%) after 2 days, a slight increase after three days, followed by a further decrease (up to 84%) after 6 days. More studies are needed to understand which depuration process parameters could be able to positive influence removal of virus from mussels.

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BIOMOLECULAR INVESTIGATION ON BALL PYTHON NIDOVIRUS IN BALL PYTHONS (*Python regius*) WITH RESPIRATORY DISEASE

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Ball python (*Python regius*), one of the most popular reptile species kept as a pet [1], is affected by a potentially fatal respiratory disease that veterinarians have been aware of since the late 1990's. The etiology of this respiratory disease is still unknown but, as with many other infections, it is probably conditioned by non-optimal environmental parameters that put the animals in a stress condition resulting in immunodepression and pathogen replication. Ball Python Nidovirus (BPNV) was detected for the first time in 2014 in dead animals with signs of respiratory disease [2,3]. After phylogenetic analyses, BPNV was assigned to the order *Nidovirales* [2,3], which includes important human and veterinary viral pathogens [4], and consequently its possible role in the ball python respiratory disease has been speculated [2,3]. Indeed, viruses belonging to the *Nidovirales* order tipically show a respiratory tissue tropism. Given there is still poor knowledge about the epidemiology and the association of BPNV with lung disease in ball pythons, it was decided to investigate the presence of BPNV in trans-tracheal aspirations collected from alive ball pythons with respiratory signs. The study group for this investigation included 32 ball pythons belonging to five breeding facilities located in Central and Southern Italy. A total of 32 trans-tracheal aspirations collected from these ball pythons with respiratory disease were analyzed. Genomic RNA was extracted from the pelleted samples and then tested using a reverse transcriptase-polymerase chain reaction (RT-PCR) assay [3]. The 220bp amplicons were secondly analyzed through direct sequencing and then the sequences submitted to a Basic Local Alignment Search Tool (BLAST) analysis to confirm specific amplification. Five out of thirty-two (15.6%) ball pythons tested positive for BPNV. This is the first report of BPNV recovered from alive captive ball pythons. Since the positive samples were collected from animals housed in the same breeding facility this may appear as a singular outbreak. Unfortunately, the real prevalence of the virus in the ball python population is still unknown making any data comparison impossible and further epidemiological surverys will be beneficial to better understand the ecology of this virus. Also, the precise host range of the virus as well as its disease causality and the routes of transmission remain undefined.

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THE USE OF VETERINARY MEDICINAL PRODUCTS IN MINOR SPECIES

Salvatore Macrì

Ministero della Salute D.G.S.A.F.

The report describes all of the rules related to the use of veterinary medicines in the event of reduced or lack of availability in the Italian territory. In particular, it provides a short description of the procedures to authorize veterinary medicinal products for the market (1), of the use of exemptions under Article 10 and 11 of Legislative Decree 193/2006 (1), of the stabulogeni vaccines (2), of procedures for the import of veterinary medicines (3), and of the term 'minor species' that is referred to in categories that enjoy special facilities with the authorization for the placing (4). In addition, the report also introduces potential new regulations introduced by the proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL relating to veterinary medicines and electronic prescriptions.

(1) DECRETO LEGISLATIVO 6 aprile 2006, n.193 Attuazione della direttiva 2004/28/CE recante codice comunitario dei medicinali veterinari. (2) D.M. 17 marzo 1994 n. 287. (3) Decreto del Presidente della Repubblica 8 febbraio 1954, n. 320. (4) EMEA / CVMP / 477/03 –FINALE.

EFFECT OF 1,3-1,6 B-GLUCAN ON NATURAL AND EXPERIMENTAL DEFORMED WING VIRUS INFECTION IN NEWLY EMERGED HONEYBEES (*Apis mellifera ligustica*)

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The European honeybee (*Apis mellifera ligustica* L.) is the most managed pollinator species. Honeybees have recently suffered extensive losses mainly due to exposure to pesticides, malnutrition and pathogens, including bacteria, fungi, mites and viruses. Among honeybee's pathogens, viruses play an important role in honeybee diseases (1). Deformed Wing Virus (DWV) is associated with honeybee colony losses. DWV can be transmitted vertically and persist in the bee colony as asymptomatic and symptomatic infections with clinical signs consisting of crumpled wings and a bloated abdomen. Symptomatic infections leading ultimately to the collapse of the colony are observed in apiaries infested by Varroa destructor which supports replication of DWV to high titers. During the first stage of the infection DWV is detected in the abdomen, at later stages presence of an actively replicating virus is also detectable in the head (2). The aim of this study was to investigate the effect of 1,3-1,6 β-glucan, a natural immune-modulator molecule, on honeybee response to both low-titer natural and high-titer experimental DWV infection. To date there are still few studies done to know the effect of ß-glucans on the immune response of honeybees. The investigation on their possible nutraceutical antiviral therapy may represent an innovative alternative to the use of synthetic chemical substances also for other diseases, with important benefits for the welfare of animals, humans and a positive impact on the environment. Two parallel experiments were performed; newly emerged honeybees, which were sampled from a Varroa-free apiary and harbored a low endogenous DWV viral titer where fed with 1,3-1,6 ß-glucan at a concentration of 0.5% and 2% respectively. Bees feed a 0.5% and 2% 1,3-1,6 ß-glucan diet were divided in three experimental cohorts and each of those was subjected to one of the following experimental treatments: no injection, injection into the haemocel of a high-copy number DWV suspension (experimental DWV infection) and PBS injection into the haemocel (physical injury). Control bees fed a ß-glucan-free diet were subjected to the same treatments. Twenty-five small cages with a maximum of 30 honeybees per cage were kept at 28°C for 13 days. Viral load was measured by qRT-PCR performed on RNA extracted from abdomen and head on honeybees. Insects were monitored daily and those found dead were collected to calculated the survival rate. Results indicated that administration of 0.5% ß-glucan to infected honeybees was associated with a significant reduction DWV viral load and with a significant increase of the survival rate suggesting that this natural immune modulator molecule might contribute to increase honeybee resistance to viral infection by restraining viral replication ultimately prolonging the honeybee lifespan.

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ANTIMICROBIAL CONSUMPTION ANALYSIS IN FOOD-PRODUCING ANIMALS: THE UMBRIAN EXPERIENCE

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The European Commission has published guidelines to stimulate a more rational antimicrobial use in veterinary medicine and to monitor antibiotic use in food-producing animals [1]. The EMA has proposed a novel approach based on the *Defined Daily Doses* (*DDD*) to quantify antibiotic use [2]. The aim of this study is to evaluate antibiotic consumption in Umbria in 2014 and 2015 [2,3]. All the prescriptions for food-producing animals were collected and indicators for consumption and prescriptive appropriateness were evaluated. Antibiotic consumptions are expressed both per animals (DDDs/1000 animals-die) and per farms (DDDs/1000 farms-die). In 2014, 16890.05 DDDs/1000 animals-die and 7323605.26 DDDs/1000 farms-die were used in Umbria; in 2015 2085.99 DDDs/1000 animals-die and 699819.39 DDDs/1000 farms-die. A reduction of -87.65% (per animals) and -90.44% (per farms) was seen in 2015, connected to the decrease of antibiotic use in swine (-22.30% DDDs/1000 animals-die and -31.14% DDDs/1000 farms-die). A similar behavior was seen for critically important antimicrobials for human medicine (CIAs). Swine and bovine were the most treated animals (DDDs/1000 animals-die). Swine, poultry and aquaculture were the species with the highest antibiotic consumption per farms. An increase in antibiotic consumption was seen in poultry (+350.28 DDDs/1000 animals-die) and aquaculture (+66.97 DDDs/1000 farms-die) and in the small ruminants CIAs consumption (+138.14 DDDs/1000 animals-die and + 143.87 DDDs/1000 farms-die). Prescriptive appropriateness was higher in bovine (48.32% of correctly dosed treatments - CDT), small ruminants (61.27% CDT) and food-producing horses (71.07 CDT and was lower in poultry (25.73% CDT) and rabbit (27.21% CDT). As to animal age, 66.72% of antibiotic prescribed DDDs in 2015 were for adult animals; prescriptive appropriateness was lower in young animals (32.80% CDT vs 47.25% CDT in adult animals). A significant association was found between young animal and 1) the use of CIAs (OR: 3.65; IC95%: 3.35-3.98; p<2.2e-16), 2) the prescriptive inappropriateness (OR: 1.83, IC95%: 1.67-2,01; p<2.2e-16) and 3) the use of oral route (OR: 2.38, IC95%: 2.17-2.61, p<2,2e-16). The 95.40% of prescribed DDDs in 2015 was administered orally (64.97% in water and 30.43% in premixes). In 67.91% of the treatments administered orally there were dosage mistakes (61.47% water, 64.87% premixes). A significant association was found between oral administration and prescriptive inappropriateness (OR: 2.47; IC95%: 2.27-2.69; p<2.2e-16). This study is the first in Italy aimed to quantify antibiotic consumption, based on DDD, in an entire Italian region. Critical points in the antibiotic therapies seems to be young animal treatments, oral route and mass therapies. These aspect should be strictly controlled and correctly performed to minimize risks for the public health. Future developments are the evaluation of an each farm antibiotic use profile, which could be helpful for the farm antibiotic risk profile.

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ANTIMICROBIAL DRUG RESISTANCE AND VIRULENCE FACTORS IN *Escherichia coli* FROM ITALIAN DAIRY CALVES WITH DIARRHEA DURING THE PERIOD 2011-2015

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Antimicrobial resistance is a worldwide health problem in veterinary such as in human medicine (1). Calf diarrhea caused by *Escherichia coli* is one of the most important economic loss in dairy farm. A retrospective study was carried out on antimicrobial resistance profile and virulence genes in *E. coli* strains isolates during 2011-2015. Seven hundred and forty-six *E. coli* strains were isolated from carcasses, feces and rectal swabs of calves with diarrhea in farms located in Northern Italy. The isolates were tested for susceptibility to 18 antimicrobials using a Kirby-Bauer disk diffusion assay. Sixty-one of them were also analyzed for virulence genes (LT I, STaP, STb, F4, F5, F6, F41, F18 e STX2e) by specific PCR assays. Fisher's exact test was used to study the statistical relationship between virulence gene coding F5 and antimicrobial resistance. Persisting and high prevalence values of resistant *E. coli* strains were observed during the study for several antimicrobials: Penicillin and Tiamulin (100%); Doxycycline, Sulfadiazine and Tetracycline (90%); Amoxicillin, Flumequine and Kanamycin (80%); Aminosidine, Danofloxacin, Enrofloxacin, Trimethoprim+Sulfamethoxazole (70%) and Gentamicin (60%). The prevalence of resistance to Cefquinome was 40%. Furthermore a significant decreasing resistance trend was observed during the study period for Amoxicillin+Clavulanic acid (from 81% in 2011 to 65% in 2015), Ceftiofur (52% to 32%) and Colistine (53% to 15%). A significant increasing resistance trend was observed for Apramycin (from 30% in 2011 to 55% in 2015). Seven hundred and forty-five out of 746 *E. coli* strains were resistant to at least three antimicrobials of different classes (1;2;3) and defined MDR (Multidrug-Resistant). Six hundred and fifty-five out of 746 *E. coli* strains were resistant to 4 antimicrobial classes: Penicillins, Pleuromutilin, Sulphonamides and Tetracyclines. F5 virulence gene was observed in 24 isolates (39%) and, in 14 of them, F5 was associated with StaP and F41. In *E. coli* F5 positive strains we found a significant difference in antimicrobial resistance for Danofloxacin (p<0.0004), Enrofloxacin (p<0.008) and Flumequine (p<0.0022) than in F5-negative strains. The results of this study suggest that the high prevalence of *E. coli* MDR strains in dairy calves with diarrhea is an important issue in animal and public health. The use of targeted antimicrobials for strictly necessary periods could significantly reduce the risk of antibiotic resistance also for Critically Important Antimicrobials recommended in international control programs (OIE, 2015; FAO,2008; WHO, 2012; EMA, 2014; WHO, 2014; ECDC/EFSA/EMA, 2015; EFSA/ECDC 2015).

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HEALTH MONITORING OF ANIMALS BRED IN THE OPEN FARMS OF THE CAMPANIA REGION

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A recent review on the prevalence of zoonotic bacteria in organic and conventional farms of cattle, pigs and poultry in the United States and Europe showed no differences between the presence of zoonotic agents and the type of the farm management [1, 2]. This study was undertaken both to evaluate the presence of zoonotic infectious agents not included in the National Control Planes for the animals presents in Farms open to public (FoP) and, consequently, to emphasize the importance of the role of the veterinary doctor with training in interspecies relationship in this context. From September 2014 to July 2016 thirty-two FoP of Campania Region have been chosen basing on the size farm population (at least 25 subjects from three different species) and then sampled. In each farm, in respect of animal welfare, rectal and cloacal swabs were collected in order to isolate *Salmonella* spp., Shigatoxins producing *Escherichia coli* (STEC) and thermotolerant *Campylobacter* by cultural and molecular methods. A total of 800 animal divided in dogs (34), cats (45), horses (82), donkeys (91), cattle (127), sheep (62), goats (73), pigs (41), *leporidae* (173), hen-house (32) and "other samples" such as eggs and other animal species (40) were sampled. In our study, all farms were positive to STEC and/or thermotolerant *Campylobacter*, conversely they were all time negative for *Salmonella* spp. In particular, a total of 124/800 (15.5%) strains of STEC were isolated primarily from poultry and donkeys followed by other animal species. Finally, 127/800 (15.87%) strains of thermotolerant *Campylobacter* were isolated primarily from poultry followed by other animal species [48/127 (37.79 %) *C. jejuni* (mainly from donkeys) and 79/127 (62.20%) *C. coli* (mainly from poultry)]. All positive animals to STEC and thermotolerant *Campylobacter* were clinically healthy. The healthcare monitoring carried out in FoP of Campania region, where the animals are raised primarily for zooanthropological purposes, has provided interesting data increasing the international scientific literature in the field of zoonoses. In this study some of the major gastrointestinal zoonoses linked to animals in the FoP were considered as well as the role of the veterinary doctor in the prevention and control of zoonotic diseases and in health education of the operator in the FoP. In fact, both the operators and visitors (families, school groups, children, elderly etc.) often underestimates the potential health risks caused by contact with FoP animals.

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Malattie parassitarie

PETS AND MINOR SPECIES

SAFETY AND EFFICACY OF THE CONCURRENT TREATMENT OF DOGS WITH FRONTLINE TRI-ACT® AND NEXGARD SPECTRA®

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Dogs are exposed to a plethora of parasitic arthropods and at risk of pathogen transmission in many epidemiological settings. This imposes the need for a combination of different products to achieve a large spectrum protection [1]. This is the case, for instance, of geographical areas where tick-borne diseases, canine leishmaniosis (CanL) and heartworm disease (HW) occur simultaneously. Despite theoretically there are no impairments in combining preventative strategies that use different molecules and mode of action, their mixture may represent a threat to animal health. In this study, we evaluated the safety and the efficacy of the concurrent monthly use of Frontline Tri-Act® spot-on (fipronil 6.76% and permethrin 50.48%) and NexGard Spectra® chewable tabs (afoxolaner 1.9% and milbemycin oxime 0.4%) in dogs during a period of six months.

A total of 41 healthy dogs living in an area of Sicily endemic for CanL and other vector borne diseases (VBDs) were included in the study at the beginning of *Leishmania* transmission season. Included dogs were ELISA (SNAP 4Dx Plus, IDEXX) negative to relevant vector-borne pathogens and for circulating microfilariae. Dogs, but no six, were also seronegative to *Leishmania*, and some (58.5%) were naturally infected by internal nematodes and/or ectoparasites. Animals were treated with the two testing products at days 0, 28, 56, 84, 112, 140 and followed-up for safety and efficacy in the interim and at the study end (day 168).

No adverse events related to the two products, neither local skin reactions in the sites of application of the spot-on were observed during the study period. Efficacy against internal parasites 14 days after first treatment was: 100% for *Toxocara canis, Toxascaris leonina* and *Capillaria aerophila*; 99.9% for *Trichuris vulpis* and 66.1% for *Ancylostoma caninum*. These dogs are hunting dogs living in kennels. They are continously infected by *Ancylostoma* with migrating immature stages. The 14 day post-treatment period could not avoid appearance of new adult *Ancylostoma* and egg shedding. Twenty-four hours after first treatment, 95.8% (23/24) of the ectoparasite infested dogs were free from fleas and ticks, and no new infestations were observed in treated dogs until the end of the study with exception of day 28 in which fleas were found in 17.1% of the animals. Blood and serum samples collected on day 168 were tested for vector-borne pathogens as at the inclusion and no new seroconversions or circulating blood microfilariae were observed which translates into a 100% protection.

Concomitant use of Frontline Tri-Act® and NexGard Spectra® in dogs for six months was safe and well tolerated. The combination was also effective in preventing relevant VBDs including CanL over one transmission season. This combination could be regarded as a safe and effective strategy for prevention of CanL and other VBDs such as HW in geographical areas where the risk of transmission overlaps.

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EFFECT OF DIFFERENT TEMPERATURES ON VITALITY OF *Aelurostrongylus abstrusus* (RAILLIET, 1898) FIRST-STAGE LARVAE AND ON THEIR CAPABILITY TO DEVELOP INTO INFECTIVE THIRD-STAGE LARVAE

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Aelurostrongylus abstrusus is a nematode lungworm featured by an indirect life-cycle that involves gastropods as intermediate hosts. Its presence and spreading is strongly connected with environmental conditions. In particular, the environmental temperature influences the distribution of the gastropods and, probably, also the survival of *A. abstrusus* in the extra host phase of its life-cycle. The aims of this study were to i) investigate the survival rate of *A. abstrusus* first-stage larvae (L1s) at different temperatures either in water or in faeces and ii) to test the ability of L1s stored in water to develop into the infective stage (L3s) in the mollusk host. Additionally, a mathematical model was exploited to describe the survival dynamics of L1s.

All the faeces emitted in a day by a naturally *A. abstrusus* infected cat were collected homogenized and divided into 2 polls. A first poll was split into 48 aliquots that were assigned to 4 groups and stored in dark conditions at different temperatures (group 1, -20°C; group 2, 4°C; group 3, 28°C; group 4, 14±1°C). Every 7 days, an aliquot of faeces from each group was analysed by Baermann technique and the sediment observed microscopically. A second poll was processed by Baermann technique, and the retrieved L1s were suspended in water and divided into 136 vials. Vials were divided in 4 groups and stored in dark conditions at -20°C, 4°C, 28°C and 14±1°C (groups A, B, C, D, respectively). Every 7 days, from each group, 300 µL from a vial were microscopically examined to detect alive L1s. Differences in the mortality rate among groups were tested by ANOVA. To evaluate the capability of L1s to develop into L3s, 5 Cornu aspersum snails for each group were infected at the beginning of the study, and every 21 days until alive L1s were detected. After 18 days the snails were sacrificed and processed through a peptic digestion, and the recovery rates registered.

The greater L1s survival time in the faeces was observed for the group 2 (63 days), whereas L1s remained alive 28 days in group 3, and 21 days in groups 1 and 4. The survival time of the L1s stored in water was significantly greater ($p<0.0001$) for the group B (231 days), while for the groups C, D, and A it was 70, 42, and 28 days, respectively. According to the mathematical model the survival rate of L1s at -20, 14 and 28°C exhibits an exponential decay trend, whereas it is quite different at 4°C, where the L1s' dynamics is characterized by three different phases: an initial decay, a stationary phase and another decay phase. The data indicate that the survival of the A. abstrusus L1s is negatively correlated with temperature. The L1s belonging to the group A lost the capability to develop into L3s after 21 days, whereas those belonging to C and D developed until 63 and 21 days, respectively. Conversely, L1s of group B continued to molt into L3s until 189 days.

Results presented show that *A. abstrusus* is more resistant to environmental temperature variations than other species of metastrongyle [1;2]. This capability to adapt to different environmental conditions may explain the wide distribution of this species.

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THE USE OF OZONE AS RESTORATION METHOD AGAINST CONTAMINATION OF BEEKEEPING MATERIAL WITH *Nosema ceranae*

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The spore-forming microsporidia *Nosema ceranae* is considered an emergent and relevant parasite of western honey bees *Apis mellifera*. It has been present in Europe since at least 1993 and has been spreading worldwide through commercial movements of bees, hive products and beekeeping material [1]. Wax and combs contaminated with spores of *N. ceranae* are an important source of infection. The goal of this work was to evaluate the resistance of *N. ceranae* spores to various ozone exposure times, both in experimental conditions and in naturally infected wax-combs. Spore viability was evaluated, by fluorescent microscopy, using Sytox-Green [2]. Spore mortality in experimentally infected wax combs reached 78.18% after 10 hours of ozone exposure. A significant reduction in spore viability was observed after the first 60 minutes of treatment (mortality 20.25%). In naturally infected combs, spore viability was reduced of 17.92% after a 6-hours treatment period. Ozone has proven to be effective in reducing *N. ceranae* contamination on wax combs, but to increase the efficacy of ozonation in naturally contaminated material it is important to arrange the combs as to maximize the surface directly exposed to ozone and to increase the exposure time until a plateau in spore mortality can be observed.

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Angiostrongylus vasorum IN STRAY AND OWNED DOGS: THE TREND IN SOUTHERN ITALY

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Angiostrongylus vasorum is a metastrongylid nematode that resides in the right side of the heart and pulmonary arteries of dogs and foxes and is responsible of canine angiostrongylosis. As a disease with a substantial animal health impact, canine angiostrongylosis remains a high priority for clinicians and researchers. A.vasorum does have a worldwide distribution [1]. The spread of this parasite in the last years is due to some factors as climatic changes, the presence of foxes acting as reservoirs in urban areas, the increased travelling of dogs owners whith their animals, and also the awareness of practitioners due to the availability of more accurate diagnostic methods [2]. The present study adds further data on the distribution of A. vasorum in the Campania region of southern Italy after a description of a fatal case of canine angiostrongilosis in 2014 and a cross-sectional survey performed in 2015 that revealed a prevalence of 13.2% in kennels [3-4]. Indeed, the aim of the present study was to evaluate the prevalence of A.vasorum in the years 2015 and 2016 in both stray and owned dogs from the Campania region. Specifically, a total of 1499 fecal samples (656 from owned dogs and 843 from stray dogs) were collected in the 5 provinces (Avellino, Salerno, Benevento, Caserta, Naples) of the region. Copromicroscopic analyses were performed by the FLOTAC technique, having an analytic sensitivity of 2 larvae per gram (LPG) of feces, using a zinc sulphate-based solution (specific gravity = 1.20) [5]. *A. vasorum* was detected in 29 of 1499 (1.9%; 95% Confidence Interval, C.I.=1.3-2.8%) samples examined. The prevalence was 1.1% (7/656; 95% C.I.=0.5-2.3%) in owned dogs and 2.6% (22/843; 95% C.I.=1.7-4.0%) in stray dogs. The positive samples were tested also by the Mini-FLOTAC technique providing comparable results (unpublished data). The present study represents the first survey on the presence of *A. vasorum* in stray and owned dogs in the Campania region. The results showed that the prevalence in owned dogs was slightly lower than that detected in stray dogs (1.1% vs 2.6%). In conclusion, canine angiostrongylosis is present in the Campania region as in other areas of Italy. Therefore, coprological diagnosis for detecting larvae of *A. vasorum* should be included in the routine veterinary practice.

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CIRCULATING GENOTYPES OF *Toxoplasma gondii* IN PIEDMONT REGION

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Toxoplasma gondii is a zoonotic apicomplexan potozoan, recently considered re-emergent like other food-borne parasites [1]. The meat-borne transmission of *T. gondii* seems to be a common infection routes [2]. *T. gondii* in Europe is genetically characterized by three main clonal genotypes, with a lesser prevalence of atypical genotypes [3]. The aim of the study was to evaluate the distribution of *T. gondii* genotypes circulating in domestic and wild animals in Northwest Italy by PCR-RFLP. We analysed 65 skeletal muscle samples derived from cattle (*Bos taurus*) (n=11), pig (*Sus scrofa domesticus*) (n=15), fox (*Vulpes vulpes*) (n=18), roe deer (*Capreolus capreolus*) (n=3) and wild boar (*Sus scrofa*) (n=18). Samples were processed by using a nested PCR of 6 RFLP markers: alt. SAG2, GRA6, 5'SAG2, BTUB, C22-8 and SAG1 [4]. PCR products were sequenced to perform in silico digestion by using the free online software NEBCutter. Out of 65 samples tested, 43 produced amplicons and were genotyped (6 cattle, 15 pigs, 14 wild boars, 7 foxes and a roe deer). Thirty-one samples showed Type I alleles at least at one locus (p=72.09%; CI95% 57.31%-83.25%), while Type II and III alleles were present in two (p=4.65%; CI95% 1.28%-15.46%) and three (p=6.98%; CI95% 2.40%-18.61%) samples respectively. Seven samples (p=16.28%; CI95% 8.12%-29.97%) showed mixed Type alleles. Although genotypes prevalence did not differ statistically between species, wildlife showed a greater genetic variability than livestock with a higher prevalence of atypical genotypes (X2=4.10; p=0.04). This is the first T.gondii genotyping study carried out in Northern Italy on both wild and domestic animals from the same area, showing unexpected high prevalence of genotype I and atypical genotypes. In other European countries, genotype II is the most common, with a prevalence ranging between 50% in Spain [5] to 100% in France [6;7]. Results suggest a risk for human health, because genotype I and atypical genotypes have been associated to a more severe symptomatology, especially in immunocompromised patients and in newborns [8]. It is likewise important to note the higher genetic variability of T.gondii in sylvatic species, and the potential role of wildlife as a source of atypical genotypes.

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Posters

SCIENZE BIOMEDICHE

SOFIVET

EXPRESSION OF AQUAPORINS (AQPS) IN TESTES OF NORMAL AND CRYPTORCHID DOGS: PRELIMINARY DATA

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The expression, biological importance and translational significance of aquaporins (AQPs) have been the object of intense exploration in various body districts. The testes are functionally compartmentalized organs where both spermatogenesis and testosterone biosynthesis occur. In particular, recent studies evaluating testicular physiology in relationship with male fertility have supposed a possible involvement of AQPs in regulating transepithelial fluid movement directly related to spermatozoa maturation [1]. To date, several AQPs have been detected in the male reproductive tract of laboratory animals [2, 3] while few data are reported in domestic animal species. The present study aimed at evaluating AQPs distribution (AQP1, AQP7, AQP8 and AQP9) in the testis of adult normal and cryptorchid dogs by using immunocytochemistry and western blotting analyses. In particular, testis samples obtained after surgery by normal and cryptorchid adult dogs (no. 5 for each animal group) were immediately immersed in fixative (for immunocytochemistry) or snap frozen at -20°C (for western blotting) until further analysis. Immunohistochemistry and western blotting technical procedures adopted for AQPs immunolocalization and expression were previously reported [4].
The immunohistochemical analysis indicated that AQPs- immunoreactivity (IR) was found in the germ cells of normal testis and in gonocytes of cryptic dog. In addition, IRs were found in the blood vessels of normal and cryptorchid dog. The Western blotting results revealed for the tested AQPs the presence, in both normal and cryptorchidic dogs, of a main band of about 25-30 kDa according to the used AQP. Other upper diffuse bands were also observed at about 45 and 60 kDa, respectively, with the latter possibly representing an AQP dimer. In addition, a different pattern of band intensity was observed both in relation to the tested AQP and to the cryptorchid dog group respect to normal dog group. The obtained results confirm previous reports in rat and in mouse testis indicating different AQPs roles in cell volume regulation (CVR). These results open the way for future investigations focused on AQPs regulation with the identification of more roles also in combination with other complexes.

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INFLUENCE OF GENTLE TOUCHING BEFORE SEPARATION FROM THE OWNER ON DOG BEHAVIOUR AND PHYSIOLOGY

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Adult domestic dogs, during separation from the owner in an unfamiliar environment, usually show behaviours indicative of discomfort and attempt to regain proximity [1]. Traditionally, for the treatment of dogs showing separation-related problems, owners were advised to reduce affiliative behaviours towards their dog and to desensitize dogs to the rituals related to departure. However, previous literature indicate a positive effect of human physical contact and interaction in decreasing dogs' level of stress in many situations, assessed through endocrinological, physiological, and behavioural parameters [2, 3]. The aim of the current study was to assess if dog behaviour and physiology during a brief separation from the owner is affected by being gentle touched before owner's departure. The sample was formed by 10 dogs (6 females and 4 males, 1-11 years old, of different breeds and sizes), not displaying separation anxiety nor used to be stroked before being left alone. Each dog was tested twice, one week apart, in an unfamiliar outdoor environment using two slightly modified versions of the same behavioural test (NGT, no gentle touch, and WGT, with gentle touch) which differed only for the first episode. Episode 1: the owner held the dog on the leash. In the NGT test , the owner spent one minute talking with the experimenter, without touching or interacting with the dog, and maintaining a relaxed, neutral attitude. In the WGT test, the owner spent one minute gentling stroking the dog, 30 seconds per body side, and in the meanwhile chatting with the experimenter. Episode 2 (separation): the owner left the dog with the experimenter and reached a hidden location, where he/she stayed for three minutes. Episode 3 (reunion): the owner came back and spent one minute holding the dog on the leash and talking with the experimenter. Saliva cortisol was measured 20 minutes after the start of separation and heart rate was measured immediately before and after both tests using a phonendoscope. Dog behaviour during episode 2 was analysed through continuous sampling measuring the duration of 16 behaviours grouped in: stress signals, calmness, vocalisations, social behaviours towards the stranger, and seeking for the owner. All data were analysed by Wilcoxon test ($p<0.05$). Saliva cortisol after the two tests did not differ, while heart rate showed a marked decrease after WGT test ($p=0.093$) and did not change after NGT. Dogs showed calm behaviours for a longer duration in WGT than in NGT (lying down + exloration, $p=0.049$); no statistically significant difference was observed for seeking for the owner ($p=0.610$), stress signals ($p=0.959$), and vocalisations ($p=0.499$). Due to the limited number of subjects and the lack of statistical differences in the measured physiological parameters, these results have to be considered cautiously. However, the longer display of behaviours indicative of calm together with the decrease of the heart rate seem to suggest that gently stroking the dog before leaving may make dogs calmer during a brief separation from the owner.

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ACUTE TOXICITY OF VETERINARY SULPHONAMIDE MIXTURES IN *Daphnia magna*

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Sulfonamides (SAs) are among the most widely used antibacterials in veterinary medicine. Many of the available Veterinary Medicinal Products containing SAs are marketed as premix or oral solution to be added to feed or water. With these VMPs, many animals may be treated for preventive purposes and this can result in high environmental load of the drugs. The acute toxicity of SAs to D. magna is low, with the exception of Sulfaguanidine (Half maximal Effective Concentration (EC50) 6.21 mg/L) [1]. However, as SAs occur in the environment not as a single drug but usually together with other compounds of the same family, it is of interest to evaluate the ecotoxicity of their mixtures. Aim of this work was to evaluate the toxicity of SAs mixtures in D. magna. The organisms were maintained as indicated elsewhere [2]. Chemicals were of the following purity: Sulfadiazine (SDZ) 99%, Sulfaguanidine (SGD) 99%, Sulfamerazine (SMA) 99%, Sulfadimethoxine (SDM) 98%, SMZ (Sulfamethazine) 99%, Sulfaquinoxaline (SQO) 95%. Acute toxicity tests were performed according to the OECD Guideline 202. Binary mixtures were prepared taking into account the EC50 of individual compounds. Ternary and quaternary mixtures to be assayed were based on results obtained with binary mixtures. Each compound was added to the mixture as a fraction of its EC50 corresponding to the total number of compounds to be added to the mixture. In this way, following the principle of Concentration Addition [3], a 50% effect was to be expected from each mixture after 48h incubation.Therefore, any detected effect >50% was considered as an indication of synergy. Where this indication was strong (>90% effect) the mixture was tested in 5 scaled concentrations, in order to identify the EC50 of the mixture. Synergies were statistically verified using the method of Tallarida [4]. Six binary mixtures, out of the 15 assayed, showed more than additive interaction. The following had more than 90% effect: SDM+SGD (100%); SMA+SDZ (97.5%); SGD+SDZ (92.5%); SQO+SDM (92.5%). Their EC were: 116.5+2.5 mg/L; 73.0+79.7 mg/L; 1.6+57.6 mg/L; 46.7+99.6 mg/L, respectively. Three ternary mixtures were tested and all showed more than additive interaction. The following had more than 90% effect: SDM+SGD+SDZ (100%); SGD+SDZ+SMA (100%). Their EC50 were: 57.7+1.3+44.7 mg/L and 1.3+46.7+128.8 mg/L, respectively. Two quaternary mixture, out of the three assayed, showed more than additive interaction with 100% effect (SDM+SGD+SMA+SDZ and SGD+SDZ+SQO+SDM). Predicted No-Effect Concentrations calculated by applying an Assessment Factor of 1000 to the EC50s were >40 ug/L, with the exception of SGD (>1 ug/L), indicating that current level of contamination should have no impact to the crustaceans. However, detected synergies show that the concept of concentration addition is not precautionary for the evaluation of the aquatic toxicity of SAs mixtures.

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LEVELS OF TRACE ELEMENTS IN MUSCLE OF RED SWAMP CRAYFISH (*Procambarus clarkii*, GIRARD 1852) IN DIFFERENT ITALIAN REGIONS: PRELIMINARY OBSERVATIONS

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The red swamp crayfish (*Procambarus clarkii*) is one of the most diffuse crustacean species in the world also because it is invasive and tolerant to the environmental conditions, due to the flexibility of its biological cycle. This species has been introduced in Europe in the seventies of the last century and nowdays it is cultivated and consumed as food in several Italian regions. The red swamp crayfish has been used as indicator species to monitor the environmental quality and the contamination of the biological habitat in previous bioaccumulation studies [1]. Heavy metals can be found in water and sediments and the benthic invertebrates, such as crayfish, are exposed to these contaminants. Cadmium (Cd) and lead (Pb) are non essential elements and can create adverse effects on animal and human health due to their potential toxicity and bioaccumulation in food chain. On the contrary, zinc (Zn) and copper (Cu) are essenzial elements, needed for biological activities in trace amounts, but may have toxic effects at higher concentrations. The objectives of the present study were to evaluate Cd, Pb, Cu and Zn levels in muscle of *Procambarus clarkii* collected from different sites and to assess the health risk related to human consumption. Samples of red swamp crayfish (*P. clarkii*) were caught between May and July 2016 in two sites of Campania region, at Villa Literno (n=20) and Cancello Arnone (n=20), in one site of Emilia Romagna region, at Campotto (n=20) and in one site of Toscana region, at Padule di Fucecchio (n=20). After capture, the specimens were weighed and the total lengths were measured. Then they were frozen at -20°C until the analysis. The abdominal muscle from each animal was separated and homogenized. Aliquots of each sample were digested in ultrapure 65% HNO_3 and H_2O_2 in a microwave digestion system. Cd, Pb, Cu and Zn concentrations were determined by atomic absorption spectrometer (GF-AAS). Mean values of the Cd and Pb concentrations were 0.011 and 0.190 mg/kg in Campotto, 0.005 and 0.049 mg/kg in Fucecchio, 0.005 and 0.010 mg/kg in Villa Literno, 0.005 and 0.015 mg/kg in Cancello Arnone. Mean values of the Cu and Zn concentrations were 10.251 and 35.87 mg/kg in Campotto, 2.766 and 12.880 mg/kg in Fucecchio, 8.628 and 26.673 mg/kg in Villa Literno, 22.580 and 53.263 mg/kg in Cancello Arnone. The results obtained in the current study show low levels of Cd and Pb in all samples analyzed and largely below the Pb and Cd maximum levels established by the European Commission for muscle meat of crustaceans (Reg CE 1881/2006) and were indicative of low risk for human consumption. The levesl of Cu and Zn do not appear to be highly elevated compared to studies reported in the scientific literature [2]. Ongoing studies on trace elements in a greater number of *P. clarkii*, in other biological and environmental samples (i.e digestive gland and sediments) and in other geographical areas, will provide more information on the role of this species as indicator of environmental contamination.

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MEASUREMENT OF PLASMA CYTOKINES IN A RAT IN VIVO MODEL TO EVALUATE HONEY-BASED MEMBRANE EFFECTS IN ABDOMINAL SURGERY

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Peritoneal adhesion following abdominal surgery is a major concern for human and veterinary patients. This could be considered an exceeding effect on healing process leading to attaching of adjacent organs with complications like bowel obstruction, infertility for female, pelvic pain, anastomotic leakage and mortality [1]. Interleukin (IL) -1beta and IL-6 [2] are involved in acute-phase response and their levels have been monitored in peritoneal fluid to predict intrabdominal after surgical procedures [3]. Several strategies are nowadays under investigation to prevent adhesions, and one of them is the use of honey-based membranes to be applied directly at surgical sites [4]. The aim of the present study was to evaluate the plasma levels of the mentioned cytokines in Sprague Dawley rats undergoing laparotomy, after a cecal abrasion induced scraping the surface of caecum. The study was ethically approved, according to Italian and European laws. At the moment of the surgery, rats were randomly divided into two groups: controls (N=22)and treated (N=29) with the honey-based membranes. Blood was collected from the caudal vein using K-EDTA tubes, prior the procedure and after 6, 24, 72 hours, 7 and 14 days. Aliquots of plasma were evaluated with ELISA commercial kits and data were analyzed using Graph pad (Prism) software, using Kruskal Wallis and Dunn's post-test ($p<0.05$). Controls showed stable levels of IL-6 till 14 days after surgery, when a significant decrease was highlighted. Treated rats demonstrated a decreasing of IL-6 levels from the day of the surgery till day 14. The comparison between the two groups did not show any significant difference, except at day 14. The measurement of Il-1 beta in control groups shown constant levels till day 14, when a significant increase was highlighted. Treated group had stable levels, without any significant difference. The comparison between the groups was similar to that of IL-6. The absence of significant differences between the two groups could be due to the high individual variability. As it has been demonstrated that interleukin levels increase after laparotomy, the data suggest that the honey-based membranes have a moderate anti-inflammatory activity and it is possible to appreciate a trend of decreasing levels of interleukins in treated group. At the end of the observation period, rats were euthanized and samples of adhesions, where present, were submitted to histology. Correlation between interleukin plasma levels and histological score of adhesion are under investigation.

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EVALUATION OF A NEW TECHNIQUE TO CULTIVATE EQUINE HOOF EXPLANTS

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Lamintis represents a challenge in equine medicine: etiopathologic mechanisms are not well known and new investigation methods, such as ex vivo experiments might be useful. The aim of the present study was to evaluate which culture medium could be better to cultivate hoof tissue explants (HTE) collected post mortem. Distal limbs were collected at commercial abattoir from horses designated for meat production. Health conditions were checked prior the slaughtering. Limbs were brought to the Department of Veterinary Sciences of Turin in ice and then scrubbed with clorexidine. Section were trimmed removing the lateral and medial walls and cutting sagittal slides. Hoof strips of 0.8*1.5 cm in thickness were obtained, and included 10-12 lamellae of the inner part of the hoof wall epidermal laminae, dermal laminae and the outer part of the bone. Sections were incubated in 6-well plates with 5 ml of the two different media: Dulbecco's modified Eagle's medium (high glucose-DMEM) adding only antimicrobial/antimycotic solution (penicillin, streptomycin and amphotericin B, 2%) and L- glutamine(2%) or DMEM+ with antibiotic/antimycotic solution, L-glutamine and fetal bovine serum (20%, FBS). All plates were incubated at 5% CO2 and and 37°C, for 24 and 48h. At the different time points, HTE integrity was assessed by a Tensile strength Test: one end of the section was fixed and the other was attached to a digital force trasducer. Tension was applied until detachment of the hoof from the lamellae occurred. Measurements were performed in triplicate. HTE were also fixed in formalin and embedded in paraffin wax. Sections of 3µm thickness were stained with hematoxylin and eosin (H/E) and Periodic acid Schiff (PAS). Basement membrane pathology were analyzed according to Pollit (1996). At 24h, histological sections of explants cultured in DMEM+ demonstrated firm adhesion between epidermal basal cells and the basal membrane with intact cells. In explants treated with DMEM alone, the junction was less tight and cells presented post mortem alterations. At 48h histological sections of DMEM+ were similar to those of 24h, while DMEM sections revealed a separation between the basement membrane ad the basal cells. At 24h, mean value of tensile strength was 0.7 kg for DMEM samples while the DMEM+ sections were virtually impossible to separate. At 48h, DMEM samples mean separation force was 0.6 kg and more than 1 kg for DMEM+ samples. HTE culture offers an attractive ex vivo model to study laminitis trigger factors. In the present study we were able to establish a test to analyze separation force between the dermal ad epidermal lamellae. Stained sections is an useful method to investigate tissues during laminitis. It appears clear that the use of the right medium is at the basis of the entire procedure, in order to maintain cells alive. In our study, we demonstrated that DMEM+ could be useful to maintain HTE cells viable and stable, to confirm by the Tensile strength Test and by histological examinations.

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RNIV

IMMUNOLOGICAL PARAMETERS OF DOGS WHEN KENNELLED: A PRELIMINARY STUDY

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Shelter poor environment and an inappropriate management can be detrimental to dogs especially when housed for lengthy periods of time. Therefore, welfare indicators are crucial to establish the standards for managing sheltered dogs. Previous studies, in sheltered dogs, evaluated mostly acute stress response and, moreover, very few studies have assessed the effects of kennelling on immune parameters since stress modulates immunocompetence. The aim of this study is to assess a set of immune parameters useful to indicate sheltering adaptation in order to identify possible poor welfare in dogs. A follow up of 12 mixed-breed dogs, housed in a shelter of Lazio Region, was set on the basis of their age, their gender and health conditions (unhealthy animals were excluded), from the entrance in the kennel to eight weeks of stay. Dogs were maintained in indoor spaces with an outdoor exercise paddock. Peripheral blood samples were collected once every week both in plain tubes and with K3-EDTA from the jugular vein and immediately sent to the laboratory. A total leukocyte count as well as the lymphocyte subsets ratio CD4/CD8 were performed. Innate immune response was assessed by complement activity and lysozyme serum levels. From the entrance, during the first eight weeks of staying, leucocytes and lymphocytes mean count showed physiological values as well as CD4/CD8 ratio. On the contrary, complement activity and lysozyme registered respectively lower and higher serum levels than reference species range. Nevertheless, no significant difference was observed during the period of study. Considering the dog's age, only complement resulted significantly lower in younger animals ($p<0.05$). Regarding the dog's gender influence, a significant difference was observed between males and females in serum lysozyme levels as well as in leucocytes, lymphocytes count and in CD4/CD8 ratio in at least two samplings. Since further investigations are still in progress in order to understand the role of sheltering on individual immune response, the interpretation of immune measures, especially during chronic stress, could represent a valid tool to improve dog management and identify possible disease susceptibility.

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GENE EXPRESSION STUDY IN A WIDELY USED CELL LINE: MDCK

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Madin-Darby Canine Kidney (MDCK) are cell lines widely used as a models to studying the characteristics of epithelial cells (1), viral infection of cells and in vaccine production (2); however, little is known about MDCK gene expression of parameters involved in the innate immunity response, in DNA repairs, in cell cycle regulation, in the ability to secrete physiologically cytokines, or in response to infectious or non-infectious stressor. Owing to these gaps, that make it difficult to develop standard protocols to produce a vaccine or to study the host pathogen interaction, the aim of our study was to evaluate the basal level of protein release and gene expression of pivotal molecules in the innate immune response and cell cycle regulation. To these purpose, we developed a RT-Real Time PCR to detect the expression of the genes of interest, a selected set of 41 immune-related and epithelial gene transcripts: the immune-related group were TNFA, iNOS, STAT5, IFNG, IL1B, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL15, IL16, IL17, IL18, IL23, IL27, MYD88, NFK/p65, TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, TLR10, MD2 and CD14, and the epithelial group were CD44, CXCR4, RAD51, p53, HPRT1, PTEN, Erb2, B2M, GAPDH, B-ACT. MDCK cells were grown to confluence, then washed with PBS and lysed using RTL reagent. Cells were tested at 33rd, 34th and 40th passages; each experiment was repeated ten times. Total RNA extraction, retro-transcription and set-up of RT-PCR reactions were done as previously described (3). GADPH was used as housekeeping gene; RT-PCR analyses were performed in a CFX96 Real-time System. All genes under study were expressed with the exception of IL4, IL10, IL15, IL17, IL27 and IFNG. In particular, IL1B was expressed in 16 out of 18 samples with a DCt of 19.4±1.5, IL2 in 16 of 18 wells (DCt of 20.0±1.2), IL12 in 14 of 18 samples (DCt of 21.2±0.7), while TNFA was unexpressed in 4 of 18 wells (DCt of 20.6±1.2). Regarding TLRs, we obtained: TLR2 expressed in 17 of 18 samples (DCt of 19.4±1.5); TLR4 in 16 of 18 wells (DCt of 18.4±0.6); TLR7 in 12 of 18 samples (DCt of 20.8±2.1); TLR8 in 6 of 18 wells (DCt 21.0±2.3); TLR-9 in 17 of 18 samples (DCt of 20.8±1.3); TLR10 in 14 of 18 analysed wells (DCt 20.6±1.8). The others genes under study were expressed in all samples. Our results, outline the expression of TLR4, MD2, CD14 and TLR5, suggesting the sensibility of MDCK to invasion and penetration of bacterial strain. Moreover, the expression of others TLRs (TLR3, TLR7, TLR8 and TLR9) explains the sensitivity of this cell line to viral infection. In conclusion, our study demonstrate the constitutive expression in MDCK of genes involved in the innate immunity response and cell cycle regulation; then provide the basis to develop usage standard protocols.

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PRELIMINARY STUDY ON THE USE OF GAMMA-INTERFERON TEST IN THE CONTEXT OF BUFFALO BRUCELLOSIS DIAGNOSIS AS A DIAGNOSTIC DEEPENING TOOL

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According to current regulations, Complement Fixation Test (CFT) is the reference assay for the diagnosis of brucellosis (1). The use of the gamma-interferon (γ-IFN) test to diagnose the disease on water buffalo can assess the reactivity of samples as a confirmatory or complementary strategy as described by Adone *et al*. (2) for cattle. The paper describes the use of the gamma-interferon (γ-IFN) test in comparison with official serological tests.
Serum and heparinised blood samples from brucellosis-free (BRC-F) (n=29) and brucellosis-infected (BRC-I) (n=150) herds were collected from the caudal vein and delivered to the laboratory within six hours, within the Italian national plan for the control of brucellosis. Rose Bengal Test (RBT) and CFT were carried out according to the official procedures (1-2). Blood samples (1 mL) were stimulated with Brucellergene OCB (Synbiotics Europe, France) (40 U per well) (3) using the stimulation with phosphate-buffered saline (PBS) as negative control. The γ-IFN production was assayed by using the BOVIGAM TB kit (Thermo Fisher Scientific Inc., Waltham, MA USA) according to the manufacturer's instructions.
The concordance value for γ-IFN with respect to CFT was 100% and 68% for BRC-free and BRC-infected herds respectively. The sensibility and specificity for BRC-infected herd with respect to CFT were 98% and 51% respectively. Therefore the γ-IFN test reveals a greater number of individuals come in contact with *Brucella* spp. but not necessarily positive to the official tests.
The γ-IFN test can be a valuable tool for deepening the diagnosis of brucellosis in particular for early identifying, within an infected herd, the subjects come into contact with *Brucella* spp. The γ-IFN test can be a strong instrument to eradicate the outbreaks.

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Scienze Cliniche - CLINICA MEDICA

RADIAL AND LONGITUDINAL STRAIN AND STRAIN RATE ASSESSED BY SPECKLE-TRACKING ECHOCARDIOGRAPHY IN DOGS WITH DILATED CARDIOMYOPATHY

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Two-dimensional (2D) speckle tracking echocardiography (STE) is a new angle-independent ultrasound technique based on tracking of speckles within the myocardium on 2D grayscale images that allows studies of myocardial deformation. STE is a repeatable and reproducible method for assessing LV strain (St) and strain rates (SR) which describes the magnitude of deformation and the rate of deformation, respectively. The combination of these indices with conventional echo-Doppler variables provide a new approach for accurately quantifying canine systolic function (1). In human patients with dilated cardiomyopathy strain is reduced in all its 3 components (radial, longitudinal and circumferential) (2). In dogs with experimentally induced cardiomyopathy - by implanted pacing system - a decreased systolic LV strain was documented (3). Dogs with naturally occurred MCD was previously retrospectively studied but using a TDI based strain (4).
To our knowledge speckle-tracking strain has not yet been used to assess myocardial dysfunction in spontaneous canine DCM.
The aims of this prospective study were to evaluate the Global St and SR in dogs with idiopathic or tachycardio-induced dilated cardiomyopathy compared with clinically healthy dogs and to verify the correlation with the conventional echocardiographic indices of DCM.
Nine dogs with evidence of naturally occurred dilated cardiomyopathy (idiopathic or tachycardia-induced) were prospectively recruited during a screening aimed to select dogs to be enrolled in a gene therapy study. The aim of this study was to reduce myocardial cells apoptosis by a coronary artery infusion of a AAV9 transgene. In each dog a conventional echocardiographic evaluation was performed and LV function indices (FE Simpson, FE m-mode, FS%) were calculated along with the other MCD criteria. Global radial and longitudinal St and SR were assessed. A group of 9 healthy dogs (homogeneous for breed) was also studied as control group.
The four chamber longitudinal global strain scanned by left apical view was -8.18% (values ranging from -3.38 to -11.94 with 3.06 of standard deviation), the radial global strain was -11.63% (values ranging from -4.66 to - 20.72 with 5.28 standard deviation).
Assessed by STE, LV function appeared to be decreases in diseased dogs compared to healthy dogs and a correlation was registered with the conventional indices of ventricular function.
Results of this study suggest that STE could be used along with the conventional B mode and M mode echocardiography to better evaluate the ventricular performance in dogs with MCD and suggest it could have a prognostic value in the follow-up of treated dogs as well as it could have an important role to detect canine DCM at a preclinical stage.

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EVALUATION OF CLINICAL WELFARE IN DAIRY COWS AND MEDITERRANEAN BUFFALO BASED ON THE OUTPUT OF A 3-DIMENSIONAL ACCELEROMETER (RUMIWATCH®)

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Change of animal behavior is one of the most important criteria for assessing animal welfare and health. Parameters of animal behavior can be used to build up an early disease warning system aiming to gain a higher health standard, above all during the transition period when a negative energy balance may favourite the onset of metabolic diseases. Although this concept is finding a widespread dissemination in dairy cows [1], it is still far of being implemented in dairy Mediterranean buffalo (MB) [2] where nowadays, some traditional cow metabolic diseases begin to be diagnosed. Considering these premises, the aim of the current study was to compare in dairy cows versus buffaloes behaviors indicative of animal welfare based on the output of a 3-dimensional accelerometer and a halter equipped with a pressure sensor [RumiWatch® (RW), ITIN+HOCH GmbH, Fütterungstechnik, Liestal, Switzerland]. Ten, healthy, pre-calving cows and 10 MB heifers were enrolled. All animals were monitored for 5 days (2 days-adaptation time, 3 days-acquisition phase). The following activities were measured: “lying bout”, “walking bout”, “standing bout”, “stand up”, “lie down”, “number of strides”, “lying time”, “walking time”, “standing time”, “upright time” for the Rumiwatch® pedometer; otherwise “rumination time”, “no. of cuds”, “no. chews during rumination”, “no. chews/cud”, “eating time” for the Rumiwatch® halter. Preliminary results comparing the two species revealed similar results regarding "lying time" (cows: 9.9h±2.4 SD; MB: 9.5h±2.5 SD), “standing time” (cows: 13.1h±2.2 SD; MB:11.6h±0.9 SD), “walking time” (cows: 56.7min±22.8 SD; MB: 61.7h±13.6SD), “upright time” (cows: 14.0h±2.6 SD; MB: 12.6h±1.0 SD), “stand up” (cows: 10.6times±3.1 SD; MB: 8.0times±2.3 SD), “lie down” (cows: 10.5times±3.1 SD; MB: 8.3times±2.5 SD), “lying bout” (cows: 10.5times±3.1 SD; MB: 8.3times±2.5 SD), “number of strides” (cows: 1483.5strides±626.4 SD; MB: 1341.5strides±311.6 SD), “eating time" (cows: 270.1min±103.9 SD; MB: 335.4min±299.6 SD), as well as for “no. chews during rumination” (cows: 27443±6319.4 SD; MB: 29083.9±3212.9 SD), “no. of cuds” (cows: 459±108 SD; MB: 497.3±72 SD) and “no. chews/cud” (cows: 59,1±3.7 SD; MB: 59.2±4,1 SD). A significant difference was found for “standing bout” ($P<0.05$; cows: 167.5 times ±58.5 SD; MB: 230.1 times±38.9 SD), “walking bout” ($P<0.05$; cows: 159.3 times±59.2 SD; MB: 226.1 times±40.4 SD) and “rumination time” ($P<0.05$; cows: 438.7min± 100.7 SD; MB: 542min±33.5 SD). To the best of the authors’ knowledge, this is the first investigation comparing behaviours indicative of clinical welfare between cows and Mediterranean Buffaloes. The results suggest that the novel device RW (pedometer and halter) allows to objectively establish these parameters, elucidating at the same time some differences between the two dairy species useful to improve and correctly differentiate the respective breeding systems. We expect that it will finally be possible to establish specific cut-off values related to different diseases relevant for the two species.

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INVESTIGATION OF HUMAN TNF-α-308G/A SINGLE NUCLEOTIDE POLYMORPHISM IN DOGS PRESENTING IDIOPATHIC INFLAMMATORY BOWEL DISEASE OR FOOD RESPONSIVE DIARRHEA – PILOT STUDY

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Idiopathic inflammatory bowel disease (IBD) and food responsive diarrhoea (FRD) and are two common canine chronic enteropathies (CEs) [1-3]. Even if the pathogenesis of IBD is not yet clearly defined, it is probably due to environmental factors, microbiome, mucosal immune system dysfunctions, and to the genetic susceptibility of the host [1,2,4]. The authors hypothesize that the presence of the TNF-α-308G/A single nucleotide polymorphism (SNP) in dogs affected by CEs could be of aid in predicting the severity of such diseases, or even a possible predisposition, similarly to what previously reported in man for IBD [5]. In 2007 it was described that coding sequences for TNF-α in man (*Homo sapiens*) are similar for the 90.8% to those of *Canis familiaris* [6]; by using a human genetic test (GENOKIT®, registered trademark of BIOAESIS srl.), we collected buccal swabs in 13 dogs previously diagnosed with IBD (n=5) or FRD (n=8) [7]. Genomic DNA was extracted from the buccal swabs of the 13 samples. The TNF-α-308G/A SNP was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCRRFLP) assay. The enzyme digestion of the amplified product of sample 5 FRD gave the heterozygotes result: the genomic DNA of dog 5 FRD has both the TNF-α-308G allele and the TNF-α-308A. The subsequent sequence analysis confirmed the presence of both the guanine and the adenine in the corresponding -308 position. In canine medicine no studies correlating the TNF-α gene's polymorphisms and CEs were ever performed [8,9]. On the contrary, some studies investigated TNF expressions in dogs CEs, even if with discordant results [1,10-14]. The detection of the TNF-α (-308G/A) polymorphism only in one dog (and only in one of the two swabs) is probably due to the fact that only in this patient we obtained an adequate amount of DNA from the buccal swabs. Unfortunately, this single finding (gene and polymorphism) (however, the latter, for the first time in the dog) did not allow us to make any considerations about the correlation between its presence and the other variables considered (CIBDAI [15], albumin concentration, histopathology). Nevertheless, in our opinion this pilot study paves the way for future studies in this direction, possibly performed on a lager cohort of dogs, of selected breeds, by also including different substrates (e.g. blood), to confirm whether the test performed in the present study could be used successfully in dogs (for both gene and polymorphism), and to confirm the eventual presence of the polymorphism in diseased (or healthy) patients.

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ANALYSIS OF SERUM PROTEINS USING CAPILLARY ELECTROPHORESIS IN NICASTRESE GOAT

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Nicastrese goat is an autochthonous and endangered breed farmed in Catanzaro district (Italy). Considering the little information with respect to laboratory data about this breed, we decided to focus on electrophoretic patterns of serum proteins, a tool that could be used to rapidly check pathological condition. Electrophoretic reference values of other species (sheep) may not be fully applicable because we expect an influence of species, breed, environment, and management. In this study we present the total protein concentration, serum protein fractions, and albumin/globulin ratio measured by capillary electrophoresis in Nicastrese goat.

The study included 50 female goats between the 5th and the 6th lactation, raised in a semi-extensive system. All animals were clinically healthy, and were treated for endoparasites twice a year. Sampling was conducted during routinary functional control. Blood samples, collected from the external jugular vein using Vacutainer tubes with no additive, were allowed to clot at room temperature (20°C) and serum was separated by centrifugation at 1500 *g* for 10 minutes. The serum total proteins concentration was determined by an automated biochemistry analyzer Dimension EXL (Siemens, Healthcare Diagnostic srl). Serum concentrations of albumin, globulins, and γ-globulins, were assessed by an automated capillary electrophoresis system (Minicap, Sebia, France).

The serum total proteins were 7.3 ± 1.1 g/dL and capillary electrophoresis clearly identified six protein fraction: albumin 3.25 ± 0.52 g/dL; α1-globulins 0.62 ± 0.13 g/dL; α2-globulins 0.56 ± 0.11 g/dL; β1-globulins 0.13 ± 0.05 g/dL; β2-globulins 0.12 ± 0.05 g/dL; γ-globulins 2.67 ± 0.62 g/dL. Finally albumin/globulin ratio was 0.82 ± 0.15.

Mean values of total proteins were within the physiological range for goats [1]. Albumin and globulin fractions were in agreement with values reported by Kaneko [2] and Nagy [3]. The differences in number of protein fractions identified in our study compared to those of other Autors [3,4] could be related to the different techniques used. The albumin/globulin ratio of Nicastrese goat was lower than values reported by Kaneko [2] but in agreement with the values reported for Girgentana [4]. Our results contribute to the knowledge of serum proteins fractions in healthy Nicastrese goat and could be used for monitoring health status and improving the management and conservation of this autochthonous breed.

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HEPATOPROTECTIVE AND ANTIOXIDANT FUNCTION OF FERMENTED METHIONINE AND *Silybum marianum* IN FELINE INFLAMMATORY LIVER DISEASE

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Feline inflammatory liver disease (ILD) encompasses a group of acquired inflammatory disorders including cholangitis and less commonly hepatic parenchymal inflammation (hepatitis). In cats it is one of the most common abnormality detected in feline liver biopsies [1]. The causes of almost all feline ILD have not been determined, but it is suspected that infectious agents or immune mechanisms may underlie the inflammatory response. In experimental liver disease models, methionine metabolites such as S-adenosyl-methionine have shown considerable hepatoprotective effects [2]. This effect has been attributed to its role in the control of intracellular GSH levels. Also Silybum marianum (SM) is considered to have hepatoprotective and antioxidant functions [3]. This study was designed to evaluate the ability of fermented methionine associated with SM to decrease oxidative stress in 20 adult domestic cats with ILD. Ten cats (T group) were daily orally administered with a formulation based on fermented methionine and Silybum marianum (1 tab each 5 kg b.w.). Ten cats, whose owners did not give consent for any supplemental therapies, were selected from the clinical database and served as control (C group). Hematochemical, biochemical and oxidative stress parameters were evaluated at 0, 15, 30, 60 and 90 days. The serum total oxidant levels and antioxidant capacity were assessed by d-ROMs (reactive oxygen metabolites derivatives) test and BAP (biological antioxidant potential) test, respectively [4]. Data were analyzed using Kruskal-Wallis and Wilcoxon rank sum test ($P<0.05$) using R software. Leukocyte, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were lower ($p<0.05$) in T group than C group at day 90. Bilirubin, gamma-glutamyl transferase (GGT) at days 30 and 90 were lower ($p<0.05$) in T group than C group. In group T, ALT, AST and d-ROMs values significantly decrease at day 90 ($P<0,05$) compared to T0. In the same group BAP levels significantly increase at the end of treatment ($P<0.05$). Altogether, these findings suggest that the formulation based on fermented methionine and SM acts on two principal pathways involved in the defence of hepatocytes, specifically against oxidative stress and inflammation. Because cats appear to be particularly susceptible to oxidative stress [5], this supplement may be a beneficial part of therapeutic regimens for ILD. Further investigations are ongoing to confirm these preliminary results.

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GLAUCOMA AND LENS SUBLUXATION IN A CROSSBRED PULI X PUMI DOG: A CASE REPORT

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The Pumi is a rare sheep-herding terrier breed dog selected in Hungary from the Puli breed mixed with French and German herding dogs since the 17th century. Primary Lens Subluxation (PLL), a painful and blinding inherited eye condition that affects many breeds of dog, is common in Pumis [4]. We present a case report of a unilateral glaucoma associated to lens subluxation in a crossbred Puli x Pumi. A 9.5 years old crossbred Puli x Pumi male dog was referred to the University of Messina VTH for painful and blind OS. Previous therapy (Timolol maleate ophthalmic gel) was ineffective. The owner excluded any ocular blunt trauma. The dog was clinically healthy. Menace reaction, dazzle and pupillary light reflexes were negative in OS and positive in OD. STT values were 20 mm/min OS and 17 mm/min OD. IOP was 63 mmHg OS and 18 mmHg OD. Complete eye examination revealed blepharospasm, epiphora, episcleral engorgement, diffuse endothelial corneal edema, and midriasis in OS associated with dorsal aphakic crescent and prolapsed vitreous. Ultrasound imaging confirmed the lens subluxation in OS. No abnormal gonioscopic findings were recorded in OS and iridocorneal angle was not evaluable because of the corneal edema. Diagnosis of glaucoma caused by PLL and mechanical obstruction of aqueous outflow by vitreal debris was made. An oral (acetazolamide for a week) and topical (Dorzolamide plus Timol maleate and iperosmotic solution) therapy was used. Therapy lasted for 4 months without acceptable improvement of OS condition. Then, various treatments have been proposed to the owner: enucleation, intraocular prosthesis, and pharmacologic destruction of ciliary body through intravitreal injection of 25 mg of Gentamicin sulfate and 1 mg of Dexamethasone. Disruption of ciliary body was finally decided. Topical therapy with Brinzolamide and Dexamethasone SID was used in postoperative period (2 weeks). After a week, the clinical condition of OS was improved, and IOP was decreased (9 mmHg). Rechecks in the following 2 months showed in OS the restoration of the corneal transparency, the reduction of the volume of the ocular globe, and no signs of inflammation. 12 month later the OS appeared smaller than the contralateral, IOP was very low, no conjunctival hyperemia or episcleral congestion were present, corneal edema was present near the limbus, and the intraocular structures were not recognizable. An entropion of the inferior eyelid caused by the reduced volume of the ocular globe was treated with Dexpantenol 5% gel to prevent corneal irritation, and a surgery to correct entropion was proposed. In many breeds, PLL results from a single base change mutation in the gene ADAMST17 [1]. Among the various breeds, Terrier and their crosses have a higher PLL frequency than others [2]. Data from the Orthopedic Foundation for Animals on the results of the test on PLL carriers breed, showed for Pumi the result of 7% [3]. Because PLL is common in Pumis [4] and displacement of the lens in the other eye usually occurs weeks or months after the first luxation, routine screening for hereditary eye disease before breeding is advised in this crossbreed dog.

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Scienze Cliniche – SICV

COMPARISON BETWEEN RADIOGRAPHY AND ULTRASONOGRAPHY IN THE EQUINE THORACIC DISEASE: A RETROSPECTIVE STUDY

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Thoracic ultrasonography (US) and radiography (RX) are reported to be complementary imaging modalities in the evaluation of the lower respiratory tract in horses [1-2]. A consistent improvement in the quality of RX and US machines and images is reached and few recent studies are available in horses. The aim of the study was to compare thoracic RX and US findings in horses. US and RX images, obtained in 26 horses referred to the Veterinary Hospital of Perugia from 2014 to 2017, have been reviewed by one observer unaware of the clinical condition; only the images obtained at admission were included. Images were reviewed retrospectively for presence/absence of pathological findings, related differential diagnosis, localization (cranial, middle and caudal lobes), detection of pleural fluid, pneumothorax and/or diaphragmatic hernia. Pathological findings considered in the RX images were: diffuse localised opacities (caudodorsal or caudoventral), diffuse generalised opacities, one/multiple discrete opacities [3]. Considered US abnormal findings were: presence of parenchymal consolidation/comet tails, thickening of the pleura, atelectasis, anechoic cavitary lesions with absence of normal pulmonary structures, multifocal hypoechoic/echogenic masses [1]. The differential diagnosis assumed for each abnormal US and RX finding were those reported in literature [1,3].
In 2/26 cases pleural effusion was detected without parenchymal abnormalities. In 20/26 horses, the number of differential diagnosis was higher for the US images; in 4/26 cases was higher for RX images. The localization of the abnormalities between US and RX matched in only 7/26 cases, completely differed in 11/26; the spread of abnormalities appeared higher in RX images and US images in 6/26 and 1/26 horses, respectively. Ten/26 cases had pleural effusion; both US and RX revealed pleural effusion in 6/10 horses whereas in 3/10 was detected only on RX and in 1/10 only in US images. Pneumothorax was identified radiographically in 3/26; US revealed atelectasis in 1/3. In this study, US has been shown to be superior to RX for detection of abnormalities, but with a greater number of differential diagnosis. RX have shown a higher ability in the assessment of the severity and spread of abnormalities and in the detection of pneumothorax, which was difficult to recognize with US if not associated with consistent atelectasis. In contrast with previous studies, in 3/10 horses pleural fluid was recognized only with RX; it is likely to depend on a wrong interpretation of radiopacity confined in the cranioventral area of the lungs. Data reported here confirm the importance of RX and US as complementary diagnostic tools in the evaluation of the equine thorax.

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ANAESTHETIC EFFECTS AFTER CRANIAL AND CAUDAL INTRAVENOUS ADMINISTRATION OF ALFAXALONE IN *Trachemys scripta scripta*

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Alfaxalone is a synthetic anaesthetic-neuroactive steroid, employed in chelonians, due to smooth induction time, a rapid recovery time [1]. Chelonians own a renal portal system (RPS), essential to avoid ischemic damage [2]. The RPS makes challenging the drugs administration in numerous reptile species [3]. The aim of this investigation was to compare the anaesthetic effects of alfaxalone administered intravenous (IV) in two different sites, cranial (CR) and caudal (CA). Our hypotheses was that RPS should negatively influence alfaxalone anaesthesia. Twenty *Trachemys scripta scripta* were randomly assigned to two groups (CR, CA administrations). A dose of 5 mg/kg of alfaxalone was administered through cervical dorsal sinus (CR group), while through ventral coccygeal vein (CA group). Induction time (IT), tracheal tube insertion time (TTiT), surgical plane of anaesthesia (SPA), and full recovery time (FR) were recorded. Heart rate (HR) and respiratory frequency (RR) values were assessed every 2 minutes until recovery. Quality of sedation (SS) was recorded as 0 to 4. In the CR group, mean IT, TTiT, time of SPA, FR were 0.68±0.37, 1.04±0.60, 23.82±6.47, 30.12±9.52 minutes, respectively. The mean SS achieved was 4.9±0.32. In the CA group, mean IT, TTiT, time of SPA, FR were 4.63±4.44, 6.83±6.75, 22.27±4.66, 27.97±6.76 minutes, respectively. The mean SS achieved was 3.3±0.82. HR, RR were statistically significant lower in CR group. Administered doses were suitable for tracheal tube insertion in 18/20 turtles. IT and TTiT was delayed in the CA group, undoubtedly due to the renal/hepatic extraction rate modified by RPS activity. Contrariwise, this outcome cannot be considered clinically important, because achieved SS was appropriate in both groups. Authors can admit that, CA administration of alfaxalone can be use without risk, but then an increased dose is needed, in order to achieve a better hypnosis, reducing IT and TTiT.

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TREATMENT OF INCREASED INTRACRANIAL PRESSURE IN NATURALLY TRAUMATIC BRAIN INJURY: COMPARISON BETWEEN MANNITOL AND HYPERTONIC SALINE: A PILOT STUDY

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Increased intracranial pressure (ICP) is an important cause of secondary brain injury. Mannitol and Hypertonic Solutions of NaCl (HSS) are the gold standard to treat intracranial hypertension in case of traumatic brain injury (TBI). Aim of our study is to compare the effect of equimolar doses of Mannitol 18% and NaCl 3% solution in decreasing ICP in severe TBI. The study protocol was approved by the local Institutional Animal Care and Use Committee. This is prospective randomized study that involved stray animals admitted at the Veterinary Teaching Hospital between October 2010 and May 2015 with TBI and MGCS score ≤ 8 after hemodynamic stabilization. After extracranial therapy animals were anesthetized and Magnetic Resonance Imaging (MRI) was scheduled. Direct ICP was measured with Codman® Microsensor ® and cerebral perfusion pressure (CPP) was calculated . Data were measured at T1 and after 5-30-60-90-120 minutes (T2-T3-T4-T5-T6) after hyperosmolar therapy. CASE 1: 2-year-old, 4.2 kg male cat presented with MGCS 5. MRI showed signs of elevated ICP; suspected cerebellar hemorrhage, cerebellar herniation, secondary compression of the brain stem. T1: ICP 48±2 mmHg and CPP 1±7 mmHg. The subject received NaCl 3%. T2: ICP and CPP unchanged. Fifteen minutes later brain herniation through the burr hole was observed; craniectomy was extended. T3: ICP 117±3 and CPP 15±8 mmHg. Because of deterioration of the patient's clinical condition, the cat was euthanized. CASE 2: 7 years old, 4.4 kg male adult cat with MGCS 8, with multiple lesions. MRI showed increased ICP, severe brain stem lesion, right hemisphere contusion, damage to the right extra cranial soft tissues. T1:ICP 27±3 mmHg; CPP 31±11 mmHg. Cat was treated with NaCl 3%. T2: ICP value 16±2 mmHg; CPP 46±7 mmHg. T3: ICP 14±1 mmHg; CPP 30±6 mmHg. T4: ICP 13±2 mmHg; CPP 81±13 mmHg. T5: ICP 13±2 mmHg; CPP 58±8 mmHg. T6: ICP 19±5 mmHg; CPP 63±15 mmHg. CASE 3: male mixed dog, of 8.4 kg, 6 years old with MGCS 7 with multiple lesions. MRI pointed out elevated ICP, severe impairment of the brain stem, right hemisphere contusion without mass effect and extra cranial soft tissues lesion. T1: ICP 21±1 mmHg, CPP 41±10 mmHg. The patient was treated with Mannitol 18%. T2: ICP value 17±2 mmHg; CPP 80±10 mmHg. T3: ICP 37±5 mmHg; CPP 54±2 mmHg. T4: ICP 34±4 mmHg; CPP 58±12 mmHg. T5: ICP 31±4 mmHg; CPP 89±4 mmHg. T6: ICP 32±3 mmHg; CPP 94±7 mmHg. To the author's best knowledge, this is the first report comparing, in dogs and cats, MRI findings of raised ICP and direct ICP measurement. In this study MRI findings, suggested raising of ICP and localized the site and the extension of lesions. Elevated ICP were confirmed by direct measurements. Both Mannitol and NaCl 3% caused immediate decrease of ICP and rose in CPP compared with pre-treatment but ICP reduction was more prolonged after bolus with NaCl 3%. Further studies are required to better define the effects of these drugs on decreasing ICP in TBI patients.

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BRONCHIECTASIS IN A DOG: RADIOLOGICAL FINDINGS AND 18 MONTHS FOLLOW UP

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Bronchiectasis is defined by the presence of permanent and abnormal dilation of the bronchi. This usually occurs in the context of chronic airway infection causing inflammation. Productive cough is the main clinical signs. Although bronchiectasis is reported as a rare condition, recently it has been diagnosed in 14% of dogs that had bronchoscopy performed for respiratory diseases. Bronchiectasis is most commonly diagnosed through thoracic radiography or computed tomography. Four patterns of bronchiectasis have been described: cylindrical (the most common form in dogs, cats, and people), saccular, cystic, and varicose. A 7-year-old, 23 kg neutered female Husky-cross dog was evaluated for chronic productive coughing and exercise intolerance. A diagnosis of severe diffuse mucopurulent bronchopneumonia associated with severe bronchiectasis was made. The initial radiographic findings included right middle lung lobe consolidation and severe saccular dilatation of the main cranial lobar bronchi bilaterally with concurrent presence of intra-luminal gas and fluid/soft tissue content. The sequential imaging studies obtained over the course of one year, documented the progression of this condition, with periodic recurrence of mucus/fluid material within of the dilated bronchi alternating with periods in which the bronchial lumina, had little material within. The radiological findings included saccular dilatation of the main bronchi with a luminal content that would cyclically alternate between gas and fluid, correlated with the cyclical waxing and waning of the clinical signs.

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Scienze Cliniche - SIRA

PRELIMINARY GENETIC EVALUATIONS IN TWO XX SEX REVERSAL DOGS

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Genetic mechanisms underlying the development and the functionality of reproductive organs are still little known. The analysis of individuals carrying sexual developmental disorders (DSDs) due to genetic causes can improve the knowledge about these mechanisms and may allow to set up tests for early diagnoses of intersex [1]. This condition, when the malformations are not so evident or only internal genitalia are involved, is often diagnosed only when the malformed organs develop pathologic conditions like strong inflammations or cancer lesions. DSDs have been often dignosed in dog with a wide range of phenotypes.
Aim of this work is to describe two DSDs cases with similar phenotype in two different dog breeds: a 9 month old French bulldog (Case 1) and a 1 year old American Staffordshire Terrier (Case 2). Both dogs showed female phenotype, abnormal ano-genital distance and enlarged clitoris with penis bone. Case 1 was submitted to laparotomy during which abdominal gonad with an uterine horn ending in an epididimus structure and one gonad herniated in the inguinal canal were observed and removed. Histological analyses showed that both gonads are atrophic testicles. Cytogenetic and genetic analyses showed a normal female karyotype (2n=78; XX) and no SRY gene. In Case 2 abdomianl ultrasound examination highlighted the presence of two ovotestis-like structure fixed in the abdominal cavity caudally the kidneys. Cytogenetically the dog showed a normal female karyotype (2n=78; XX) and no SRY gene has been found. Interestingly Case 2 shows an high aneuploidy rate (2n≠78 = 14%). Both cases have been classified as true hermaphrodite [2], a condition that, up to now, has been reported in at least 28 breeds and 1 mix breed. Previous analyses ruled out SOX9 and RSPO1 gene mutations as cause of this type of DSD in the dog [3]. However many genes are involved in the development of gonads, such as WNT4, FoXL2, BMP15, DHH [4] thus further gene analyses are necessary to uncover the causes of the this type of DSD.

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EFFECTS OF SUPPLEMENTATION WITH HIGH-POLYPHENOLS EXTRA-VIRGIN OLIVE OIL ON KINETIC SPERM FEATURES AND SEMINAL PLASMA OXIDATIVE STATE IN DOG

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Sperm membrane contains high levels of polyunsaturated fatty acids and the antioxidant function of seminal plasma is fundamental for the equilibrium between free radicals (ROS) production and oxidizing activity [1]. Polyphenols are plant secondary metabolites with marked antioxidant activities and several studies showed their beneficial effects [2]. The aim of this work was to evaluate the effects of administration of two specific extra-virgin olive oil (EVOO) having different polyphenols levels, on canine spermatozoa kinetic parameters and seminal plasma oxidative state. The study was conducted on 12 clinically healthy owned dogs, of different breeds, aged 2-7 years old and 5-48 kg bw. All subjects had normal semen parameters [3] and were previously submitted to clinical and ultrasonographic evaluation of prostate and testis to exclude pathologies. Dogs were divided into two groups: experimental group (EG, n=8), supplemented with EVOO rich in polyphenols (254 ppm, Coratina cultivar); control group (CG, n=4), fed EVOO low in polyphenols (138 ppm, Cima di Bitonto cultivar). The oil was administered daily per os (1 ml/3 kg bw), before meal. All dogs were fed the same commercial dry food. Semen collection was made twice at 15 days distance, as dual control (T01 and T02), and then at 30 (T1), 60 (T2), 90 (T3) days (day 1: 1st EVOO administration). Semen concentration and kinetic parameters were measured with CASA system (IVOS 12.0, Hamilton Thorne) evaluating: sperm total count; sperm motile (MOT%); progressive motility (PROGR%) and its fractions (rapid, medium, slow, static); straight-line velocity (VSL, µm⁄s); curvilinear velocity (VCL, µm⁄s); average path velocity (VAP, µm⁄s); amplitude of lateral head displacement (ALH, µm); beat cross frequency (BCF, Hz); straightness (STR%); linearity (LIN%). On seminal plasma (3rd canine semen fraction), ROS concentrations and biological antioxidant potential (BAP) were tested with a free radical analyzer (Free® Carpe Diem). Data were analysed using repeated measures ANOVA (CoHort Software Inc., USA); differences were considered significant at $P<.05$. From results, no differences were found for sperm MOT, VSL, VCL, VAP, ALH, BCF, STR and LIN, and all semen sample parameters resulted always in physiological range values. A progressive enhancement of PROGR% was observed in EG (T0 and T1 vs T2 and T3; T2 vs T3, $P<.01$). In EG, PROGR% resulted improved at T3 compared to CG ($P=.032$). In EG, ROS levels were higher in T1 vs T0 ($P<.01$), T0 vs T2 ($P<.001$), whereas at T3 were lower (T1 vs T3, $P<.05$; T2 vs T3, $P<.001$). The ROS raise may be related to a major prostate cellular efficiency, leading to more ROS production, followed by the restore of basal metabolic activity. The BAP test did not reveal differences between groups. These preliminary results highlight the positive effects of polyphenols on PROGR% in healthy dogs. Our findings must be considered as preliminary; however, our results provide interesting evidences that need further studies.

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NON INVASIVE PROCEDURES OF CHEMICAL STERILIZATION IN THE DOG: THE INTRATESTICULAR VS INTRAEPIDIDYMAL INJECTION OF CALCIUM CHLORIDE IN ALCOHOL. LONG-TERM EFFECTS ON FERTILITY AND ANATOMO-FUNCTIONAL ALTERATIONS

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Nonsurgical methods of sterilization could yield positive impacts on canine overpopulation [1]. Previous researches have shown intratesticular injection of calcium chloride dihidrate at 20% in 95% ethanol (CaCl2) to be a promising alternative to surgery [2].
The aim of this study was to compare epididymis vs intratesticular injection of CaCl2, to assess their feasibility, side effects, long term effect on fertility and anatomo-functional alterations.
40 dogs were divided into 4 equal groups and lightly sedated. Based on the scrotal width, a dose of CaCl2 was administered via intratesticular injection (group A) or in the epididymis (group B) . The experimentally treated animals were compared to a control group receiving saline injection only, via intratesticular injection (group C) or in the epididymis (group D). Injections in he epididymis were eco-guided. The treated animals were examined at 0, 3, 6, and 9 months for sperm production, blood levels of testosterone, and side effects.
After administration of CaCl2 in the testicle (group A) or epididymis (group B) aspermia and azoospermia respectively were achieved for at least 9 months. Dogs of control groups C and D were still normospermic. Testosterone levels significantly decreased (still at the low end of physiological range) following treatment with CaCl2 in group A, sexual activity disappeared. Testosterone kept at baseline level for the groups B, C and D. No adverse effects were noted.
Performing this procedure was not easy when injecting in the testicle; it needed a little practice to be performed. The injection in the epididymis was very challenging due to the small anatomical dimension, flexibility of structures and better if echographically guided. It is very important to avoid the CaCl2 seepage not to have side effect such as testicular and scrotum necrosis [3]. This is more likely to occur injecting the epididymis.
We confirm literature data that a single, bilateral intratesticular injection CaCl2 is a reliable method for induction of sterilization in the dogs [2]. This approach showed long-term efficacy and reduced sexual behavior with a durable reduction of testosterone, as compared to baseline levels, and reduced aggressive and sexual behavior. Sterility was also achieved if injected in the epididymis but no drop in the serum testosterone level occurred. Moreover, performing the intraepidydimal injection is time consuming as orchiectomy; this makes it a not recommended technique.
The chemical sterilization by an intratesticular injection of CaCl2 alone might provide an effective, efficient alternative to surgical castration.

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PLACENTAL DEVELOPMENT IN THE DOMESTIC CAT: HISTOLOGICAL AND ULTRASOUND INVESTIGATION

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In spite of comprehensive placental classification, no exhaustive studies of placental development during different stages of pregnancy in the Domestic Cat are present in literature. The aim of this study was to evaluate placental histological changes in relation to ultrasound appearance during the course of the entire pregnancy. Thirty-five pregnant queens were recruited for this study. Pregnancy was previously confirmed by ultrasound evaluation using a linear probe 7-10 Mhz. Date of mating was not noted. Pregnancy data was estimated through specific parameters according to the study of Zambelli et al. (2002) [1]: gestational sac diameter and total length of chorionic vesicles for early stages; crown-rump length of fetus for older stages. All the queens underwent ovariohysterectomy and once the uteri were removed, morphologic assessments were recorded before fixation in 10% neutral buffered formalin solution. Samples were processed after embedding in paraffin, stained with E&O Standard Staining Protocol and 5µm thick-sections were microscopically analysed. Queens were divided in six groups according to pregnancy data: Group A (n=5) pre-contact phase; Group B (n=6) 15-19 days post coitum (p.c.); Group C (n=9) 20-29 days p.c.; Group D (n=3) 30-39 days p.c.; Group E (n=7) 40-49 days p.c.; Group F (n=5) 50-60 days p.c. The ultrasound uterine evaluation has provided useful information of gestational age even before the placenta develops. Placental ultrasound appearance does not undergo noteworthy modifications during the entire course of pregnancy. At the histological examination the endometrium shows a highly proliferative activity even before contact with trophoblast occurs. The process leading to placental development is carried out gradually and the placenta shows marked morphological changes according to fetal development. Histologically, three overlapping layers forms the placental thickness: the most relevant zone is lamellar; below there is the junctional zone at the level of which detachment from the endometrial glandular layer takes place during parturition. The most significant histological events occur into the lamellar zone. In a limited group of samples (15%) multiple foci of mineralization have been found into the lamellar zone. The latter have never been outlined for the queen so far. Comparable findings have been described and identified as placental calcification in human and mouse placenta [2,3]. Further studies and specific staining procedures are required to determine their origin and role. In conclusion the placenta is an evolving organ that adapts gradually to the growing demands of foetus. Ultrasonography can be considered a useful tool for monitoring pregnancy and placenta developments in the Domestic Cat as well as maternofetal blood flows. Histological examinations allow identification of events like calcification and other modifications occurring during the placental growth.

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RECONSTRUCTION OF A NECROTIC PENIS AND USE OF AN AMINO ACID SOLUTION TO FAST THE WOUND HEALING IN A DOG

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A 'Volpino Italiano' breed male dog, aged 10 years, was visited for the prolapse and necrosis of the penis. At clinical examination, the dog appeared depressed, anorexic and the body temperature was 39.2°C. The glans and 2/3 of the penis were brown in color and necrotic. The owner referred that in the previous days the dog was seen continuously licking his penis. He also indicated that around 10 days earlier the patient tried to mate a bitch much taller than the male. This probably provoked a skin trauma which leaded to a build up of dead tissue and necrosis of glans and penis. The veterinary team decided to surgically remove the necrotic tissue [1]. The anesthesiological protocol adopted was: premedication with Acepromazina (0.02 ml/kg iv), induction with Propofol (4 mg/kg iv); maintenance with Isofluran. Analgesia was achieved by Tramadol (4 mg/kg iv). During the surgery a catheter was inserted to preserve the urethra. The necrotic tissue, 1 centimeter thick, was removed by Metzenbaum scissors, exposing the underlying living tissue. An antibiotic ointment (gentamicin 0.1%) was applied on all over the penis. The preputium was used to cover the penis using a purse-string suture [2] and an Elizabethan collar was worn for ten days to avoid self trauma. After the surgery an antibiotics (amoxicillin 25 mg/kg) and antinflammatory therapy (robenacoxib 0.5 mg/kg) was administer for 10 days. Additionally, a therapeutic gel 'Aminogam Gel' was applied for 10 days (7 days within the preputium-made purse and 3 days spread on the penis surface); It contains four amino acids (glycine, leucine, proline and lysine) and sodium hyaluronate. Literature [3] indicates that Aminogam is used to accelerate the post-surgical wound healing process of the soft oral tissues (after teeth extraction, oral laser surgery with secondary healing without direct suture of the surgical wound, and after dental implant insertion). Absence of post-operative infections was also observed. The Aminogam Gel promotes angiogenesis in the vascular proliferation and has the capacity to induce, in human fibroblasts, the expression of an angiogenic cytokine, namely Vascular Endothelial Growth Factor. For all the reported reasons and for the structural and histological affinity between oral and genital mucosa, we tested Aminogam Gel on the dog's penis mucosa to evaluate the wound healing after removing the traumatic lesion. The dog started to ate again 48 hours post-surgery and the clinical condition improved significantly. At the purse-string suture removal (7 days) an almost complete regeneration of the penis mucosa was noticed. Total repair of the penis was detected 10 days post-surgery even if at the end its dimension resulted thinner than in previous condition. Aminogam Gel can be considered a valid auxiliary drug to fast the wound healing after penis surgery.

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AIPVET

GOOGLE GLASS IN THE VETERINARY FORENSIC PATHOLOGY

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Photography is an important component of documentation in veterinary forensic pathology (2). Pictures are taken primarily to make the forensic reports easier to understand for the layman, especially in court proceedings (1-2). Many reflex cameras and mobile phones with photographic capabilities can be used for this purpose. However, these devices need to be used by qualified personal with knowledge of photography and basics of veterinary forensic pathology in order to take clear and understandable pictures and to minimize distortion and misleading information (1). Usually this assignment is delegated to veterinary forensic pathologists themselves because there are not professional figures formed for this purpose. These limits cause an excessive workload for the pathologist with consequently lengthening of the time request to perform the necropsy. In this context, a camera that could be used hands-free to take the pictures and capture the details would mark an innovation in this field. In this study, we tested the new device Google Glass, a smartphone designed in the shape of a pair of eyeglasses with 5.0 Mpx integrated camera and ability to take pictures and record a video with simple voice commands. The aim of the study was to determine the feasibility of the Glass use in veterinary forensics pathology assessing the usability aspects and the quality of the photographic documentation compared with an entry level reflex camera (Nikon D3200). A total of 12 forensic necropsies of 3 different species (4 dogs, 4 cats and 4 cattles) were performed by 2 veterinary pathologists (AC, GP). They performed 2 necropsies of each species; half of which using Google Glass device and the other half using reflex camera Nikon D3200. For each examination we measured the time required to perform the necropsy. Also, at the end of each necropsy performed with Google Glass, pathologists were asked to give their opinion about the positive and negative aspects of this device. In addiction, 3 forensic pathologists (OP, RF, SP) evaluated the images taken by both devices for image quality using a 5-point Likert scale. The parameters assessed were color discrimination, region of interest and sharpness. Statistical evaluations were performed to evaluate the difference in image quality between both device. We found significant reduction in the time of necropsy of post-mortem examination performed with Google Glass compared with reflex groups ($p<0.001$). Moreover, on the basis of interviews conducted at the end of the post-mortem examination, voice control with hands-free operation have been reported as useful. However, the images taken by Glass ($n=150$) during necropsy received significantly lower ratings than those acquired by reflex camera for region of interest and sharpness, especially for the close-up photos of the injuries ($p<0.001$). This findings suggest that Glass is usable for acquiring images for documentation in veterinary forensic pathology. However, in this step of development, the low quality of the Glass pictures and the absence of zooming capabilities make the device not suitable for taking pictures of small anatomical details.

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ADVANCES IN ENVIRONMENTAL BIOMONITORING IN CAMPANIA REGION: THE CRIUV EXPERIENCE

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Since 2011, the Regional Center for Veterinary Urban Hygiene (CRIUV) performs an environmental biomonitoring activity through necropsy on sinantropic animals in Campania Region; the epidemiological investigation is focused mainly on dog which, in urban and suburban areas, can be considered a good animal sentinel [1]. The main task of the activity is to obtain data on causes of death, and study the prevalence and frequency of chronic, degenerative and neoplastic diseases possibly associated with environmental pollution. From January 2011 to December 2016, 1144 necropsies were performed on stray and owned dogs lived and died in Campania Region. Dogs were grouped by age (1 to 4 in ascending order) and the cause of death was established and categorized by organ system (OS) and pathological process (PP). Samples of liver and kidney were collected from animals died from malignant neoplasm or chronic/degenerative diseases to check the presence of heavy metals (Cd, Pb, Hg, As) by Atomic Absorption Spectroscopy. All data were georeferenced and the spatial distribution of dogs was compared with the legal and illegal dumping sites in Campania Region. Whithin the studied population, inflammation (20.2%) and infectious diseases (16.9%) affecting the respiratory and gastrointestinal systems were the main cause of death in young dogs, whilst cardiovascular and urogenital systems were the most involved in adult and aged dogs. Trauma (17.7%) was a frequent cause of death for dogs of groups 1 and 2 and declines steeply thereafter. Tumors were the fourth cause of death in the whole studied population (15.8%); the main organ system involved by neoplasia as cause of death were the hemolymphopoietic and the vascular systems, with multicentric lymphoma (25.5%) and hemangiosarcoma (18.6%) as the most frequent diagnosed tumors. Traces of al least one heavy metal were found in 68 out of the 69 selected cases (98.5%), with lead and cadmium as the most representative ones. Pb was found in the liver of 51 dogs (73.9%) while Cd was present in the kidney of 63 dogs (91.3%). By the way, heavy metals concentration detected in tissues of dogs included in the current study were generally unable to exert acute toxic effects. Unlike previous studies [2,3] this is the first work that encompasses the frequency of causes of death, that evaluates the levels of heavy metals in kidney and liver, and analyses the frequency of tumors and chronic/degenerative diseases in such a wide population of dogs. Furthermore, Geographical Information System (GIS) data were used to identify areas at risk for pollutants exposure in public health.

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MASS MORTALITY OF *Cornu aspersum* IN ITALIAN SNAIL FARMS: AN HISTOPATHOLOGICAL SURVEY

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Cornu aspersum is one of the commercially most important snail species in Europe, also recognized as an emerging vector of Snail Borne Diseases (SBD) affecting both humans and animals worldwide [1]. In this context, basic knowledge of the biology and pathology of these invertebrates is fundamental to perform both effective farm management and sanitary preventive actions linked to control measures. For that reasons, we performed a histopathological survey on diseases recorded in Cornu aspersum in intensive breeding farms. We examined samples from 13 snail farms experiencing mass mortality events (90-100% loss in 2017) from Northern, Central and Southern Italy. In the examined snails (about 150 samples), lesions were mainly localized at Kidney and Digestive Gland (DG), and were predominantly characterized by regressive changes (degeneration and necrosis) and massive inflammation (infiltrative, nodular and/or encapsulation type), depending on the geographical provenience and examined farm. In most cases the lesions were associated to *Rickettsia*-Like Organisms (RLO) (70%) and protozoan infections (30%), along with a consortium of both Gram+/- Bacteria. In affected individuals, RLO localized predominantly at DG level. Two morphological types of RLO-associated lesions were observed: in samples from Southern Italy (Sicily) RLO localized in Digestive Cells (DC) only and were associated to regressive changes of DG; on the contrary, in samples from central (Latium) and northern (Lombardy) Italy, RLO localized selectively in Calcium Cells (CC), and were accompanied by infiltrative/nodular inflammation of DG. Protozoan infection occurred mainly in the kidney in Latium samples, with consequent massive infiltrative and nodular/capsular inflammation of the organ. The recording of RLO is of particular concern in terrestrial gastropods: they are emerging pathogens in marine bivalves and in non-pulmonate gastropods (*Haliotis* sp.), associated to mortality events. The RLO, along with the bacterial consortium and the observed protozoan, related with the most relevant lesions recorded, suggest they could have a pathogenic role in the mass mortality events in the studied snail farms in Italy. To our knowledge this study represent the first baseline record of pathology and diseases observed in snail farms in Italy. The relevance of the data is discussed in comparison to those recorded in other snail farms in Europe [2].

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FIRST DETECTION OF PAPILLOMATOUS LESIONS AFFECTING A WILD POPULATION OF ITALIC BARBEL (*Barbus plebejus* BONAPARTE, 1839) AND EUROPEAN BARBEL (*Barbus barbus* LINNAEUS, 1758) IN NORTHERN ITALY

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Skin tumors are recorded in many fish species. They are observed in wild and bred animals and are reported as Idiopathic Epidermal Proliferation (IEP) [3] related to the detection of the unclear etiology. Epidermal hyperplasia and papilloma were described in many cyprinids as the European barbel [2].
The hyperplasia and papilloma were associated to different virus infections and other factors as pollution and mechanical stimulation [1]. Particularly, lesion incidence may increase in a stressed fish-population.
The aim of the present work is to describe a case of skin proliferative lesions, like "papilloma", occurred in a wild barbel population in the Adda River.
The affected population was detected by a local sportive fisherman that attended the river in the tract between Olginate and Brivio countries. This place is sited between the Lecco Lake and Robbiate dump. The affected fishes were firstly detected in January 2016 and increasing thereafter, but it was impossible to quantify the percentage. Variations were observed during the year, probably connected to fish migration. Different cyprinid species are present in the river, in particular, there are two different species of barbel: the Italic barbel (*Barbus plebejus*) and the imported European barbel (*Barbus barbus*) and, probably, their hybrids. No other fishes, except for barbels, were affected and none notification of the disease was reported In Italy before.
In march 2017, three barbels were sampled and processed for different exams: necropsy; histopathology; parasitological examination of skin, gills and gut scrubs by direct light microscopy observation; bacterial examination of brain, liver, kidney and spleen by cultural detection on solid medium; and viral detection of skin and a pool of internal viscera (kidney, brain, heart and spleen) through isolation on fish cell lines (EPC and BF2) followed by confirmation with transmission electronic microscopy.
During necropsy, proliferative papilloma-like lesions were observed and they were mainly localized on the fins and hips of the fishes. Their size varied from 5 to 70 mm. Parasitological examination evidenced a weak infestation only of the skin with *Piscicola geometra*. Cultural detection using solid medium did not evidence bacterial growth. Viral investigations were negative, no cytopathic effect has been detected on cell monolayer and no viral particles have been observed by electronic microscopy. Histopathological investigation of the skin lesions showed hyperplasia and multiple finger-like projections of thickened epidermal cells supported by fibro-vascular connective tissue (papillomatous lesions). Mucous cells were also present in the center of papillomatous folds.
Although many investigations were performed, the etiology of the case was unresolved. Moreover some environmental elements were identified as predisposing factors responsible for the onset of the disease.

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HISTOPATOLOGICAL FINDINGS IN ATTENUATED *Salmonella Typhimurium* MONOPHASIC VARIANT VACCINATED PIGLETS

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Salmonella Typhimurium and its monophasic variant are increasingly responsible of food borne infections in humans. The main source of infection is pork meat. Indeed, the subclinical disease in swine is the cause of the transmission of the infection into the human food chain [1].
Aim of this study was to evaluate the alterations of the intestinal mucosa of pigs immunized with an attenuated vaccine against *S. Typhimurium* monophasic variant ΔznuABC (mSTΔznuAB) and experimentally infected with homologous and heterologous strains of *S. Typhimurium*. A histopathological assessment was performed in order to evaluate the effects of vaccination and infection on different intestinal tracts.
Twenty weaned piglets were divided in 4 groups: 5 piglets were vaccinated with mSTΔznuAB and infected with virulent *S. Typhimurium* monophasic variant (mST) (group A), 5 piglets were vaccinated with mSTΔznuABC and infected with virulent *S. Typhimurium* (ST) (group B), 5 piglets were infected with virulent ST (group C) and 5 piglets were infected with virulent mST (group D).
At day 20 after infection, piglets were euthanized and samples of tonsils, ileocecal lymph nodes, spleen, ileum, caecum and colon were collected for histological evaluation. A numerical value based on the degree of lesions was assigned to each examined intestinal tract, considering epithelium, submucosa and Peyer's patch conditions, congestion and lesion patterns. Haemorrhages, congestion and lymphoid tissues were also scored in tonsils, ileocecal lymph nodes, and spleen. Statistical analysis was then performed by Two-Way Anova for Repeated Measures or by Kruskal-Wallis tests, assuming statistically significant differences when $p<0.05$.
Histologically, a diffuse epithelial conglutination was revealed in all the examined intestinal tracts and in all groups, associated to vascular congestion and lymph nodes depletion. Only in one case (group C, ileum) necrosis was revealed. Mucosal lesions were significantly more severe in caecum and colon of groups B and C. Differences were detected neither in lymphocyte amount and distribution, nor in mucosa and submucosa hemorrhages. No differences were revealed in spleen or lymph nodes for each of the examined parameters. In tonsils, a significantly higher congestion, as well as a higher activation of lymphoid follicles, were revealed in group A in comparison to group D.
Although mSTΔznuABC vaccine is able to reduce immune system colonisation and faecal shedding of homologous and heterologous virulent strains, the pattern of histological lesions was not clearly altered in the examined tissues. Only in group A tonsils appeared more reactive than in unvaccinated group.

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REDUCTION OF ALZHEIMER'S DISEASE BETA-AMYLOID PATHOLOGY BY MODULATING THE GUT MICROBIOTA IN A TRIPLE TRANSGENIC MOUSE MODEL

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Gut microbiota has a proven role in modulation of some neurodegenerative diseases progression suggesting the use of probiotics in preventive or therapeutic procedures (Bhattacharjee and Lukiw (2013); Wang and Kasper (2014)). In the present study, a novel probiotic formulation (SLAB51) was administered to a triple-transgenic mouse model of Alzheimer's disease (AD), named 3xTg-AD, and their respective wild types (WD). The main aims of this research were to get a better knowledge about modulation of the gut-brain axis upon administration of SLAB51 and to investigate the potential beneficial effects on memory deficits, amyloid plaques deposition, and neuronal apoptotic index. Eight weeks old male mice (n=60) were organized in a treated group (administered for 4 months with SLAB51 in water) and a control group (administered with water). Animals were tested for behavioural tests: The open field (OF), The novel object recognition (NOR) tests, The passive avoidance and The elevated plus maze test (EPM). Afterwards, animals were sacrificed and brains collected, weighted and macroscopically evaluated. Brain samples were treated for histological investigation, then stained to analyze Aβ peptides deposits using Congo red assay and immunohistochemical methods (anti Aβ1-42 peptide antibody). Behavioral tests revealed that SLAB51 exerted a beneficial effect on memory deficit in AD mice. Interestingly, the brain weight of probiotic-treated mice showed no changes whereas in control animals it was significantly decreased. Macroscopic evaluation showed a decline in the cortical thickness of untreated mice that was instead significantly reduced in treated group. In addition, ventricular dilatation observed in untreated animals, showed a decreasing upon the probiotic administration. Histological investigation revealed that SLAB51 contributes to a consistent reduction in the amount of brain Aβ. Congo red staining evidenced a significant reduction in extracellular amyloid deposits, associated with low staining of somata and processes of hippocampal pyramidal cells from Ammon's horn, or in granule cells from dentate gyrus, especially in the AD mice treated samples. Moreover, data were validated by the immunohistochemical results that showed higher amounts of amyloid deposits in the untreated mice than in control ones. This study suggests the beneficial effect of SLAB51 in counteracting brain damages typical of Alzheimer's disease.

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IMMUNOHISTOCHEMICAL DETECTION OF VIRAL ANTIGENS FROM DIFFERENT MORBILLIVIRUS-INFECTED SPECIES

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Morbilliviruses are lympho-epithelio-neurotropic RNA viruses infecting several species. Morbilliviral infections have been reported in domestic and wild animals [1,2], including aquatic mammals [3,4]. A recent entry into the *Morbillivirus* genus is represented by *Feline Morbillivirus* (FeMV) [5]. This study was aimed at evaluating the immunohistochemical (IHC) reactivity of tissues from different *Morbillivirus*-infected species to a panel of anti-morbilliviral antibodies (Abs). The brain and lung of *Morbillivirus*-infected wolves (*Canis lupus*), badgers (*Meles meles*), foxes (*Vulpes vulpes*) and striped dolphins (*Stenella coeruleoalba*), along with the kidney of *Morbillivirus*-infected badgers, kidney and urinary bladder from FeMV-infected cats (*Felis catus*), were investigated by means of IHC and reverse transcription (RT)-PCR for *Morbillivirus*. Three different Abs were used in IHC, a commercially avaible monoclonal Ab against the nucleoprotein (N) antigen of *Canine Distemper Virus* (CDV); an anti- FeMV N protein and anti-*Peste des Petits Ruminants Virus* (PPRV) polyclonal Ab. The three concerned Abs were able to recognize viral antigens, more or less intensely, in all tissues from the 5 *Morbillivirus*-infected species under study with the exception of the anti-CDV Ab, which did not provide positive results on kidneys and urinary bladders from FeMV infected cats. Based upon the herein reported data, we can assume that the positive IHC reactions obtained following utilization of the three Abs on tissues from the 5 *Morbillivirus*-infected species under study were the likely result of the antigenic cross-reactivity among the viruses infecting these species. It is our additional belief the aforementioned antigenic cross-reactivity relationships were enough to justify the positive immunolabeling achieved when using the anti-FeMV Ab on tissues from the *Morbillivirus*-infected animals under study. The same did not appear to be true *viceversa*, since the kidney and the urinary bladder of FeMV-infected cats showed negative IHC results when challenged with the anti-CDV Ab. In this respect, being the anti-FeMV and the anti-CDV a polyclonal and a monoclonal Ab, respectively, it seems reasonable to believe that the reaction's sensitivity was enhanced by the anti-FeMV Ab, in a similar manner to what observed with the polyclonal anti-PPRV Ab, while the reaction's specificity was increased by the anti-CDV Ab. Investigations of this kind, which may enhance both our level of diagnostic capability against *Morbillivirus* infections and our knowledge of their pathogenetic features, are warranted.

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ANITSCHKOW CELLS IN VEAL CALVES AND BEEF CATTLE CORONARY ARTERIOSCLEROSIS: POTENTIAL ROLE IN THE DEVELOPMENT OF VASCULAR DEGENERATION

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Anitschkow cells are stromal cells firstly observed in myocardium and cardiac valves of human patients. In veterinary medicine they have been reported in normal and degenerated mitral valves and myocardium of pigs, horses, dogs and cattle[1]. Their origin and role remain controversial and still debated. The present study represents the first description of morphological, immunohistochemical and ultrastructural features of Anitschkow cells in coronary arteries of cattle. A total of 25 male veal calves (6-9 months old) and 17 male beef cattle (10-24 months old) were included in the present study to investigate the coronary arteriosclerotic process. Samples of interventricular septum, papillary muscle, ventricular free wall and atrium were collected, fixed in 10% buffered formalin solution and submitted to histological evaluation (Haematoxylin & Eosin, Weigert Van Gieson and Alcian Blue stains). Stenotic and non stenotic pathological vessels were separately evaluated for Anitschkow cells identification. The severity of Anitschkow cells infiltration was semiquantitatively scored as follows: 0 (no cells), 1 (0—25% cells), 2 (25-50% cells) and 3 (more than 50% cells). Immunohistochemical (vimentin, desmin, actin, cytocheratin) and ultrastructural investigations were also performed on selected samples. GraphPad Prism software was used to perform statistical analysis (Mann-Whitney U test, $P<0.05$). Arteriosclerosis of the intramural coronary arteries was observed in all animals (100%). Anitschkow cells were detected in both calves (60%) and cattle (76%). They were characterized by a typical nucleus with a "caterpillar" appearance in the longitudinal section and an "owl-eyed" appearance in the cross one. Anitschkow cells scores showed no significant differences ($P>0.05$) between stenotic and non stenotic vessels, thus suggesting no influence of these cells on the temporal evolution of the arteriosclerotic changes. Immunohistochemical investigations revealed vimentin, desmin and actin positivity. At ultrastructural examination, Anitschkow cells revealed contractile elements in their cytoplasm. According to the literature Anitschkow cells may originate from muscular cells of the vessels wall [2], macrophage-histiocyte [3], pericytes, endothelial cells or fibrocytes [4]. The immunohistochemical and ultrastructural findings of the present study suggest that they may represent modified muscle cells probably originating from the tunica media of the coronary arteries. The exclusive detection of Anitschkow cells in the coronary walls allows hypothesizing a potential involvement in the pathogenesis of intramural coronary arteriosclerosis, even if further investigations are need to confirm this hypothesis.

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EFFECTIVENESS OF SLAB51 PROBIOTICS AND *Moringa oleifera* LEAF MEAL FOR TREATMENT OF COCCIDIOSIS IN BROILERS

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Coccidiosis is the major parasitic disease of poultry and it is caused by protozoa of the phylum *Apicomplexa*, family *Eimeriidae* [1]. Drugs and vaccines are the two main control measures for this disease however, due to concerns on prophylactic drugs use and the high vaccines cost, alternative methods are needed [2]. The use of probiotics is now preferred to antibiotics in poultry industry [3]. Also herbal preparations could be an alternative to treat coccidiosis in poultry and Drumstick tree (*Moringa oleifera*) is indicate for its effectiveness [4]. Aim of this study is to formulate a new poultry diet, integrated with probiotic blend SLAB51 (Sivoy®, Mendes SA, Switzerland) or *Moringa oleifera* leaf meal, to avoid parasitic overgrowth and the usual administration of anticoccidic drugs. Three groups (M=Moringa; P=probiotics; C=control) of 150 chickens each, with naturally acquired coccidia infection, were studied. All groups were breeded and feeded in the same conditions. Group M received *M. oleifera* leaf meal added to the food (5%) from day 60 and Group P received SLAB51 probiotics in water (7.5 Billion/50ml), from day 1. At day 120, poultry were slaughtered and gut samples were collected for histopathology. Intestinal morphology (IM) was evaluated: sections from duodenum, ileum and cecum were used to measure the height of villi, the depth of crypts, and the ratio villi/crypts. At the level of cecum, thickness of the lamina propria was measured. Lesions due to coccidia replication were evaluated according to Lesions Score (LS) criteria [5]. The scoring system was standardized for a scale of 0 to 4 [6]. Data on growth performances and mortality were recorded. Lesion Score (LS) and oocysts count (OC=oocysts/microscopic field) in different intestinal tracts were used to evaluate the different groups. In group P, LS, OC, IM, ponderal increase, final weight and mortality reduction were statistically significant, compared to group C. In group M, only LS, OC and mortality reduction resulted statistically significant. *M. oleifera* leaves had a positive effect against the coccidiosis but had no effect on the IM, ponderal increase and final weight. In general, group P showed better results than group M. SLAB51 probiotics seem to have a protective activity in poultry coccidiosis, and an addictional auxinic activity, preserving IM and integrity. In conclusion, *M. oleifera* leaf meal and SLAB51 probiotics can be useful to formulating a diet that avoids the use of anticoccidial drugs in broilers.

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Angiostrongylus vasorum: AN INCREASING THREAT FOR WOLVES POPULATION OF CENTRAL ITALY?

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Adult *Angiostrongylus vasorum* resides in the heart and pulmonary blood vessels of dogs and many wild carnivores, and terrestrial gastropods, slugs and snails, are its intermediate hosts (1). In Italy, *A. vasorum* infection was reported with increasing frequency in dogs and foxes and now it is considered endemic throughout the country (2). In the different host species, *A. vasorum* infection can be asymptomatic or cause respiratory and circulatory disorders, at times causing disseminated infections of various severity (3). Between February 2012 and December 2016, 25 wolves found dead in Lazio Region (Central Italy) were submitted for necropsy; their genotype was ascertained by means of microsatellite markers (4). Teeth were examined to group the subjects in juveniles (<12 months) or adults (>12 months). Multiple organs were sampled for histopathology and, when adult worms and/or larvae were seen in lungs, other organs were processed to evaluate larval dissemination. Molecular identification of parasites in lungs was performed using two primers targeting the ITS2 region (5). Obtained sequences were compared with those available in the GenBank using nBLAST tool. No genetic admixture with dogs for any of the 25 wolves was evidenced. Seven wolves (28.0%) had nematode larvae in lung sections and in two of them, adults were visible in pulmonary arteries. In 4 animals, larvae were also detected in other organs. All 7 positive wolves died for traumatic bone fractures. Lungs were congested with multifocal areas of consolidation in 5 wolves and only one had a focal whitish and firm nodule; the other 2 wolves lacked gross lesions in lungs and other organs. Microscopically, a multifocal to coalescing piogranulomatous pneumonia were observed, with few multinucleated giant cells surrounding eggs or larvae; adults were detected in arteries of two subjects. Larvae were also observed in the brain of 3 subject, in 2 kidneys, and in a mediastinal lymph node, surrounded by mild to moderate inflammatory infiltrate. DNA sequencing, performed on 7 PCR positive samples, resulted in sequences with an identity of 99% (query coverage of 100%) with the ITS2 region of *A. vasorum* (accession number EU627597.1). *A. vasorum* is known to be at times highly pathogenic in dogs and able to cause significant pathology in foxes (2). Evidences reported in the present study, would confirm the pathogenic potential of *A. vasorum* in wolves, especially in juvenile animals, although death of these wolves was ascribable to fatal injuries. The surprisingly high prevalence reported in the present study is similar to prevalences reported in foxes (2) indicating that natural areas of Central Italy would be particularly favourable to this parasite, being a concern in the conservation of this species.

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ANTI-SKELETAL MUSCLE ANTIBODIES IN SERA OF DOGS WITH LEISHMANIASIS

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Canine Inflammatory Myopathies (IMs) are a heterogeneous group of disorders characterized by infiltration of inflammatory cells into muscle. The most common immune-mediated IMs in dogs include the highly specialized masticatory muscle myositis (MMM); polymyositis (PM) that resembles PM in humans; dermatomyositis and extraocular myositis. In dogs, IMs can also be associated with infectious diseases caused by *Toxoplasma gondii, Neospora caninum, Ehrlichia canis, Hepatozoon canis* and *Leishmania infantum* [1]. In a previous study we showed in canine inflammatory myopathy, associated with *Leishmania infantum* infection, a pattern of cellular infiltrations and MHC upregulation compatible with an autoimmune myositis [1]. Other authors suggested that during leishmaniasis autoantibodies anti-platelet [2], anti-smooth muscle [3], anti-histone [4] etc. are produced both in dogs and in humans. The aim of this study was to investigate the presence of autoantibodies anti-skeletal muscle in sera of dogs with leishmaniasis. For this purpose, we tested 40 sera from leishmaniotic dogs with an indirect immunofluorescence on muscle sections of 5 normal dogs, 3 normal sheep and 3 normal mice. As controls were processed, in the same way, 10 sera from normal dogs. We further performed immunoblot analysis using the same sera and normal muscle proteins extract to check the molecular weight of the unknow antigen. A band to about 120 kDa was identified. We show here, that a sub-population of dogs with leishmaniasis have circulating IgG autoantibodies specific for an unknown skeletal muscle sarcolemmal antigen. These results may contribute to highlights the pathomechanism underlying inflammatory myopathy associated to *Leishmania* infection in dogs. Several mechanisms can be proposed to explain the role of infectious factors as a trigger for autoimmune disease: 1) during leishmaniasis may occur polyclonal B- or T-cell activation; 2) the protein sequence of a leishmanial protein may be homologous with autoantigen sequences (antigen mimicry); 3) secondly to infection-mediated inflammation may occur an increased immunogenicity of organ autoantigens. [1] Moreover, *Leishmania* spp. should also be considered as a possible cause in the pathogenesis of human IM, and the dog may be a model to study this condition.

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WHEN FELINE MAMMARY TUMORS LOOK "DIFFERENT"

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Feline mammary tumors (FMTs) are malignant and aggressive carcinoma that very often look the same at histology. They usually carry sad news, manifesting a high mortality rate (80%). Rarely (10-15%) they are benign tumors or low grade malignancies and in these cases the prognosis is expected to be more favorable (1). Since most cases show well described and similar morphology, additional phenotyping is usually not needed. Expression of estrogen and progesterone receptors, as well as expression of HER2, is often very low (2). Histological grading and lymph node/lymphovascular invasion are therefore the most relevant prognostic information (3). In this study, we present a subset of nine FMTs which showed unusual morphology at histology characterized by a biphasic appearance with two potential cell populations. The 9 FMTs were firslty diagnosed as "pleomorphic" malignant mammary tumors since they did not match to any specific histological subtype. A comment on the unusual morphology was included in the report suggesting further immunohistochemical analysis for a more precise classification. Immunohistochemistry (IHC) was therefore performed for cytokeratins, cytokeratin 14, cytokeratin 5/6, vimentin, p63, and calponin with an automated immunostainer. Interestingly, 4/9 FMTs showed a clearly biphasic component and, on the basis of the IHC results, they were classified as carcinosarcoma (1/9) and carcinoma-and-malignant epithelioma (3/9). In the latter, the interstitial malignant component was positive at least for two of the following markers: vimentin, p63, calponin, cytokeratin (any of 14, 5, 6). To our knowledge, carcinoma-and-malignant myoepithelioma has never been described in the cat. In addition, 1/9 FMT was a ductal carcinoma and 4/9 were instead simple tubular carcinomas, grade III. These findings show that, despite rare, unusual biphasic mammary carcinomas can be present also in the cat and in these cases IHC provides a more precise classification. Biphasic mammary tumors (two cells populations) are common in the dog (4). Canine carcinomas composed of an epithelial population and a well differentiated myoepithelial population (i.e. complex tumor) show a less aggressive behaviour than those composed of malignant epithelium associated with malignant myoepithelium (carcinoma-and-malignant myoepithelioma) or with mesenchimal tissues (carcinosarcoma) (5). Morphology, therefore, can play a significant role for prognosis and, thus, a standardized and updated classification can be relevant both for the dog and for the cat. Additionally, IHC can be necessary to precisely classify unusual FMTs and this might give supplementary prognostic information, suggest the existence of new histological subtypes, and also extend the knowledge on the role of different cells in mammary tumor development and aggressiveness. In conclusion, when a tumor looks different, it might be something new!

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IMMUNOPHENOTYPE OF SERTOLI CELLS IN CRYPTIC/ECTOPIC AND CONTRALATERAL TESTES OF YOUNG AND OLD DOGS: PRELIMINARY RESULTS

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Sertoli cells (SCs) are important in testicular physiology and during their development from an immature to a mature immunophenotype undergo changes in markers expression. In dogs, SCs express vimentin (VIM) in both mature and immature testes, while desmin (DES) and cytokeratins (CKs) are lost during foetal life [1-2]. Anti-Müllerian hormone (AMH) and Inhibin-α (INH) expression is lost few weeks after birth [3-4]. A previous study [1], realized on cryptic/ectopic testes of 1-2 year old dogs, evidenced that in scrotal testes SCs showed mature phenotype, while in cryptic/ectopic they re-expressed markers of immaturity.
To increase number of cases, and to consider both younger and older dogs, immunohistochemistry for VIM, DES, CKs, AMH and INH was performed on sections of pairs of formalin fixed – paraffin embedded testes (cryptic/ectopic and eutopic), belonging to 13 dogs. Nine dogs aged 7 months-3 years, one dog was 6 years old, and three dogs aged 10-13 years.
Except for the 3 oldest dogs, scrotal testes were normal, showing degrees of seminal epithelium development, depending on the dog age. In these testes, SCs were VIM+. All other markers tested negative with the exception of AMH, observed exclusively in two dogs aging respectively 7 months and 1 year. Scrotal testes of old dogs were atrophic, and a seminoma was present in all of them. As in younger dogs, VIM was always expressed and in 2/3 cases AMH+ SCs were also observed.
Considering cryptic/ectopic testes, necrotic seminal cells were still visible in young dogs, while seminal tubules of 6-13 years old dogs were exclusively lined by SC. In addition, in all these 4 latter testes, a tumor was observed: 1 seminoma and 3 Sertoli cell tumors. As in scrotal testes, VIM expression was observed in all cryptic/ectopic ones while CKs, DES, INH and AMH were observed in 5, 3, 2 and 10 cases respectively, independently from the age of the dog.
Scrotal testes: results suggest that, generally, in young dogs, SCs show a normal immunophenotype while markers of immaturity reappear in old dogs. However, these latter testes, because of aging and compressive seminoma, were diffusely atrophic. Re-expression of markers of immaturity is known in atrophic testes [5]. Cryptic/ectopic testes: markers of immaturity were observed in SCs of numerous cases. Such findings were observed independently from the age of the dog suggesting that, in these testes, SCs frequently show an immature phenotype. Further studies are required to understand if in cryptic/ectopic testes SCs dedifferentiate to an immature phenotype or fail to reach maturity.

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MALIGNANT PERIPHERAL NERVE SHEAT TUMOUR (MPNST) IN THE JAW OF A SARDA BREED LAMB

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Peripheral Nerve Sheat tumours (PNSTs) comprise a group of tumors arising from peripheral, cranial or autonomic nerves. In animals the World Health Organization suggested the term PNSTs to include a range of tumors originating from Schwann cells, perineural cells or fibroblasts [1]. PNSTs have been described in human [2] and in animals, including goat [3-4], cattle [5], horse [6], dog and cat [7].
Herein, we describe the histopathological and immunohistochemical findings of a PNST involving the jaw of a Sarda breed lamb.
A 7 months-old Sarda breed lamb showed 3 to 5 in diameter, solid, non-encapsulated oval mass that localized to the subcutaneous tissue of the left-rostral part of the mandibular region, deforming the incisive arch of the teeth and closely adherent to the bone. Because of his inability to feed independently, the animal was humanely sacrificed and submitted to appropriated autopsy. Representative samples from the mass were collected, fixed in 10% neutral buffered formalin, treated with histological routinary methods and examined by histological and immunohistochemical means.
Grossly, on the cut section, the mass appeared white pearlescent in color and homogeneously myxoid. Microscopically, the mass contained a diffusely distributed population of atypical spindle-shaped cells within a basophilic-myxoid matrix. Rarely, these cells were bi-nucleate or multinucleate. Focal regions resembling nerve fibers were observed at the margins of the mass together with well-differentiated bone islands, indicating the infiltration of the surrounding bone tissue.
Scattered inflammatory foci of mononuclear cells and mitotic figures were also detected. By immunohistochemistry neoplastic cells were strongly positive for Vimentin, S100 and KI-67 but constantly negative for GFAP, Neurofilaments and Cytokeratin. On basis of these histopathological and immunohistochemical findings a maligant form of PNST (MPNST) was diagnosed.
To the best of our knowledge, PNST has never been reported in sheep. In the present case, the evidence of invasivity was correlated with an high KI-67 expression indicating a malignant variant. We hypothesize that this MPNST probably arise from peripheral branches of the trigeminal nerve or facial nerve. In this regard, MPNSTs rarely involve the cranial nerves in humans [8]. So far, no immunohistochemical staining is considered specific for MPNST, although, in agreement with our results, this tumor was demonstrated to be immunoreactive for S100 in cattle [5], goat [4] and horse [6].

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FIRST DESCRIPTION OF A SKIN TRICHOBLASTIC CELLS TUMOR IN OVINE (SARDINIAN BREED SHEEP)

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Skin tumors with adnexal differentiation are commonly reported in dogs and cats, whilst only few anecdotal reports described these neoplasms in other species, including the ovine [1]. Adnexal tumors often have a complex histological appearance and immunohistochemistry (IHC) is an aid in distinguishing follicular tumors from others cutaneous neoplasms, also in humans [1, 2]. We describe the macroscopic, histologic and immunohistochemical features of two cutaneous lesions with adnexal differentiation of a female Sardinian breed sheep. A 6-year-old sheep was admitted to the Veterinary Teaching Hospital of Sassari University and treated for an exophytic cutaneous mass located in the right pinnae. Grossly, the base of the pinnae was characterized by a horn-like, exophitic, 4 x 1.5 x 1.2 cm, superficially ulcerated, whitish mass. On cut surface, multiple white-yellowish bands circumscribed two multilobulated, 1.3 x 1.2 x 0.5 cm and 3 x 1.2 x 1 cm, nodules. Formalin fixed tissue specimens were stained with hematoxylin and eosin and evaluated by IHC, using antibodies against pan-Cytokeratin (CK) (AE1⁄AE3; Dako), Vimentin (V-9; Dako) CK 5/6 (D5/16B4; Ventana), P63 (4A4; Ventana), and KI67 (MIB-1, Dako). Histologically, one lesion was characterized by multiple, enlarged, but otherwise normal sebaceous lobules clustered around dilated sebaceous ducts suggesting a diagnosis of sebaceous gland hyperplasia. Furthermore, the dermis was expanded and the adnexa were effaced by an expansive, unencapsulated and well demarcated second multilobulated nodule. This neoplasm was composed of cuboidal to spindle basaloid cells arranged in variably-sized winding cords with characteristic peripheral cells-palisades sustained by a finely, hylinized fibro-vascular stroma. The neoplastic cells, with variably distinct cell borders, exhibited scant, pale eosinophilic cytoplasm and elongated, slightly pleomorphic, euchromatic nuclei with a high mitotic count and elevated Ki-67 expression. A diagnosis of skin tumors with adnexal differentiation was supposed. IHC revealed a diffuse and strong expression of pan-CK and CK 5/6 both in sebaceous hyperplasia and in the skin tumor. P63 signals were confined in the basal layers of sebaceous gland hyperplasia and widespread detected in the nuclei of the skin tumor. Unexpectedly, vimentin was observed in approximately 20-30% of skin tumor cells with stronger signals in the more spindled cells. Based on these results, the cutaneous neoplasm was diagnosed as a trichoblastoma with atypical features due to the high rate of spindled vimentin positive cells resembling the follicular papillary mesenchymal bodies described in dogs and cats [1]. Trichoblastoma is a benign cutaneous tumor that derive from primitive hair germ and IHC studies, both in human and canine, have demonstrated the origin from follicular stem cells [1, 2]. To our knowledge, this is the first report of a skin trichoblastic cells tumors in ovine and effort should be performed in order to elucidate the prevalence of skin tumor with adnexal differentiation in this species.

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FELINE VASCULAR NEOPLASIA: MORPHOLOGIC AND IMMUNOHISTOCHEMICAL EVALUATION OF TWO, VISCERAL AND CUTANEOUS, HEMANGIOSARCOMAS

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Hemangiosarcoma (HSA) is a malignant neoplasm arising from vascular endothelial cells; it is uncommon in cats, with a reported incidence of 0.3% up to 2.0%. Feline hemangiosarcoma is commonly classified as dermal or visceral, with no distinction made between cutaneous and subcutaneous origins for dermal tumors.
The aim of this work is to characterize, morphologically and immunohistochemically, two cases of feline hemangiosarcoma.
The first case was a female, 12 years old, DSH cat that presented a subcutaneous, expansive, ulcerated and hemorrhagic neoplasm, in the interscapular region. Cytology of the nodule was scantly cellular, with severe hemodilution; there were rare individually arranged spindle cells, with finely vacuolated cytoplasm, characterized by severe anisocytosis and anisokaryosis, elevate N/C ratio and numerous nucleoli. Grossly, adjacently to the primary neoplasm, there were numerous local metastases and lungs were characterized by myriads of metastatic nodules.
The second case was a female, 11 years old, DSH cat that presented numerous, variably in size, splenic nodules. Grossly, metastatic nodules in liver and lungs were evident.
Histologically, in both cases, all the primary and metastatic nodules were characterized by unencapsulated, variably infiltrative and expansile neoplasm composed of blood-filled, variably-sized and shaped, lacunae lined by endothelial neoplastic cells; multifocally there were more dense areas of spindle cells, arranged in short interlacing bundles and streams, with scant fibrovascular stroma. There were also, large and diffuse areas of necrosis and hemorrhage, surrounded by numerous hemosiderin-laden macrophages. Particularly, cutaneous hemangiosarcoma had prevalence of densely cellular pattern, meanwhile the visceral form was more lacunar, characterized by viable neoplastic endothelial rim. In both cases, pulmonary metastases were characteristically centered on bronchial blood vessels. Spleen, liver and lung also presented numerous areas of extramedullary hematopoiesis.
Immunohistochemistry has been performed on primary neoplasms, local and distant metastases using factor VIII–related antigen, CD31(PECAM-1) and CD34; neoplastic nodules resulted positive for all three markers, with a more specific and homogeneous expression of CD31.

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HISTOPATHOLOGICAL ASSESSMENT OF DISEASE TARGET ORGANS IN A MOUSE MODEL OF PROGERIA (LMNA G609G/G609G)

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Hutchinson-Gilford Progeria syndrome is a fatal disorder characterized by accelerated aging caused by an LMNA gene mutation, which elicits production of progerin, a mutant lamin A precursor. Recently a knock-in mouse strain carrying the most frequent Hutchinson-Gilford progeria syndrome mutation (Lmna c.1827C>T; p.Gly609Gly) [1] has been generated. We collected 41 mice of this strain further genotyped as 5 wild type (WT - Lmna +/+, 27 Heterozygotes (Het.) Lmna +/- and 9 Homozygotes (Homo.) Lmna -/-) as for the mutation with the aim to assess the target organs and their changes at the clinical end point stage (i. e. irreversible physical decay measured as three subsequent weight loss measurements) or at natural death. The colony was housed in our animal facility in Ozzano Emilia and each subject was genotyped after birth. Animals were housed until the clinical endpoint (three subsequent weight loss measurements) was reached (mean values for Lmna +/-and Lmna -/- were days 284.6±64.0 and 106.1±15.6 respectively) while for Lmna +/+ suppression was planned at several steps (mean days 124.6±94.2). Then, suppression by overdose of anesthetic (isoflurane) and cervical dislocation followed. A complete necropsy and sampling for histology (fixation in formalin and embedding in paraffin) was carried out. Histology was based on the observation of hematoxylin-eosin stained sections and, only for large arteries, also on PAS stain and Alcial stains at pH 2.5 and 1. The most frequently affected organs were lung, skin, large arteries, spleen, bones. Interstitial pneumonia (3/5 WT; 19/22 Het.; 2/4 Homo.) and hypoplasia/atrophy of the spleen lymphoid tissue (1/5 WT; 5/20 Het.; 2/6 Homo.) were non-specific changes observed in all genotypes. Genotype-associated lesions were: 1) kyphosis of the cervico-thoracic spine (0/5 WT; 27/27 Het.; 9/9 Homo.), 2) alopecia associated with reduction in number of follicles, that were mainly in the catagen phase, and dermal fibrosis (0/5 WT ; 27/27 Het.; 8/9 Homo.), 3) hypoplasia/atrophy of the adipose tissue of the subcutis (1/5 WT ; 27/27 Het. ; 8/9 Homo.), 4) reduction of cells in the tunica media of aorta paralleled by an increase of slightly eosinophilic intramural substance (0/4 WT ; 14/21 Het. ; 7/7 Homo.) that had a positive alcian stain at pH 2.5 but not at pH 1. These findings reflect most of the lesions occurring in the human disease (weight loss, lipodystrophy, dermic and cardiovascular changes, bone disorders) [2]. Genotype-associated changes here observed can be divided into two groups: the first (including weight loss, osseous anomalies, kyphosis and alopecia) to be used for the objective assessment of disease onset and the second (including atrophy of the adipose tissue in the subcutis, catagen follicles, artheriopathy) to be used to evaluate severity of disease at end point. Both groups of changes should be taken into account to test the delay in the onset of the changes and the reduction of severity of the end point lesions when experimental therapies are planned [3].

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MAST CELL TUMOR AND MAMMARY GLAND CANCER: A CASE REPORT OF COLLISION TUMOR IN DOG

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Neoplasms developing at the same venue are difficult to classify because of distinct morphological characteristics that do not fit any usual cancer classification. Collision tumors, at clinical and macroscopical examination resemble a single tumor, but histological and/or immunohistochemical analysis reveals that they are constituted by two phenotypically distinct cell populations yet maintaining sharp distinct boundaries [1]. In human medicine, collision tumors have been documented in the last decade in different anatomical sites [2]. In veterinary literature, few cases have been so far described [3,4,5,6]. A case of a collision tumor, in a 12-year-old female Labrador with a mammary gland nodular lesion, is described. Histopathological and immunohistochemical examinations revealed the presence of two distinct malignant tumors. One arose from the mammary gland epithelium, the other one was composed by neoplastic mast cells. To the authors' knowledge, this is the first description of a collision tumor in the dog composed by mast cell tumor and tubular carcinoma in the mammary gland. The raising interest for collision tumors suggests to widen their knowledge and to set up a multimodal approach including surgery and targeted therapy.

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TUMOUR THICKNESS AND MODIFIED CLARK LEVEL IN CANINE MELANOCYTIC TUMOURS

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Breslow thickness is the most important independent prognostic morphologic factor in human cutaneous melanomas: thinner melanomas have usually a better prognosis than thicker ones. It is measured, with an ocular micrometer, from the top of the granular layer of the epidermis to the deepest invasive cell of the tumour [1]. Since in dogs most of cutaneous melanocytic tumours are confined to the dermis, Lacroux et al. [2], evaluating tumour thickness, found that tumours with benign behaviour were thinner than malignant ones.

In human cutaneous melanocytic tumours Clark level is used to evaluate the anatomic level of invasion through the layers of the dermis and is associated with the frequency of metastasis and death [3].

We sought to determine whether tumour thickness is associated with histological diagnosis both in oral and cutaneous tumours, whether it is possible to use a handy system for measuring it and the applicability and usefulness of Clark level in the assessment of canine melanocytic tumours.

Sixty formalin-fixed paraffin embedded samples of primary melanocytic tumours, including oral melanomas,cutaneous melanomas and melanocytomas were examined.Tumour thickness was measured from the most superficial tumour cell layer down to the deepest point of invasion, both with an ocular micrometer and a ruler that was applied on the surface of the glass slide. Moreover, since in the dog the papillary dermis is not present, we elaborated a modified Clark level considering the levels in which the dermis is divided in veterinary medicine [4]; we further stratified cases into two groups(I-III level; IV-V level), classifying modified Clark level also as a dichotomous variable.

The association of histological diagnosis with tumour thickness and Clark level was evaluated; to explore the differences between the two methods of tumour thickness evaluation, Bland–Altman plot analysis and intraclass correlation coefficient (ICC) were performed.

Tumour thickness was associated with histological diagnosis ($P=0.056$), being higher in cutaneous melanomas than melanocytomas, but it did not differ between oral melanomas and cutaneous tumours. Agreement between the two methods of tumour thickness evaluation was excellent: ICC was 0.970 ($P<0.001$) and the Bland–Altman analysis showed a bias of -0.021 ± 0.118 cm.

Both five levels ($P=0.005$) and dichotomous classification ($P=0.001$) of modified Clark level have shown to be associated to histological diagnosis.

These results suggest that, in dogs, tumour thickness could be a feature of interest, especially in cutaneous melanocytic tumours, that could be evaluated with the ruler when an ocular micrometer is not available; moreover, a modified Clark level can be useful to evaluate in cutaneous melanomas and melanocytomas reflecting the degree of tumour invasion. Whether these two morphologic factors have an independent prognostic value in canine melanocytic tumours has to be elucidated.

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MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF CANINE PHEOCHROMOCYTOMA

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The canine pheochromocytoma is the tumor of the chromaffin cells of the adrenal gland. It occurs, not frequently, without breed and gender predisposition in middle-aged dogs (9-11 years) [1], mostly as a single, slowly growing, nodular lesion of 0.5 cm to more than 10 cm in diameter, often involving the right adrenal gland and sometimes with a bilateral presentation [2]. The peculiarity of this neoplasia is that the invasiveness of the vena cava is not a prognostic factor and in the majority of the cases the prognosis is good. However the recurrence might occur many years after surgery [3], thus for this reason, prognostic criteria are important. The aim of the study is to evaluate the morphology and the immunohistochemical profile of canine pheochromocytoma with parameters and markers currently used in human medicine for diagnostic and prognostic purposes. The histological sections of 22 chromogranin-A positive canine pheochromocytomas were morphologically evaluated following a score system named PASS validated in human medicine (4). Additionally, the following antibodies were tested: ki-67, VEGF, COX-2, p53, BCL-2, c-erbB-2, S100. As a result, some of the microscopic criteria of the PASS score system revealed to be positively related to malignant neoplasms. The immunohistochemical panel demonstrated the presence of scattered S-100 positive substentacular cells in some of the tumors, as seen in the human counterpart. Furthermore there is an increased expression of ki-67, p53 and COX-2 in the malignant neoplasms. The other antibodies did not reveal any significant expression. Additional work on a larger number of cases and on their follow-up is needed to achieve cut-off values with a prognostic significance.

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UNUSUAL BIPHASIC RENAL TUMOUR IN A CAT: GROSS AND MICROSCOPIC FEATURES AND DIFFERENTIAL DIAGNOSIS

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Renal tumors with biphasic pattern are a subset of neoplasms rarely occurring in humans. Mixed epithelial and stromal tumor of the kidney (MESTK) is the term given to uncommon biphasic renal tumours, characterized by a mixture of epithelial and stromal components [1]. To the best of our knowledge, biphasic renal tumours are not described in veterinary medicine. The aim of this report is to describe the unusual gross and histological features of a renal tumour occurring in a 10-year-old, male castrated, European shorthair cat, presented with abdominal enlargement. Ultrasound examination revealed a heterogeneous mass originating from right renal parenchyma, occupying most of the caudal abdomen. CBC, serum chemistry with SDMA and urinalysis were within normal limits. Total right nephrectomy was performed. On gross examination, the affected kidney showed markedly increased volume (15x9x9 cm), with renal parenchyma substituted by a solid and cystic mass, characterized by multiple cysts of varying size, containing a clear to haemorrhagic fluid, admixed with white to greysh, solid areas, with multifocal mixoid appearance. No infiltration in the adjacent tissues or metastasis were detectable at the time of surgery. Samples of the mass were routinely processed for histology and immunohistochemistry for pan-cytokeratin (CK), vimentin, CAM5.2, α-smooth muscle actin (α-SMA). Histopathological examination revealed a neoplasm with biphasic features comprising epithelial and mesenchymal elements. The epithelial component was constituted by tubules and cysts, lined by cuboidal, well-differentiated epithelium, with frequent clear cell change. Urothelial-like epithelium also lined some of the larger cysts. The epithelial elements were variably interspersed throughout the mesenchymal component, characterized by bundles of spindle cells, multifocally surrounded by abundant mixoid matrix. Spindle cells showed slightly pleomorphic, ovoid nuclei, with occasional mitotic figures. The epithelial component showed intense and diffuse immunoreactivity for CK, CAM5.2 and vimentin, whereas spindle cells revealed intense, diffuse immunostaining for vimentin, and intense, variably distributed positivity for α-SMA. Histological and immunohistochemical findings suggested a biphasic renal tumour with features compatible with MESTK, which is tipically composed of tubules and cysts, interspersed between spindle cells showing smooth muscle, fibroblastic, or myofibroblastic differentiation. Although an aggressive behaviour was rarely reported, MESTK usually behaves in a benign fashion following surgical excision [1]. The cat is healthy after 2 months of follow-up. Differential diagnosis included leiomyomatous renal cell carcinoma, a rare human tumour composed of nests and cords of clear epithelial cells forming solid areas, tubules or papillary structures, immersed in a stroma composed of mature smooth muscle [3]. Renal leiomyosarcoma, rarely described in cat [4], was also excluded due to the biphasic pattern, the bland cytological features and variable α-SMA expression of spindle cells.

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VALIDATION OF TISSUE MICROARRAY FOR THE DIAGNOSIS OF CANINE GASTROINTESTINAL SPINDLE CELL TUMORS

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Smooth muscle tumors (STM) in the dog often occur in the alimentary tract where the main differential diagnosis is gastrointestinal stromal tumors (GIST) [1]. Since there are no reliable histological criteria to differentiate SMT from GIST, immunohistochemistry (IHC) should be applied testing the expression of CD117, smooth muscle actin (sma) and desmin, allowing the distinction of GIST (CD117+), SMT (CD117-, sma and/or desmin+) and nonGIST/nonSMT [1,2]. Tissue microarray (TMA) is a method used for the simultaneous immunohistochemical evaluation of tissue samples from a large patient cohort. The aim of this study was to validate the TMA technique for the IHC-based diagnosis of SMT, GIST and nonGIST/nonSMT. Thirty-four cases were retrospectively selected from the archive of two our institution while searching for a larger study on canine SMTs. Dual core TMAs were constructed based on previously reported method using a 3mm skin punch. CD117, sma, and desmin expression was evaluated in the full section and in the TMA cores in 34, 33 and 25 cases respectively. CD117 was expressed in 6/34 cases in the full section, and in 6/34 cases in the TMAs. Sma was expressed in 30/33 cases in the full section, and in 28/33 cases in the TMAs. Desmin was expressed in 18/25 cases in the full section, and in 15/25 cases in the TMAs. Comparing the expression of CD117, sma and desmin between the two cores of each case, the expression was concordant in 34/34 cases, 33/33 cases and 21/25 cases respectively. The sensitivity of TMA for the expression of CD177, sma, and desmin was 100%, 93.3%, and 83.3%. The specificity of TMA was 100% for the expression of all the three markers tested. Since the diagnosis of GIST relies on the expression of CD117, regardless of the expression of other markers, the results indicate that TMA allows the diagnosis of GIST with 100% specificity and sensitivity. CD117 false negative and sma or desmin false positive did not occur, indicating that TMA allows the specific diagnosis of SMT. A small number of false negative were encountered only in sma and desmin IHC. As a consequence, the diagnosis of nonSMA/nonGIST should not be based on TMA only but should be confirmed by running IHC on the full section. Based on these results TMA technique allows the evaluation of CD117, sma, and desmin with high specificity and sensitivity and represent a good method to immunohistochemically classify a large number of spindle cell tumors of the alimentary tract of the dog.

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PALATOPLASTY IN DOGS WITH BRACHICEPHALIC AIRWAY OBSTRUCTIVE SYNDROME: HISTOMORPHOLOGICAL EVALUATION OF THERMAL INJURY WITH DIODE LASER AND AIRPLASMA DEVICE

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Brachycephalic airway obstructive syndrome (BAOS) is a common disorder affecting English Bulldog, French Bulldog, Pug and Boston Terrier, which usually present overlong soft palate, stenotic nares and abnormal turbinate growth. Surgical correction of BAOS abnormality with caudal staphylectomy is common [1,2,3]. The aim of this study is the histological evaluation of thermal injury on surgical specimens, comparing the effect of diode laser and Airplasma device. Twenty dogs with elongated soft palate and suitable for surgical removal were included in this study. The owners were informed about all the procedures, and signed informed consent. Patients were randomly divided into two groups: 10 for Airplasma device and 10 for diode laser. Palatoplasty was performed using the two devices, and the surgical specimens were stapled on a wooden support and routinaly fixed in 10% formalin. Each sample was halved perpendicularly to the surgical margin, and both sections were examined. The slides were then stained with haematoxylin and eosin, and 3 images in 3 distinct field (10x magnification) were acquired, using a Nikon Eclipse E600 microscope. The depth of the necrosis (measurement unit: micrometer) induced by the two types of surgical devices was measured with an image analysis program (ImageJ) on captured images. The mean value and standard deviation were calculated on 6 measurements from each sample. All data were tested with a commercial software for normality using the Shapiro-Wilk test. The data were normally distributed and therefore analyzed with Student's T test, and considered statistically significant for values of $p<0.05$. The slides were blindly examined. Histologically, in each sample of soft palate, regardless of the device used, coagulative necrosis of the epithelia and lysis of collagen bundles on the cutting edge was found and considered related to the surgical devices. Other background lesions included different degree of edema, lymphoplasmacytic interstitial inflammation, hyperemia, lymphoplasmacytic myositis, myofibers atrophy, sialoadenitis and ductal ectasia. The mean value of the depth of necrosis was 512.36 micron for the Airplasma device and 633.95 micron for the diode laser. In conclusion, the depth of necrosis was minimal, never exceeding 1 mm, and no statistical difference ($p=0.70$) was noticed comparing the two devices. Therefore, they have similar performances.

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Produzioni Animali e Sicurezza Alimentare

THE EFFECT OF QUERCITINE ON *IN VITRO* METHANE PRODUCTION AND RUMINAL PROTOZOA DETECTED BY FLOTAC

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Quercitine, a flavonoid widely distributed in nature, is produced as secondary metabolite by several plants. Flavonoids, antioxidants able to reduce/prevent some of free radical damage, have recently acquired considerable interest in animal nutrition for changing microbial activity and fiber digestion, increasing volatile fatty acids (VFA) production and reducing methane (CH_4) emission [1]. The latter causes concern for its contribution to the greenhouse. Livestock significantly contribute to CH_4 production due to the rumen fermentation of feeds. Rumen CH_4 is produced, starting from H_2 liberated by fermentations, by methanogens bacteria and protozoa. Methanogens associated with protozoa are apparently responsible for 9-25% of rumen methanogenesis [2]. The *in vitro* gas production technique is routinely used to evaluate feed nutritional value, but recently also to measure CH_4 released by rumen fermentation [3]. FLOTAC is a multivalent, sensitive, accurate and precise quantitative technique, commonly used to detect protozoa and helminthes in human and animal faeces and urine [4]. Aim of this study is to verify the *in vitro* effect of pure quercitine, added to a ruminant diet, on microbial activity, in particular CH_4 production and protozoa count. The diet (Forage: Concentrate ratio 60:40, CP 12.8 and NDF 43.9% DM) was incubated *in vitro* alone (control) and with 150 mg of Quercitine (Sigma-Aldrich) at 39°C, under anaerobic conditions with buffered cow rumen fluid. After 24 hours of incubation pH, total gas (OMCV, ml/g), degraded organic matter (dOM, %), CH_4 (% of the total gas) and VFA (mM/g) were determined [3]. Protozoa were counted by FLOTAC [4], using 5 ml of a zinc sulfate based flotation solution (density=1.200) added to 100 µl of fermentation liquor. Data obtained were statistically analyzed (T-test; SAS System, 2014). The addition of quercitine showed no effect ($P>0.05$) on pH and OMCV, but significantly ($P<0.01$) affected dOM (55.9 vs. 51.2%), VFA (99.9 vs. 86.3 mM/g) and CH_4 (11.6 vs. 15.4%) in control and supplemented diet, respectively. FLOTAC proved useful for protozoa detection on rumen material; after quercitine addition, a consistent decrease in their number was detected ($8.0 \cdot 10^5$ vs. $1.9 \cdot 10^5$ protozoa/ml). As reported in similar study [5], quercitine confirmed to have some *in vitro* effects on microbial activity. Moreover, it should be important to identify the right quercitine amount useful to contain the environmental impact, in terms of CH_4 emission and protozoa number, without reducing feed nutritional value.

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ENDOCRINE DISRUPTOR BISPHENOL A: OCCURRENCE IN HUMAN COLOSTRUM, BREAST MILK AND INFANT FORMULA

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Bisphenol A o 2,2-bis (4-idrossifenil) propano (BPA), an endocrine disruptor (ECDs), is a synthetic phenolic compound employed in the production of polycarbonate and epoxy resins used in food containers [1]. Mothers are exposed through the diet and, during pregnancy, ECDs can be transferred to the fetus across the placenta, and to the newborn baby by breastfeeding. Infant exposure may be also the consequence of raw material contamination for infant formula [2]. BPA may have effects on mammary gland, reproductive, neurobehavioral, metabolic system, and also on the immune system. The European Food Safety Authority fixed a Tolerable Daily Intake of 4 µg/kg bw/day [3]. Effects of exposure can be particularly harmful to the fetus, infants, and young children, due to their lack of metabolic enzymes capable of conjugating BPA. The vulnerability of humans during the neonatal and prenatal periods makes the BPA intake assessment more important for infants. To evaluate the infant exposure, as biomarkers of infant exposure, BPA contamination levels in colostrums, breast milk and infant formula collected from different geographic areas were considered. Biomonitoring studies suggested higher levels of BPA in human colostrums than in breast milk. In 101 samples of colostrum, BPA was detected at a range of 1-7ng/ml (mean level of 3.41ng/mL) [2]. As a biomarker of exposure, noticeable differences were reported in human milk. In breast milk of 23 healthy women, BPA was at a range of 0.28-0.97 ng/ml (mean 0.61 ng/mL) [2]. Other studies reported BPA levels up to 6. 3 ng/mL in 60% of human breast milk samples. Liquid infant formula showed BPA levels below the permissible limit (0.1-13.2 ng/g), while powder formula showed levels (0.13-2.6 ng/g) even lower [4]. BPA finds its way into food via miscellaneous pathways. High levels of BPA in breast milk may be due to differences in exposure, lifestyle and inefficient metabolism [5]. Infrequent use of all-metal cans, a limited contact area with epoxy phenolic coatings, resistance to mass transfer between packaging and solid food can explain the low concentrations detected in powder formula[6]. Infants and children are particularly vulnerable to the effects of exposure to BPA [7]. Further studied are recommended, considering children explicitly as a relevant and vulnerable group to BPA contamination levels of human milk and infant formula.

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VALIDATION OF AN ENZYME-LINKED FLUORESCENT ASSAY (ELFA) TO DETECT *Campylobacter* SPP. IN DAIRY PRODUCTS

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Campylobacter spp. are recognized as the bacterial agents responsible for campylobacteriosis, that is the most frequently reported human gastrointestinal bacterial infection in the European Union (EU) [1]. Foods implicated in human campylobacteriosis include raw or undercooked poultry and raw dairy products. Despite the high frequency of reported cause of campylobacteriosis infection in the EU, conventional methods are cumbersome. Therefore, rapid methods for *Campylobacter* detection and quantification in food are needed. The aim of this study was to validate, according to the standard procedure (ISO16140:2003), an alternative to the reference method (ISO10272-1:2006) to evaluate the Campylobacter spp. presence in raw milk and dairy products. Milk samples collected from 16 milk vending machines located throughout the Genoa area were analyzed using two different methods, an enzyme-linked fluorescent assay (ELFA) and a RealTime-PCR assay, and evaluated in parallel against the reference method. Three milk samples testing negative for Campylobacter were spiked with a 50 CFU/mL suspension of *C. jejuni* (ATCC 29428, American Type Culture Collection - Manassas, USA). Each sample was divided into 11 aliquots and stored at 4±2°C. An aliquot of each sample was used for pH control; the others were kept refrigerated: T0 (baseline), T1 (1 h), T6 (6 h), T24 (24 h), T30 (30 h), T48 (48 h), T144 (6 days), T192 (8 days), T240 (10 days), and T312 (13 days). The samples were processed with enrichment according to ISO 10272-1:2006. The samples were analyzed using the three analytical methods previously mentioned. Regarding the validation protocol these parameters were evaluated: limit of detection (LOD), relative sensitivity, accuracy, specificity, relative detection level, inclusiveness, and exclusivity. To assess the LOD, serial dilutions were prepared from a suspension of *C. jejuni* (ATCC 29428) 0.5 McFarland in physiological solution. Each sample was contaminated with 1 mL of a bacterial suspension previously prepared. Relative sensitivity, specificity, and accuracy of method were tested in total of 104 samples 45 of which were artificially contaminated with *C. jejuni*. Between January 1st 2014 and December 31th 2016, the ELFA method was used in our laboratory to analyze 460 samples of raw milk samples collected from milk vending machines. As the RealTime-PCR assay detected *Campylobacter* DNA after several days of refrigeration, but the bacteria could not always be isolated on the plate, only the ELFA method was validated. Indeed, *Campylobacter* spp. DNA could still be detected at T312 but there was no colony growth on the plate, demonstrating that *Campylobacter* were no longer viable. ELFA detected the presence of *Campylobacter* spp. between T0 and T240. Results obtained with the ELFA showed it was compliant with ISO10272-1:2006 criteria and that the immunoassay had 100% sensitivity, specificity, and accuracy. In addition, out of the 460 samples analyzed, 5% tested positive at ELFA screening and were subsequently confirmed by isolation of the bacterial strain; in all cases *C. jejuni* was isolated. Validation according to UNI EN ISO 16140:2003 of the ELFA method suggests that it may represent a useful alternative to conventional methods for detecting *Campylobacter* spp. in official controls.

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DETECTION OF *Toxoplasma gondii* DNA IN EWES' MILK FROM UMBRIA REGION

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Toxoplasma gondii is a zoonotic Apicomplexan parasite characterized by multiple transmission routes; among others, transmission of tachyzoites through unpasteurized milk has been observed [1]. Until now, only raw goat's milk has been associated with the development of acute toxoplasmosis in human [2], but *T. gondii* DNA has been detected in other type of milk, like sheep [3]. Lots of local cheese, produced in Central Italy and exported worldwide, are made from raw sheep milk, involving a risk for consumers' health. The aim of this study was to verify the presence of *T. gondii* DNA in ewes' milk from Umbria region (Italy) by using LAMP reaction targeting the SAG2 gene. We analysed 127 milk samples collected between June to September 2016 during routine milking procedures. Prior to DNA extraction, samples were treated to avoid interference by casein [4]. Extracted DNA was used as template for the LAMP reaction, with the protocol described elsewhere [5], and positive samples were sequenced. From the same animals, also blood samples were collected for the detection of anti-*T. gondii* antibody by using MAT and IFAT techniques. LAMP results showed a positivity for *T. gondii* of 18 samples, with a prevalence of 14.17% (CI95% 9.16%-21.29%). Serological results showed 39 positive samples (p=30.71%; CI95% 23.35%-39.20%), five of which were also positive with LAMP. Positivity was not uniquely associated to just one flock. Results indicate the presence of anti-*T. gondii* antibody in almost a third of examined animals and the occurrence of parasite DNA in milk, suggesting a risk for human health. More studies must be carried out, to evaluate the vitality of tachyzoites in this matrix and the possible transmission through comsumption of unpasteurized raw milk and cheese.

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ANIV

ABSCESS DISEASE IN SMALL RUMINANT FARMS OF EMILIA-ROMAGNA REGION: A PRELIMINARY STUDY

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Abscesses are widespread in sheep and goat farms, which underlies severe economic losses such as decrease in milk production and weight gain, lameness, drugs and labour for therapies. *Staphylococcus aureus* subsp. *anaerobius* (SAAN) and *Corynebacterium pseudotuberculosis* (CPS), are causative agents of Morel's disease (MD) and Caseous lymphadenitis (CLA), respectively [1], with abscesses occurring in perilymphnodal area (MD) or within lymph nodes (CLA) [2]. Beyond animal suffering and decreased growth and production, it has to be highlighted that both SAAN and CPS are possible zoonotic agents [4][5]. There is confusion in scientific literature as to abscess disease aetiology. Awareness of MD in Italy is limited, and it is likely to be mistaken for the well-known CLA, which shows a very similar clinical picture [3]. The objectives of this study were to evaluate clinical appearance and spread of these diseases in farms of Emilia-Romagna Region and to characterize the aetiology of the clinical cases observed on farm. We visited 8 dairy goat and 2 sheep farms: 14 goats and 2 sheep aging about 7 years with abscess lesions were examined and purulent tissue samples were collected. Each sample was concurrently subjected to a bacteriological examination by the following procedures: (i) direct smear on Blood Agar plates, to be incubated at 37°C in aerobic or anaerobic atmosphere, and (ii) enrichment in liquid Brain Heart Infusion (BHI) medium, followed by biochemical characterization using Biomerieux API Staph and API Coryne test kits. Clinical examination revealed that abscesses, containing yellowish creamy pus, were mostly located in prescapular (39%) and precrural (35%) regions, as well as in supramammary region (6%); their size ranged between 1.5 to 10 cm. Microbiological results revealed, in addition to SAAN (2 samples) and CPS (10 samples), unexpected hetereogeneity of bacteria including species commonly isolated in humans like *Trueperella pyogenes, Staphylococcus aureus, Kocuria kristinae, Gemella morbillorum, Streptococcus thoraltensis, Staphylococcus caprae, Prevotella, Peptoniphilus asaccharolyticus*. A mixed infection was found in five samples. This must be taken into account towards autogenous vaccine preparation which should be supported by proper bacterial identification procedures. A strong biosafety plan is needed to prevent SAAN and CPS introduction along with infected animals, as well as abscess outbreak in livestock, and to limit environmental contamination due to spontaneus abscess breakage and bacterial spread through direct contact and vector insects.

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DETECTION AND MOLECULAR CHARACTERIZATION OF A NOVEL PUTATIVE OVINE ASTROVIRUS IN A SYMPTOMATIC LAMB (ITALY, APULIA REGION)

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Astroviruses (AstVs), family *Astroviridae*, are non-enveloped RNA viruses with an icosahedral capsid and a diameter of 28-30 nm. AstVs are classified into two genera, *Mamastrovirus* and *Avastrovirus*, that infect mammals and birds, respectively. AstVs are an important cause of gastro-enteritis in humans of all age groups worldwide, mostly infants, but they also have been associated with enteric and extra-enteric diseases in animals, including infections of the central nervous system in humans and domestic ruminants (1). The authors report a case of gastroenteritis in a lamb associated to a novel putative *Astrovirus.* The sample, collected in April 2016 from a 10 days-old lamb skowing sickness and diffuse diarrhea, was previously resulted negative for presence of the most common enteric pathogens, such as *Rotavirus, Coronavirus, Escherichia coli, Coccidia* and *Cryptosporidium parvum.* After viral RNA extraction by RNeasy Mini Kit (Qiagen), the sample was also tested for AstVs by a broadly reactive consensus semi-nested RT-PCR targeted to the RNA polymerase (RdRp) region (ORF1b) (2). The amplicon of the expected size was purified and sequenced with both primers by the BigDye terminator methods (Life Technologies). The sequences were aligned by ClustalW algorithm implemented in MEGA 6.0 and phylogeny was performed using PhyML software. The seminested PCR generated an amplicon of about 410 bp. The comparison with other deposited sequences showed a 90 % identity with a partial RdRp gene of a putative AstV isolated from feces of a monkey (*Macaca mulatta*) in Bangladesh (accession number KT599572). Furthermore, the sequence showed 79% identity with some bovine AstVs and 78% with some porcine and dromedary AstVs. No similarities with other infectious agents were found. The phylogenetic tree constructed from the nucleotide alignment showed that this ovine AstV is located between the groups of the bovine and porcine AstVs. The p-distance confirmed the intermediate position of the strain, as it was 0.245 and 0.238 with bovine and porcine AstVs, respectively. Contrarily, the similarity between our sample and an ovine AstV isolated from a sheep in Scotland was only of the 66% (accession number Y15937.2). On aggregate, data suggest that the cluster including the monkey AstV, the dromedary AstV and the ovine AstV here reported, may derive from a common ancestor with the group of the bovine and porcine AstVs, which are able to infect also other species (e.g. ovine) and to adapt to the new hosts. Considering the great diversity characterizing the *Astroviridae* family, a larger or the complete sequence of this virus is necessary to shed further light on the intriguing evolutionary history.

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ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM OVINE MILK

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Coagulase Negative Staphylococci (CoNS) represent one of the main responsible agent of subclinical mastitis in sheep [1]. They are considered as opportunistic pathogens since they are usually present in the milking environment, equipment, and teat surface. CoNS can cause persistent infections leading to an increased number of somatic cells, changes in milk composition, and reduction of production [2]. Moreover, their role as reservoir of virulence factors for other microorganisms, especially *Staphylococcus aureus*, has to be considered. This study aims at characterizing the antimicrobial resistance profiles of 73 CoNS isolates, each of them collected from the bulk tank milk of a different sheep farm located in Tuscany and Lazio (Grosseto: 44; Pisa: 16; Viterbo: 8; Lucca: 2; Livorno: 2; Pistoia: 1). Isolation was performed on Mannitol Salt Agar incubated at 37°C for 48 h. Four/five colonies unable to ferment mannitol were picked from each samples and streaked onto Tryptone Soy Agar to obtain pure cultures. Catalase and coagulase tests and Gram-staining were then performed. One isolate for each farm was then subjected antibiogram. Following the guidelines by the European Commitee on Antimicrobial Susceptibility Testing (EUCAST), the disc diffusion method was employed to evaluate the resistance against ampicillin (AM), cefoxitin (FOX), cefalotin (KF), cefotaxime (CTX), chloramphenicol (C), clindamycin (DA), gentamicin (CN), tetracycline (TE), trimethoprim-sulfamethoxazole (SXT), enrofloxacin (EN), kanamycin (K) and rifampicin (RA). After incubation at 35°C for 24 h, the diameters of the inhibithion zones were compared with the breakpoint values provided for *Staphylococcus* spp. or, when available for CoNS by CLSI and EUCAST [3, 4]. Thirty-five out of 73 isolates (48%) were susceptible to all the antimicrobials tested, while the remaining 38 isolates (52%) showed resistance to at least one antibiotic. All the isolates were susceptible to FOX, KF, CTX, SXT and ENR. The highest number of resistant isolates was recorded against AM (19/73; 26%), suggesting the frequent production of penicillases by CNS. Resistant phenotypes were also obtained against TE (9/73; 12%), DA (9/73; 12%), CN (7/73; 10%), K (5/73; 7%), RA (1/73; 1%) and C (1/73, 1%). Moreover, 10 isolates out of 73 (14%) were resistant to more than one antibiotic. This work highlighted the putative role of CoNS as reservoir of antibiotic resistance traits, despite the non-clinical source of isolation. Further studies will be necessary in order to assess the presence of antibiotic resistance genes and other virulence determinants, such as genes responsible for biofilm production, which could contribute significantly to the persistance of CoNS in the dairy environment.

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EVALUATION OF DIFFERENT PCR PROTOCOLS FOR DETECTING MAEDI VISNA VIRUS IN SHEEP WITH HISTOLOGICAL LESIONS AND POSITIVE IMMUNOHISTOCHEMICAL RESULTS IN NORTHERN SPAIN

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Maedi-Visna (MV) is a widespread disease responsible for direct losses in sheep production. MV is characterised by a slow but progressive infection in sheep, resulting in a chronic interstitial inflammation of lungs, mammary glands and joints, and in a non-suppurative encephalitis and demyelination of the central nervous system (CNS). Prevalence in Spanish Assaf dairy sheep ranges between 44% and 96% among flocks and >80% in half of them (1). Maedi Visna Virus (MVV) is characterised by a high genetic variability, which may affect the sensitivity of diagnostic tests. The aim of this work was to evaluate different PCR protocols for detecting MVV in samples from Spanish Assaf sheep with histological lesions and resulted positive by immunohistochemistry (IHC).

Archival frozen samples from 6 sheep and formalin-fixed and paraffin embedded (FFPE) samples from further 8 sheep were available for PCR. The animals were Spanish Assaf sheep submitted to the Pathology Diagnostic Service. CNS, lung, or udder showed lesions referable to MV, p28 antigen of MVV was detected by IHC, and all but 2 animals were positive by nested PCR (2). DNA obtained from frozen or FFPE samples was tested by different PCR protocols (3, 4, 5, 6, 7, 8). On the basis of preliminary results, new primers were designed and used in single or in nested PCRs in combination with other primers (2) to amplify a sequence of the LTR gene. PCR products were sequenced and compared with known sequences (6).

The best results were obtained when the products of PCR based on external primers previously described (2) were used in nPCR with the new primers (100% sensitivity). The sequences (about 800 bp) obtained by the gag-pol PCR (6) from the 2 animals positive only using the new primers showed higher identity with sequences previously found in Spanish sheep (9), although identity was lower than 90%. No products were obtained by gag-pol PCR on DNA from FFPE samples, probably because DNA degradation.

Further studies are required for obtaining more genetic sequences from samples with discordant PCR results and for studying the phylogenetic relationship among different MVV strains.

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MOLECULAR TYPING OF THE OVINE PRION PROTEIN AND IDENTIFICATION OF RARE GENOTYPES

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Ovine scrapie belongs to the transmissible spongiform encephalopathies (TSEs) or prion diseases, a group of slowly progressive and invariably fatal neurodegenerative conditions affecting man and animals (1). TSEs are characterized by accumulation in the central nervous system (CNS) of an abnormal isoform (PrPSc) of the host-encoded prion protein (PrPC)(2). The susceptibility of sheep to scrapie is influenced by the PrP genotype of the host, as well as by the strain of the agent associated with the infection (3). The ovine PrP gene is highly polymorphic; the most important variations in modulating sheep susceptibility to scrapie are those at codons 136, 154 and 171, producing six different alleles, namely VRQ, ARQ, AHQ, ARH, ARK and ARR. The ARR/ARR genotype has been associated with the highest level of resistance to the disease whereas VRQ/VRQ, VRQ/ARQ and ARQ/ARQ genotypes have been associated with susceptibility to scrapie. In November 2015 a national plan for the prevention and eradication of classical scrapie was established, based on the selection of genetic resistant animals (harbouring the ARR allele). In Campania and Calabria regions the genotyping program is carried out on male animals by IZSM (Istituto Zooprofilattico Sperimentale del Mezzogiorno) and the results are communicated to the National Data Bank for Genetic Selection (BDNSG). In the present study 6000 sheep from the region Campania and Calabria regions have been analyzed by real time pcr analysis. DNA was extracted from blood sample using the automated Qiasymphony (Qiagen) instrument and then analyzed by Real time PCR for the characterization codons 136,154 e 171 (4). Sample giving incomplete amplification were sequenced by using the big dye terminator v1.1 kit on 3500 genetic analyzer (Life technologies). Most sample (5968) were clearly characterized and exhibited common genotypes (different combinations of the alleles ARR, VRQ, ARQ, AHQ, ARH and ARK) while 32 grave incomplete amplification, and were therefore further characterized by sequencing. Twenty-nine out of 32 showed frequent genotypes, while three animals exhibited the rare genotype AT137RQ/VM137RQ associated with the heterozigosity at the codon 137. Additional studies are needed to better characterize the presence of rare mutations in internal positions between codons 136 and 171 (5)(6).

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FIRST BT EPIDEMIC IN SARDINIA DURING THE YEAR 2000: RETROSPECTIVE GEOREFERENTIATION OF THE OUTBREAKS AND EVALUATION OF THE IMPACT OF SOME CLIMATIC AND ENVIROMENTAL FACTORS IN ASL OF SASSARI

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In the year 2000 there was the first occurence of Bluetongue in Sardinia. The first oubreak in Azienda Sanitaria Locale (ASL), district of Sassari, occured in the first decade of september. At the third decade of december the number of outbreaks was about 1000. At that time location of oubreaks has been performed only at the level of single municipality, without individual georeferentiation. Moreover these data were not digital recorded. The absence of georeferantiation did not allowed a punctual correlation between location of the infected flocks and climatic and enviromental data sharing identical coordinates and times. The aim of this preliminar study was to rebuild outbreacks georeferentiation of the first BT epidemic (the only epidemic without any influence of previous immunity on ruminants population) and evaluate the possible correlations with geographic factors (altitude, water bodies, rivers, ecc.), climatic data (temperature) and temporal evolution and spread of outbreaks. We reviewed more than 3000 record cards of the oubreaks of BT in ASL, data from National Database (BDN 2014 census), maps and layers related to the district of Sassari (amministrative borders, satellite imagery, topographic maps, water bodies and rivers, ecc.) climatic data of the year 2000 from 19 weather stations provided by ARPAS. Data were recorded and analyzed using Microsoft Access and Excel, maps and layers were implemented using GIS ESRI Arcview 10.3. Coordinates of flocks were recovered a) from corresponding data of BDN, b) detecting the corresponding location on the map of toponyms of Sardinia (about 5000 features) and satellite maps, c) through interviews with veterinarians and farmers. Altitude of any farms represented the average of height contours in a buffer of 200 meters radius. In regard to climatic factors we considered the average value of a decade of average daily temperatures of each flocks at the likely day 0 of infection, i.e. 30 days before the detection of the outbreak. Presence and proximity of water bodies and rivers to outbreaks was detected integrating the correlated layers and the satellite maps. We detected 977 outbreaks in the area, 718 of these likely started in the month of September alone (clinically detected in October). The 73.79% (721 outbreaks) were located beneath 250 m. of height, the 16.06% (157) between 251 and 350 meters, 9.72% (95) between 351 and 500 meters (the environmental lapse rate ERL has an average of 6.49 K/km). Only 4 outbreaks were above this last limit. The timing of the infections had a strong correlation with high temperature. For example the weather station of Sassari (area with an overall of 280 outbreaks) reported 22.11°C – 22.48°C – 21.56°C (at 0 MASL) for the three decade of September 2000 respectively. It was also observed a strict relation between areas with large presence of water bodies and rivers and location of outbreaks. Results of this preliminary study confirm that BTV transmission, as for other arbovirus, is strongly related to suitable environmental conditions. The use of GIS can contribute not only for georeferencing “cold cases” but to better investigate vector-borne diseases.

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ISOLATION OF *Macrococcus brunensis* AND *Kocuria varians* IN DOGS SUFFERING FROM CHRONIC CONJUNCTIVITIS

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The Human Microbiome Project launched in 2008 by the National Institutes of Health, revealed a remarkably abundant and diverse community of microbial species inhabiting the human body, and the eye represents an emerging area of research [1] trying to understand possible relations between microbiota alterations and pathogenesis of ophthalmic diseases [2]. In veterinary medicine, there are only few paper focused on this topic [3,4,5]. The aim of the present study was to isolate and identify, by molecular techniques, several bacterial species from the conjunctival microflora in dogs with chronic conjunctivitis. Four owned dogs conducted at the UNICAM Veterinary Teaching Hospital for eye examination due to chronic and/or relapsing conjunctivitis, were included in the study. All subjects were submitted to complete ophthalmologic evaluation, including Schirmer Tear test and fluorescein test, slit lamp examination, direct ophthalmoscopy and tonometry, to rule out other causes of conjunctival disease. The administration of systemic antibiotic in the six months prior the visit was considered as an exclusion criteria. To obtain isolated bacterial colonies, conjunctival swabs from seven infected dog eyes were spread onto agar plates with selective and non-selective media. From each isolated colony, the bacterial DNA was extracted using Bacterial Genomic DNA Isolation kit (Norgen Biotek, Ontario, Canada); the 16S bacterial rRNA gene was amplified by PCR and purified by Nucleo Spin Extract kit (Macherey-Nagel, Dürham, Germany). Each purified DNA sample was prepared and sent to be sequenced by Eurofins MWG Operon (Martinsried, Germany). The sequences obtained from each bacterial strain were analyzed using BLAST® (Basic Local Alignment Search Tool, www.ncbi.nlm.nih.gov/BLAST). *Enterococcus faecium*, *Kocuria varians*, *Macrococcus brunensis*, *Staphylococcus aureus* and two strains of *S. equorum* were the six different bacterial strains isolated from 3 out of 7 samples (43%). Sensitivity of the isolated bacterial strains to the most frequently used antibiotics in veterinary ophthalmology: chloramphenicol, gentamycin, neomycin, and tobramycin was assessed using Kirby-Bauer method. All strains were resistant to chloramphenicol (all patients had previously been treated with it) and sensitive to tobramycin, neomycin, and gentamycin except *E. faecium* that was sensitive only to tobramycin. To the Authors' knowledge, this is the first isolation of *Macrococcus brunensis* and of *Kokuria varians* from the eyes of dogs suffering from chronic conjunctivitis. They had been previously isolated from animals (e.g. *M. brunensis* from the skin of llamas [6] and *K. varians* from the eyes of healthy donkeys [7]), but this is the first isolation from dog. Even if the low number of patients and the single isolates did not allow us to draw conclusion about the involvement of these bacteria in the disease, the isolation of *K. varians* is particularly relevant because *Kokuria* spp. is a well-known pathogen in humans, especially in compromised hosts [8].

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PRELIMINARY ANALYSIS OF TRANSFERRIN RECEPTOR GENE POLYMORPHISMS IN DOGS INFECTED BY PARVOVIRUS

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Canine parvovirus (CPV) is a small, non-enveloped, single stranded DNA virus, which replicates in dividing cells. It was unrecognized canine pathogen until 1978, when it was identified as a leading, highly contagious, potentially fatal cause of infectious enteritis in puppies. It has been continuously evolving since its discovery, with the original, less virulent strain (CPV-2) being replaced by the now ubiquitous and more virulent CPV-2a and CPV-2b strains. Another strain, CPV-2c, was discovered in Italy in the year 2000. The emergence of CPV in dogs was associated with the virus acquiring the ability to bind the canine transferrin receptor type-1 (TfR). Parvoviruses exploit transferrin receptor type-1 (TfR) for cellular entry in carnivores, and specific interactions are key to control of host range. Modifications of amino acid sequence which modify binding, can provide resistance to the carnivore parvoviruses [1].

To the aim of finding molecular polymorphisms related to the resistance for parvovirus infection, we sequenced the whole gene of Transferrin receptor in both coding and non coding regions on a total of 28 dogs.

DNA from 3 groups of dogs were collected:

Group 1) DNA from blood samples of 15 dogs which survived to outbreak of distemper and parvovirus in a kennel of Salerno province.

Group 2) DNA from different organs of 32 dogs all positive to parvovirus which succumbed to the infection (2013) from the same kennel.

Group 3) DNA from blood samples of 10 dogs from Palermo province all negative for parvovirus.

Sequencing analysis on TFRC gene were performed: on 8/15 of group 1, on 10/32 of group 2, on all 10 negative control dogs from Palermo area.

TFRC genes contains 18 exons and primers spanning all different exons and introns of TFRC gene were designed by pick primer and oligo analyzer (NCBI).

Sequencing reaction were performed using BigDye Terminator v1.1 Cycle Sequencing kit following manufacture's analysis on ABI prism 310 genetic analyzer (Applied Biosystem).

The analysis showed that all coding regions (exons) are well conserved. A single nucleotide mutation in a codifying sequence was observed only in some dog samples in the exon 16 which did not bring to aminoacid change (silent mutation). A higher polymorphisms was present in the non coding regions (introns) with more than 22 SNP (single nucleotide polymorphisms) already present in the database in the full gene. 4 new SNP and 2 new insertion present only in 3 samples of dogs which succumbed to infection were also detected. However no alternative splicing that could bring to different form of receptors seemed to be created by these polymorphisms.

Transferrin receptor is used by canine parvovirus to infect the host and some structural changes could affect the infection. In our study we could not find so far any specific polymorphisms at the coding sequence level that distinguished dogs that survived from the ones that succumbed to the parvovirus infection since the same grade of polymorphism was observed in the animals of the 3 analyzed groups. It is possible that the reason of survival might be related to other factors such as the competence of the individual immune-system. However more samples need to be analyzed based also on different canine pedigrees in order to find possible mutations in TFRC related to the survival of some dogs to parvovirus infection.

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PREVALENCE OF CANINE DISTEMPER VIRUS IN CANIDS IN CENTRAL ITALY AND FIRST IDENTIFICATION OF ARCTIC LINEAGE IN MARCHE AND UMBRIA REGIONS: A PRELIMINARY STUDY

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Canine distemper virus (CDV) is one of the most commonly virus implicated in outbreaks in wild and domestic carnivores. CDV causes severe systemic diseases which normally involves the respiratory, gastrointestinal and nervous systems. To our knowledge the literature about the real incidence of such disease is scarce, particularly in wild animals population. Recently, outbreaks of CDV have been documented in Italian grey wolves (*Canis lupus italicus*) [1], a least concerned species in IUCN Red List. Therefore, the surveillance of CDV is a priority for the conservation of the wolves and, more generally, for the protection of wild carnivores which are widespread in Central Italy, especially in the National Parks. In total, 215 samples, belonging to 148 canids for CDV presence, were analysed from November 2012 to December 2016 in the laboratory of IZSUM. Of these, 37.2% were dogs, 33% wolves and 29.8% foxes. Animals were collected in 12 different provinces of 6 Regions: Umbria, Marche, Emilia Romagna, Tuscany, Lazio and Apulia. All samples were collected from dead animals which were sent to the Diagnostic Units of Istituto Zoprofilattico Sperimentale Umbria e Marche and subjected to autopsy. The RNA was extracted from organ pools and swabs with a commercial kit, retrotranscripted to cDNA and amplified by the real time PCR with QuantiFast SYBR Green RT PCR kit (Qiagen GmbH, Hilden, Germany) using primers for a fragment of 278bp in CDV nucleoprotein (NP) gene [2]. Samples having a melting temperature (TM) value ±0.5°C versus TM value of positive control were considered positive. Moreover, samples were visualized by UV rays with GelRed TM (Biotium Inc.) after electrophoresis in agarose gels and bands of appropriate sizes were excised, extracted and sequenced. Sequences obtained (n=11) were aligned with NP gene sequences of CDV available in GenBank by MUSCLE. Molecular phylogenetic analysis (MEGA 7.0) was carried out by using Maximum Likelihood method based on the Tamura 3-parameter model. The CDV RNA was identified in 20.3% of the analysed animals. A high positivity rate was identified in dogs with 10.1% of 148 sample tested positive followed by wolves (6.08%) and foxes (5.11%). The Artic Lineage of CDV was identified in 9 out of 11 sequenced samples, in both wild and domestic canids. This strain was identified in 3 different provinces (PU, AP, PG), raising concerns given the vastness of the affected area. Two Onderstepoort strains were also identified. In conclusion, this study shows a wide CDV circulation involving different ecotypes and species in the investigated area. Further studies, based on epidemiological and genetic analysis, will be carried out in order to assess the phylogenetic correlation among the identified strains. This follow up will be important in order to highlight potential risk factors associated with the introduction of this new genotype and to better understand the role played by domestic and wild carnivores interactions in virus spreading. These additional studies should be carried out as soon as possible in order to prevent virus dissemination and to perform ad hoc vaccination campains.

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MOLECULAR TYPING OF *Staphylococcus pseudintermedius* ISOLATES FROM CLINICALLY RELEVANT CASES OF CANINE PYODERMA

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Staphylococcus pseudintermedius is a newly described species of *Staphylococcus* regarded as the main causative agent of canine pyoderma [1]; recently the first case of infection in humans was drescribed. An important characteristic of this pathogen is the high genetic identity with two other species of staphylococci, *S. intermedius* and *S. delphini* that all together are known as the *Staphylococcus Intermedius* Group (SIG) [2]. This scenario makes impossible a clear phenotypic differentiation of these three pathogens, but on the other hand, the genetic research kicks off the first molecular protocol for the identification of *S. pseudintermedius* [3]. The aim of this study was to investigate the presence of different biotypes of *S. pseudintermedius* coming from clinically relevant cases of pyoderma in dogs using three molecular methods commonly used for typing bacteria: the Ribosomal Spacers Amplification (RSA) [4], the Random Amplification of Polymorphic DNA (RAPD) [5] and the Restriction Fragment Length Polymorphism (RFLP) [6]. 46 different strains were included in this work. First of all it was performed the sequencing of all the strains in order to be sure on the presence of the interested species. The RSA technique was never applied before on this bacterium and in this work revealed a clear identification of the interested pathogen while it didn't highlight the presence of significative different biotypes. The RAPD assay showed a single cluster collecting all the interested strains, which are grouped in three different sub-clusters. The RFLP technique showed the most discriminative power, giving the opportunity to distinctly identify this bacterium. In conclusion, the use of these three different techniques allows clearly identifying *S. pseudintermedius* and observing the presence of different biotypes. In future could be interesting to couple these results with the determination of the antibiotic resistance patterns in order to verify if Multi Drug Resistant strains have particular RSA and RAPD profiles.

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CANINE DISTEMPER: DESCRIPTION OF CLINICAL CASES REFERRED TO A VETERINARY HOSPITAL IN BARCELONA, SPAIN

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Canine distemper virus (CDV) is an enveloped RNA virus, belongs to genus *Morbillivirus* and family *Paramyxoviridae* [1]. CDV has a worldwide distribution and a large host range, affecting most terrestrial carnivores. Dogs and ferrets are the only domestic species susceptible to CDV, although wildlife might serve as disease reservoirs. CDV causes a highly variable, multisystemic disease affecting mainly the eyes, skin, alimentary, respiratory and central nervous systems. The outcome of infection depends on the immune status of the animal and the pathogenicity of the viral strain. Outcomes include subclinical infection or acute disease followed by recovery, death or progression to chronic disease [2]. The aim of this study was to identify recent cases of canine distemper in dogs and to describe their characteristics. This was achieved by evaluating the clinical signs during CDV infection in dogs referred to a Veterinary Hospital (Hospital Veterinari Provença) of Barcelona, Spain, in the period December 2015 – July 2016. CDV infection was detected using an antigen detection kit and/or serological test. Information recorded for each patient includes signalment and vaccination status, method of diagnosis, clinical signs and the case outcome. Statistical analysis was performed using the MedCalc® 16.8.4 statistical software (Minitab, Inc., State College, PA, USA). Descriptive statistics were performed to analyze patient characteristics. Dogs were classified according to the outcome (alive and mortality) and data comparison between groups was performed by chi-square test. $P<0.05$ was considered to be statistically significant. Logistic regression analysis of the significant data was done to identify potential risk factors. Seventy-two dogs was included in the study; all patients were lesser than one year, 71/72 of purebreed, 7 vaccinated against CDV; 46 (63.9%) are died. Statistically significant correlations with the outcome were evidenced with the dogs size, some clinical signs, anemia and the trend of IgG titres. Neurologic symptoms are the main risk factor. The increase of canine size, the lack of generic symptoms (fever, hypothermia, depression, anorexia, weight lost, pale mucus and dehydration) and gastroenteric signs, and the increase of IgG titres resulted protective in the outcome of patient.

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FIRST IDENTIFICATION OF PANTROPIC CANINE CORONAVIRUS IN WOLVES (*Canis lupus*)

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Coronaviruses (CoVs) are RNA viruses that infect a variety of mammals, canine coronaviruses (CCoVs) include two genotypes: CCoV type I (CCoV-I) and CCoV type II (CCoV-II), with the latter being classified into two subtypes: CCoV-IIa and CCoV-IIb. A hypervirulent CCoV-IIa strain, designated pantropic CCoV (pCCoV), was described and isolated in Italy [1]. Coronavirus infection in dog is usually restricted to the enteric tract and generally produces only mild or asymptomatic enteritis. In contrast, pCCoV is associated with a systemic, often fatal disease in dogs. Currently, there are no diagnostic tests able to differentiate the pantropic from the enteric CCoV strains, so that the identification of pCCoV relies on the detection of CCoV-IIa in extraintestinal tissues. Recently published articles report an increasing number of pCCoV outbreaks in dogs, giving to this strain the role of an emerging pathogen.

Free-ranging canid populations, were only sporadically found to be infected by CCoV-I or CCoV-II [2]. However, to date cases of pCCoV infection in wild canids have not been reports yet.

The aim of this work is to report a case of pCCoV infection in a wolf that displayed a coinfection with other canine viruses.

Samples were collected from different organs of a two-year-old wolf, found dead near Avellino (Italy), and submitted to routine laboratory investigations. After sample homogenization and RNA extraction using the automated extractor QIAsymphony (Qiagen), the extracts were subjected to the CCoV detection and characterization by means of established molecular protocols [3]. The samples were also submitted to molecular detection of other viral pathogens, including canine parvovirus type 2 (CPV-2), canine adenovirus type 1 and type 2 (CAdV-1, CAdV-2), canine distemper virus (CDV), canid herpesvirus type 1 (CaHV-1) and rotaviruses.

Diagnostic testing revealed that the wolf had a triple infection caused by CCoV, CPV-2 and CAdV-2; CDV, CaHV-1 and rotaviruses were not detected. The CPV strain was characterised as CPV-2a by sequencing partial VP2 gene, whereas the CCoV strain was characterised as CCoV-IIa by using molecular assays. This virus was detected in the gut and in several internal organs, including kidney, spleen, heart, brain, eye, lymph node, ear. Therefore, the virus was characterised as pCCoV. Noteworthy, the liver and lungs tested negative.

To our knowledge, this study is the first in the world reporting a case of pantropic CCoV infection in wolves. In addition, for the first time, the detection of CAdV-1 in a wolf in Italy and of CPV in the wolf population of the Campania region, were reported.

The detection of multiple pathogens, including the emerging pCCoV, in the wolf population highlights the crucial role of epidemiological surveys in wild carnivores and free-ranging dogs living in the same geographic areas. The density and spatial distribution of free-ranging dogs, which represent the main epidemiological source for this viruses, may facilitate the virus spreading to the wolf population, with a consequent threat to the preservation of this wild carnivore.

The recent emergence in Europe of new viral strains with increased pathogenicity, strengthens the need for surveillance programs to monitor the circulation of emerging viruses in the wolf population.

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FIRST DETECTION OF PRRSV IN WILD BOAR IN CAMPANIA REGION

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Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is an enveloped, single stranded RNA virus of the *Arteriviridae* family, member of the order *Nidovirales*. PRRSV has been classified in two genotypes: North American (NA) and European (EU). Nowadays both genotypes display, with different prevalence, a worldwide distribution (1). Although PRRSV is widespread in domestic swine, there is a lack of information regarding PRRSV infection in European wild boar (*Sus scrofa*). The aim of our study was to report the presence of PRRSV in wild boar in Campania Region and to investigate the role of wild boar in the circulation of the virus. In fact, anomalous episodes were registered in June 2015 when a small piglet was found abandoned and dead in the province of Avellino; in September 2016 a young wild boar (almost 1 year old) was found dead in the same province and, in October, another wild boar (almost 1 year and a half) was found with rear paralysis symptoms in the province of Salerno. All 3 animals were submitted to our Institute for laboratory examination. In details, twenty-five mg of lung samples were homogenized and 200 µl of the supernatant underwent nucleic acid extraction (Qiagen). Samples were analyzed in triplicate by Real Time RT-PCR with a multiplex PRRSV RT-PCR Kit (Qiagen) that allows the simultaneous detection of the NA, EU and highly pathogenic (HP) genotypes. All tests were performed with a 7500 Fast Real-Time PCR (Applied Biosystems) according to manufacturer's instructions. Organ samples were investigated for different microbiological and virological parametres but only lung samples resulted positive to a Real-Time RT PCR test for PRRSV (EU genotype). Sequencing and phylogenetic analysis on these samples are still in progress. From a serological investigation conducted at our Institute during 2014, 2015 and 2016 on 7,283 sera samples, a prevalence of 0.8% of PRRSV was observed in our Region indicating the presence of the virus among wild boar. Interestingly, all the investigated animals came from a restricted geographical area and, in particular, the piglet and the dead wild boar came from two close municipalities (Montefusco and Quindici respectively) thus suggesting the probable circulation of the virus among domestic pigs and wild boar in Campania. PRRSV is recognized worldwide as an impacting pathogen in swine production. Currently there is no evidence that wild boar are a reservoir of PRRSV (2). Nevertheless, wild boar may be likely to be infected by domestic swine through occasional direct or indirect contact. Furthermore, wild boar can act as a reservoir for infectious diseases of domestic pigs (3). Indeed, further study are needed to support the role of wild boar as a possible natural reservoir for PRRSV.

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WHAT IMPACT HAS REPTILE-ASSOCIATED SALMONELLOSIS IN ITALY? PRELIMINARY RESULTS OF AN EPIDEMIOLOGICAL STUDY IN THE PIEDMONT REGION

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During the last decade, reptiles have become increasingly popular pet animals. However owners are not often aware of reptile-associated salmonellosis (RAS). In USA, RAS are regularly notified and represent the 6% of sporadic salmonellosis [1]. Few information are available about the European (and Italian) situation, because a uniform system of notification is not currently in use [2].
The aim of the present study was to estimate the impact of RAS on the population affected with salmonellosis in Piedmont, an Italian region.
To estimate the impact of RAS in Piedmont (Italy), two epidemiological studies were performed. The first on the population afferent to the Hospital "Città della Salute e della Scienza" of Turin: a telephone survey was administered to every patient with salmonellosis. Patients who reported reptile contact were asked questions about where the exposure occurred and, if the reptile was kept as a pet, about the animal husbandry. Patients owner of reptiles were asked to submit animals' and habitat samples (swabs, faeces and water) in order to perform further analysis. The study was then broadened to the entire Piedmont, thanks to the involvement of the local hygiene services: a question about reptile exposure was added to the form used for salmonellosis investigation. In case of a positive answer, the previously described survey was administered to the patient, if willing.
Between January 2015 and December 2016, 28 interviews were administered to patients of the Hospital "Città della Salute e della Scienza" of Turin. Three cases reported the exposure to turtles, two of them as unique risk factor (at relatives' house and by a lake, respectively). The third owned a turtle, but reported other risk factors and refused to submit specimens.
Between May 2016 and March 2017, local regional hygiene services sent 148 answers to the question about reptile exposure: nine were affirmative. Of these, two people refused to further participate to the study and one patient reported the contact with a wild tortoise along with alimentary risk factors. The other six subjects owned pet turtles and agreed to submit samples. In one case, *S. Pomona* was isolated from turtles' cloacal swabs and water. In another case, monophasic variant of *S. Typhimurium* (1,4,[5],12:i:) was isolated from environmental samples: the PFGE patterns of these isolates and of the patient's one were identical. In a third case, *S. Lome* was isolated from turtles' cloacal swabs. The remaining three tested negative.
These preliminary data show that exposure to reptiles should always be considered as a risk factor in salmonellosis cases. Even though some animals may test negative for *Salmonella* spp., they can not be excluded as cause of RAS due to intermittent shedding [3]. To prevent the disease, a comprehensive One Health approach is essential. An edutainment project has been released by the authors (http://www.scuolachannel.it/unrettileperamico) to raise awareness in children of young age and their family.

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MICROBIOLOGICAL SURVEY IN SICILIAN POND TURTLES (*Emys trinacris*, FRITZ ET AL., 2005)

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Emys trinacris (Fritz et al., 2005) is an endemic pond turtle of Sicily [1]. Whereas other species of turtles were object of studies and evaluations about prevalence of microbiological agents, no data are available in this native specie. Some Authors referred about the importance of the anthropogenic factors and the presence of alien species on the ecology of *Emys trinacris* [2]. Aim of this study was a bacteriological and virological survey in *Emys trinacris*. For this purpose, turtles were captured by double-entry nets in 14 different sites of Sicily, selected according to bibliographic researches [3], from June to October 2016. Cloacal swabs (virological and bacteriological) were collected from 82 turtles. DNA was extracted from virological swabs using a commercial kit (DNeasy Blood & Tissue kit - QIAGEN). A nested PCR for Herpesviridae was performed using primers targeting the highly conserved sequence within the DNA polymerase gene [4]. Bacteriological investigations were performed to research *Pseudomonadaceae, Enterobacteriaceae*, including *Salmonella* spp., and *Campylobacteraceae*. For the isolation of *Enterobacteriaceae* and *Pseudomonadaceae* the swabs were initially seeded on Agar MacConkey and incubated at 37°C for 24h. Subsequently, the obtained colonies were seeded on BHI Agar and incubated at 37°C for 24h. For isolation of *Salmonella* spp., samples were enriched in growing mediums (Buffered Pepton Water, Selenite Cistyne, Rappaport Vassiliadis), seeded in specific cultural mediums (Xylose Lysine Desoxycholate Agar, Brilliant Green Agar) and incubated at 37°C for 24h. For the isolation of *Campylobacter* spp. the samples were enriched in Preston broth and seeded in Karmali agar, incubated at 37°C in microaerofilia. Biochemical tests were performed using API 20 E test, incubated at 37°C for 24h. All samples resulted negative to *Herpesviridae*. Bacteriological assays evidenced the presence microorganisms included in the genera *Campylobacter, Aeromonas* and *Enterobacteria* (*Escherichia coli, Salmonella, Klebsiella, Citrobacter*). Only one sample resulted positive for *Salmonella Richmond* 6,7; y; 1,2, serotyped by White-Kauffmann-Le Minor scheme [5]. Results evidenced a low microbiological risk in *Emys trinacris*, due to absence of specific viral pathogens and low number of bacterial colonies. These data seems to be connected to good quality of water. These preliminary data encourage further researches, with the improving of the number of samples, sites and pathogens investigated, to establish the effective state of conservation of the populations of the sicilian Turtle.

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PREVALENCE AND ANTIMICROBIAL RESISTANCE OF BACTERIAL ISOLATES FROM *Caretta caretta* OF THE TYRRHENIAN SEA

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In the marine context, methods to assess ecosystem health involve specific marine wildlife species, regarded as sentinels. Sea turtles, for their biological and ecological characteristics, are valid sentinels of near shore environments [1]. Most of the bacteria reported in sea turtles are not considered pathogens for them, but some species could present a potential zoonotic threat for other marine animals and humans [2]. Thus, this study was aimed at performing a bacteriological survey on live *C. caretta* from the Tyrrhenian sea, in order to assess the health status of this population and their ecosystem, focusing on the prevalence and antimicrobial susceptibility of various bacterial species and on identifying potential zoonotic pathogens. A total of 35 *C. caretta* were sampled, collecting two oral and two cloacal swabs for each animal. Bacterial isolation was performed through different procedures; all isolated strains were identified through their phenotypic and biochemical characteristics and subsequently submitted to antimicrobial susceptibility test using the disk diffusion method. Microbial cultures resulted in a mixed growth. Gram-negative were predominant: *Pseudomonas* spp., *Vibrio* spp., *S. putrefaciens group*, *A. hydrophila* and different Enterobacteriaceae species were isolated. In contrast, *Salmonella* spp. and *Campylobacter* spp. were never recovered. Regarding Gram-positive isolates, both coagulase negative and coagulase positive staphylococci were detected; the latters were identified as *S. aureus*. The bacteria isolated in this study have been previously recovered from ill sea turtles; microbial infections have been reported in many systems, at different degrees of severity. Some species are involved in opportunistic diseases in other marine animals and humans [3]. Bacterial isolates showed the highest rates of antimicrobial resistance to Ampicillin, whereas the lowest to Amikacin. The strains that showed resistance to the greatest number of antimicrobials were *M. morganii, Citrobacter* spp., *S. aureus* and *P. aeruginosa;* two strains of *C. freundii* were found to produce Extended Spectrum Beta-Lactamase. Some similarities with another study, conducted on *C. caretta* in southern Italy [4], suggest similar selective pressures provoked by the use of antimicrobials and induce concerns about the dissemination of resistance in marine wildlife. In conclusion, this study highlights the role of sea turtles as carriers of potential zoonotic agents and as sentinels of their ecosystem.

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CHLAMYDIOSIS IN ORNAMENTAL CHICKENS (*Gallus gallus*) IN ITALY

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Until recently, *Chlamydia psittaci* was considered to be the only aetiological agent of avian chlamydiosis, but two new avian species, *Chlamydia avium* and *Chlamydia gallinacea*, have recently been described, with *C. gallinacea* most frequently detected in poultry. The aim of this study was to explore the occurrence of *C. psittaci* and *C. gallinacea* in ornamental chickens in Italy. Cloacal swabs were taken from 160 asymptomatic ornamental chickens reared in 16 family farms. Samples were tested by a *Chlamydiaceae*-specific real-time polymerase chain reaction (rt-PCR) targeting a region of the 23S rRNA gene conserved among all *Chlamydiaceae* [1]. Samples with Ct values <40 were considered positive and reanalyzed by a *C. psittaci*-specific rt-PCR targeting the incA gene [2] and with enoA-based rt-PCR for *C. gallinacea* [3]. The ompA gene of *C. psittaci* or *C. gallinacea* positive samples was amplified [4] and sequenced, to evaluate the percentage of intraspecies nucleotide similarity. Twenty-four of the 160 (15%) samples from chickens reared in nine farms, were *C. gallinacea*-positive. Then, 13 chickens from the two farms where a higher number of chickens tested positive, were sampled to attempt chlamydial isolation, obtaining eight *C. gallinacea* and one *C. psittaci* isolates. *C. gallinacea* was confirmed to be the endemic chlamydial species in chickens. A high intraspecies diversity was detected, with 12 different *C. gallinacea* sequence types. The isolation of *C. psittaci* and the detection of *C. gallinacea* circulating in backyard farms pose a public health problem. Unlike *C. psittaci*, the zoonotic potential of *C. gallinacea* has been suggested, but not confirmed, until now. However, mainly before the common use of molecular assays, the diagnosis of some human cases of chlamydiosis could be been stopped at genus level, disregarding other potential etiological agents. Breeding of ornamental chickens might expose the farmers to zoonotic risks because of the close farmer-animal contact. Moreover, whereas the principles and practices of on-farm biosecurity may be familiar to commercial farmers, hobbyists and backyard farmers may not be aware of the steps required to keep infectious diseases.

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Campylobacter coli AND PET BIRDS

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The incidence of campylobacteriosis have increased in both developed and developing countries over the last 10 years [1]. Several avian species are considered the main reservoirs of *Campylobacter* spp. [2,3]. Nevertheless, current scientific knowledge on the presence of *Campylobacter* spp. in pet birds is scarce. To address this lack of information, the present study was undertaken with the aim to evaluate the presence of these microorganisms in pet birds bred in southern Italy. To achieve this goal, 14 bird farms located in the Campania region (southern Italy) were visited. In each farm, bird farm population ranged between 20 to 100 animals belonging to the families of *Estrildidae, Fringillidae* and *Psittacidae*, from which 33, 28, and 27 pooled faecal samples were collected respectively. Specifically, eighty-eight cages housing a total of 225 captive birds were examined. The cage was used as an epidemiological unit, and each cage housed from 1 to 5 birds. Each sample was stored in Amies Charcoal Transport Medium at 4°C, transported to the laboratory, and analyzed within 2 h of collection by cultural and molecular methods. A total of 12/88 (13.6%) cages were positive for *Campylobacter* spp. which was identified as *C. coli*. In particular, 7/33 (21.2%) cages came from *Estrildidae* and 5/27 (18.5%) cages came from *Psittacidae* family. The twenty-eight cages coming from *Fringillidae* family were consistently negative. our results demonstrate that *C. coli* may be found in the intestines of apparently healthy pet birds which could be considered as a further potential carrier of *C. coli* for humans and other companion animals. The adoption of good hygiene practices when handling pet birds should be, therefore, promoted.

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COMPARISON OF DIFFERENT METHODS FOR THE IDENTIFICATION OF *Staphylococcus pseudintermedius* AND *Staphylococcus aureus* STRAINS OF ANIMAL ORIGIN

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The increasing interest for staphylococci in Veterinary medicine highlights the need to have an accurate bacterial identification. Furthermore, the control of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) [1] requires a dual approach aimed at reducing antimicrobial consumption and preventing transmission between animals and from animals to humans or viceversa. In this study, 24 clinical isolates were firstly identified as "Staphylococcus aureus/Staphylococcus intermedius if of animal origin" by API Staph method. Later, this identification was compared to Vitek 2 and Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) identification methods. Molecular profiling, using the species-specific nuc genes [2], identified 14 isolates as *S. pseudintermedius* and 10 isolates as *S. aureus* confirming the MALDI-TOF-MS results. Moreover, genetic profiles of methicillin resistance were also carried out by PCR to assess the detection of mecA and mecC genes in all the identified isolates [3]. Precisely, 21% of *S. pseudintermedius* and 10% of *S. aureus* isolates harboured mecA gene, whereas none of the isolates revealed mecC gene. We conclude that MALDI-TOF-MS method is the most accurate method for the identification of *Staphylococcus pseudintermedius* and *Staphylococcus aureus*, that should be used in the veterinary routine clinical practice for a proper bacterial identification. The current investigation also highlights a higher presence of methicillin resistance among *S. pseudintermedius* than *S. aureus* strains of animal origin.

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ONE HEALTH: COMPARISON BETWEEN ANTIBIOTIC-RESISTANT *E. coli* STRAINS FROM CANINE AND HUMAN UTI (2014-2016)

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Urinary Tract Infections (UTIs) are one of the most commonly encountered bacterial infections both in small animals and in human patients [1, 2]. *Escherichia coli* is responsible, alone, for 30-50% of canine UTIs and until 80% for uncomplicated human UTIs [1, 2]. The emerging problem with multidrug resistant bacteria and the possibility of a zoonotic transmission of genes encoding for antibiotic-resistance have increased awareness of antibiotic use and misuse in pets [3]. The aim of this study was to compare bacterial isolation rates between canine and human urine samples and to compare antibiotic-resistance profiles in *E. coli* strains isolated in both species. Bacterial isolation and identification, both in 304 examinated dogs (Veterinary School - Università degli Studi di Milano) and in 12,839 human urine samples (San Paolo Hospital, Milano) was performed using microbiological standard methods. Bacterial isolation was performed by streaking a known amount of urine (10-200 microliters) on blood agar plates (TSA containing 5% of sheep blood, Microbiol, Italy); the incubation parameters were 24 h at 37°C under aerobic conditions. Isolates were identified by morphology, Gram stain, catalase and oxidase activities, growth on selective media and, sometimes, using a commercial identification kit. The strains were tested for susceptibility to different antimicrobial molecules by the disc diffusion method (Kirby-Bauer sensitivity test); after incubation at 37°C overnight, the result was interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute (reference #4 is referred to veterinary pathogens) [4]. Positive urinocultures achieved 25-30% for all the samples (canine and human) and *E. coli* was the most isolated bacterium, with a lower prevalence in canine samples than in human ones (44% vs 56%). The antimicrobial susceptibility of canine *E. coli* showed higher resistance to amoxicillin-clavulanic acid (53%), third generation cephalosporins (52%), nitrofurantoin (32%). Thirteen Strains were Multidrug Resistant (MDR) and among these 5 were Extensive Drug Resistant (XDR). In humans, *E. coli* strains showed higher resistance to ampicillin (64%), fluoroquinoles (42.5%) and cotrimoxazole (35%). In this study we evidenced important rates of antibiotic-resistance in *E. coli* strains from dogs more than in human ones. Particularly, it is alarming the appearance of MDR and XDR canine strains.

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MONITORING OF BOVINE VIRAL DIARRHOEA VIRUS INFECTION IN WILD RUMINANTS AND IN CATTLE BY PCR ON FAECAL SAMPLES: A PRELIMINARY STUDY

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Bovine Viral Diarrhoea Virus (BVDV) is a Pestivirus responsible for severe economic losses in cattle farms and it has also been discovered in some wild ruminants. Blood, milk, saliva, ear notch and tissue samples are usually used for diagnosis of BVDV infection in cattle [1]. However, these samples could be not easily collected when wild ruminants or beef cattle are tested.
The aim of this work was to investigate if BVDV can be detected by PCR in fecal samples from wild ruminants and cattle.
Fecal samples (n=60) were collected in 2 bovine farms with a history of seropositivity for BVDV-1 (A, n=40; B, n=20). In addition, faecal samples from red deer (*Cervus elaphus*, n=16), Appennine chamois (*Rupicapra pyrenaica ornata*, n=13), fallow deer (*Dama dama*, n=6), and roe deer (*Capreolus capreolus*, n=5) were collected from the environment. RNA was obtained from pool samples (10 individual samples = 1 pool) of bovine faeces and from individual samples from wild ruminants. Aliquots of faecal samples were stored at 4°C and tested after 3 months. Real time PCR for Pestivirus [2,3], nested PCR for BVDV [4] and PCR for 5'-UTR sequencing [5] were carried out.
BVDV-1 was detected in all 4 pool samples from cattle in farm A, in 4 samples from red deer, in 3 samples from chamois and in one sample from fallow deer. Positive results were obtained also by the faecal samples stored and tested after 3 months. The sequences obtained showed highest identity with BVDV-1 types 1a and 1c.
These preliminary findings suggest that faecal samples can be used for monitoring the molecular epidemiology of BVDV-1 in cattle and in wild ruminants. This approach allows to perform diagnosis of BVDV-1 infection in situations where is not possible to catch nor to kill the animals, such as in protected environments.
Further investigation are required for evaluating the sensitivity of the method and for detecting BVDV-1 types infecting wild ruminants in Italy.

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MOLECULAR SURVEY OF *Mycobacterium tuberculosis* IN WATER BUFFALO HERDS OF THE CAMPANIA REGION

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Bovine tuberculosis is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. It is a member of the 'tuberculosis complex' along with *M. tuberculosis, M. africanum, M. microti, M. pinnipedii* and *M. caprae*. In many countries bovine tuberculosis is a major infectious disease among cattle, other livestock species and certain wildlife populations. Among domestic ruminants, water buffalo can also be affected mainly by *M. bovis* and *M. caprae*. In Southern Italy tuberculosis has not yet been eradicated and there is an increase of new cases. After death, infection is diagnosed by histopathological and bacteriological techniques. Mycobacterial culture is the gold standard method for confirmation of infection but it requires at least 8 weeks to get bacterial growth. So rapid nucleic acid methodologies, such as the polymerase chain reaction (PCR), may support the classical microbiology providing results in shorter time [1]. We investigated the presence of *Mycobacterium tuberculosis* complex (MtbC) in water buffaloes breed in the Campania region. We carried out a molecular screening on 177 water buffalo lymph nodes for the detection of MtbC within the Regional plan for the control of TB. Lymph nodes were collected from different body sites of the same animal during necropsy and processed both by classical microbiological method [1] and molecular technique. DNA was extracted from homogenized tissues and the insertion sequence IS6110 was amplified by real-time PCR [2,3,4]. Among the analyzed samples, 9 gave positive results both to the microbiological technique and the molecular analysis. Two samples allowed the isolation of *Mycobacterium* but resulted negative to the Real-Time PCR detection of MtbC. Molecular analysis gave no false positive results among all the analyzed samples. We also carried out end point PCRs [5,6] on samples from MGIT broth culture to identify *Mycobacterium* species. These PCRs allow the identification of both species belonging to the MtbC and *M. avium*. The presence of MtbC was confirmed in all the positive broth cultures analyzed and in one case we were able to identify the species represented by *M. bovis*. *M. avium* was never detected in the analyzed samples. Molecular methods for detection and characterization of MtbC proved to be highly effective screening tools supporting the microbiological technique within the plan for the water buffalo TB eradication in the Campania Region.

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REDUCTION OF *Salmonella typhimurium* MONOPHASIC VARIANT SHEDDING BY ADMINISTRATION OF ORGANIC ACIDS AND PHYTOCHEMICAL IN PIGS

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Salmonella Typhimurium (including *S. Typhimurium* 1,4,[5],12:i-), associated to other enteric pathogens and to other stressful factors, causes the Post Weaning Diarrhea in piglets. This disease is responsible for economic losses, for development of chronic infection and, thus, for the introduction of zoonotic bacteria into the slaughterhouse [1]. It is largely demonstrated the importance of controlling infection in farm for preventing disease in humans [2]. Furthermore, the increment of antibiotic resistance strains determines the necessity to find new control strategies, as administration of vaccines [3] or organic acids [4]. The aim of this trial was to assess the effects of organic acids and phytochemicals on controlling *S. Typhimurium* 1,4,[5],12:i- shedding.

Animals were divided in three groups. In group A (460 piglets), animals were feed with a base diet added of additive (organic acids and phytochemicals) at the concentration of 1kg/t. In group B (460 piglets), additive was added to the water at the final concentration useful to obtain a final pH value equal to 4.5 in relation to water hardness. The last group (460 piglets) was the control group. Microbiological analyses were conducted on feces and environmental swabs to evaluate *Salmonella*-shedding in a representative number of animals (15 ear-tagged piglets) of each group. The sample timing was: a week before arrival (T0) and at day 0 (T1), 15 (T2), 22 (T3), 43 (T4) and 57 (T5) after arrival. Serum samples were collected a week before arrival (T0) and at day 0 (T1), 15 (T2), 43 (T4) and 57 (T5) after arrival to evaluate antibody titers against *Salmonella* sp.

Results demonstrated that environmental swabs were positive before piglets arrival and after cleaning procedures. The shedding evidenced that animals were negative from breeding farm and started to shed *Salmonella* since day 15 post weaning. In treated animals (groups A and B), the shedding rapidly reduced and difference with control group was statistically significant at T5. The antibody titers reached the peak at the end of the trial, when bacterial load was extremely low especially in treated groups (A and B).

In conclusion, all pens were contaminated with *S. Typhimurium* 1,4,[5],12:i- and animals generally got infected at post weaning, one week after their arrive. Probably, the reason is that site 2 is a re-adapted farm which has severe structural problems. Nevertheless, the shedding rapidly decreased in treated groups (A and B) and bacteria load in feces was statistically different in comparison to control group at T5.

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ANTIMICROBIC - RESISTANCE IN *E. coli* ISOLATED FROM SHEEP: PRELIMINARY DATA

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Antimicrobial-resistance is a major public health problem, but there are no so many studies about its spread in sheeps. The province of Macerata (Italy, AREA VASTA 3) is particularly dedicated to sheep farming, so the monitoring of the antimicrobial resistance prevalence in this species is crucial. Thirteen farms were included in the study, 20 rectal swabs were made on clinically healthy animals in each farm. The swabs were submetted to bacteriological examination and *E. coli* eventually isolated were identified with RapiD 20 E (bioMérieux). Colistin sensitivity of all strains was tested through diffusion agar technique and the resistant ones or with intermediate susceptibility were tested with further 10 antibiotics. The 17 strains with lower susceptibility to colistin were also tested in MIC and submitted to the research of mcr-1 gene in PCR. Overall, 164 *E. coli* were isolated, among the early 59 resistant or partially susceptible to the colistin, only 4 (6.8%) showed intermediate susceptibility to colistin again, 31 (52.6%) were resistant and 16 (27.1%) partially susceptible for cephalothin. The 17 strains with lower susceptibility to colistin, were all susceptible to colistin in MIC and they were devoid of mcr-1 gene. The strains of *E. coli* isolated in the only biological farm in which roaming pasture is not carried out were susceptible to all tested antibiotics. Excluding cephalothin, 7 strains (11.9%) showed resistance to at least one antibiotic, value which is almost triple compared to previous studies (1). According to other publications (2), the spreading agar technique does not seems to be the correct tool to evaluate resistance to colistin, since the same strains tested twice with Kirby-Bauer tecnique showed different susceptibility and they were all susceptible in MIC. However, unlike other essays (2), the agar diffusion tecnique overestimate the resistance to colistin, compared to the MIC. Although other authors suggest to use ampicillin as a prototype molecule for amoxicillin + clavulanic acid, there is no perfect overlapping of the antibiotic resistance profile: 3 ampicillin-resistant strains and all susceptible to amoxicillin + clavulanic acid. EUCAST discourage the use of cefazoline in the antibiogram for Enterobacteriaceae. In this study, the cephalothin which is the same a first-generation cephalosporin, was used and the poor susceptibility to this molecule confirms the EUCAST indications. The farm owning pastures has no resistance to antimicrobials. This could suggest that rambling pasture is a risk factor for antimicrobial-resistance onset in a flock. Finally, it would be desirable to check the antimicrobilal treatments administered to the animals in the last years and the purchase of animals from other farms. In addition it would be useful to include in the study, farms with intensive production systems too.

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Malattie parassitarie

HARD TICKS INFESTATION OF DOGS AND CATS IN NORTHERN ITALY

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Changes in the endemic foci of tick populations and invasions of tick species to new areas have become evident in Europe, leading to changes in the epidemiology of tick-transmitted diseases. However, data about tick infestations of pet animals are limited in Northern Italy. The occurrence of tick vectors in dogs and cats was investigated by using a veterinarian based sampling strategy. From March 2015 to April 2017, 291 hard ticks infesting 80 dogs and 21 cats were collected by veterinarians at clinical examination and subsequently identified by morphological features in the Laboratory of Parasitology of DIMEVET (Department of Veterinary Medicine, University of Milan). Dogs and cats acquired tick infestation in 12 different provinces sited in 4 four Regions of Northern Italy (Piedmont, Liguria, Lombardy, Emilia-Romagna). Four species of hard ticks were identified: *Rhipicephalus sanguineus* s. l. (N=191, 65.6%), *Ixodes ricinus* (N=72, 24.7%), *Ixodes hexagonus* (N=21, 7.2%) and *Dermacentor reticulatus* (N=7, 2.4%). Ticks were mainly adults (259/291, 89.0%), followed by nymphs (29/291, 10.0%) and larvae (3/291, 1.0%); nymphs belonged to *R. sanguineus* (13/29, 44.8%), *I. ricinus* (4/29, 13.8%) and *I. hexagonus* (12/29, 41.4%), while larvae were exclusively *I. hexagonus*. *I. ricinus* were collected both from dogs and cats while *R. sanguineus* and *D. reticulatus* were collected exclusively from dogs; *I. hexagonus* feed only on cats. During the study period, hard ticks fed on domestic carnivores in 11/12 months (no ticks were found in November); from cats, no ticks were collected also in February, October and December. *R. sanguineus* was found on dogs from March to September (7/12 months); during the study period the average number of *R. sanguineus* collected was: 1 in March, 13 in April, 50 in May, 4.5 in June, 4 in July, 0.5 in August and 0.5 in September. *I. ricinus* fed on domestic carnivores in 11/12 months; the average number of *I. ricinus* collected was: 1 in January, 1 in February, 2 in March, 4 in April, 9 in May, 7.5 in June, 0.5 in July, 5.5 in August, 1 in September, 1 in October and 0.5 in December. *I. hexagonus* was found on cats in January, April, June and July; the average number of ticks collected in each month was 0.5, 3.3, 3 and 2 respectively. *D. reticulatus* fed on dogs in February, April and May; the average number of ticks in each month was 0.5, 1.3 and 1 respectively. Results of this acarological investigation showed that dogs and cats in Northern Italy are exposed to tick bites in all seasons of the year and at least 4 different species are involved in infestations. Pharmacological prophylaxis against ticks should be prescribed taking in account not only pets' life styles but also effectiveness against detected species and their presence in different periods of the year. In addition, all the four identified species fed not only on domestic carnivores but also on humans [1, 2], so the same risk for tick bites observed in domestic carnivores throughout the year exist also for people.

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A SEROLOGICAL STUDY OF EXPOSURE TO TICK-BORNE PATHOGENS IN DOGS FROM NORTHERN ITALY

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The distribution of tick-borne pathogens and their ticks vector has increased in many parts of the world, including Europe [1]. Dogs are exposed to several tick-borne pathogens and may act as reservoir hosts for human vector-transmitted infectious agents. Since the close relationship between humans and their pets, dogs are considered as sentinel animals to assess the risk of human infection, then regional exposure patterns can be readily established through epidemiological investigations using dog sera. The aim of the study was to determine the exposure to five selected tick-borne pathogens, *Babesia canis*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Rickettsia conorii* and *Borrelia burgdorferi* and the risk factors associated with the seropositivity in dogs in northern Italy.

From March 2015 to January 2016 blood samples were collected from 303 healthy dogs randomly chosen from veterinary clinics and kennels located in Lombardy region. Dog serum samples were analysed with a commercial IFAT (MegaScreen®FLUO) which detect IgG antibodies against the tick-borne pathogens. A questionnaire administered to veterinarians provided data on individual history of dogs. Generalised linear and multivariate models were performed to evaluate risk factors statistically associated to the serological positivity (SPSS v.20).

One hundred and fifty-five dogs (51.2%) were positive for at least one pathogen; *A. phagocytophilum* (26.1%) had the highest seroprevalence followed by *E. canis* (22.1%), *R. conorii* (22.1%), *B. burgdorferi* (13.9%) and *B. canis* (9.5%). Out of 155 positive dogs, 87 had antibodies against more than one pathogen. Risk factors significantly related to seropositivity were the regular walking in a large green area (OR=16.638), and the mixed breed (OR=0.55). The use of acaricide products among the prophylactic measures throughout the year was identified as a protective factor (OR=0.348).

The study highlighted the exposure of selected arthropod borne pathogens in dogs in northern Italy. Particularly, these dogs were frequently exposed to *A. phagocytophilum*, *E. canis* and *R. conorii*. In contrast, there was infrequent exposure to *B. canis*. The association of dog seropositivity with the walking in a green area (Groane Regional Park) could be related to a foci of *Dermacentor reticulatus* ticks in this periurban park. *D. reticulatus* is a main vector of *B. canis* and can also transmit *Rickettsia raoultii* and *A. phagocytophilum* [2]. Further, the highest seropositivity in dogs of mixed breeding could be explained by the presence in this groups of kennelled dogs from southern Italy, an area where high seropositivity to tick-borne pathogens are usually recorded. Finally, the use of acaricides throughout the year seems reduce the risk of infection in contrast to the administration of the drugs only in the tick activity season; it should be a consequence of the presence in the study area of *D. reticulatus* which adult stages are active also in winter.

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A REGION-WIDE SURVEY IN AOSTA VALLEY FOR TICK PRESENCE AND TICK-BORNE DISEASES

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Ticks are important vectors of many viruses, bacteria and protozoa that can cause serious infections in humans and animals. Parasites of the Genera *Babesia*, *Theileria*, *Anaplasma* and *Ehrlichia* are emerging tick-borne pathogens [1]. The rapidly changing epidemiology of vector-borne diseases is becoming a global public health/veterinary concern that needs active surveillance [2]. The aim of this study was to survey the distribution of ticks in Valle d'Aosta (Northwestern Italy) and to analyze the collected ticks for the presence of *Babesia* spp., *Theileria* spp., *Anaplasma* and *Ehrlichia* spp. A total of 368 ticks were collected by environmental dragging between May and December 2016 in 34 different areas. Each sampled area had specific characteristics of altitude, solar exposure and land cover. Tick were morphologically identified and then pooled together depending on species, life-cycle stage and area of detection. Eighty-six pools were formed and analyzed. Total genomic DNA was extracted from pooled ticks and tested by PCR for the presence of protozoal and bacterial pathogens [3, 4]. Two tick species were identified: *Ixodes ricinus* (59 adults, 292 nymphs, 15 larvae) and *I. hexagonus* (2 adults). The results had shown that 13 (P=15.12; CI95% 9.05% - 24.16%) samples were positive for *Babesia* spp / *Theileria* spp and 17 (P=19.77%; CI95% 12.72% - 29.40%) samples were positive for *Ehrlichia* spp/ *Anaplasma* spp. Our results indicate that *I. ricinus* is the most abundant species in Valle d'Aosta and that ticks are commonly founded in woods and shadowed areas. Our findings extend the knowledge of the tick's geographical distribution and support the conclusion that vector is important to the transmission of pathogenic micro-organisms in urban and suburban areas. Moreover, tick coinfection with multiple pathogens was found to occur frequently, which poses a serious challenge for diagnosis and appropriate treatment.

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A CASE OF BOVINE TRYPANOSOMIASIS CAUSED BY *Trypanosoma theileri* IN SICILY

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Trypanosoma spp is an unicellular parasitic flagellate protozoan. In Europe, *T. theileri* has been described in Ireland, Scotland, England, Belgium, Germany, Poland, France, Spain. Data on its occurrence in italian cattle is not well documented. However, two cases occurred cattle farmed in Sicily were described in 2000. The life cycle of *T. theileri* is not fully understood, but transmission occurs by the bite of vectors belonging to the *Haematopota, Hybomitra, Tabanus* and *Culicoides* genera, *Hyalomma anatolicum anatolicum* ticks and probably also the *Ixodes* genus. *T. theileri* is usually considered a non-pathogenic species, although some clinical signs have been described in cattle population. These includes anemia, fever, weight loss, abnormal behaviour, leucocytosis, mainly within lymphocytes B, abortion, neonatal death, difficulty in milking. Moreover, it was supposed to be an etiological agent of the suppurative encephalitis and cerebrospinal meningitis. In this work a case of *Trypanosoma theileri* infestation in cattle in Sicily is described. The presence of live, mature and immature parasitic forms within cell cultures was observed after collection of hematopoietic cells from the bone marrow of a crossbreed bovine female of about 3 year old reared in a farm in the province of Messina (Sicily). In order to identify the parasite, a Semi-nested PCR, amplifying a 600 bp fragment of the 18S region, was performed as described by Odongo et al. (1), while the animal was subjected to a clinical examination. Complete blood count (CBC) and culture were carried out on whole blood; PCR and anatomo-histological examination were performed on organs (spleen, liver, brain, lymph nodes) collected after slaughtering. The same sampling and analysis were carried out in a second animal, a young adult male reared in the same farm. The clinical examination showed only a growth delay. The CBC revealed, in both cases, a slight leukocytosis with an increase in both lymphocytes and neutrophils. The total number of red blood cells were within the range. However, the mean corpuscular volume was reduced, probably in relation to a regenerative process as it was supported by the slight increase of spleen observed at the gross examination and the histological detection of a proliferative activity of the white and red splenic pulp. No other macro and microscopic alterations were detected. Sequencing analysis of the 18 S segment, identified the parasite as the flagellated protozoan *Trypanosoma theileri*. Affected ruminants infected by this species of *Trypanosoma* are generally asymptomatic even in the most severe cases of infestation. However, the unusual finding of its DNA in brain, testis and bone marrow raises new questions about the long-term clinical evolution of this parasitosis and it's possible sexual transmission. This is the second report of the presence of this trypanosomiasis in Sicily. However further epidemiological investigations should be carried out in order to clarify the true prevalence of this parasite and the vectors involved in its spread and maintenance.

S. Odongo, V. Delespaux, M. Ngotho, S.M. Bekkele, S. Magez. Comparative evaluation of the nested ITS PCR against the 18S PCR-RFLP in a survey of bovine trypanosomiasis in Kwale County, Kenya. *Journal of Veterinary Diagnostic Investigation*. 2016, Vol. 28(5), 589-594.

DETECTION OF *Streptococcus equi* DNA IN *Rhipicephalus bursa* TICKS FROM SOUTHERN ITALY

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Ticks are obligated ecto-parasites of wild and domestic animals and occasionally humans. They have veterinary interest because vectors of different viruses, bacteria and protozoa. Among the most common species in Italy and Europe there are *Ixodes ricinus* and *Rhipicephalus* spp. The tick-borne diseases epidemiologically relevant in our country are: Lyme disease, rickettsiosis, relapsing fever, tularemia, meningoencephalitis and ehrlichiosis.

Recently, the extensive characterization of the tick-associated microbiome faces the possible role of ticks in the transmission of additional pathogens, which are known to be transmitted by other arthropod vectors.

Through the Denaturing Gradient Gel Electrophoresis (DGGE), we analysed the bacterial community of seven ticks belonging to three species from different areas of Italy: no. 2 *Rhipicephalus sanguineus* (Lombardia), no. 2 *Rhipicephalus bursa* (Campania) and no. 3 *Ixodes ricinus* (Marche). The samples were analysed selecting the highly variable V3 region of the bacterial 16S rRNA gene as target. The dominant DGGE bands were purified and sequenced allowing the identification of the bacteria present in each samples.

Burkholderia sp., *Coxiella*-like endosymbiont, unc. *Clostridium*, *Rickettsia peacockii*, unc. *Staphylococcus* and *Xanthomonas* sp. were detected, as already described in ticks. Moreover, *Streptococcus equi* subsp. *zooepidemicus/ruminatorum* was identified in *R. bursa* collected from buffalos living in rural areas of Southern Italy (the high genetic identity does not allow the distinction of these subspecies, that is usually achieved by biochemical tests on cultured bacteria). The DGGE outcome was confirmed by multiplex-PCR in other *R. bursa* samples collected from buffalos, ponies and goats from the same area. In particular, no. 6 positive samples over no. 15 analyzed were detected (no. 2 from buffalos and no. 4 from ponies).

S. equi is a complex-species including *S. e. equi*, *S. e. zooepidemicus* and *S. e. ruminatorum*: *S. e. equi* is the ethological agent of strangles, a highly contagious and serious infection of horses, whereas *S. e. zooepidemicus* and *S. e. ruminatorum* can cause several infections in both animals and humans. All these pathogens are known to be transmitted through direct contact.

The DNA detection of *S. e. zooepidemicus/ruminatorum* in *R. bursa* is particularly interesting since to our knowledge this pathogen was not previously detected in any tick species. Thus, in-depth studies are worthy to assess an effective competence of ticks as vectors of *S. equi* subspecies, since if a potential role of *R. bursa* in their transmission will be confirmed, new perspectives in the control of these zoonoses will be opened.

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COLLABORATING WITH A ZOO-SAFARI: A PARASITOLOGICAL CHALLENGE

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Zoos are an ex-situ form of conservation were animals are breed in confined areas for recreational exhibition, educational or research and conservation purposes. Parasites and infectious diseases have become a major concern in conservation of endangered species as they can lead to mortality, dramatic population declines and even contribute to local extinction events. Some studies have showed that gastrointestinal parasites of wild animals in captivity included zoonotic species and rise public health concerns. According to Italian law (D.Lgs 73/2005), zoos housing domestic, wild and exotic animals are required to address both animal health and welfare. The last two words are suggestive for parasites control mainly in a more extensive space where many animals of different species share the same environment as in safari park. The study was carried out to know the prevalence of gastrointestinal parasites of captive animals at Zoo Safari Ravenna in the Emilia-Romagna region. Fecal samples were collected in the biennium 2014/2016. The first year were recovered 94 samples, the second one 99. The seemingly low number of samples is due to the difficulties of getting the fecal material by a well known individual subject. In fact, we considered of little interest to analyze a fecal pool, much easier to get but poorly indicative; in this safari park many animals are not confined to a specific area but are free to move throughout the park area. The totality of fecal samples were screened using classical qualitative and quantitative analyzes. The species were grouped by theriological affinity in 3 groups: poligastric (18 species) and monogastric (4 species) herbivores and carnivores (2 species). In the first year, out of 94 fecal samples examined, 70 were positive for parasitic eggs/oocysts of different species indicating an overall prevalence of 74.5%, of which 4/17 carnivores 23.5%, 54/59 (91.5%) poligastric and 12/18 (66.6%) monogastric herbivores. The second year, out of 99 samples 56 were positive with a prevalence of 56.6% for gastrointestinal parasites, 6/16 (37.5%) among carnivores, 40/65 (61.5%) ruminants and finally 10/18 (55.5%) monogastric herbivores. Parasites recovered were: coccidia; *Toxascaris leonina* and *Parascaris equorum*; gastrointestinal strongyles and *Nematodirus* spp.; *Capillaria* spp. and *Trichuris* spp. McMaster examination showed an aggregate distribution (high proportion of parasite is concentrated in a few host individuals) of parasitic fauna inside the area. The Chi-square test revealed a significant difference ($\chi 2=6.05$; $P<0.05$) between the overall prevalence of two considered periods. We like to think that this result may be due to our surveillance activities. This experience fit into poorly investigated context. If a significance of our challenge must be find, this is the continuous involvement of veterinarians that laid the foundations for a more strictly collaboration between Zoos and University. Proves it the publication of a scientific paper [1] and an amazing skeleton that now enriches the "Department of Veterinary Medical Science Anatomy Museum", realized with a dead wallaby.

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STUDY OF BACTERIAL AND FUNGAL POPULATIONS IN LOGGERHEAD SEA TURTLE (*Caretta caretta*) EGGS FROM EASTERN SICILY COASTLINE

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Caretta caretta is the most common species of turtle living in the Mediterranean basin, however dangers of various nature threaten them. Despite the increasing reports of nesting sites in the Mediterranea Sea, sea turtles are still considered from the IUCN a least concern species.
The purpose of this research was to investigate the occurrence of pathogen microorganisms in *C. caretta* eggs in nests from Eastern Sicily shores.
During 2016, 272 eggs, 7 dead newborns and 12 sand specimens collected from three different nests were analyzed to the *Centro di Referenza Nazionale sul benessere, monitoraggio e diagnostica delle malattie delle tartarughe marine*, at the *Istituto Zooprofilattico Sperimentale della Sicilia*. For the bacteriological and mycological analysis, both hatched and unhatched eggs were pooled in groups from 5 to 7 based on their location in the nest obtaining 11 pools of hatched eggs and 17 pools of unhatched eggs. Bacteriological analysis was performed using Alkalin Pepton Water broth (APW) incubated at 25°C for 24-48 h, then each pool was seeded on Blood agar and in selective and differential Agar. Isolated bacterial colonies were identified macroscopically and biochemically with API System. Fungal culture, achieved on Sabouraud dextrose agar, was firstly observed by optical microscope and secondly the PCR and ITS sequencing was used to confirm the results.
No bacteria have grown except for *Aeromonas hydrophila* (5.63%) which was isolated from both the eggs and the sand of only one nest. Molecular analysis confirm the presence of *Fusarium solani* (15.44%) and *Fusarium oxysporum* (13.60%) from both eggs and dead newborn specimens. *Aspergillus fumigatus* (8.08%) and *Aspergillus flavus* (6.98%) were isolated from all the type specimens including sand.
This study would indicates the presence of *F. solani* and *F. oxysporum* as a cause of the hatching failure of sea turtle eggs accordingly with previous reports. The occurrence of *Fusarium* spp. could not be due to environmental contamination, given the absence of the fungus in sand samples. In the next future, a large scale study including wider areas could significantly contribute to better understand the causes of threatening for *C. caretta* at their first stage of development.

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VETERINARY ACTIVITIES OF WILDLIFE RESCUE AND REHABILITATION CENTER OF NAPOLI DURING 2016

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Wildlife Rescue and Rehabilitation Center (CRAS) Federico II of Naples is a non-profit center that gives wildlife a second chance at life. It was established by the Executive Decree n. 94 of 06.05.2010. As for other CRAS, the goal of this center is to reintroduce in nature, whenever possible, wildlife that has undergone medical care and rehabilitation in the Center [1].
The aim of this study was to describe the main reasons of hospitalization and the related follow-up results. A detailed analysis of the wild animals' histories arrived at the CRAS Federico II in 2016 was performed. A total of 1682 animals were admitted to this CRAS. In particular, 1584 belonged to various species of birds, 32 were reptiles, and 66 were mammals. Among the birds, 326 were birds of prey and 538 were passerines. Most hospitalizations occurred in the months of June and July. Trauma was most frequently diagnosed in these animals and occurred in 38% of the animals, 22% of animals were admitted because were immature, 21% were sequestered animals, 10% were medical cases and 9% were admitted for other causes. With reference to the final outcome: 58% of the animals were released, 11% and 25 % had to be euthanized or died respectively, 4% is still hospitalized and 2% was entrusted. In this CRAS, epidemiological investigations on infectious and parasitological agents, especially those with a possible zoonotic impact, were also conducted. In this context, 148 intestinal contents were collected from birds of prey carcasses to isolate *Campylobacter* spp., *E. coli*, and *Salmonella* spp. which showed a prevalence of 33.1%, 6.8% , and 6.8%, respectively. Furthermore, 145 samples of faeces were collected from animals admitted to the center to detect endoparasites showing a positivity of 68% for endoparasites mainly represented by *Capillaria* spp., *Centrorhynchus* spp., Ascarididae and Eimeriidae families. We may conclude that a CRAS plays an essential role on an environment, because the wildlife may act as sentinels of ecosystem health in order to supply significant information about the health status of the natural environment where animals live, which is itself connected to public health issues.

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ANALYSIS OF THE ROLE OF THE GOLDEN JACKAL *Canis aureus* IN THE EPIDEMIOLOGY OF CARDIO-PULMONARY DIROFILARIASIS IN SERBIA

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Cardio-pulmonary dirofilariasis is a vector-borne disease sustained by a nematode named *Dirofilaria immitis*, or heartworm. It is transmitted by mosquitoes of the genuses *Culex, Aedes, Anopheles, Culiseta spp.*, and the adult parasite can be found in the pulmonary arteries and right heart chambers. Dirofilariasis in Serbia is still widespread with prevalences of 12.3% in the canine population (Tasić *et al.,* 2012), but there are still few studies on the role of wild canids; i.e. Penezić *et al*., 2014 who determined a mean prevalence of 7.3% in the golden jackal from 2009 to 2013. This work therefore aims to better determine the epidemiological role of the golden jackal *Canis aureus* by analysing 64 infected individuals which were legally hunted, spanning 2009 to 2017, and recovering all possible parasites through necropsy. The worms were preserved in 96% alcohol, and the following data was collected: parasitic load, no. of male and female heartworms, and length of the females. Also, embryogenic dynamics in the female macrofilariae were analysed, using the embryogram technique as described by Lok *et al*., 1988, only in those samples where the female/s were in the presence of at least one male filaria. The results showed, in the 64 infected jackals, an overall prevalence of 9.6% (IC 95%: 7.3%-11.8%), with a median parasitic load of 2 (range: 1-42), an average female nematode length of 20.8 cm (±5,0), and a percentage of monosexual infestations of 45.3% (IC 95%: 33.1%-57.5%). The embryogenic dynamic analysis resulted in 17.4% (IC 95%: 8.0%-26.8%) normal embryograms (progressive caudo-cranial maturation, with no absent stages or withheld microfilariae). The mean length of normal female filariae was 25.7 (±3.6). We can conclude that, due to the small number of female dirofilariae with normal egg maturation patterns, resulting in a poor capacity of transmitting the disease, the golden jackal is not the most apt host for the parasite, and does not therefore play a major role in this disease's epidemiology.

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FIELD USE OF MINI-FLOTAC TO DIAGNOSE GASTROINTESTINAL PARASITES IN ZOO MAMMALS

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Zoological gardens maintain large and diverse collections of wild and endangered animal species and develop programs to promote the well-being of these species [1]. Parasites have been shown to be a significant cause of morbidity and mortality in mammals kept in zoological collections [2]. The present study was conducted to evaluate the use of Mini-FLOTAC [3] for the first time in zoo mammals kept in four zoological gardens in central and southern Italy. From June to August 2016, 70 fresh composite samples (pools) were collected by Fill-FLOTAC from different mammal species (31 *Artiodactyla*, 9 *Carnivora*, 1 *Chiropthera*, 4 *Diprodotondia*, 1 *Hyracoidea*, 9 *Perissiodactyla*, 10 Primates, 1 *Proboscidea* and 4 *Rodentia*) at the zoological gardens of Aprilia (8 pools), Lanciano (17 pools), Napoli (16 pools) and Pesco Sannita (29 pools). Each pooled faecal sample was thoroughly homogenized and three Fill-FLOTAC devices were filled: two aliquots for the Mini-FLOTAC technique (2 grams each) and one aliquot for the FLOTAC technique (5 grams). The Mini-FLOTAC technique was performed in situ (at the zoo's veterinary department) using a field portable microscope. The other aliquot was transported to the laboratories of the Department of Veterinary Medicine and Animal Productions, University of Naples Federico II and processed using the FLOTAC dual technique. For both Mini-FLOTAC and FLOTAC two different flotation solutions were used: FS2 (Sodium Chloride, specific gravity=1,200) and FS7 (Zinc Sulfate, specific gravity=1,350). Out of the 70 pools analyzed, 29 (41.4%) resulted infected by at least one parasite. Most animals showed mono-parasitic infections (n=27); co-infections were found in 2 samples with 2 different species of helminths and protozoa. The FLOTAC detected a higher number (29/29=100%) of parasitic infections compared to the Mini-FLOTAC (25/29=86.2%); the 4 samples negative with the Mini-FLOTAC showed an EPG values <5, lower than the detection limit of this technique (5 EPG). Considering data obtained with the FLOTAC, as regards geographical location, a higher prevalence of parasites was found in central Italy (Lanciano 58.8% and Aprilia 50%) compared to southern Italy (Pesco Sannita 37.9% and Naples 37.5%). Several genera of helminths and protozoa were detected in mammals at the four zoos. With regard to nematoda, gastrointestinal strongyles were the most frequent (21.4%) followed by *Trichuris* (14.3%), *Parascaris* (5.7%), *Capillaria* (2.9%) and *Nematodirus* (1.4%). To protozoa, most samples were positive to *Blastocystis* (4.3%), followed by *Giardia, Eimeria* and *Entamoeba coli* (1.5%). Our results suggest that Mini-FLOTAC is a user-friendly technique, produces reproducible results, and is particularly useful for monitoring and surveillance, where large numbers of faecal samples must be rapidly, yet reliably, examined; it ensures the high safety of the operators, through the use of the Fill-FLOTAC device that acts as a closed system.

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DETECTION OF SELECTED PATHOGENS IN WILD RODENTS: A COMPARISON BETWEEN ISLAND AND CONTINENTAL ECOSYSTEMS

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Domestic and wild rodents are known as the most abundant and diversified order of mammals and have a key role in the ecological food chain and in the transmission of parasites and pathogens to other animals. There are more than 24 different infectious agents directly or indirectly transmitted by rodents to humans [1]. The aim of this study was to evaluate presence and prevalence of different pathogens in rodents, captured at 2 locations: i) Pianosa island (Italy) an island that has been uninhabited and farming has stopped since 20 years ago and with few wild animal species; ii) Natural Park of Los Alcornocales (Spain) a natural area in which several wild species are present and with climatic conditions similar to Pianosa. Thirty-one rodents (*Rattus rattus*, *Mus* spp.) where captured on the island and 46 were captured at the National Park. Specific PCR protocols to detect *Leishmania infantum*, *Babesia* sp., *Theileria* sp., *Anaplasma* sp., *Ehrlichia* sp., *Toxoplasma gondii* and *Neospora caninum* were applied [2,3,4]. Total genomic DNA from spleen and skeletal muscle was extracted and used as template for PCR. Positive samples were purified and sequenced. Obtained sequences were compared to the ones available in GenBank to confirm pathogen identification. None of the rodents tested positive by PCR for neither *T. gondii* or *N. caninum* while *L. infantum* was detected in 8 rodents from Pianosa (25.81% CI 13.7 - 43.25%) and 17 individuals from the National Park (36.96% CI 24.52 - 51.4%). *Babesia* sp./*Theileria* sp. were detected in 15 rodents from Pianosa (48.39% CI 31.97 – 65.16) and 37 rodents from the National Park (80.43% CI 66.83 - 89.35%). *Anaplasma* /*Ehrlichia* sp. were found in the spleen of 2 rodents from Pianosa (6.45% CI 1.79 - 20.72%) while all rodents from the National Park were negative. Sequencing confirmed the presence of *L. infantum*, *B. divergens*, *B. microti* and *Theileria* sp. in Pianosa island. In the National Park we detected *L. infantum* and *B. capreoli*.
Understanding the role of rodents in the epidemiology of vector-borne diseases and of *T. gondii* and *N. caninum* is a relevant issue for improving disease managment and pathogen control. Comparative studies carried out in ecological contexts were the main reservoir/definitive hosts are nort present can give useful insights on the epidemiology of these parasites.

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EVALUATION OF THE EFFECTS OF COMMERCIAL AND NON-COMMERCIAL SUPPLEMENTS ON *Nosema ceranae* IN *Apis mellifera*

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Nosema ceranae is a microsporidian parasite of honeybees that infects the epithelial layer of ventricolus and midgut of adult *Apis mellifera* bees causing digestive disorders and shortening the bee life span [1]. Transmission of *Nosema ceranae* in honey bee colonies is mainly by the faecal-oral route in which pathogens are spread through fecal contamination by infected hosts to uninfected bees. Adult bees ingest *Nosema ceranae* spores by eating contaminated food and by cleaning up faecal material from infected bees [2]. The aim of the study was to evaluate the effects of commercial and home-made supplements on *N. ceranae* infection pattern in *A. mellifera*. Supplements were added to sucrose syrup and supplied in single dose of 3.5 kg. We analyzed 4 apiaries (for a total number of 232 honey bee colonies known to be infected with *Nosema spp*.) and we divided them in 5 homogeneous groups: three treated with different commercial supplements: Vitafeed Gold®; Nosevit Plus® and Apiherb®; one treated with 5% v/v vinegar (used for sucrose inversion), and one control group (supplied with sucrose syrup only). Honey bee colonies were analyzed after 15 (T1), 30 (T2), 45 (T3) days following the administration of supplements, by taking 50 bees from each colony. For each sample, 20 adult bee's abdomens were mechanically homogenized in 20 ml of 0.9% saline solution and then examined by light microscopy to quantify *Nosema ceranae* spores on a Burker chamber. Generalize Linear Mixed Models were applied to assess the efficacy of each treatment in relation to *N. ceranae* spore load (output variable). The apiary was used as random effect in the model. Results showed a statistically significant reduction in *Nosema ceranae* spores number only in one apiary, in both the groups treated with Apiherb® and vinegar. In conclusion, we observed the efficacy of two supplements in reducing the numbers of *Nosema ceranae* spores. Particularly interesting is the efficacy of the vinegar, a very low cost and easily available supplement that is routinely used for sucrose inversion. More studies must be carried out to understand how infestation of N*osema ceranae* is affected by seasonality, and it would also be tempting to test different vinegar concentration.

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PROTOZOAN PARASITE AND *Anaplasmataceae* INFECTION IN WILD BADGERS: AN EPIDEMIOLOGICAL SURVEY

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The European badger *Meles meles* is extensively distributed and a relatively common throughout Europe [1]. Wildlife and wild carnivores especially, play an essential role in the epidemiology of several vector-borne diseases as well as in maintaining a sylvatic cycle of *Toxoplasma gondii*. The present study was aimed to investigate the occurrence of parasitic protozoa and of bacteria of the Family *Anaplasmataceae*, namely *Leishmania infantum*, *Hepatozoon canis*, *Toxoplasma gondii* and *Anaplasma/Ehrlichia* spp. in wild badgers from Northwestern Italy (Piedmont Region). Total genomic DNA which was extracted from spleen (≈10 mg), skeletal muscle and Central Nervous System (CNS) (≈25 mg) collected from 52 badgers that were road-killed from 2010 to 2017. Specific PCR protocols for *L. infantum*, *Anaplasma/Ehrlichia* spp., *H. canis* and *T. gondii* were used [2,3,4]. A total of 26 [26/48] individuals was found infected with *L. infantum* (P=54.17%, CI95% 40.29 - 67.42%) and 29 tested positive to *Anaplasma/Ehrlichia* spp. PCR (P=60.42%, CI95% 46.31 – 72.98%). All spleen samples tested negative for *H. canis* (P=0.00%, CI95% 0.00 – 7.41%). All samples of CNS [0/30] and skeletal muscle [0/52] tested negative for *T. gondii*. To our knowledge this is the first report of badger natural infection with *L. infantum* and *Anaplasma/Ehrlichia* spp. in Italy. Considering the high prevalence of infection for both *L. infantum* and *Anaplasma/Ehrlichia* spp., we deem necessary to further investigate the role of free-ranging badgers in the epidemiology of these vector-borne diseases.

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Mystery Case

UNUSUAL CASE OF SHARPER POINT OF THE SHOULDER IN A PRE HORSE

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A 5 year old, PRE, gelding, weighing approximately 520 kg was referred for left forelimb (LF) lameness of 1 month duration. The horse was bought in Spain 1 month before admission without the pre-purchase examination. Since the arrival, the owner noted an irregular gait in front.

At physical examination, the horse showed a prominent point of the shoulder of the LF associated with a diffuse muscle atrophy of the biceps brachii, brachialis, supraspinatus, descending pectoral and extensor carpi radialis muscles. The horse had also a smaller LF foot, an upright pastern-hoof axis bilaterally, reduction of the range of motion of the fore fetlocks and positive retraction test of the LF.

At dynamic examination, the horse showed a reduction of the cranial phase of the stride and mild shoulder joint instability (sweeny) of the LF at walk; he was 2/5 lame at trot in straight line and the lameness was exacerbated on hard ground with the limb inside. Flexion tests of the distal limb of the left and right forelimb exacerbated severely the baseline lameness.

Baseline lameness was abolished by the low 4 point diagnostic analgesia after the abaxial nerve block did not result in any changes. Residual lameness was detected on the LF, which was exacerbated on soft ground with the limb outside the circle.

Radiographic examination of the fetlock and shoulder identified a small osteophyte at the dorsomedial articular surface of the proximal phalanx of the LF fetlock and presence of only one groove in the sulcus intertubercularis of the humerus; the distal part of the supraglenoid tubercle had some remodelling. Ultrasonographic examination of the fetlock did not identified significant abnormalities; sonographic evaluation of the shoulder was able to detect hypoplasia of the minor tubercle of the left humerus, medial luxation of the proximal tendon of the biceps brachii, which has an abnormal rounded shape, and mild bicipital bursitis.

The definitive diagnosis of the left shoulder abnormality was dysplasia of the sulcus intertubercularis (hypoplasia minor tubercle) and medial luxation of the biceps brachii.

The dysplasia of the sulcus intertubercularis is a rare and congenital abnormality described in several breed, and has a pathognomonic physical appearance characterized by a sharp and prominent point of the shoulder as consequence of the medial luxation of the biceps brachii and the shoulder joint varus conformation. This abnormality induces several biomechanical changes because the biceps brachii is part of the stay apparatus of the forelimb (shoulder extension); the consequences are higher during locomotion as the varus conformation of the shoulder puts more stress on the infraspinatus and deltoideus muscles during the stance phase, the luxation decreases the extensor function of the biceps brachii on the shoulder and its flexor function on the elbow joint during the swing phase.

Dysplasia of the sulcus intertubercularis can be considered a mixed lameness, with mechanical and pain components. There is no treatment for this condition and the level of athletic activity of horses is variable with some horses performing at high level before and after the diagnosis.

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NAPOLEONE AND HIS "MYSTERIOUS" COUGH

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Napoleone, a 2 years old Boxer, was referred at a local veterinarian for a recent history of apathy and moderate cough. The dog lived outdoor in a garden in the suburb of Perugia (Central Italy). The owners reported that no prophylaxis was ever performed for dirofilariosis, while permethrin spot-on products were seasonally administered.

At physical examination body temperature was out of range (39.6°C) and chest auscultation revealed respiratory sounds suggestive for bronchitis. The dog was treated with antibiotics (amoxicilline + clavulanic acid), corticosteroid at anti-inflammatory dosage and a mucolytic compound (carbocysteine). After a slight short-lived improvement, the dog was hospitalized for a severe loss of appetite, weakness, persistent cough with rare episodes of haemoptysis. Haematological exams were performed showing a mild normocytic, normochromic and regenerative anaemia, in association with marked leucocytosis and moderate thrombocytopenia. Radiographic examination of the thorax showed a diffuse, interstitial to alveolar pattern with an enlargement of the main pulmonary arteries in ventro-dorsal view. The dog was tested for circulating heartworm antigens (Heartworm IDEXX Snap Test®), scoring weakly positive. However no microfilariae were detected at the modified Knott's technique.

Despite the first clinical suspect, based on the laboratory and diagnostic imaging findings, was an occulted case of cardiopulmonary filariosis, Napoleone was subjected to echocardiographic examination and was also tested for the detection of *Angiostrongylus vasorum* antigens by Angio Detect™ Test (IDEXX). No echoes compatible with adult parasites in situ were recorded at the ultrasound examination, however *A. vasorum* circulating antigens were detected. This finding was further confirmed by the recovering of L1 larval stages in faeces analysed by Baermann's technique. *Angiostrongylus vasorum* (*Nematoda, Metastrongyloidea)* is a snail-borne parasitic disease affecting the heart and pulmonary arteries of wild and domestic canids. In the last 10 years, several factors have contributed to the geographical expansion of this nematode from traditionally endemic areas (e.g. France, UK and Denmark) to canine populations of central and southern Europe, including Italy [1,2]. Clinical signs in dogs affected by *A. vasorum* may evoke a whole range of other diseases, including cardiopulmonary filariosis [3]; moreover several Authors showed that diagnosis of occulted dirofilariosis is frequently misdiagnosed with angiostrongylosis because a cross-reaction may occur by using of commercial kits for the detection of circulating *D. immitis* antigens [3,4].

On the basis of these overall findings a final diagnosis of angiostrongylosis was issued and the occulted cardiopulmonary filariosis was excluded. Napoleone was immediately treated with 2 doses of a spot-on solution containing imidacloprid 10%/moxidectin 2.5% (Advocate®, Bayer Animal Health) 15 days apart, showing a rapidly recovery of the clinical signs.

The simultaneous use of different specific diagnostic tools (e.g. antigenic tests, modified Knott's and Baermann's techniques) is the recommended approach in dogs with suspected *D. immitis* and/or *A. vasorum* infection, especially in areas where an overlapping distribution of both the parasites is documented as in Central Italy.

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WHAT IS RESPONSIBLE FOR AN UNUSUAL CASE OF MYOSITIS OCCURRING IN A BRITISH HORSE?

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In June 2016, a 9-year-old Hunter gelding was referred to a veterinary surgeon of Newmarket (UK) for progressive swelling on right forelimb, extending to right forelimb and chest; blood sample showed marked elevation of globulins, muscular CK and AST enzymes, and low red blood cell. Antibiotics and steroids were given; after 5 weeks the swelling reduced in size but two locally extensive lumps were still present underneath the subcutaneous tissue/muscle on left side of chest. Transcutaneous surgical biopsies from the affected lumps were performed and the specimens were processed for histopathological examination.
Microscopic examination of the muscle biopsies showed multifocal to coalescing inflammatory infiltrates composed by eosinophils, epithelioid macrophages and giant cells, surrounding a necrotic area and embedded in reactive fibrous tissue with newly formed capillaries; muscle fibers were degenerated; encysted parasites, consistent with *Sarcocystis* spp., were observed within the intact myofibers. To identify the species of *Sarcocystis* occurring, DNA extraction was carried out from paraffin-embedded sections of muscle tissue using DNeasy Blood and Tissue Kit (Qiagen). The complete 18S rRNA gene was amplified by PCR with a pair of generic apicomplexan 18S rRNA-specific primers [1]. The amplified products were purified and sequenced; the sequences were compared with those available in GenBank™ using the Nucleotide-Nucleotide Basic Local Alignment Search Tool (BLASTN), and analysed using Molecular Evolutionary Genetic Analysis version 7.0.20 (MEGA7) software. BLAST analysis showed an identity score of 99% with reference sequences from *Sarcocystis moulei* (a.n. KC508513.1) and *S. gigantea* (a.n. KC209733.1). The sequence obtained has been deposited in the NCBI database under the accession no. KY594259. Based on the skeletal muscle histological and biomolecular findings a diagnosis of multifocal eosinophilic granulomatous myositis caused by *S. moulei* and/or *S. gigantea* was formulated and Baycox® was added to the therapeutic plan with recovery of the clinical signs.
The presence of sarcocysts in equine skeletal muscles has been considered to be an incidental finding and there are just sporadic associated reports of myositis [2]. *Sarcocystis moulei* and *S. gigantea* (Order *Eucoccidiida*, Family *Sarcocystidae*) are very close species of Apicomplexan protozoans that usually form a sister clade in phylogenetic analysis. *Sarcocystis moulei* is usually associated with goat as intermediate host however *S. gigantea* is considered a sheep specific species and both are considered not pathogenic in the origin species [3]. These findings can suggest that some *Sarcocystis* species have a wider intermediate host choice than previously thought; moreover, it is possible to speculate that horses could represent an alternative intermediate host for these two species in single or in mixed-infection and that they may induce severe myositis in this “new” host.

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NALA: A CASE OF CHRONIC HEPATITIS ASSOCIATED WITH CANINE LEISHMANIOSIS

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A 3 years-old, spayed female, Labrador Retriever was presented for weight loss, inappetance, vomiting and dullness. On physical examination the dog showed a mildly altered level of consciousness. A complete blood cell count (CBC) and a serum biochemical analysis were performed. CBC revealed a moderate normocytic hypochromic anemia. Biochemical profile revealed increased values of liver transaminases: Aspartate transaminase (AST, 70 UI/L), Alanine transaminase (ALT, 356 UI/L) and Gamma Glutamyl Transpeptidase (GGT, 16 UI/L). Abdominal ultrasonography was also performed and revealed a moderate enlargement of liver and kidneys. Fine needle aspiration of liver showed a poor cellularity with rare hepatocytes admixed with scattered lymphocytes, plasma cells and macrophages. Liver biopsies were collected and stained with haematoxylin and eosin for histological evaluation. A panel of histochemical stains was also performed. Histochemical panel comprised: Periodic acid - Schiff (PAS) for the evaluation of polysaccharides storage; Masson trichrome for the evaluation of fibrosis; Perl's Prussian Blue stain for the assessment of iron accumulation; Rubeanic acid stain for the assessment of copper accumulation. Haematoxylin and eosin revealed mild to moderate infiltration of inflammatory cells consisting of a mixed populations of macrophages (sometimes haemosiderin-laden), lymphocytes and plasma cells, hepatocyte vacuolation and small foci of coagulative necrosis. Numerous small granulomatous to pyogranulomatous foci scattered randomly through the parenchyma were also observed. Macrophages were often expanded for the presence of numerous intracytoplasmic 3-4 □m, round to oval protozoa showing 1-2 µm diameter and a basophilic nuclei with adjacent perpendicular basophilic kinetoplasts (amastigotes). A mild periportal fibrosis was observed with Masson trichrome stain. A moderate accumulation of PAS positive material was detected within the hepatocytes. No copper or iron deposition within the hepatocytes was demonstrated, respectively, with rubeanic acid stain and Perl's Prussian blue stain. The morphological diagnosis was hepatitis, macrophagic, subacute, diffuse, and moderate with multifocal hepatocellular degeneration and necrosis. The etiology was consistent with *Leishmania* spp. infection (Hepatic leishmaniasis). Hepatic lesions have been poorly characterized both in natural and experimental *Leishmania* infection in dogs[1]. Rallis et al. identified three main histological patterns based on the severity of the lesions. However, no correlation was found between histopathological pattern and breed, sex, age, clinical manifestations, serum biochemical profile or parasite load in the hepatic tissue[1]. The present case highlights the importance of including leishmaniasis as a differential diagnosis for hepatic chronic injury in dogs. Moreover, we hasten to add the fundamental role of histopathology as the gold standard method for the definition and grading of a morphological and etiological diagnosis of canine hepatic leishmaniasis.

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