SOCIETÀ ITALIANA
DELLE SCIENZE VETERINARIE

ATTI
LXX Convegno SISVET






Joint meeting

REEV-Med
XVI Convegno S.I.C.V.
XIV Convegno S.I.R.A.
XIII Convegno AIPVet
XIII Giornata Studio So.Fi.Vet.
III Convegno RNIV

13 -16 Giugno 2016

Viale delle Scienze
edificio 19
Palermo

SISVet

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EDIFICIO 19

Università degli Studi di Palermo
Viale delle Scienze

Palermo, 13-16 GIUGNO

2016

Con il patrocinio di:

In collaborazione con:


Istituto Zooprofilattico Sperimentale della Sicilia
"A.Mirri"


STUDIORUM UNIVERSITAS
MESSANAE


REEV-Med
Réseau des établissements
d'enseignement vétérinaire
de la Méditerranée


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Saluto e relazione del Presidente al

70° Convegno SISVet

Cari amici e colleghi,

benvenuti al 70° Convegno della SISVet. Quest'anno il Simposio accoglie i convegni e le giornate di studio della SICV (Società Italiana di Chirurgia Veterinaria), AIPVet (Associazione Italiana dei Patologi Veterinari), SIRA (Società Italiana di Riproduzione Animale), SOFIVet (Società di Fisiologia Veterinaria), RNIV (Rete Nazionale di Immunologia Veterinaria) e l'Assemblea Generale del REEV-Med (*Mediterranean Network of Establishments for Veterinary Education*).
Sono in programma 232 lavori scientifici sotto forma di comunicazioni orali e posters, oltre a cinque workshops, due tavole rotonde e quattro *Main lectures*. Inoltre siete tutti invitati alla "*Mistery Case Evening*" del 15 Giugno, in cui saranno presentati casi misteriosi di patologia, clinica e parassitologia. Nell'occasione l'Istituto Zooprofilattico della Sicilia offrirà ai partecipanti una grigliata che contribuirà a rendere gradevole un'interessante serata culturale.

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 2016, un premio da 1000 € per ogni sessione scientifica. I premi sono destinati alle comunicazioni che saranno pubblicate su riviste indicizzate, mentre il miglior poster di ogni sessione sarà premiato con l'iscrizione gratuita al convegno del 2017. La RNIV ha bandito due borse da 500 € ciascuna per favorire la partecipazione al Convegno SISVet 2016 a giovani ricercatori.

Una importante novità è rappresentata dall'accordo che il Consiglio Direttivo ha appena siglato con l'Editor della banca dati CABI Publishing grazie al quale gli atti dei Convegni SISVet saranno indexati e le comunicazioni e relazioni dei workshop e tavole rotonde verranno inseriti *full text in CAB Abstracts/Full-Text Repository*.

La cena sociale si svolgerà presso il famoso ristorante di Natale Giunta a Castello a Mare, una delle più belle *location* di Palermo.

Sarà attivo anche il Tavolo Tecnico per la costituzione della Federazione delle Società Scientifiche Veterinarie Italiane con la partecipazione dei Presidenti delle principali Società Scientifiche Veterinarie italiane e dei rappresentanti dei SSD che non fanno riferimento a Società Scientifiche.

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 agli Sponsor e agli Enti patrocinatori: all'Università degli Studi di Palermo, all'Istituto Zooprofilattico della Sicilia e al Dipartimento di Scienze Veterinarie di Messina. 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.

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

Benvenuti a Palermo

Bartolomeo Biolatti

Presidente SISVet

Seventy years of SISVet

Marco Galloni and Patrizia Peila

Approaching the expiration of the seventy years since the constitution of the Italian Society of Veterinary Sciences, it seems appropriate, interesting and even enjoyable to look back on this long elapsed time and ideally we want to reconnect our telling to those considerations that, at the expiration of the fiftieth anniversary, the then President prof. Franco Monti wanted to gather in an elegant volume.

The origins of the Society (now customarily referred to by the acronym "SISVet") date back to the first years after World War II, when, during the meeting of the Deans of the Faculties of Veterinary Medicine, held on the occasion of the National Congress of Veterinarians in Florence on November 28-29, 1946, the creation of a "Scientific Society of Veterinary Medicine" was announced; promoted by a committee made up of professors of various Faculties, among which we can remember Elio Barboni and Valentino Chiodi, both of them teachers at the University of Perugia, who where the most passionate supporters, the initiative was very well received by the participants, and so the official establishment of the "Italian Society of Veterinary Sciences" was determined in Bologna, at the Faculty of Veterinary Medicine, on June 1, 1947. The constitutive act, a deed written by the notary dr. Giusto Gondoni (July 18, 1947), was followed by a decree of the President of the Republic (April 6, 1948), with which the Society was erected to a charitable trust, and it approved the articles of partnership, which, together to the guidelines, governed since the beginning the organization and activities of the Society. The articles of partnership and guidelines were then repeatedly partially revised to adapt them to the changing needs of the Association and of the Italian Veterinary Medicine; the current ones are now available on the web site SISVet.

The Society's organs were the President, the Executive Council, the Scientific Committee, the General Meeting, the Board of Auditors and the Board of Arbitrators.

The first President of the Italian Society of Veterinary Sciences was Pietro Gherardini, professor of pathological anatomy, while the above mentioned prof. Barboni, veterinary pathologist experienced in parasitology, was the first secretary. In the Board of Directors were present, in addition to the Chairman and the Secretary, the Vice-President, prof. Luigi Montroni, professor of pathology, the cashier-treasurer, prof. Plinio Bardelli, Director of the Animal Disease Prevention Institute of Padua and two advisors: prof. Paul Girotti, President of the Italian National Veterinary Association (ANVI) and prof. Philip Usuelli, President of the CNR Veterinary Commission.

As you can see, from the beginning the different souls of the veterinary world (universities, research institutes and profession) were well represented among the Society executives. Similarly, also the various disciplines of the Veterinary Medicine emerged from the composition of the first Scientific Committee: prof. Valentino Chiodi (normal anatomy), prof. Luigi Leinati (pathology), prof. Attilio Mensa (surgery), prof. Giuseppe Borgatti (physiology and therapeutics), prof. Ugo Pagnini (food inspection), prof. Sebastiano Paltrinieri (clinics), prof. Igino Altara (infectious diseases), prof. Pietro

Sartoris (obstetrics), prof. Arturo Magliano (animal husbandry). All of them were true teachers recognized in their respective fields, names often linked to treaties present even today in all libraries, which represent a fundamental value, an example to propose to the generations that will follow over the years.
Among the SISVet presidents, academics who sometimes also headed the Animal Disease Prevention Institutes, we remember prof. Dino Desiderio Nai, who won the gold medal of merit of public health just when he began his term, in 1964.
Members of SISVet (honorary and ordinary) have always gathered at the meeting at least once a year, usually in conjunction with the scientific congress. The ordinary member status has always been attributed, subject to prior application to the President and payment of the annual membership fee, to all Italian veterinarians, to graduated in other fields but involved in veterinary science, to the organizations and associations interested in veterinary science and pursuing goals similar to those of SISVet. Recently, in order to promote the membership, particularly for young unstructured researchers (PhD students, research fellows, fellows, students of the Schools of Specialization and Master), several initiatives, such as the reduction of membership fees, have been implemented.
The SISVet initially had a "travelling" seat, because the activities related to the Bureau, as well as those of secretary, both scientific and administrative, were being held at the Presidency. But in 1975, during the annual conference, this practice was questioned by prof. Bruno Romboli, then President of the Society. The growing number of members, the burden of publication of Acts (expected by the end of each conference) and the maintenance of relations with similar Italian and foreign scientific societies representing critical issues, which could be overcome by establishing the SISVet registered office in Brescia, at the “Fondazione Iniziative Zooprofilattiche e Zootecniche”. This Foundation, which boasted twenty-year activity at the time, seemed fit for the purpose, as it had been established in order to "promote, encourage and subsidize initiatives to improve the livestock production and health defense with particular regard to the study, experimentation and scientific research in animal husbandry and veterinary industry". An agreement was then signed for the relocation of the SISVet secretariats in the registered office in Brescia and that statement, valid for five years from 1976, was extended and still is valid, as far as administrative secretary is concerned. Our Society has shared the cultural vitality of the Foundation and its continuous technological update, which led to transfer much of the activity of information on the network and, starting from 2004, to publish and distribute on CD-ROM the acts of Congresses.
We believe that almost all the members experienced as young researchers the presentation of the results of their early researches in the sections of the SISVet congress, a "baptism of fire" faced, often with fear, before recognized masters from which came approvals but also, not infrequently, acute and constructive critic caveats. The first conference of SISVet took place from 11 to 13 October 1947, in Perugia; the capital of Umbria since then hosted the event several times, the last of which just last year. The choice of venues has always responded to criteria of impartial distribution throughout the country, not neglecting the tourist aspects of geographical realities with which the members could get in contact. In particular, we note that the dinner of the 2014 conference was held at the Natural History Museum of the University of Pisa.
The annual congress has always been the place dedicated to the divulgation and discussion of the results of scientific researches in the veterinary field, as well as the

meeting ground between academia and profession. In the first years the scientific communications were associated with relationships, sometimes conferences, which characterized the activities of SISVet until 1972. In the following years, the congress was enriched of round tables; since then, there always was at least one, flanked recently (when not replaced) by one or more workshops on hot topics (environmental pollution, animal welfare, BSE, veterinary education, the future of the profession, GMOs just to name a few).

From a detailed analysis of the topics covered in the conference, especially in panel discussions and workshops, it emerges that the SISVet has managed, over time and in equal measure, to be the bearer of both the requests from the academic world and the issues of practical order posed by professional veterinary world. Training and education have always been one of the themes that characterize the life of the Society. While in Italy the obligation of continuing education for health professionals has been enshrined in law since 1992 and the National Program of ECM dates back to 2002, in 1978 Prof. Giovanni Ballarini, now Professor Emeritus of the University of Parma, placed emphasis on the need for ongoing education. Further evidence of interest of SISVet in this area were the round table in 1993 "Objectives, teaching methods and assessment of learning in a modern vision of veterinary teaching" and in 2012 "Veterinary Teaching Hospital: training, research and territory", focused on the crucial topic of the update of teaching and the requests presented to the Faculty by an ever-changing society, even in the human-animal relationship. This last topic of discussion was taken up, two years later, by prof. Gaetano Oliva, in his speech "The veterinary hospital, role in teaching and relationships with the NHS", on the occasion of a round table, held in Pisa, to discuss with the social parts about the hypothesis of a new degree program in Veterinary Medicine. In addition to prof. Oliva in this event, organized by SISVet in agreement with the Conference of Directors, were present dr. Gaetano Penocchio, President of the National Federation of Italian Veterinary Orders (FNOVI), prof. Massimo Castagnaro (ANVUR) and prof. Attilio Corradi, Chairman of the Conference of Directors.

Albeit from a different perspective, namely that of the university-professional relationship, the training was also discussed at the round table of 2003. At that conference, dr. Penocchio, on behalf of FNOVI, reiterated the need for the university system to better respond to the training needs of a veterinarian, taking into account European standards.

Emphasis on professional updating supported by new technologies was placed, however, during the conference "ICT, Telemedicine and knowledge networks in Veterinary", held in Pisa in 2008 by SISVet, in collaboration with the Italian Society for Telemedicine (SIT) and with the Italian Association of Telemedicine and Medical Informatics (@itim)) and sponsored by FNOVI. It is worth stressing that it was the first initiative on this subject in the veterinary field.

Another recurring theme in the conferences was that of food safety, related to livestock production: the discussion on health and hygiene controls on food of animal origin in order to protect public health, which was repeated several times since the first round tables, leads us to conclude that our Society had perceived the importance of the inspection of food long before the transposition in Italy, in 2007, of the EC Directive on the HACCP protocol.

Of course the SISVet congresses could not miss moments of confrontation on the diagnostic and therapeutic techniques: the attention was brought on infectious diseases, on surgical techniques, on issues of reproduction, introducing also ethological aspects, such as in "The animals city: health and relationship problems ", in 2005. We can therefore say that the SISVet has represented, since the last war, the most important but also the most dynamic reference for the entire veterinary world, hosting in its workshops as speakers even two Nobel prize winners for medicine and physiology: Daniel Bovet , biochemist Nobel prize for medicine in 1957 for his research on curare-like and the biochemist Ernst Boris Chain, who was awarded the Nobel prize for physiology and medicine in 1945 for studies of penicillin, in collaboration with Alexander Fleming and Howard Vincent Florey.The Society has therefore taken on a role similar to that which, in other times, was characteristic of the Royal Society and Italian Veterinary Academy, that was active for half a century between 1858 and 1912, in times when science and profession were making fast and important progress, as documented in the publications of the period. There have been countless examples that demonstrate how the SISVet events engage in wider cultural landscapes: for example in the '70s, in the wake of the rapid changes in social roles, there was a serious reflection on the presence of women in Veterinary Medicine; this happened, due to M. Cipolletta and C. Marini, in 1977, just the year in which was promulgated the law on equal treatment between men and women in matters of work. We also remind the ECM conference "Unitary Veterinary Medicine (1861-2011)" held in 2011 in Rome at the Ministry of Health, a tangible sign of the participation of the class in the commemoration of the 150th anniversary of unification of Italy. The intense day of study and the volume that ensued from it have pointed out all the aspects in which the activities of the Italian veterinary doctors were explicated, moreover they have especially shown the impact on civil and economic life, emphasizing the cultural value and the capacity for analysis and innovation. Organized by the Society in collaboration with other organizations, including the FNOVI and veterinary society of historical studies (CISO-Veterinaria), the initiative gained the recognition of the President of the Republic Giorgio Napolitano, who awarded the Presidential Medal.

Over the years there were also quite unusual and original scientific contributions, such as the reports submitted by prof. Roberto Piazza and colleagues from Torino in 1995 and 1996 dealing about vocal emissions of Sardinian sheep; these studies have recently been revised and presented in specific and interdisciplinary scientific events, enjoying a lively interest.

We report two more particular initiatives, proposed at SISVet annual congress in 2011:

- the presentation of the exhibition "1980-2010: 30 years of veterinary medicine of disasters". The exhibition recalled the activities carried out by the veterinary services in natural disasters, particularly in the event of earthquakes, focusing on the figure of Adriano Mantovani, parasitologist with strong interests in the social field, who was the pioneer in the planning and management of veterinary public health operations during more serious disasters
- the award ceremony of the first edition of the Veterinary Literary Cenacle. This essay contest, sponsored by the Region of Calabria and reserved for graduates in Veterinary Medicine and related disciplines, was divided into three sections (fiction, non-fiction, poetry) and the works, on any subject, could be unpublished or already published. The

winner was prof. Franco Mantelli for the fiction, with a new book entitled "I, my patients and their owners".
A new way to stimulate the curiosity and the critical spirit of the participants at the congress was, last year, the introduction of the "mystery case evening", which was an opportunity to present, in an informal way, infrequent and problematic cases in pathology, clinics and parasitology.
In 2013, under the chairmanship of prof. Bartolomeo Biolatti, the SISVet aims to renew, dealing with specific objectives, such as aiming to strengthen the relations between the different souls in the world of Veterinary Sciences; pointing to internationality, taking care not to neglect relations with emerging countries; helping in improving the quality of research and teaching; making more and more visible the documentation regarding the activity of the Society.
The SISVet intends to refine and strengthen relationships and collaboration with the new Italian Scientific Societies, born in recent years in the veterinary environment because of the need to create more homogeneous areas of discussion among researchers in specific disciplines. With this aim, a technical committee with the presidents of these companies was created for founding the "Federation of the Italian Scientific Veterinary Societies"; moreover, since some years, the annual SISVet congress hosts parallel scientific sessions, held by the Italian Society of Veterinary Physiologists (SOFIVet), the Italian Society of Veterinary Surgeons (SICV), the Italian Society of Animal Reproduction (SIRA), the Italian Association of Veterinary Pathologists (AIPVET), National Association of Veterinarians Morphologists (ANMV), the Italian Network of Veterinary Immunology (RNIV).
In the perspective of internationalism, the joint meeting between the SISVet and the Réseau des Etablissements d'Enseignement Vétérinaire de la Méditerranée (REEV-Med) of 2013 demonstrates the commitment of our Society in the development of a collaborative program between the schools of Veterinary Medicine of the Mediterranean countries.
The SISVet must create a synergy with the University, the Ministry of Health, the Animal Disease Prevention Institutes, the local health authorities, but also with the professional practitioners, in order to contribute to the training and promotion of the quality of the research carried out by young fellows. To achieve this goal, facilitations have been provided for participation in conferences, calls for both awards and scholarships, destined to scientific communications published in indexed journals. Subject of a recent workshop was the comparison between the assessments made about veterinary Schools from Italy by international structure EAEVE and Italy's ANVUR, both of them being very important judgments for the consequences on the academic and financial level. In addition, in 2015, SISVet participated to the Joint Assembly with representatives of the CUN-Area 07, of AISSA, the Conference of Agriculture and Veterinary Medicine Conference, to discuss the new national scientific rules for professors selection.
We want to ideally continue the work of those who came before us and conclude this brief reconstruction of the history of the Society remembering what the then President prof. Franco Monti said at the 50th Conference, when he proudly called SISVet the "first and most prestigious Italian Society established in the context of Veterinary Sciences ".

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PROGRAMMA GENERALE

SISVET 2016

LUNEDÌ 13 GIUGNO

11.00-13.00 **Seminario**: "Ricordando Orazio Catarsini" (AULA 9)

13.30 – 14.30 Registrazione (ATRIO Edificio 19)

14.30 – 17.00 Workshop 1: Le Scienze Forensi in Medicina Veterinaria (**AULA 8**)

17.00 – 18.30 Comunicazioni scientifiche, lezioni magistrali – sessioni parallele SISVet, AIPVet, So.Fi.Vet., S.I.C.V., S.I.R.A., RNIV

17.30 – 18.15 **AIPVET - Lettura Magistrale:** "Metodologie e protocolli delle autopsie" (AULA 8)
Dr. Lorenzo Ressel

18.30-19.30 Assemblee delle Società Scientifiche

20.00 *Welcome Party* (Giardino all'italiana – *Villa d'Orleans*)

MARTEDÌ 14 GIUGNO

08.30 – 10.30 Comunicazioni scientifiche, lezioni magistrali, ecc. – sessioni parallele
SISVet, AIPVet, So.Fi.Vet., S.I.C.V., S.I.R.A., RNIV

8.30 - 10.30 Conferenza dei Direttori di Dipartimento, incontri con rappresentanti CUN (AULA SEMINARIO C)

8.30 -10.30 Tavolo Tecnico Federazione Società Scientifiche Veterinarie

10.00 - 10.30 *Coffee break* - **Sessione poster n.1**

10.30-11.30 **Inaugurazione del 70° Convegno SISVET** (AULA MAGNA POLITECNICO)

11.30 – 13.30 Workshop 2: La valutazione EAEVE nei paesi del bacino mediterraneo (**AULA MAGNA POLITECNICO**)

13.30 – 14.30 *Pausa pranzo* (Museo dei motori)

14.30-15.00 **Lettura Magistrale:** "SISVet Accademia per una sintesi dei mille saperi in una società liquida" (AULA MAGNA POLITECNICO)
Prof. Emerito Giovanni Ballarini

15.00 – 17.00 Workshop 3: Le biotecnologie applicate alle discipline cliniche veterinarie – SICV, SIRA, Clinica Medica (**AULA 9**)

15.00 - 17.00 Comunicazioni scientifiche, lezioni magistrali – sessioni parallele SISVet, AIPVet, So.Fi.Vet., S.I.C.V., S.I.R.A., RNIV

15.00 **Scienze Biomediche** - **Lettura Magistrale:** "Appettite regulation in health and disease" (Aula Seminario B)
Prof. James Sartin

15.00 - 17.00 **REEV-Med General Assembly** (AULA MAGNA POLITECNICO)

17.00 - 17.30 Coffe break - **Sessione poster n.2**

17.30 – 19.30 **Tavola Rotonda RNIV**: The innate immune response to non-infectious stressors: human and animal models **(Aula Seminario A)**

17.30 - 19.30 Comunicazioni scientifiche, lezioni magistrali – sessioni parallele SISVet, AIPVet, So.Fi.Vet., S.I.C.V., S.I.R.A.

20.30 **Cena sociale presso Castello a Mare, Palermo**

MERCOLEDÌ 15 GIUGNO

8.30 – 10.30 Comunicazioni scientifiche, lezioni magistrali, ecc.– sessioni SISVet, AIPVet, So.Fi.Vet., S.I.C.V., S.I.R.A., RNIV

8.30 - 9.00 **AIPVET - Lettura Magistrale** "L'immunoterapia: una prospettiva applicabile per il melanoma del cane" (**AULA 8**)
Prof.ssa Federica Cavallo

10.30 – 11.00 Coffee break - **Sessione poster n.3**

11.00 – 13.30 **Workshop 4**: Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria (**AULA 11**)

13.30 – 14.30 *Pausa pranzo* (Museo dei motori)

14.30-15.00 **Lettura Magistrale** "Pathogenesis of Bluetongue" **(AULA 12):**
Prof. Massimo Palmarini

15.00 - 17.00 Comunicazioni scientifiche, lezioni magistrali– sessioni parallele SISVet, AIPVet, So.Fi.Vet., RNIV

17.00 – 17.30 Coffee break - **Sessione poster n.4**

17.30 - 18.30 Comunicazioni scientifiche, lezioni magistrali – sessioni parallele SISVet, AIPVet, So.Fi.Vet., S.I.C.V., S.I.R.A., RNIV

17.30 **Tavola Rotonda RNIV:** Attualità e prospettive di sviluppo del saggio interferon-gamma per la diagnostica veterinaria **(Aula Seminario A)**

18.30-19.30 Assemblea Soci SISVet **(AULA 10)**

20.30 ***Mistery Case evening (IZS Sicilia)***

GIOVEDÌ 16 GIUGNO

9.00 – 13.00 **Workshop 5**: Emergenza specie esotiche (**AULA 11**)

WORKSHOP

E

MAIN LECTURES

Workshop 1

Le Scienze Forensi nella Medicina Veterinaria

Some challenges in Forensic Veterinary Pathology

Lorenzo Ressel

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Forensic veterinary pathology is defined as the application of knowledge of veterinary pathology to the elucidation of evidence for the Courts[1]. In Europe, as well as in the US, this is an emerging field and the increase in forensic cases submitted to veterinary pathologists is likely due to the rising incidence of animal abuse and neglect coupled with the new attention of society on animal welfare and demand that perpetrators are prosecuted[2]. The section of Veterinary Pathology, University of Liverpool represents one of the leading institutions for forensic veterinary pathology in Europe with approximately 10 years of experience on field, and more than 100 forensic cases yearly distributed among a wide variety of different species[3]. A percentage of these cases are followed-up by Pathologist's attendance to Court. The forensic necropsy is always a complete and thorough examination and is characterised by high level of meticulous data recording, measurements and in depth interpretation of findings. Often, information from related disciplines (e.g. normal anatomy and physiology) are vital in order to produce a high standard report and correctly interpret pathological changes. Samples collected during the post mortem examination are usually submitted to further tests (histology, parasitology, toxicology, and forensic entomology) in order to confirm, but also rule out, pathological conditions.

The forensic report represents the heart of the forensic veterinary diagnostic process, and the document that constitutes evidence in court. Such report must be detailed, tightly associated with a comprehensive photographic documentation, and, at the same time, easy to interpret for the layman (nonprofessional). When the evidence provided in the forensic report is not agreed from the prosecution or the defence, the veterinary pathologist is summoned to attend Court in order clarify the contents of the report,

and/or discuss findings which may have been challenged by another expert witness (Veterinary Pathologist). A comprehensive training in all the aspects of the forensic activity is advised for the veterinary pathologist facing this task.

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Workshop 1

Le Scienze Forensi nella Medicina Veterinaria

Forensic entomology and veterinary: an emerging synergy

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The association between decomposing bodies and insects has been a well-known topic since antiquity. This theme has been reported in numerous holy and literary books as well as in paintings and sculptures. The presence of insects on dead bodies has created many legends and beliefs, however the development and modernisation of the scientific method, the confutation of the spontaneous generation theory and the development of technology such as the invention of the microscope at the end of the 16th century, gave birth to a new forensic science: forensic entomology. This discipline applies the knowledge of insects to legal cases, in both civil and criminal law.

During the decomposition process, insects are responsible for the removal of important parts of the soft tissues of the body and the alteration of other tissues such as bones and hair. The feeding activity of insects has two consequences: 1) insects can be used to obtain useful information about the circumstances surrounding death; 2) they can produce alterations and modifications of the body that can be misinterpreted as a result of a violent act before death.

Experiments of insect colonization and body decomposition have been performed using pigs as human model. In general, insects do not distinguish between animal decomposing carrions and human decomposing cadavers, so if it is possible to use animal as human model for decomposition/insect colonization, it is as well possible to transfer, mutatis mutandis, the knowledge developed in the past on human cases to animal cases.

Only few articles have been published on the application of the entomological approach to animals in forensic cases (Canada, Italy, Northern Europe) but the interest on the subject is progressively increasing. In fact, the study of the insects collected from the carcass and from the "crime scene" can provide useful information not only on the time since death estimation but as well about the carrion transfer, concealment and presence of drugs or other chemicals.

It is worth mentioning that the "forensic entomology" previously described, the so-called medico-legal, is only one of the three branches of this discipline with store-product forensic entomology and urban entomology. These two sciences deal with the discovery of responsibility in case of insect infestation on food, human goods and buildings.

The entomological approach requires qualified people and strict protocols in the collection, preparation and study of the specimens. A proper collection and storage are anyway the condition sine qua non for any further analysis. All the people involved in crime scene analysis and corpse/carrion inspection, both human and animal, have to know the basic element of a correct collection and preparation. In Italy, the GIEF (Gruppo Italiano per l'Entomologia Forense) provides support and education to all the people interested on the disciple. Further information about the association can be found on the website is: http://www.giefitalia.org/it/home/.

Workshop 1

Le Scienze Forensi nella Medicina Veterinaria

Diagnostic imaging in Veterinary Forensic Medicine

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Diagnostic imaging techniques give information about morpho-structural and functional features of the anatomical regions examined. The nature of the information depends on the physical medium used: atomic density and thickness of the tissues – X-rays (Radiography, Fluoroscopy, CT); echogenicity (i.e. variety in acoustic impedance) – ultrasound (Ultrasonography, Elastography); proton susceptibility and molecular complexity – high magnetic fields and radiofrequency (MRI). All the above mentioned techniques are currently used in Human Forensic Medicine. The use of X-rays in a legal debate dates back to 1896, less than one year after their discovery by Prof. Roentgen.[1] To date, Diagnostic Imaging plays a crucial role in Forensic Medicine and an "International Forensic Radiology and Imaging Society" was founded.[2]

In the Veterinary Medicine, forensic sciences are moving their first steps but already in 1980, in dogs suspected died from a gunshot, a complete radiographic examination of carcasses was recommended before the necropsy.[3] Nowadays, postmortem radiographic examination represents the basic to rule out bullets or skeletal lesions in forensic investigations[4] and a new method called "virtopsy", based on CT and MRI, is becoming more and more relevant.[5] In the Veterinary field, the most frequent reasons that need the diagnostic imaging techniques are: neglect, abuse or cruelty acts, malpractice debates, ascertaining cause of death, determining of age in traded puppies. Other fields in which veterinary diagnostic imaging proved its usefulness are the archeo-zoological studies[6-8] and food frauds unmasking.[9]

Since clinical and postmortem radiology are not the same, the radiologist should be aware about the cases and the imaging procedures have to be discussed with the

pathologist to ensure the best results. It is also important to remember that the role of veterinary radiologist is decisive in helping the pathologist to recognize the signs of animal abuse since it has been demonstrated that perpetrators of a violence in the home is likely to abuse both pets and family members.[10]

Currently, there is no established curriculum but, probably, as in human medicine, in the next future, a new subspecialty in veterinary radiology will born.

In conclusion, the best advantage of imaging techniques is the non-invasiveness however, on the other hand, their diffusion in the veterinary forensic medicine may be prevented by the high cost that is the main disadvantage, particularly of the advanced tomographic devices (CT and MRI).

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Workshop 1

Le Scienze Forensi nella Medicina Veterinaria

Forensic genetics in crimes against animals

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Public awareness about wildlife conservation and pet care has raised dramatically in recent decades. One consequence of this new sensitivity is, for example, the acknowledgment of animal cruelty and illegal killing as crimes not against human morality (which can be offended when looking at any form of cruelty to other living creatures), rather as crimes against the animals themselves, eventually regarded as sentient beings. Animals, however, are still common victims of heinous crimes, like cruelty to domestic animals, smuggling and poaching of wild endangered species, at risk, or anyway subject to protection. Currently, forensic investigations in crimes against animals can rely on many scientific disciplines, which are widely used in human forensics but are only recently landed in animal forensics, such as molecular genetics, i.e. DNA analysis. Biological samples from the injured/killed animal are often the only evidence collected at the crime scene, and DNA analysis is the only option for investigators to solve the case and assign the responsibility for that crime. Forensic genetics can provide valuable help in solving diverse types of crimes against animals. Just to give a first example, I mention the use of dog and cats in fur industry, which is a legal practice in Asia (China in primis) and a thriving market towards the Western countries, but it is an illegal activity in the European Union, where production, marketing, import/export of fur items from dog and cats are officially prohibited since several years. Dogs and cats are the most popular and beloved companion pets. Consequently, people outside Asia find it unacceptable to farm these animals for their furs, nor do they want to inadvertently buy products containing such fur. In this regard,

the Italian Ministry of Health officially charged the Centro di Referenza Nazionale per la Medicina Forense Veterinaria at the Istituto Zooprofilattico Sperimentale of Latium and Tuscany with developing a method for the identification of animal species in fur products, in order to comply with the EU laws, and to help stopping this trade. Forensic genetics is also widly used in investigations against wildlife poaching. Although wild animals are strictly protected by law in the national parks of Italy, poaching remains a perennial problem with protected or even highly endangered species succumbing regularly to snares, poisoned baits, and the hunting rifle. The latest DNA-based technologies today make it feasible to identify both the species and single individuals from only trace amounts of their genetic material. This is fundamental when only parts of animal body (e.g. pieces of meat, feathers, hairs, blood spots) are seized and matches between evidentiary and suspect samples are carried out to establish whether they come from same species and/or same individual. The genetic relatedness among individuals, for example to verify their birth in captivity (e.g. in exotic species), and their gender, can be also determined by DNA typing from animal traces. In conclusion, prosecution of crimes against animals is a complex forensic activity, which however, can now rely on valid "unconventional weapons", like the recent genetic techniques. In animal forensics, as well as in human forensics, DNA analysis can be decisive to establish a link between the victim and the suspect with a far greater degree of confidence.

Workshop 2

EAEVE evaluation of the Establishments for Veterinary Education on the Mediterranean basin

Moderatore:
André Laurent Parodi, *Honorary President of REEV-Med*

11.30 – 11.45 The revised "SOP" and the Consultative Visit of the Establishments which are not member of EAEVE
Stephane Martinot, *Vice-President of EAEVE*

11.45 – 12.00 The activities of OIE in the field of veterinary education with particular focus on the Mediterranean basin
Jean-Philippe Dop, *OIE Deputy Director General*

Key points of the evaluation process: most frequent major deficiencies and the way to correct them
12.00 – 12.25 Curriculum
Ehab A. Abu-Basha, *Dean of Faculty of Veterinary Medicine of Jordan University of Science and Technology (Jordan)*
Gualtiero Gandini, *Department of Veterinary Sciences, University of Bologna, (Italy)*

12.25 -12.50 Facilities and equipment
Samir Ben Romdhane, *Veterinary School of Sidi Thabet (Tunisia)*
Marc Gogny, *Dean of Veterinary School, Alfort (France)*

12.50 – 13.15 Animal resources and teaching materials of animal origin
Lysimachos G. Papazoglou, *University of Thessaloniki (Greece)*
Mohammed Ouassat, *Dean of Veterinary School of Rabat (Maroc)*

13.15 – 13.25 Discussion

13.25 Conclusion
Noursaid Tligui, *REEV-Med President*

Workshop 3

Le biotecnologie applicate alle discipline cliniche veterinarie

A Decade of advances in microbiota investigations

Patrizia Brigidi

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The gastrointestinal tract harbours one of the most complex microbial ecosystems, the intestinal microbiota[1]. The comprehensive genome of these microbial populations is estimated to have a far greater genetic potential than the host genome itself. This intestinal bacterial ecosystem has gained increasing interest from the scientific community because of its demonstrated involvement in the aetiology and/or outcome of different physio-pathological conditions. Indeed, the microbial counterpart provides essential features the host has not evolved, including enhancement of the digestive efficiency and modulation of energetic homeostasis, vitamin synthesis, competitive barrier against colonization/invasion, development, education and function of the immune system, strengthening of the GIT epithelium impermeability, detoxification of xenobiotics, central nervous system modulation and endocrine system modulation[2,3].

A comprehensive and detailed view of the gut microbiota, in terms of phylogenetic composition, as well as functional potential, is essential to understand dynamics and possible mechanisms of the cause/effect relationships between gut microbiota and host health/pathology. In the last few years metagenomic has emerged as one of the most powerful sequence-driven approaches to study this complex ecosystem, allowing to obtain an accurate microbial identification as well as to identify and annotate diverse arrays of microbial genes that encode many different biochemical and metabolic functions of the gut microbiota. The efforts in this direction have been smoothed by the implementation of next generation sequencing (NGS) platforms[4]. Functional metagenomics NGS-based studies revealed that the microbiota plasticity is strategic for several aspects of the host biology, addressing the different immunological and metabolic

needs at various ages, and adjusting the ecosystem services in response to different lifestyle, physiological states or diets.

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Workshop 3

Le biotecnologie applicate alle discipline cliniche veterinarie

Evolution and actual state of veterinary regenerative medicine

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"Regenerative medicine is a branch of translational research in tissue engineering and molecular biology, which deals with the process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function. This field holds the promise of engineering damaged tissues and organs via stimulating the body's own repair mechanisms to functionally heal previously irreparable tissues or organs.
Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely implanting them when the body cannot heal itself. If a regenerated organ's cells would be derived from the patient's own tissue or cells, this would potentially solve the problem of the shortage of organs available for donation, and the problem of organ transplant rejection.
The term "regenerative medicine" was first found in a 1992 article on hospital administration. […]
Regenerative medicine refers to a group of biomedical approaches to clinical therapies that may involve the use of stem cells. Examples include the injection of stem cells or progenitor cells obtained through directed differentiation (cell therapies); the induction of regeneration by biologically active molecules administered alone or as a secretion by infused cells (immunomodulation therapy); and transplantation of in vitro grown organs and tissues (tissue engineering)." (https://en.wikipedia.org/wiki/Regenerative_medicine)

Earlier, the concept was that adult tissues had no intrinsic potential for regeneration, and that somatic cells did not have the capacity of differentiation. A paradigm shift arrived with the postulation of the mesengenic process, claiming that there were Mesenchymal Stem Cells in adult tissues, which were able to differentiate into a variety

of different tissues, like chondrocytes, tenocytes, adipocytes, neurons, and others, and could probably be used to regenerate the respective tissues (1, 2)(1, 3).
Veterinary science paralleled basic sciences in this field, and in 1998, the first publication on the isolation of MSC from equine bone marrow arrived (4).
At that time, the horse served as an animal model for cartilage repair studies, and mainly chondrocytes or modified, genetically engineered chondrocytes were used attempting to regenerate hyaline cartilage.
Tendon tissue regeneration on the basis of MSC was first focused in 1998 when a study on the use of MSC in a rat Achilles tendon defect model was published (Young 1998).
While the application of regenerative principles, especially MSC, was motivated by use of the horse as an excellent animal model in orthopedic research, this changes when in 2003, the first report on the application of MSC for the treatment of a clinically diseased equine superficial flexor tendon appeared (5). This publication stimulated veterinary research groups worldwide to focus on regenerative medicine with an emphasis on tendon regeneration.

That same year, the intra-articular application of MSC was reported to have high potential even for the regeneration of excised meniscus (6, 7), and this may be the start of attempts to use MSC as an intra-articular therapeutic principle by simple injection. Since then, equine joint disease was the target of some clinical studies (8–10).
Joint disease equally is the target of cell therapy strategies in dogs. Here, other than in the horse. joint pathologies dominate the clinical arena and are the main focus of therapy. Often, the stromal vascular fraction of adipose tissue was used to treat osteoarthrosis in hip and elbow joints, and positive results reported include a positive influence on consumption of NSAIDs.

During the same period of time, non-MSC regenerative therapeutics equally gained interest and were more and more applied. This refers to the use of bone marrow aspirate as a transplant for the treatment of diseased M. interosseous medius. Blood derived therapeutics became equally popular.
Platelet Rich Plasma was trialed to improve bone healing in animal models of mandibular defects. As this addresses tissue regeneration, PRP was first applied in horses to stimulate regeneration of tendon tissue. Autologous Conditioned Serum was originally developed as a therapeutic for human osteoarthritis, and, in analogy, preferably used to treat osteoarthritis in horses as well.

Experimental data have been published on the effect of several of the regenerative therapies, and often, positive effects have been described. As an example, tendinopathy has been induced in horses using collagenase to produce the defect. eqbmMSC, BMMNC, fibrin and saline as a control were applied and compared. eqbmMSC and BMMNC led to a good quality of healing, while fibrin or saline did not support healing the same way in terms of biochemical and histologic parameters (11–13). This points towards the possibility for further improvement of regenerative therapy as well as to the need for further investigation into the mechanisms of action of MSC and their interaction with soluble factors.

While clinical data is not abundant for these therapeutic strategies, and blinded clinical trials do barely exist, positive results together with the overall hype for regenerative medicine stimulated the wide use of all of the regenerative therapies. So far, the only double blinded study using horses with naturally occurring disease, and investigating MSC treatment effects until the tissue level, was presented by Smith and co-authors. However, the horses had to be sacrificed for the completion of the study, and this shows where obstacles for ideal clinical studies may be (14).

However, having had a quick start in regenerative therapies, veterinary medicine, like any other discipline dealing with regenerative therapies, needs to direct itself more towards basic research again, in order to create a foundation for the so far successful attempt to introduce regenerative therapeutic strategies (15). Characterization of MSC and tracking of cells in vivo are amongst the steps which have been undertaken in this direction recently (16, 17).

Laboratories and companies offered services and products with VetCell® in the UK probably being the first offering the isolation, propagation and formulation of bone marrow derived MSC for horses and later for dogs. Other companies developed materials and tools to be used for the production of regenerative therapeutics on site. In the beginning, centrifuges, syringes and filters were developed for the instant production of mainly PRP and ACS. Recently, systems were offered for the on site isolation of stromal vascular cells from adipose tissue for immediate application (e.g. InGeneron®).

Therefore, veterinary regenerative therapeutics can be autologous or heterologous in source, they can be produced on site or be sent to a laboratory and returned to the veterinarian for application, even trespassing national borders.
It is important in this context to take into consideration that, while these therapeutics appear very safe, pharmaceutical regulations apply for any material, which is used in animals with the intention to heal.
Therefore, there is a need for clarification as to the situation of the treating veterinarian when using MSC or other regenerative medicines for therapeutic purposes (18).

Legal framework for regenerative therapies in veterinary medicine in the European Union

Contrary to medicinal products for human use veterinary pharmaceuticals are neither governed by the so called Advanced-Therapy Medicinal-Products Regulation (ATMP, Regulation (EC) No 1394/2007) nor by the Tissues and Cells Directive (**Directive 2004/23/EC)** of the European Union. Both, only apply to pharmaceuticals for human use. The ATMP-Regulation, which sets regulations for market approval, monitoring and pharmacovigilance for tissue engineering products, gene therapy and somatic cell therapy products for human pharmaceuticals, discloses veterinary medicinal products since the ATMP-Regulation only refers to European Directive 2001/83/EC (community code pertaining pharmaceuticals for humane use), which only regulates medicinal products for human use. The European Tissues and Cells Directive discloses pharmaceuticals for veterinary use since the scope of the Directive is limited to human medicinal products by art. 1 of the Directive. Therefore, the Tissues and Cells Directive is setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution only of human tissues and cells.

However, regenerative pharmaceuticals for veterinary use are already governed by the current legislation of the European Union. The production and market authorization of regenerative pharmaceuticals for veterinary use are mainly governed by Directive 2001/82/EC and Regulation (EC) No 726/2004, respectively. Directive 2001/82/EC includes provisions governing the production, placing on the market, distribution and use of veterinary medicinal products. Since Directive 2001/82/EC does not discriminate between “classic” pharmaceuticals and regenerative pharmaceuticals as it is true for pharmaceuticals for human use, unlike in the sector of pharmaceuticals for human use,

there is no special regulation for regenerative pharmaceuticals for veterinary use. Regenerative pharmaceuticals for veterinary are rather treated regularly as (veterinary) pharmaceuticals. If pharmaceuticals for veterinary use contain cells or if cells were used to produce such pharmaceuticals Directive 2001/82/EC states special documentation responsibilities for the manufacturer.

Basically no veterinary medicinal product (exceptions do not apply to cell-based products) may be placed on the European Union market without a marketing authorization. Therefore, no veterinary medicinal product may be administered to animals unless a marketing authorization has been issued, except for the purpose of product testing. Where there is no medicinal product for a condition, Member States may exceptionally, in order to avoid causing unacceptable suffering to the animals concerned, permit the administration to non-food-producing animals of medicinal products for human use.

The authorization is issued by the competent authority of the Member State concerned or, where the centralized procedure established by Regulation No 726/2004 applies, by the European Medicines Agency (EMA). Contrary to cell-based medicinal products, market authorization for veterinary medicinal products are regularly granted by a competent authority of a member state of the European Union and not by the European Union. However, exceptions apply to such cell-based pharmaceuticals for veterinary use which are manufactured by means of recombinant DNA technology, controlled expression of genes coding for biologically active proteins in prokaryotes and eukaryotes including transformed mammalian cells, and hybridoma and monoclonal antibody methods. In such cases, the relevant medicinal product has to be evaluated by EMA and granted by the European Union if the pharmaceutical meets the European requirements for safety and quality for such pharmaceuticals.

The difference between a market authorization granted by the European Union and a market authorization granted by a national authority is the legal range. Whereas an European Union granted authorization covers the market of the entire European Union, national granted authorizations only cover the state in which the authorization has been granted. Therefore, if a regenerative medicinal product for veterinary use is granted in one of the European Union's member states, that product cannot be used in another member state, since it does not have market authorization there. If the pharmaceutical manufacturer wants to distribute its pharmaceutical product in other member states,

either it has to apply for an additional market authorization in that particular state, or the holder of an authorization asks for this authorization to be recognized in other countries of the European Union (so called mutual recognition procedure). Furthermore, according to art. 3 (2) lit. a) of the European Union's Regulation (EC) 725/2004 under certain circumstances pharmaceutical manufacturers have the option to apply for a centralized market authorization by the European Union if the medicinal product is not covered by the mandatory centralized market authorization process.

Legal status of animal regenerative medicines

According to art. 1 No 2 of the European Union's Directive of medicinal products for veterinary use, regenerative medicinal products are medicinal products since they are substances or combinations of substances presented as having properties for treating or preventing disease in animals; or since they are substances or combinations of substances which may be used in or administered to animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

The essential regulatory challenge regarding regenerative products for veterinary use is the discrimination between regenerative products for single use, e.g. in a treatment attempt or for purposes of basic research, and such products which need a market authorization. Regularly, a market authorization will be mandatorily needed, if the treatment and/or administration of the regenerative product is not based in making scientific/medicinal findings, but in healing attempts, abstract from a single case. Therefore, regenerative products, directed to cure animals, regardless if the product used is autologous or allogeneic, are medicinal products. This is true for autologous products, since the classification of a medicinal product as a medicinal product cannot only be justified by the character of the product itself, but also to the manufacturing process to guarantee quality and safety of medicinal products. When autologous medicinal products for veterinary use are manufactured on a routine base within a certain process, to cure an a-priori undefinable number of animals this speaks for the fact, that such medicinal products have to be classified as medicinal products for veterinary use. Therefore, the mentioned statues governing veterinary medicinal products apply to such products.

Single case treatments

Single case treatments with regenerative medicinal products do not need market authorization since the pharmaceutical regulations only apply to pre-manufactured medicinal products, products, which are manufactured regularly large scale.

Therefore, making scientific/medicinal findings has to be the leading purpose to fall into the scope of a single case treatment regime. Such single case treatments with regenerative medicines is performed under informed consent by the animal owner. Sever legal problems will occur, if there will be regenerative medicines which have official market authorization in one member state of the European Union or in a state outside of the European Union and where such medicinal products are asked to be imported to a member state of the European Union, where the questioned product does not have market authorization, to be used in a single case treatment. Since this products was once classified as a medicinal products, one has to suppose, that a single case treatment is not possible in that case, since the product has no market authorization in that county where it should be administered but should also be seen as medicinal product.

Furthermore, within the single case treatment approaches one has to distinguish between animal experiments and the classical single case treatment with the attempt to cure. Regularly, animal experiments need an official permission by a local competent authority, whereas a single case treatment does not. In a fast evolving field such as the field of regenerative therapies the correct handling of these questions will challenge practitioners.

Routine treatment

The routine use of regenerative medicines for therapeutic purposes requires market authorization of this veterinary medicinal product. If such a market authorization is necessary, the applicant has to undergo the regular procedure of pre-clinical and clinical testing of the new medicinal products. Additionally, its medicinal product has to be manufactured under Good-Manufacturing-Practice (GMP) conditions.

Future perspectives for the legal framework

The European Medicines Agency is aware of the evolving field of regenerative medicinal products for veterinary use and the fact, that such medicinal products unlike medicinal products for human use are not governed by special status of the European Union. Therefore, the European Medicines Agency proposed to explore to what extent the current veterinary medicines legislation can cover these cell-related medicinal products

for veterinary use, and to what extent new legislation is needed. However, it is clear to the European Medicines Agency that the human legal framework cannot be implemented equally in the veterinary field (cf. EMA/CVMP/463298/2010, p. 19).

Conclusion

- Regenerative Medicine has a past, present and future in veterinary medical applications.
- Regenerative therapy in veterinary medicine touches a field where regulatory changes will most probably occur.
- Any regenerative product is by definition a veterinary medicinal product.
- Any veterinary medicinal product requires market authorization.
- The only ways of legally treating animals using regenerative therapies at the time being are (a) in the context of a scientific study, or (b) when the regenerative medicinal product is produced in the clinic of the treating veterinarian under his direct responsibility, and applied by the treating veterinarian under his direct responsibility, as long as there is no such product in the EU having obtained market authorization.

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Workshop 3

Le biotecnologie applicate alle discipline cliniche veterinarie

The global mission of mammalian assisted reproduction

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Mammalian embryology has obtained considerable reputation during the past decades and is regarded now as one of the most developed branches of biomedical sciences with impressive impact on animal breeding and human reproduction. Although achievements are unquestionable, the advancement seems to slow down due to structural, administrative, financial setbacks as well as lack of innovative thinking. These tendencies may endanger the accomplishment of ambitious goals and delay realization of intrinsic possibilities of applied mammalian embryology.

This review is an attempt to focus attention on these problems and call for changes in structure as well as mentality.

The purpose of this work is to critically review the past 25 years of mammalian embryology, in order to answer the question of whether this discipline has fulfilled its role. Firstly, terms used in the title should be clarified. *Mammalian,* in this context, includes humans, domestic and wild animals, but excludes laboratory animals such as rats and mice. *Embryology* as used in assisted reproduction is restricted to the laboratory work involving oocytes and embryos. Sperm technologies are not discussed since artificial insemination in domestic animals developed to a large-scale industry before the period of interest. The clinical part of human infertility treatment is a related but different discipline with its own approaches, instrumentation and specialists; therefore it is discussed here only in a general sense. *Global* refers to a discussion that encompasses the status of embryology and overall efficiency of different methodological approaches around the world. Finally, *mission* means goals that are realistic, achievable and possibly promised, and meet demand and expectations.

This analysis is self-critical, sometimes provocative, and conclusions do not necessarily agree with some widely held views. The intention is to challenge those views, and provoke thoughts and debates that may help eliminate obstacles to progress and accelerate development. It is beyond the goals of this review to suggest specific changes in the structural, financial and legal frameworks of research and development in embryo laboratories. However, to overcome the barriers, and to accelerate advancement changes are inevitable.

Reconsidering the frames seems to be indispensable in the laboratory and clinical work, to replacing atavistic dogmas and obsolete techniques with open minded approaches. This does not necessarily mean more complicated or more expensive procedures: in fact, a seemingly nonsense change in protocols may double the outcome[1], and ridiculously simple technique may result in much higher efficiency and may also open the way to automation[2,3]. However, the possibilities are still far from exploited. A fresh start with the mentality and courage of the great pioneers of the 70's and 80's would be needed for human and domestic animal embryologist to fulfil our destiny.

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Workshop 4

Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria

One Health: a model of integration between the health professions and influences on veterinary medicine

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The battle against antibiotic resistance is a war being fought everywhere and that, in a One Health view, relates the man, the Animals and the Environment. EFSA and ECDC tell us that every year in Europe there are about 4 million infections caused by germs that are resistant to antibiotics. There are 37,000 cases of death with an economic loss, in terms of health resources, which amounts to 1.5 MLD of Euro. The Istituto Superiore di Sanità says that the last surveys in Italy show approximately 280,000 patients affected with an average of deaths ranging between 2 and 3% per year. Italy has long been very engaged in this field, to the point that during the six-month EU presidency in 2014 a dedicated event called "Fighting Antimicrobial Resistance: smart weapons against smart microorganism. One Health and Global Security Agenda as a support for the struggle against anti-Microbial Resistance" was organized. This was not, however, a sporadic initiative; One Health approach is in fact a theme that has marked all the initiatives of the Ministry of Health and the Italian Health Service, which from the apex to the base see doctors and veterinarians at work daily in the service of this system.

There are three areas of action: farm animals, animal by-products and waste. In the first field, plans of Epidemiological-surveillance, of bio-security, of use and consumption of the veterinary drugs, of detection of their use and of production of medicated feed have been prepared and encouraged. In the second field a national plan for the detection of drug residues in food matrices and in the third one a sampling plan for the isolated in animal by-products, including manure.

Ultimately, it is appropriate to emphasize the need for the creation of a greater awareness in all areas affected by the issue, from that of human medicine to the veterinary health; for the latter, I expected, in fact, more attention to animal therapy through a registered and prudent use of antibacterial drugs, a careful evaluation of production processes that contribute to the spread of treatment resistant bacteria, without neglecting the awareness and the educational role of citizens to this inescapable problem.

Workshop 4

Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria

Antimicrobial resistance: actions and interventions of risk management in the international and european context

Loredana Candela

Directorate General for Animal Health and Veterinary Medicine -Ministry of Health

Since their discovery, antimicrobials have played an essential role in the treatment of infections in humans and animals, improving significantly the population health.

This progress is now fading due to the increasing of microbes resistant to antimicrobial agents.
Antimicrobial resistance is a natural biological phenomenon of adaptation of some organisms that acquire the ability to survive or grow in the presence of a concentration of an antimicrobial agent that is usually sufficient to inhibit or kill microorganisms of the same species. The capacity of resistance occurs by genetic mutations or by acquiring, from other organisms, the "pre-established resistance genes".

However, in the last few years, this phenomenon has amplified and accelerated by an excessive and improper use of the antimicrobial agents, both in human and veterinary medicine, exerting strong selective pressures on the microbial population, resulting in:

- loss of efficacy of therapies;
- greater likelihood and severity of the disease;
- greater spread of disease;
- Reduction of productivity and efficiency of breeding.

In the veterinary field, improper use of antimicrobial agents (eg. inappropriate prescribing, poor adherence to treatment, failure to comply with the indications for use, permanence of their residues in feed for non-target species due to phenomena of carry-

over etc.) may be a potential risk factor for the selection and the spread of resistant bacteria, whether commensal and zoonotic ones. Their transfer from animals to humans can occur either directly by contact and consumption of food of animal origin that indirectly through more complex environmental contamination cycles.

However, it is necessary to deepen the impact that the use of antimicrobials in animal husbandry has on human health. In fact, the mechanism by which the resistance can be transferred to humans and the extent of the threat that this poses to human health is still unclear (1).

Ministry of Health has worked for years at national, European and international level, in the promotion and adoption of measures aimed to:

a) Promoting a "correct and rational use" of antimicrobial agents;
b) Improving animal health through a proper livestock health management and the implementation of biosecurity measures, on the basis of the principle that "Prevention is better than cure";
c) Strengthening the monitoring of consumption of veterinary medicinal products through the use of informatics tools in the different steps of distribution and the use of the veterinary drug in the zootechnical field;
d) Identifying and collecting useful indicators for the categorization of farms depending on the level of risk (health, animal welfare and veterinary drug consumption) for a better effectiveness of planning controls;
e) Supervising and monitoring the antimicrobial resistance;
f) Raising awareness and informing people and professionals on the Antimicrobial resistance theme and on the importance of the appropriate use of antimicrobial agents (2).

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Workshop 4

Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria

Prudent and responsible use of veterinary drugs in poultry sector, experience and interventions in the european and national context

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Today the topic of antimicrobial resistance is one of the most important challenges in public health worldwide due to several causes, including the use of the drug in veterinary medicine. Defense of public health, Safeguard of therapeutic arsenal and livestock industry protection are the main aspects to consider in approaching this sensitive issue.

The most recent data (WHO, ESVAC) have drawn attention to the One Health approach and the importance of a progressive and rationalized use of antibiotics at all levels, including animal breeding, to protect and safeguard human health.

The Italian poultry sector pays particular attention to this issue, taking into account implications on the health of consumers, protection of animals and quality of products, so decided to play its role in a responsible and proactive way.

Following the path taken by other countries, such as Denmark and Holland, Companies associated in to Unaitalia have drawn up a "National Plan for the responsible use of veterinary medicine and fighting against antibiotic resistance in poultry sector" in collaboration with the Italian Society of Avian Pathology (SIPA) and with the approval from the Ministry of Health, who spread the plan throughout the whole Country.

The high level of integration in the poultry sector allows a fast and accurate data collection on the antibiotics consumption, as well as a capillary and precise

implementation of the proposed strategies within the Plan to reach gradually the reduction of 40% of the use of these drugs by 2018.

Increased attention to biosecurity measures and animal welfare, together with good managing practices at farm level, implementation of specific restrictive measures on the use of certain drugs and vaccination plans are the main measures contained in the Plan, applied on a voluntary basis. This Plan is currently applied by the great majority of the Unaitalia Companies and covers 83% of Italian broiler's production and 95% of those of Turkey.

Workshop 4

Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria

Epidemiological role of the slaughterhouse in controlling the spread of antibiotic-resistant microorganisms

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Antibiotic resistance is a global, constantly increasing concern and it represents one of the major threats to public health. In European Union several studies aimed to highlight the role and impact of veterinary drugs in farms as a factor able to select antibiotic resistant bacteria are ongoing (CVMP strategy on antimicrobials 2016-2020).

The actual criteria of assessment, management and mitigation of risk connected to the production of food of animal origin require interventions able to "anticipate" health prevention strategies. In particular, this concept regards those related to the first stages of the production process (breeding) that have to be constantly linked with the inputs of the pathological lesions coming from the slaughterhouse.

In such a view the results of a pilot project regarding the modernization of inspection pigs are reported herewith. As a whole 231,673 pigs belonging to 1,832 batches from 636 farms were inspected. The farms were then classified as a function of the prevalence of each single lesion. In brief, the study highlights the importance to carry out a categorization of farms as a function of the level of risk associated with the health status of the animals. Such a classification has to be aimed at a reduction in impact of pharmacologically active substances and to contribute to a final inspection judgment effectively expressed in a global approach optics.

Workshop 4

Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria

Antimicrobial resistance: new frontiers of international research

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At present, the significant increase of AMR bacterial pathogens in food-producing-animals and Humans, rises a serious concern worldwide. Among animal reservoirs, poultry has been described as one of the most affected with reported occurrence of antimicrobial resistant (AMR) *E. coli* higher than 90 %[1]. In Humans, four million patients are estimated to acquire healthcare associated infections every year in Europe, with 25 000 human deaths due to AMR pathogens[2]. In this frame, a rapid prediction of their antimicrobial resistant phenotypes may significantly boost the control of infectious diseases and the overall comprehension of antimicrobial resistance spread and distribution.

Whole Genome Sequencing (WGS) is a particular application of high throughput sequencing technologies providing the complete DNA sequence of an organism's genome at a single time. Briefly, the DNA extract of the microbial isolate is fragmented and then ligate with adaptors. All fragments are then sequenced in parallel. After sequencing, software tools are used to perform the de novo assembly[3, 4]. Since the introduction of high throughput sequencing in recent years, a sharp drop on the overall sequencing costs allowed an exponential increase in studies based on this technology.

WGS data are currently used for the rapid prediction of AMR phenotypes using software tools able to perform an *in silico* search of hundreds of known and putative new AMR genetic determinants (genes and mutations)[5]. Although, a high concordance has been described between *in silico* predicted and phenotypic susceptibilities, caution should be

taken on interpretation of *in silico* results strongly linked to the comprehensiveness and up-to-date of the related antimicrobial resistance databases[5, 6].

Besides, these tools were effectively applied for the discovery of new AMR determinant genes. With this WGS- approach the new plasmid-mediated colistin resistance gene mcr-1 was first described in China in *E. coli* and *K. pneumoniae* strains isolated from Humans and food-producing animals in November 20157. Colistin belongs to polymyxins, a class of antibiotic discovered in 1947, which acts disrupting the outer membrane of gram negative bacteria. Since November 2015, the molecular basis of colistin resistance was believed to rely exclusively on chromosomal point mutations in specific regions (pmrA/B and phoP/Q) leading to structural changes (ParR–ParS system) of the outer membrane in *Klebsiella*, *E. coli* and *P. aeruginosa*[8]. These mutations are not stable after several passages in vitro and cannot be transferred horizontally suggesting in the past a low risk of rapid spread of colistin resistance. Since the discovery of *mcr-1*, researchers all over the world have initiated a retrospective study on already available gram-negative whole genome sequence data for an *in silico* search of the *mcr-1* gene. In few months thousands of genomes have been screened leading to the identification of the gene in gram-negative bacteria isolated in more than fifteen countries belonging to four different continents[9]. According to these studies, the gene is worldwide distributed and is often found on plasmids harbouring other AMR determinant genes including genes coding for carbapenemases and extended spectrum β-lactamases. The co-resistance to colistin and other unrelated drugs is of particular concern since, the use of those drugs might select for bacterial pathogens resistant to not only the drug used but also to colistin. Recently the worldwide emergence of colistin-resistant bacteria in humans without colistin usage as been described[10]. These studies suggests a high risk of rapid spread of colistin resistance in the next future in contrast to what was generally thought since November 2015.

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Workshop 4

Resistenza agli antimicrobici nella catena alimentare, prospettive e contributi della Medicina Veterinaria

Surveillance of antimicrobial resistance in veterinary public health in Italy according to EU legislation

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In Italy, monitoring of antimicrobial resistance (AMR), i. e. monitoring of resistant major food-borne pathogens and commensal opportunistic bacteria and AMR determinants in farmed animals and food of animal origin, is carried out according to current EU legislation (Dir. 2003/99/EC, Dec. 2013/652/EU) and EFSA guidelines.

At present, monitoring of AMR in food animals in Italy and EU is conducted, by reporting antimicrobial susceptibility testing on a combined set of representative isolates of major zoonotic (e. g. *Salmonella*, *Campylobacter jejuni*) and commensal indicator bacteria (mandatory monitoring for *E. coli*, voluntary monitoring for *Enterococcus faecalis/faecium*). The study design includes mandatory monitoring of *Salmonella* spp. isolates from samples analysed according to the National Control Programmes on Salmonellosis in poultry, and *Salmonella* isolates from HACCP and own checks on carcasses at slaughter (according to Reg. 2073/2005/EC). Moreover, it includes surveys at slaughter every other year in a representative sample of epidemiological units of: broilers and turkeys, or fattening pigs and bovines <12mo, and meats thereof at retail. This scheme of surveys allows to check for the presence and prevalence of Campylobacter, *E. coli* (including *E. coli* resistant to Extended-spectrum cephalosporins) from these sources, in order to gather a representative set of isolates to be tested and reported for AMR by Member States to the EU Authorities (i. e. EFSA, according to the EU Commission Decisions).

In Italy data are produced, stored, interpreted and commented by the National Reference Laboratory of Antimicrobial Resistance (NRL-AR) for Italy (http://195.45.99.69/crab/), at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT, Rome, Italy), and approved on a yearly basis by the MoH, before they are sent to EFSA.

Salmonella isolates and metadata from National Control Programmes in poultry and isolates and metadata form passive laboratory monitoring on animals and foodstuffs are sent to the NRL-AR Italy by the Istituti Zooprofilattici Sperimentali (IZS) laboratories network (http://www.iizzss.it/), which actively collaborates with the NRL-AR. The study design of the surveys at slaughter and at retail is done in collaboration with the Ministry of Health, Veterinary/Food Safety general directions. The NRL-AR coordinates the shipping of samples from surveys taken by the Veterinary Services (VS) on the Italian territory, and analyses all samples. Then performs the identification of pathogens of interests for the monitoring programme, and performs the antimicrobial susceptibility testing. Relevant metadata are retrieved from the database of samples taken by the VS, which is kept by IZS Abruzzo e Molise, on behalf of the MoH (SINVSA web-oriented system). Finally, AMR data and metadata are analysed, interpreted, commented and reported to the National (MoH) and EU (EFSA) Competent Authorities by the NRL-AR on a yearly basis.

These data and comments are published yearly on the National Zoonoses Report Italy, on the EU Summary Report on Antimicrobial Resistance (by EFSA), and in the National Reports, all available as online documents (e. g. http://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=2476).

Some examples of the most relevant issues of AMR monitoring in the primary productions and food of animal origin in Italy will be presented.

Workshop 5

Emergenza specie esotiche

The exotic species trade: not only a conservation issue

Luca Brugnola
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The changes in the abundance of a selected number of species can be used as an important indicator of the ecological health of the planet.

The Living Planet Index reflects changes in the state of biodiversity on the planet, using the size of the trend of 9,014 populations belonging to 2,688 species of vertebrates of different biomes and regions. This index shows a 28% decrease of global biodiversity between 1970 and 2008[1].

In 2013, the IUCN Red List found that the animal endangered species categorized as CR (Critically Endangered), EN (Endangered) and VU (Vulnerable) are 23,250 (29%) on 79,837 evaluated (only 5% of the described species)[2].

At the root of all the problems plaguing, directly or indirectly, biodiversity is the unsustainable use of natural resources, as a result of the exponential growth of the world population and the uneven distribution of economic and energy resources[3].Along with the destruction and degradation of habitats, international trade in wild species of flora and fauna is one of the main causes of the world biodiversity's depletion.

The total value of turnover relating to products of wild animal species is estimated at between 350 and 530 million dollars a year and, besides this, it must be considered also the amount of illegal trafficking, difficult to control and estimated in 23 billion dollars a year[4]. The Washington Convention is an international agreement whose purpose is precisely to prevent the uncontrolled trade threatening the survival of wild species of flora and fauna, ensuring, therefore, the conservation and sustainable use of biodiversity.

Animals and plants are traveling rapidly from major “producer” countries, depository of a large share of the world's biodiversity (South America, Central America, Africa and Asia), to "consumer" countries, where wealth and welfare are most widespread (North America, Europe, East Asia, Middle East), also passing through “transformer” countries (Africa, Eastern Europe and Asia).

Unfortunately, plants and animals often handle other more or less undesired guests (viruses, bacteria and macroparasites) which may constitute a sly danger not only for domestic and/or wild animal species, naturally present in the recipient countries, but also for humans who come in direct and/or indirect contact with them.

1,407 is the number of known biological agents of disease in humans; out of these, 58% (816) is transmitted from animals and 13% (177) is considered emerging or re-emerging. Out of the 177 emerging or re-emerging pathogens, 130 (73%) are agents of zoonoses. Emerging infectious agents in the 1940-2004 period were 335 (25% of known pathogens) of which 60% (202 pathogens) is represented by agents of zoonoses and 43% (144 pathogens) is transmitted by wildlife[5].

The risk of introducing pathogens, together with animals or plants, clearly increases in terms of logical probability when individuals whose health status is virtually unknown (specimens from illegal trafficking or caught from the wild) are marketed and when epidemiological situations in the origin countries are little or not known.

The health risk is therefore analyzed in relation to legal and illegal trade in live animals affecting Italy in the period 2009-2014, species involved, origin of the specimens and related origin countries.

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Workshop 5

Emergenza specie esotiche

New introduced vector species and the role of the OIE reference centres

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Vector-borne diseases (VBDs) are transmitted by arthropod vectors such as ticks, mosquitoes and sandflies. Zoonoses are vector borne infectious diseases affecting both humans and animals. Prevalence of many VBDs, is increasing worldwide, while other VBDs are emerging or reappearing in countries where they have never been found or were considered eradicated. For this reason, they are considered emerging diseases.

VBDs distribution is closely related to the one of their vectors that is affected by environmental and climate changes, population movements, trade, urbanization. Introduction of exotic vector species can contribute to new pathogen introduction or may affect dissemination of already present pathogens. In any case, adaptation and establishment of a vector in a new area increase the risk of new diseases.

The aim of this talk is focusing the attention to the possible implications related to new vectors species introduction in a new area.

At the Istituto Zooprofilattico della Sicilia three OIE Reference centres for vector-borne diseases are present (OIE Reference Centre for Babesiosis, for Theileriosis and for Leishmaniosis). They are involved in the implementation of vector sustainable control strategies, assistance in surveillance for both carriers and diseases, diagnosis and case management.

Some of the factors affecting vector distribution can be limited using prevention and control measures for animals and vectors. However, more complex it is the case of unlawful introduction of animals or the movement of wild animals and migratory birds.

Just to provide some examples, on 2008 an illegal traffic of tortoises from Northern Africa was discovered in Sicily. The intervention allowed the recovery of about 1400 live *Testudo graeca* individuals. The animals were infested by *Hyalomma aegyptium*, a tick species rarely found in the Italian territory, but that can be occasionally found due to illegal

importation of its hosts. *H. aegyptium* is a vector for several pathogens as *Rickettsia aeschlimannii, R. africae* and *Theileria annulata*.

Climate changes could also allow *Rhipicephaline* adaptation to European transalpine climate with the consequent spread of diseases such as babesiosis, theileriosis, anaplasmosis and rickettsiosis. The similarity in the habitat could enable the tick *Hyalomma anatolicum anatolicum* to establish in our territory. The tick is widely distributed in central Asia, Middle and Near East, Arabia, southeastern Europe and north Africa and is a vector of CCHFV, *Theileria* and *Babesia* species, *Anaplasma marginale* and arboviruses.

As concerning sandflies, the introduction of exotic species in our territory could determine new *Leishmania* species introduction, as for example *Leishmania major*, the etiologic agent of a cutaneous leishmaniasis spread in Central and North Africa, Middle East and Central Asia.

Control of new vector species introduction is therefore essential to improve risk prevention and the health status for both animals and humans.

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Workshop 5

Emergenza specie esotiche

Rickettsial diseases with emphasis on Rocky Mountain Spotted Fever

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The *Rickettsidae* of veterinary and human interest include *Anaplasmataceae* and *Rickettsiaceae* and are obligate intracellular "parasitic" microorganisms. Mitochodria are closely related. *Anaplasmataceae* include the genuses *Ehrlichia*, *Anaplasma*, *Neorickettsia* and *Wolbachia*. The genus Rickettsia[1] belongs to *Rickettisaceae*. *Coxiella* is morphologically similar to *Rickettsia*, but not genetically and physiologically. They are transmitted by arthropods, including chiggers, ticks, fleas, lice and have specific geographic locations and induce diseases with specific names. There are 3 groups: spotted fever group (SFG), typhus group (TG), scrub typhus group (STG)[1]. They multiply within the endothelial cells. This is associated with vascular wall necrosis, inflammation leakage and thrombosis, rhexis and petechial or large hemorrhages. Degenerative changes in muscles frequently occur in Rocky Mountain spotted fever (RMSF). Our studies[2] in mice indicate that in mice bacteria localize within circulating monocytes, macrophages, hepatic Kupffer cells and Iba1 detection indicates a prominent increase of macrophages in liver and spleen and monocytosis. Lesions include multifocal, mild to severe hepatic necrosis with formation of microabscesses and microscopic pyogranulomas, splenic necrosis and neutrophilic splenitis. In humans symptoms include fever, headache, rash, malaise, lymphadenomegaly. Severe weakness, often with cough, respiratory distress, and sometimes emesis follow. Gangrene may develop, hepatomegaly, splenomegaly and renal disease may occur, and blood pressure may fall dangerously low causing shock and demise. *Rickettsia conorii*, transmitted by *Ripicephalus sanguineus*, causes Mediterranean spotted fever (MSF) in humans in Mediterranean countries, Sub Saharan Africa and Asia and has been identified via

serology and PCR as cause of febrile illness in dogs in Southern Italy and its Islands[3]. Rocky Mountain spotted fever (RMSF)[4,5] is a disease of humans and dogs caused by *Rickettsia rickettsii* endemic in large parts of the Americas and it is transmitted primarily through the bites of infected ticks such as *Dermacentor variabilis, D. andersoni, Amblyomma, Rhipicephalus sanguineus*. The pathogenic role in dogs of genetically similar agents, such as *R parkerii*, is unknown. Dogs are sentinels for *R. rickettsii* infection in humans. Clusters of disease are frequently reported in defined geographic areas, and temporally associated infections may be seen in both dogs and their owners. The pathogen is acquired by larval and nymph stages of ticks while feeding on infected vertebrate hosts and is also passed vertically from female ticks to progeny via transovarial transmission. About 1% of *Dermacentor* spp ticks harbors *R. rickettsii*, even in areas considered highly endemic. In highly enzootic regions of Arizona where *R. rickettsii* is transmitted by the brown dog tick, as many as 5% of ticks may be infected. Seroprevalence in dogs from endemic areas may range widely (4% to 77%, crossreactivity with other *R.* may impair values). Transmission via blood transfusion should be considered. Direct transmission from dogs to humans has not been reported, although human infection may occur after contact of abraded skin or conjunctiva with tick hemolymph or excreta during removal of engorged ticks from pets. It is rarely diagnosed in cats. Clinical signs include fever (39.2°C to 40.5°C), oral, ocular genital, retinal petechiae and ecchymoses, edema of extremities, late-stage necrosis (acryl gangrene). Other findings in dogs may include evidence of abdominal pain, anorexia, or both; altered mental status (signs of depression, stupor); myalgia, polyarthritis, or both; vestibular deficits (circling, head tilt, or nystagmus).There may be cough or dyspnea and thoracic radiography typically reveals diffuse interstitial densities (pneumonitis). Clinical laboratory findings are hypoalbuminemia, moderate leukocytosis (minimal left shift), and thrombocytopenia. Platelet counts usually range from 25,000 to 250,000/µL, hypoalbuminemia from widespread damage to the vascular endothelium and subsequent intercellular leakage. Diagnosis is usually based on clinical recognition and serology; the latter requires comparison of acute- to convalescent-phase serology, so is only helpful in retrospect. Etiologic agents can generally only be identified to the genus level by serologic testing. PCR and immunohistochemical analyses may also be helpful. If ehrlichiosis or anaplasmosis is suspected, a buffy coat may be examined to identify characteristic intraleukocytic morulae. Doxycycline is the treatment of choice 5–10 mg/kg/day, PO or IV, for 10–21 days. Tetracycline at 22 mg/kg, PO, tid for 2 weeks is also effective.

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Workshop 5

Emergenza specie esotiche

Infectious and parasitic diseases in non traditional pets

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Non-traditional pets include exotic animals, defined either as imported, non-native species or species that originally were non-native but now are bred in Europe; indigenous wildlife; and wildlife hybrids (wildlife crossbred with domestic animals producing offspring known as hybrids) (1). The definition of non-traditional pets includes certain species of mammals (e.g. rabbits, ferrets, rodents) and birds (especially parrots), reptiles and amphibians. Infectious and parasitic diseases of non-traditional animals include both typical diseases of the species and zoonoses. Concerning infectious diseases, rabbits can be affected by rabbit hemorrhagic disease caused by a Calicivirus or by myxomatosis, a Leporipoxvirus-induced disease but also by potential zoonotic agents as *Salmonella* spp. and *Encephalitozoon cuniculi* (2). The main infectious diseases of ferrets are distemper and epizootic catarrhal enteritis as well as influenza which may be transmitted from ferret to human and vice versa. An important disease of parrots is the psittacine beak and feather disease caused by a circovirus which attacks cells of the immune system and those cells that produce the feathers and the beak. Parrots may serve as a reservoir of zoonotic agents as *Campylobacter* spp., *Salmonella* spp. and *Chlamydophila psittacii*. Reptiles and amphibians may act as a reservoir of zoonotic agents as *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (3,4). One of the most serious diseases of snakes, primarily boas and pythons, is the inclusion body disease which affects the nervous system and other organs and has been recently associated with Arenavirus infections. Amphibians can be infected by *Pseudomonas* spp. and *Aeromonas* spp. which have been indicated as the causal agents of the red leg syndrome.

Similarly, non-traditional pets may harbor a variety of ectoparasites (e.g. lice, fleas, ticks and mites) and endoparasites (protozoa, nematode, cestoda and trematoda); some of them may have a zoonotic potential as *Giardia, Cryptosporidium, Dirofilaria* and other helminths and arhtropoda. Interestingly, co-infections by viruses/bacteria and parasites may occur in non-traditional pets. As an example, a significant positive correlation between *Salmonella* spp. and oxyurids was demonstrated in turtles (5). However, while knowledge of the husbandry and veterinary care of non-traditional pets is increasing, still little information is available on the presence and prevalence of parasitic diseases.

Most importantly, diagnosis and control of parasitic infections are often neglected in pet exotic animals. For these reasons the multivalent FLOTAC and Mini-FLOTAC techniques (6,7) were recently introduced also in exotic pet medicine in order to improve diagnostic performance of parasitic infections.

FLOTAC has been validated as to detect parasitic infections in pet lizards and snakes (8), ferrets (9), squirrels (10), rodents (11), and other exotic pets. Regarding small mammals, surveys performed in southern Italy by FLOTAC showed the presence of intestinal parasites in 30% of pet ferrets (infected by ancylostomids and *Sarcocystis*), 18% of pet squirrels (infected by *Dicrocoelium dendriticum, Syphacia, Strongyloides* and other nematoda). Furthermore, FLOTAC and Mini-FLOTAC techniques have been recently used for the diagnosis of parasitic elements and yeast infection (*Macrorhabdus ornithogaster*) in pet birds (12).

In conclusion, it is mandatory to collect data and to study in depth the epidemiology and clinical significance of infectious and parasitic diseases in non-traditional pets using accurate methods in order to improve diagnosis and better planning control measures.

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Workshop 5

Emergenza specie esotiche

Reptile associated salmonellosis: well-known disease but of unknown relevance

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During the last decade, reptiles have become increasingly popular pet animals. However owners are not often aware of the sanitary risks associated with hosting turtles, bearded dragons, iguanas, and similar. Reptile associated salmonellosis (RAS) is a well known zoonosis: it has been reported in humans since 1943[1]. *Salmonella* organisms occur naturally in the gastro-intestinal tract of many reptiles as a part of their normal microbiota[2]. Animals do not usually show clinical signs, but scatter the pathogen with feces, infecting other individuals and contaminating their environment[2]. *Salmonella* shedding is intermittent, causing troubles to identify healthy animals from carrier ones[3]. Thus all pet reptiles need to be considered as an infection hazard to people[1]. Transmission can be direct or indirect. Sporadic indirect contacts (at relatives or friends houses or animal shops) are enough to cause the illness[3]. Categories at major risk are children under 5 years of age, elderly people and immunocompromised people. In USA, RAS are regularly notified and represent the 11% of sporadic salmonellosis in subject younger than 21 years of age[4]. Few information are available about the European (and Italian) situation, because a uniform system of notification is not currently in use[5].

The aim of the present study was to perform an epidemiological study to estimate the impact of RAS on the population affected with salmonellosis afferent to the Hospital "Città della Salute e della Scienza" of Turin. Another goal was to evaluate the risk of exposure to *Salmonella* in public places, such as shops, fairs and exhibits.

To perform the epidemiological study, a telephone survey was administered to every patient with salmonellosis in order to characterize demographics, clinical severity, exposure to reptiles and other risk factors. Patients who reported reptile contact during the exposure period were asked questions about where the exposure occurred and, if the reptile was kept at home as a pet, about the animal husbandry. Patients owner of reptiles were asked for their willingness to submit stool and habitat samples in order to perfom further analysis.

To evaluate the risk of exposure to *Salmonella* in public places, observations of human visitors' and traders' behaviors, related to potential hygiene and pathogen transfer issues, were conducted. In details, visitors and trades were observed and the direct or indirect contacts with a presumed contaminated source, and the subsequent touched items, were recorded. Moreover the presence of in situ public health warnings and safety protocols was assessed.

Twenty-one interviews were administered between January 2015 and March 2016. Seven patients were females and 14 males, of age ranging between one to 80 years. Two cases reported, as unique risk factor, the exposure to turtles, at relatives' house and by a lake, respectively. One other case owned a turtle as pet at the time of the illness, but reported other risk factors too, such as having eaten cold cuts, raw shellfish and raw milk or raw milk cheeses. The patient refused to submit specimens from the animal or the environment.

To evaluate the risk of exposure to *Salmonella* in public places, three shops, three fairs and one exhibit were visited between April 2015 and April 2016, for an amount of observational time of 400 minutes. During this time frame, 61 contacts were reported, mainly indirect (58) and performed by visitors (54). The most touched item, after the contacts, was body/bag (30 times), followed by other people (10), eyes/nose/mouth (7), other items (10) and smart-phone (3). Public health warnings and safety protocols were never noticed.

The preliminary data recorded in this study shows that exposure to reptiles should always be considered as a risk factor in salmonellosis cases, given also the ease with which people can come into contact with contaminated sources. More data will be produced in order to confirm and give consistency to these results.

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Workshop 5

Emergenza specie esotiche

Exotic pets: pathology, welfare, public health. A new challenge for the italian Public Health Institutes - Istituti Zooprofilattici Sperimentali

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Reptiles, birds, rodents, small mammals are the new pets. In recent years, the exotic or unconventional animals have become an increasingly important presence both as pets and as a type of farming. To recognize their state of health, what are the indicators, what are the health problems, the implications as potential carriers of zoonotic agents, diagnostic methods, health standards, are just some of the skills to improve and to put available to owners and breeders.

EURISPES data show that Italian people having an animal is increasing. 22.5% of the population in Italy, 55% of households, has decided to adopt an animal, 9.3% of Italians has two, 4.1% have three and 7.4% have more than three pets. The best friend is the dog (60.8%) followed by cats (49.3%), then are fish and turtles (both 8.7%), birds (5.4%), rabbits (5.2%), hamsters (3.1%) and exotic animals (2.1%) . The 41.7% of people has animals to fill the loneliness; 18.5% to have someone to care. The northwestern and central Regions. From the point of view of the Veterinary Public Health concern, it is important to define the information based analysis instruments, the possible zoonotic implications, due to the close relationship between man and animals. Regarding the exotic pets, the risk of disease agents are still little known or even unknown, taking into consideration some key factors: the importation of animals with unknown health status, from foreign Countries, the possibility of illegal trade of animals (in fact, the data show that Italy is among the most active Countries in the international trade of animals), the relative ease of acquiring exotic animals via e-commerce[2]. Literature describe investigations in order to understand the reasons for the adoption of a dog or a cat or, at most of the canaries, but there are very few scientific data describing the psychological

motives on the choice of an unconventional or even exotic animal. In today's world there is an easy access to new and exotic destinations, and to new and fascinating animal species, but it is necessary the need to ask the question whether these animals can be kept as pets and what are the needs in terms of care, of welfare of both proprietary and animals[3].

The diagnostic laboratories of the Veterinary Public Health Institutes in Italy (Istituti Zooprofilattici Sperimentali - IIZZSS), have the task to protect human health and in the course of decades, since they were set up, while maintaining the skills and functions identified since their institution, they have done the natural evolution aimed at meeting the needs of public health as necessary and the challenge of non-conventional animals, their health care and the welfare conditions warranty is one of challenges, to fit their purpose. The goal is to protect the human health as well as animals and environment. The diagnostic capacity of zoonoses is a key factor. are the most involved[1].

Zoonoses involve infections and infestations transmissible from animals to humans. Zoonoses are a major global threat. Exposure to zoonotic pathogens exists in various settings including encroachment on nature; foreign travel; pet keeping; bushmeat consumption; attendance at zoological parks, petting zoos, school 'animal contact experiences', wildlife markets, circuses, and domesticated and exotic animal farms. Under ascertainment is believed to be common and the frequency of some zoonotic disease appears to be increasing. Zoonoses include direct, indirect and aerosolized transmission. Improved awareness of zoonoses in the society, including hospital environment may be important to the growing need for prevention and control[4]. There is a significant need for the promotion of awareness and management of zoonoses and this is one of the functions of the IIZZSS.

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Main Lecture

Appetite regulation in health and disease

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Appetite is a complex process with multiple neural, hormonal and metabolic inputs. Within the hypothalamus there are two classes of neurotransmitters regulating appetite, the orexigenic neurotransmitters (Agouti-related protein, neuropeptide Y, orexin, melanin concentrating hormone) and the anorexigenic neurotransmitters (such as α-melanocyte stimulating hormone). In addition, hepatic oxidation signals and motility and chemical signals from the GI tract are communicated to the brain stem via the vagus and sympathetic nerves and integrated along with hypothalamic inputs at the nucleus tractus solitarius. In addition, hormonal signals, such as leptin, cholecystokinin, Ghrelin and many others, may also interact with the hypothalamus and the nucleus tractus solitarius to control feed intake. A series of studies were developed to examine the major stimulatory neurotransmitters for their role in controlling feed intake in ruminants. After determining which neurotransmitters affected feed intake, a series of studies were initiated to examine the effects of endotoxemia on appetite control mechanism in sheep. Elevated leptin concentrations in plasma were examined as a potential mechanism to explain the inhibition of appetite in disease, but leptin concentrations were unchanged utilizing endotoxin, parasitism and *Salmonella* infection models in farm animals. Therefore, studies were initiated to examine the proopiomelanocortin neuron and the melanocortin-4 receptor as a target for disease-associated reductions in feed intake. Although in the sheep, proopiomelanocortin gene expression is unchanged after endotoxin, the blockage of melanocortin-4 receptors with agouti-related protein prevents the feeding inhibition characteristic of endotoxin, suggesting a role for the melanocortin receptor in mediating feed intake inhibition in disease. Since melanocortin-4 receptor agonists have been designed to cross the blood brain barrier and increase feed intake, the possibility exists that these antagonists may be utilized to maintain feed intake in critically ill patients and thus enhance animal welfare and productivity in farm animals.

Main Lecture

SISVET, ACCADEMIA: per una sintesi dei mille saperi in una società liquida

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Testimone di una cultura frantumata

Al fin del cammin di mia vita, mi ritrovai per una selva oscura, che la diritta via era smarrita potrebbe essere una glossa dell'incipit dantesco per una breve premessa ad alcune considerazioni che riguardano lo stato della nostra conoscenza in un travagliato periodo culturale del quale sono al tempo stesso partecipe e testimone.

Dopo il Liceo Classico, nel 1945 m'iscrivo nella Facoltà di Medicina Veterinaria dell'Università di Bologna presso la quale, il 18 luglio del 1947 viene fondata la Società Italiana delle Scienze Veterinarie, ma nell'ottobre del 1946 partecipo a Roma al Congresso Scienza di Filosofia, percorrendo in continuazione un difficile cammino di raccordo tra la cultura scientifica e quella umanistica che nel 1959 Charles P. Snow infiamma con il suo celebre pamphlet Le due culture.

Siamo in un quasi magico periodo nel quale le scienze sperimentali stanno esplodendo in potenza e in innovazione e al tempo stesso si frazionano in discipline sempre più specialistiche, in un processo ancor oggi in un pieno e sempre più rapido svolgimento. Queste, a mano a mano che vanno formandosi, sempre meno dialogano tra di loro e usano linguaggi di difficile se non impossibile comprensione da parte di una società civile che pur ne accoglie le applicazioni pratiche che si diffondono con ritmo incalzante e occupano tutti gli spazi della vita.

Di pari passo quelle che erano le scienze umanistiche cadono in una crisi sempre più profonda che non pare trovare vie d'uscita, nonostante i molti e diversi tentativi, non ultimi quelli di una loro sempre più specialistica scientificizzazione che le porta, anch'esse, a una progressiva frantumazione.

Un incontro o scontro tra le due culture frantumate che mi trovo continuamente dover affrontare non tanto nella ricerca scientifica specialistica della mia carriera

universitaria, quanto in quella della didattica e formazione di allievi e che si acutizza quando, per quasi nove anni, inviato dal Governo Italiano alla Comunità Economica Europea a Bruxelles debbo contribuire a costruire un dialogo tra le conoscenze scientifiche e le condizioni sociali in tutta la loro complessa varietà culturale, che mi viene largamente ampliata dai numerosi viaggi in Asia e nelle Americhe. Una complessità che cerco di affrontare e superare con il pensiero sviluppato dall'antropologia nelle sue diverse suole, non ultima quella strutturalistica.

L'estrema, stimolante difficoltà sta nella complessità e soprattutto nel frazionamento delle mille discipline scientifiche e umanistiche é alla base della presente Società Liquida e dei Non Luoghi.

I mille saperi della Società Liquida e dei Non Luoghi.

Nei miti con le idee rappresentate e descritte dai loro dei, gli antichi costruiscono, identificano e tramandano prescrizioni che ordinano, regolano e fondano il mondo sociale umano, in un sapere che, anche se mutevole con le diverse popolazioni e dissimile di periodo in periodo, si prolunga per millenni, e che di recente ha visto un pericoloso se non esiziale stravolgimento.

Antica era la coesistenza non conflittuale ma dialogante tra il sapere razionale e quello emozionale, in equilibri che si sono prima incrinati e poi tragicamente rotti, e sono stati sostituiti da derive che da pericolose sono divenute quasi distruttive di una cultura che, in ogni società, e per essere tale, deve essere unitaria, anche se nel suo interno multipla e dialogica.

L'unità della conoscenza e del sapere si é progressivamente frazionata con il sorgere delle discipline sperimentali, iniziate nel Rinascimento italiano con il metodo galileiano. Queste discipline hanno avuto un indubbio successo, ma non sono state sufficientemente integrate tra loro e soprattutto non sono state inserite in un più vasto e unitario pensiero umanistico, come invece avveniva in chi le ha inventate, Galileo Galilei, e in coloro che nei primi tempi le hanno accolte e sviluppate.

Decisivo per il mancato dialogo tra le discipline scientifiche e del fallito processo d'integrazione é stato un tragico capovolgimento di rapporto tra sapere e tecnica. In un passato sempre più lontano e che salvo sempre più rare eccezioni sembra quasi perduto, le tecniche nascevano e si sviluppavano nell'ambito di un pensiero unitario. Oggi invece singole tecniche, sempre più specialistiche e autoreferenziali, si sviluppano autonomamente. In questo modo si vengono a creare nuovi, singoli frazionati saperi, che tali non possono essere perché spezzati, segmentati e caoticamente inseriti in un mondo culturale che, per questa su suddivisione, suscita le incertezze della modernità e le paure dell'attuale postmodernità.

In un recente passato il già citato Sir Charles P. Snow ha segnalato il dilagante pericolo di Due Culture, mentre oggi stiamo assistendo all'incontrollata crescita di mille scienze, grandi e piccole, ognuna delle quali parla linguaggi diversi, tra loro non

comunicano e nel loro insieme formano un'oscura selva dantesca.

In questa selva delle mille discipline scientifiche quello che la tradizione diceva non é più vero, ma soprattutto il vero e il giusto cambiano di momento in momento e da luogo a luogo, dando origine a una Società Liquida (Zygmunt Bauman) che si espande in Non Luoghi (Marc Augé).

Nel periodo delle ultime due generazioni, dal 1950 al 2010, questa selva oscura si é straordinariamente sviluppata e senza freno continua a aumentare per l'azione incontrollata di Atena, dea di una conoscenza razionale che non basta all'uomo, e per l'assenza equilibrante di Dioniso dio del sapere contaminato.

Una veterinaria frantumata in una società che cambia

Laureato in Medicina Veterinaria nel 1949 sotto la guida del Prof. Albino Messieri, uno dei fondatori della SISVET, con una tesi sperimentale svolta nella unificante disciplina della Clinica Medica, di una Facoltà di Medicina Veterinaria, unitaria nelle sue principali discipline morfologiche, anatomopatologiche, cliniche.

Dopo una breve permanenza presso la Clinica Medica Veterinaria dell'Università di Bologna, passo in quella delle Università prima di Camerino e poi per approdare e rimanere in quella di Parma. Un periodo di due generazioni, oltre sessanta anni durante i quali, anche per lo svilupparsi di sempre più sofisticate tecnologie, ma anche per i profondi se non radicami cambiamenti sociali e economici, la Veterinaria subisce un processo di frammentazione, anzi di poliframmentazione.

Nelle Facoltà di Medicina Veterinaria si assiste a una progressiva frantumazione delle discipline scientifiche che divengono sempre più specialistiche, erodendo se non in diversi casi distruggendo il potere unificante e di sintesi delle grandi linee di pensiero che i Maestri della Veterinaria avevano costruito, sviluppato e difeso. Una frantumazione che s'aggrava ulteriormente negli ultimi tempi, con la scomparsa delle facoltà e l'assoluta inefficacia unificante del Corso di Laurea.

Un analogo, per certi aspetti necessario processo, avviene con la costituzione di società scientifiche specialistiche veterinarie.

Allo stesso modo anche la pratica veterinaria si frantuma, scindendosi secondo le specie animali, come il Servizio Sanitario Nazionale che si suddivide in tre aree.

Drammatica, se non a volte quasi tragica é la conseguenza della poliframmentazione che, di fatto, rende difficile il dialogo e soprattutto rende difficile, se non quasi impossibile, arrivare a sintesi culturali e operative, quali sono richieste – sia pure spesso inconsciamente – da una società che da una parte lei stesso é frammentata e liquida, e che al tempo stesso sta profondamente cambiando, ha perso i tradizionali rapporti con il mondo animale e é alla ricerca di una nuova zooantropologia, che non può scaturire da una miriade di discipline specialistiche e dal diffondersi di controculture

antiscientifiche.

Cultura e controcultura antiscientifica

Tra i paradossi in tutti gli aspetti della vita contemporanea, vi é l'emergere e il diffondersi delle controculture e tra queste anche la controcultura scientifica.

Evidenti e sotto gli occhi di tutti sono i fenomeni di controcultura che vanno dalle varie forme di arte, al modo di vivere, vestire e di mangiare, alla medicina e cura delle malattie, al rapporto con gli animali e che si esprimono in modi diversi e continuamente cangianti.

Le controculture sono state diversamente interpretate, anche nei loro aspetti positivi di evoluzione sociale.

Per quanto riguarda le controculture scientifiche, più propriamente antiscientifiche, é da rilevare che sono sempre esistite fin dai tempi di Galileo Galilei e degli Illuministi, ma che non hanno mai fermato il progresso scientifico.

Senza entrare sulle profonde e spesso inconsce motivazioni delle controculture antiscientifiche, e non limitandoci a un loro inquadramento nella società borghese della quale stiamo assistendo alla scomparsa, é qui necessario segnalare il ruolo che ha avuto e continua ad avere la frammentazione delle scienze, ma soprattutto la generalmente scarsa capacità comunicativa di chi opera in queste.

Il linguaggio delle scienze diviene necessariamente e progressivamente sempre più specialistico, man mano che progredisce il loro differenziamento e sviluppo.

Per questo, i linguaggi tra le diverse scienze si distaccano e rendono non sempre facile un dialogo tra le diverse discipline, contribuendo alla frammentazione della conoscenza scientifica, che agevola la nascita delle controculture antiscientifiche.

Altrettanto, se non ancor più importante é il distacco tra il frammentato linguaggio delle mille discipline scientifiche, non di rado con una rilevante componente matematica o simbolica, e il parlare della gente comune.

Per questo si generano equivoci e fraintendimenti favorevoli alla nascita, sviluppo e soprattutto strumentalizzazioni da parte di controculture antiscientifiche.

In proposito basta pensare al concetto di rischio che nel linguaggio scientifico é una probabilità che deve essere espresso in termini matematici, mentre nel linguaggio comune é confuso con quello di pericolo e quasi di certezza.

Atena Dea di una sempre più potente e frazionata conoscenza tecnologica razionale

Atena, figlia di Zeus è la dea della sapienza, delle tecniche, delle arti di lavorare i metalli in tutti i vari tipi di artigianato, venerata poiché a lei sono dovute le invenzioni e

le tecnologie di ogni tipo, di pace e di guerra e fra coloro che la invocano, aiuta solo chi usa l'astuzia (metis) propria di personaggi come Odisseo. Astuzia e furbizia sono le doti che Atena dona e al tempo stesso esige dai suoi protetti.

Atena è accompagnata da Nike, dea della Vittoria tecnica e nella tarda età classica il legame fra le due idee di tecnica e di successo é così forte che si fondono nell'unica divinità Atena Nike, spesso raffigurata come un'Atena alata e dallo sguardo acuto, alla quale è dedicato l'omonimo tempio sull'acropoli di Atene.

Atena Nike, con le sue sempre più nuove, raffinate e differenziate arti tecniche oggi manifesta il suo potere sia in guerra sia in pace, soprattutto quando associa la fredda razionalità all'astuzia e alla furbizia, e quando collabora con Efesto. É quest'ultimo il dio di molti fuochi, non ultimo quell'odierno atomico, é anche il dio dell'energia bruta di struggitrice.

Atena Nike, dea guerriera, armata e con ali che le permettono di spostarsi e operare in tutto il mondo, ieri ancor più oggi opera in tutto il mondo nell'epoca attuale anche attraverso anche le organizzazioni sovranazionali e le imprese multinazionali. Sotto l'aspetto culturale, Atena Nike rappresenta l'idea operante di una sempre più potente, ma frazionata conoscenza tecnologica razionale.

In una concezione sempre più specialistica, nell'epoca attuale vediamo generarsi e diffondersi un'incessantemente crescente serie di discipline specialistiche che, se da una parte danno risposte tecniche a vecchi, nuovi e sempre mutevoli bisogni umani, al tempo stesso non forniscono risposte sicure e soprattutto non danno indirizzi di percorsi di vita individuali e sociali e non danno significato al grande mistero della vita.

Questo, perché il potere di Atena Nike non é più contemperato dalla azione di Dioniso, Dio dei poteri contaminati.

Dioniso Dio dei saperi contaminati

Dioniso, dio dell'estasi, della liberazione dei sensi e di un sapere emotivo, per la saggezza antica rappresenta l'essenza del creato nel suo perenne fluire, lo spirito divino di una realtà smisurata, l'elemento primigenio del cosmo, l'irruzione spirituale, ossia l'esistenza intesa in senso assoluto e nel frenetico flusso di vita che tutto pervade.

Dioniso, il giovane figlio di Zeus, ci riporta allo Spirito dionisiaco che Friedrich Nietzsche contrappone allo Spirito apollineo, che Georg Wilhelm Friedrich Hegel riporta alla conoscenza del Vero, mentre Kàroly Kerényi ricorda che dove regna Dioniso la vita si rivela irriducibile e senza confini.

Dioniso rappresenta lo stato di natura dell'uomo, presente anche di quello più civilizzato e dimensione originaria insopprimibile, che può emergere ed esplodere in maniera violenta se è repressa, anziché compresa e incanalata correttamente.

Una dimensione umana, che deve essere ricuperata soprattutto quando ci siamo perduti nel bosco sempre più fitto e disordinato delle scienze, quasi senza eccezione poco sapienti e sempre più tecniche senza un'anima.

Dioniso é Dio dei saperi, soprattutto di quelli contaminati perché contaminare é mettere a contatto e mescolare – da tag o tang – nel bene e oggi, da quando Dioniso é fuggito, anche nel male.

Contaminare é un bene e la strada maestra dello sviluppo del sapere, del vero sapere che é scienza, cioè sapere, che non può assolutamente derivare dal suo opposto, la separazione.

La selva oscura dei mille saperi

La postmoderna conoscenza razionale, scientifica, ipertecnologica, estremamente frazionata da una parte, non ha più il necessario bilanciamento di un sapere emotivo, umanistico, artistico e unitario di cui l'uomo ha bisogno.

La selva di pur sfavillanti, singole discipline tecnologiche non di rado di vita effimera, é nel suo complesso oscura, caotica e priva di un cammino o anche di singoli e pur provvisori sentieri che aiutino l'uomo a percorrerla.

Per questo, anche se viviamo sempre più a lungo, numerosi e ricchi, siamo anche sempre più soli, incerti e paurosi. Mentre dilagano le incertezze e le paure, riusciamo a combattere le malattie del corpo ma non quelle dell'anima perché il dominante pensiero razionale e tecnologico che nutre il corpo non si associa al pensiero dionisiaco che nutre l'anima.

Esemplare é quanto sta avvenendo in questi ultimi tempi nell'alimentazione umana.

Mai come oggi, per opera delle tecniche e di razionali trattamenti di produzione e controllo, abbiamo a disposizione cibi abbondanti, buoni e soprattutto sicuri. Al tempo stesso mai come oggi dilagano i dubbi e le paure alimentari, che si riflettono sul loro uso, ma che soprattutto generano ansie e malattie dello spirito, avvelenando l'anima.

Non é certamente la razionalità di Atena Nike che deve intervenire, e neppure una sempre più spinta spettacolarizzazione del fenomeno alimentare, ma abbiano bisogno del pensiero emotivo di Dioniso e della sua azione di contaminazione tra i diversi saperi che sono stati dispersi nelle diverse discipline che, direttamente o indirettamente si occupa di alimenti e costumi alimentari.

Accademie luoghi di un sapere condiviso

Le Accademie sono come le lucciole, brillano nel buio o nella penombra e, come

ricorda James Hillman (Hillman J. – An Essay on Pan – 1972 - trad. italiana - Saggio su Pan – Adelphi Edizioni, Milano, 1977), nell'attuale Società Liquida dei Non Luoghi, quando la visione dominante che tiene assieme un periodo della cultura s'incrina e la coscienza regredisce in contenitori più antichi, cercando fonti di sopravvivenza che offrano anche basi di rinascita, abbiamo bisogno di strutture di collegamento culturale quali erano e rimangono le Accademie.

L'Accademia si svolse e si affermò nel suo significato e nella sua funzione specifica moderna in Italia nell'età del Rinascimento, prima quale libera unione di sapienti e poi come associazione vera e propria di dotti con norme e leggi fisse, con lo scopo precipuo di coltivare disinteressatamente le discipline letterarie, scientifiche e le belle arti.

Accademia, parola che evoca un luogo del sapere, studio, eccellenza e che prendeva nome dai giardini dedicati all'eroe Academo o Ecademo, un ginnasio o una palestra per educare e sviluppare il corpo, prima di diventare una scuola per la mente, un luogo pubblico che fu concesso a Platone, un maestro, per insegnare a un gruppo ristretto di allievi, tra i quali Aristotele. Insegnare non è un termine adatto all'Accademia, perché evoca un modo formalista di trasmissione di un sapere, mentre nell'Accademia tutti insieme sunphilosophein, cercano insieme la saggezza.

Anima di ogni Accademia primigenia non è la trasmissione di un sapere precostituito, ma una ricerca continua e condivisa di una conoscenza che non si può trasmettere, ma soltanto costruire o ri-costruire in continuazione, quasi come una fiamma che quando è nata può nutrirsi di sé medesima.

Un'Accademia che deve occuparsi non solo di idee, ma anche di cose e di problemi, al tempo stesso generali e pratici, e tra questi e non ultimo del piacere, perché ricerca e vita sono due aspetti da tenere strettamente e perennemente congiunti.

La cultura è in crisi? O sono in crisi i valori che la contraddistinguono? Oppure non riusciamo a comprendere i nuovi valori che si stanno affacciando e tentano di sostituire quelli del passato, nel continuo processo di cambiamento e di evoluzione delle società, indipendentemente dalle nostre sensibilità, delle nostre abitudini, delle nostre opinioni e soprattutto dei nostri gusti?

Mai tema come quell'ora indicato, ampio ed insieme diversificato, al tempo stesso generale e pratico, è di vitale importanza per la nostra società, anche per la somma di conseguenze soprattutto in relazione alla contaminazione dei saperi e al dialogo tra le diverse culture.

In un momento di globalizzazione dei mercati e al tempo stesso di espansione delle disuguaglianze che creano una crisi di governance globale, senza abbandonare le microstorie e partendo anzi da queste nella loro molteplicità, anche per l'alimentazione dobbiamo riconoscere la necessità di una storia di lunga ricuperando una tradizione storica europea orientata al futuro, di longue durée come quella originalmente proposta da Fernand Braudel.

Questo compito richiede l'impegno di studiosi capaci di guardare al passato con occhi nuovi, che possono spiegare le cose da dove e come accadevano, esaminando con precisione i dati e gli eventi anche alla luce del presente, in un più ampio quadro dei grandi avvenimenti di lunga durata, al fine di servire la società nel formare un pensiero responsabile sul nesso tra passato, presente e futuro, con un metodo che possa essere anche quasi una ricetta per un cambiamento della ricerca storica e per una previsione del futuro.

Una ricerca che un tempo fu delle Accademie storiche e che deve ritornare a esserlo per le nuove Accademie d'oggi.

Il pensiero accademico condiviso nella selva dei mille saperi per una Nuova Veterinaria

Antica era la coesistenza non conflittuale ma dialogante tra il sapere razionale e quello emozionale. L'unità della conoscenza e del sapere si é progressivamente frazionata con il sorgere delle discipline sperimentali, iniziate nel Rinascimento italiano con il metodo galileiano.

Determinante del mancato dialogo tra le discipline scientifiche e del fallito processo di integrazione é stato un tragico capovolgimento di rapporto tra sapere e tecnica.

In un passato sempre più lontano e che salvo rare eccezioni sembra quasi perduto, le tecniche nascevano e si sviluppavano nell'ambito di un pensiero unitario, oggi invece singole tecniche, sempre più specialistiche e autoreferenziali, si sviluppano autonomamente nella già citata scissione delle Due Culture di Sir Charles P. Snow e nell'incontrollata crescita dei mille saperi, grandi e piccoli, ognuna dei quali parla linguaggi diversi, senza unità culturale.

La postmoderna conoscenza razionale, scientifica, ipertecnologica e frazionata, non ha più il necessario bilanciamento di un sapere emotivo, umanistico, artistico e soprattutto unitario di cui l'uomo ha bisogno e per questo, anche se viviamo sempre più a lungo, numerosi e ricchi, siamo anche sempre più soli, incerti e paurosi.

Mentre dilagano le incertezze e le paure, riusciamo a combattere le malattie del corpo ma non quelle dell'anima perché il dominante pensiero razionale e tecnologico che nutre il corpo non si associa al pensiero dionisiaco che nutre l'anima.

Esemplare é quanto sta avvenendo in questi ultimi tempi nell'alimentazione umana. Mai come oggi, per opera delle tecniche e di razionali trattamenti di produzione e controllo, abbiamo a disposizione cibi abbondanti, buoni e soprattutto sicuri. Al tempo stesso mai come oggi dilagano i dubbi e le paure alimentari, che si riflettono sul loro uso, ma che soprattutto generano ansie e malattie dello spirito, avvelenando l'anima.

Mai come oggi una società frantumata e liquida chiede alla veterinaria un'unità culturale che aveva e l'aveva portata ad altissimi livelli culturali e sociali, e che oggi può

essere ricuperata solo attraverso un pensiero accademico unificante.

Altrettanto avviene nei rapporti che la nostra specie ha con il mondo in cui vive e in questo con il mondo animale. Tutte le zooantropologie tradizionali del passato sono state spazzate via, anche l'ultima che era stata faticosamente costruita dalla veterinaria tra la fine del milleottocento e la prima metà del millenovecento.

Una nuova zooantropologia, nella quale dare le risposte ai quesiti, problemi e dubbi sollevati dalla moderna società postmoderna, possono trovare la loro giusta collocazione i mille saperi tecnico-scientifici, non può scaturire da questi singolarmente presi, ma solo da un loro confronto e dialogo che può avvenire solo nell'ambito di una Accademia che si rifaccia ai principi costitutivi ancora valevoli oltre di duemilacinquecento anni.

Senza rinunciare, anzi valorizzando in una nuova dimensione la grande ricchezza dei mille saperi tecnico-scientifici razionali, solo in un livello di sintesi superiore il pensiero unitario accademico, di tipo dionisiaco anche emotivo e necessariamente di contato e contaminante tra le diverse discipline, può creare tra queste una conversazione capace di produrre una nuova sintesi.

La SISVET accademia per una Nuova Sintesi

É nella Società Italiana delle Scienze Veterinarie, Accademia nel senso più profondo e vero e quale l'avevano pensata, ideata, voluta e fondata i nostri Maestri, che con l'apporto di tutti e superando ogni frantumazione, bisogna costruire una Nuova Sintesi.

Una Nuova Sintesi, sulla quale tracciare la diritta via necessario per ricostruire una Nuova Veterinaria e una Nuova Zooantropologia, capaci di dare valide e coerenti risposte, e soprattutto certezze, non solo per il corpo della nostra società, ma soprattutto per la sua anima, permettendole di superare i dubbi e le paure che, anche nei riguardi agli animali, la travagliano in questa nostra epoca postmoderna.

Main Lecture

Pathogenesis of Bluetongue

Massimo Palmarini

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Bluetongue is one of the major infectious diseases of ruminants. The disease is caused by bluetongue virus (BTV), a dsRNA virus transmitted by *Culicoides* biting midges. Similarly to what observed in other arbovirus infections, animals affected by bluetongue can show a variety of clinical signs ranging from a mild febrile illness to severe hemorrhagic disease. The variability of the clinical outcome as a result of BTV infection is due to a variety of factors related to the mammalian host (e.g. species, breed, age, immune status) and the virus itself (virulence of serotype/strain etc.). I will present both published and unpublished data on virus and host factors affecting the pathogenesis of bluetongue. In particular, I will focus on the early events of BTV infection. I will show data demonstrating that BTV induces a transient immunosuppression in the infected host and offer new perspectives in understanding the pathogenesis of arbovirus-induced hemorrhagic fevers.

COMUNICAZIONI ORALI

XIII Giornata di Studio
SOFIVET

SCIENZE BIOMEDICHE

FACE DISCRIMINATION IN HORSES: INTERSPECIFIC RECOGNITION AND CORTISOL RESPONSE

Giulia Ragonese, Cristina Cravana, Esterina Fazio, Pietro Medica and Adriana Ferlazzo

University of Messina

Horses have good capacities for discrimination learning (Murphy & Arkins, 2007). The hypothesis that horses are able to recognize faces' images of conspecifics from faces' images belonging to other species was tested. Furthermore, in order to estimate the potential stress due to the execution of experimental tests, serum cortisol was measured. Eight adult horses have been used. The testing apparatus consisted of a large rectangular wooden panel with two trapdoors. The stimuli consisted of twenty prints of colour pictures. Half of the pictures represented horses' faces; the other stimuli represented faces of other domestic animals. After familiarization procedure, the animals were submitted to a two-choice discrimination task, in order to establish the basic pattern of responding differentially to one of two stimuli. The criterion for success in all experiments was for the horse to make at least 8 correct choices per session in two consecutive sessions. All subjects were submitted to two experimental tests (Experiment 1 and Experiment 2). Each experiment included a training test and a generalization test. During the training test of Experiment 1 one photograph of a horse, S+, and one photograph of a pig, S–, were used. During the Generalization test, 10 faces of different horses and 10 faces of other domestic animals were used. During the Experiment 2 the faces of other domestic species were S+ and the faces of horses were S–. In all the Experiments each session consisted of 10 trials. Blood samples for determination of cortisol concentrations were collected from the jugular vein on three days before starting the familiarization procedure and 30' after the last daily trial. During the Training test, the average number of sessions needed to reach the criterion was 5.4 ± 2.5 and 5.4 ± 1.4, in Experiment 1 and Experiment 2, respectively. During the Generalization test all the subjects reached the criterion in two sessions, in both the experiments. There was a statistically significant difference between the number of sessions required in the Training phase and in the Generalization phase in Experiment 1 ($p=0.001$) and in Experiment 2 ($p=0,0003$). Post-test cortisol levels compared to baseline showed

significant difference (p=0,025) only in the Familiarization phase. In conclusion, the results show that face Species discrimination using two-dimensional picture occurs in the Horse. Furthermore, it seems that the subjects perceived pictures as real objects and that there was not a difficulty in recognising images belonging to faces of “horse” from faces “not horse”. Familiarization phase only influenced cortisol levels of horses. This response maybe could be associated with a novelty stimuli of performance in this learning tasks.

DYNAMICS OF THE SWINE BLOOD-BRAIN BARRIER: QUALI-QUANTITATIVE ANALYSIS OF THE CEREBROSPINAL FLUID AT DIFFERENT TIME POINTS.

Domenico Ventrella[1], Luca Laghi[2], Francesca Barone[1], Alberto Elmi[1], Noemi Romagnoli[1] and Maria Laura Bacci[1]

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The dynamics regarding the maturation of the blood brain barrier (BBB) in the swine are still to be completely clarified and timed. This species is nowadays acknowledged as one of the most accurate preclinical model for developing and testing therapeutic approaches to be translated to the pediatric medicine, with particular focus regarding the neurology field [1]. Since one of the biggest issue in translational medicine is overlapping developmental timing patterns between the testing and the final species, it is extremely important to have accurate knowledge regarding the BBB maturation in biomedical piglets. Cerebrospinal fluid (CSF), due to its physiology, might represent and indirect instrument for a better understanding of the above mentioned processes [2], and is an excellent specimen for 1H NMR spectroscopy for quali-quantitative analysis [3]. The aim of this study was to analyze the composition of young piglets' CSF (5, 30 and 60 days old) trying to relate the results to BBB maturation. Piglets enrolled in the study were commercial hybrids coming from the same breeding facility divided into 3 age groups: P5 (5 days old; n=17), P30 (30 days old; n=18) and P50 (50 days old; n=9). All animals were enrolled as negative controls or as pre-treatment individuals in different protocols approved by the Italian Ministry of Health. CSF collection was performed from the Cisterna Magna under general anesthesia. Samples, stored at -80°C, were thawed and centrifuged before analyses. 1H-NMR spectra were recorded at 298 K with an AVANCE III spectrometer (Bruker, Milan, Italy) operating at 600.13 MHz. Molecules concentrations that significantly varied between different time points were looked for by means of the Mann-Whitney U test. A probability lower than 0.05 was considered significant, adjusted for multiple comparisons through Bonferroni correction. We were able to observe 30 molecules above the quantification limits. The concentration of 11 molecules was significantly different between P5 and P50. The P5-P30 comparison was significant for 8

molecules, the P30-P50 for 4 molecules. The increasing trend of some compounds may be related to active processes within the Central Nervous System (CNS) and to higher blood concentration of the compound itself, while the decreasing one is probably due to the progressive maturation and selectiveness of the BBB. Data doesn't allow to identify a definite age for complete maturation of the BBB as suggested by differences between P30 and P50: studies on older animals are needed. However, the work provided valuable data regarding the physiological description of the swine CSF, providing new knowledge about such an important animal model.

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2. Di Terlizzi R, Platt SR. The function, composition and analysis of cerebrospinal fluid in companion animals: part II - analysis. Veterinary Journal. 2009; 180:15-32.
3. Musteata M, Nicolescu A, Solcan G, et al. The 1H NMR profile of healthy dog cerebrospinal fluid. PLoS One. 2013; 8:e81192.

A FIRST STEP TOWARD THE IDENTIFICATION OF MAMMALIAN SPERMATOZOA ITERACTOME.

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The study of unexplained infertility of male origin is one of the most important challenges in the reproductive medicine of post genomic era. Unfortunately, despite the continuously growing effort of researchers, it remains unresolved[1]. This causes the worsening of living conditions and of physical and psychological wellness of a large number of couples and determines the rising of healthcare costs for National Health Services.

Recently, the availability of high throughput technologies, the so called –omics, has opened new perspective in clarifying this issue and posed new problems, related to the management of big data.

On this basis, the adoption of a computational modelling-based strategy could offer a reliable tool to manage the continuously growing data on male gametes physiology[2,3].

Here, we realized a computational model representing the whole ensemble of molecules present in spermatozoa, linked by their interaction.

As first, we collected the data from papers indicized in PubMed referred to proteomic studies of sperm biology. Then, staring from the list of identified proteins, we carried out a pathways reconstruction analysis (Reactome FI). All the obtained pathways were used to realize a network-based computational model by using Cytoscape 3.3, called Mammalian Sperm Interactome (MSI), constituted by nodes, representing the molecules, linked by their interactions.

Whole MSI is constituted by 7052 nodes, 15587 links, and 104 connected components. In particular, we identified a Main Connected Component (MCC_MSI) that accounts for 6525 nodes and 14944 links. The analysis of MCC_MSI showed that it is characterized by a scale free topology that follows the Barabasi-Albert (BA) model. The number of links per node (the node degree) follows a power law, with a negative exponent ($y = a\ x^{-1.639}$, $R2 = 0.826$), and the clustering coefficient (cc), which is a measure of the network

tendency to form clusters, is low (cc = 0.152) and unrelated with the node degree (R2 = 0.259). In addition, MCC_MSI displays a small world architecture: the averaged no. neighbours, which represents the mean number of connection of each node, is 3.337 and the characteristic path length, which gives the expected distance between two connected nodes, is 7.227.

The analysis of MSI network topology has led us to infer important characteristics of the biological system:

• the network is robust against random failure: indeed a random damage has the higher probability to affect the most frequent nodes, i.e. the less linked ones, with negligible consequences on network topology;

• the messages will spread within the networks quickly and efficiently, thus allowing to male gametes to adapt efficiently to the intra and extracellular stimuli.

In addition, it will be possible to identify the nodes that shows a higher level of control within the networks, thus potentially offering new perspectives in the study of molecular target for diagnostics and therapeutics of male infertility of unknown origin.

1) Bernabò et al., BMC Syst Biol. 2010, 4:87.
2) Bernabò et al., OMICS. 2015, 19(11):712-21.
3) Bernabò et al., OMICS. 2014, 18(12):740-53.

OBJECTIVE VISION ASSESSMENT IN IODOACETIC ACID MODEL OF SWINE RETINAL DEGENERATION

Francesca Barone[1], Domenico Ventrella[2], Alberto Elmi[2], Jose Fernando Maya Vetencourt[1], Walter Sannita[3], Fabio Benfenati[1] and Maria Laura Bacci[2]

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The most common inherited disease that leads progressively to loss of night vision up to bilateral blindness is retinitis pigmentosa[1] (RP). The iodoacetic acid (IAA) model of swine retinal degeneration[2] was created as an alternative model to the costly and time-consuming transgenic approach. The aim of this study was to characterize the IAA model through electrophysiology, in particular electroretinography and Visually Evoked Potential electrophysiology.

The use of animal in this study was regulated by a protocol approved by the Italian Ministry of Health (art. 7, D. Lgs 116/92). Seven animals were anesthetized with the following protocol: Tiletamine-Zolazepam 5mg/kg and sodium thiopental 5 mg/kg, then were intubated and anesthesia was maintained with isoflurane. Iodoacetic acid (IAA) 12 mg/kg was injected trough the auricular vein. Erg and Vep analysis were performed for both right and left eyes before (baseline) and 4 weeks post IAA injection (post IAA) under general anesthesia with the same protocol. Flash and pattern electroretinogram (fERG, pERG) and flash and pattern visual evoked potential (fVEP, pVEP) were recorded to evaluate visual function in terms of rod and cone photoreceptors, ganglion cells and visual cortex activity. Flash stimuli were produced by a stroboscopic lamp in which the light stimulus has a maximum duration of 5 milliseconds and a brightness of 1.5-3 cd s m-2; pattern stimuli consist of a set of horizontal/vertical black and white bars that reverse their position without modification of the global luminance, but with changes due to alternation of the bars with frequency of 2 Hz.

We recorded differences between baseline and post IAA, in particular the cone response (fotopic Erg) was maintained while the rod response (scotopic Erg) was decreased after the treatment. We also recorded a reduction in Perg and vep stimuli after the treatment.

The ISCEV adapted protocol for Erg and Vep stimuli was consistent and gave good quality results concerning the description of the model and future applications (e.g. artificial retina implants). The results are confirming the degeneration of the photoreceptors in particular the rods. Pattern Erg and Vep stimuli and Flash Vep stimulus were successfully recorded in swine for the first time; the IAA (12 mg/kg) seems to alter ganglion cells interrupting the signal from the photoreceptors to the cortex.

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ANTI-PROLIFERATIVE EFFECT OF OVINE WHEY COLOSTRUM IN TWO MODELS OF HUMAN HEMATOLOGICAL NEOPLASTIC DISEASES: A PRELIMINARY STUDY

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Ovine colostrum is a complex biological fluid composed principally of proteins, carbohydrates, lipids, and ions (1). In the colostrum, the dominating proteins are immunoglobulins, but it also contains less represented protein species, endowed with anti-microbial, anti-inflammatory, and anti-cancer activity, which are indispensable for the normal development of lambs (2, 3). In the last years, several studies reported the anti-tumor effects of bovine whey proteins in human colon and mammary pre-clinical tumor models, highlighting a possible therapeutic use to treat or mitigate the effects of cancer (3).

The aim of this work was to evaluate anti-tumoral activity of ovine whey colostrum in two models of human hematological neoplastic diseases: chronic myelogenous leukemia and B cells lymphoma.

To this end, colostrum samples (OWC-1 and OWC-2) were collected immediately after parturition from 2 Sarda sheeps and whey was obtained by ultra-centrifugation, and filtration in order to sterilize it. Whey total proteins were quantified in triplicate by Bradford assay (Bio-Rad protein assay, Bio-Rad Laboratories) using BSA (Bovine Serum Albumin, Sigma Aldrich) as standard. K562 (Human chronic myelogenous leukemia) and DeFew (human B cell lymphoma) cell lines were seeded at $1x10^5$ cells/ml and after incubated with increasing amounts of single whey (0 - 6 – 12 – 24 μg/μl). OWC-1 and -2 anti-proliferative effects were evaluated at 24 and 48 hours from stimulation by cell count using hematological analyzer ADVIA 2120 (Siemens Healthcare). All experiments were performed in triplicate and data analyzed by two way analysis of variance (ANOVA) using Holm-Sidak multiple comparisons test (Sigma Plot vers 12.3). The level of significance was determined with a $p\text{-value} < 0.05$. At 24h from stimulation, OWC-1 and -2 did not show anti-proliferative effects in both cancer cells lines at all dose tested (0h

vs 24h: $p>0.05$). Differently, at 48h OWC-1 inhibits both cell lines at all doses tested (0h vs 48h: $p<0.05$), whereas OWC-2 was active against De Few at all doses tested, but inhibits K562 cell line growth only at 24 μg/μl. The IC50 (50% inhibitory concentration) confirm the less efficacy of OWC-2 compared with OWC-1 in both cell lines (DeFew IC50(OWC1)= 8.4 μg/μl against IC50 (OWC2)= 13.1 μg/μl; K562 IC50(OWC1)= 10.1 μg/μl against IC50 (OWC2)= 16.2 μg/μl). In DeFew cell line, OWC-1 and -2 show a IC50 lowest than in K562 cell line. These results demonstrate the anti-proliferative effect of ovine whey colostrum on cell line models of two hematological diseases. Further experiments will clarify (a) the colostrum biochemical components and (b) the molecular mechanisms responsible of the anti-proliferative effect of ovine whey colostrum.

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IMMUNOPHENOTYPICAL CHARACTERIZATION OF CANINE MESENCHYMAL STEM CELLS DERIVING FROM SUBCUTANEOUS AND PERIVISCERAL ADIPOSE TISSUE WITH A PANEL OF DIRECT SPECIES-SPECIFIC ANTIBODIES

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Mesenchymal stem cells (MSC) find a practical application in regenerative medicine and tissue engineering. In order to design and execute sound experimental and clinical studies, their immunophenotypical characterization of is fundamental. The scarce availability of species-specific antibodies to canine antigens has hampered the immunophenotypical characterization of canine MSC. The aim of our work was to select and create a panel of species-specific direct antibodies readily useful for canine MSC characterization. MSC were isolated from perivisceral and subcutaneous adipose tissue samples collected during regular surgeries from 8 dogs with stable systemic conditions. Peripheral blood samples for the isolation of peripheral blood mononucleate cells, used as positive control for flow cytometrical analysis were taken from 3 healthy dogs. All samples were collected following owner consent under standard ethical and sterile conditions. Single color flow cytometric evaluation of CD29, CD34, CD44, CD45, CD73, CD90 and MHC-II expression was measured on MSC (P3) from both sources using a panel of 7 direct anti-canine antibodies. Data expressed as mean and st. dev. analyzed with statistical software SPSS 16 IBM were considered statistically significant for $p<0,05$. All antibodies reacted with the control group. The immunophenotypical characterization of MSC deriving from both sources revealed two largely homogenous cell populations with a very similar surface protein profile: CD29(+), CD44(+), CD73(+), CD90(+), CD34(-), CD45(-) and MHC-II(-) with no statistically significant differences among the sources. Our results allowed the identification of a panel of 7 primary antibodies suitable for canine MSC immunophenotyping and present for the first time a comparison between the immunophenotypic profile of canine MSC deriving from subcutaneous and perivisceral adipose tissue. The similarities between the two cell populations are

reinforced by the study of in-vitro cell morphology, differentiation ability in various phenotypes and furthermore, by RT-PCR analysis of a wider panel of MSC markers that confirm flow cytometrical results as both cell populations resulted positive for CD90, CD73, CD105, CD44, CD13, CD29, Oct-4 gene and negative for CD31 and CD45 expression. The substantial equivalence between the two sources has practical consequences on clinical applications, giving the opportunity to choose the source depending on the patient needs. Our results contribute to routine characterization of MSC populations grown in vitro, a mandatory process for the definition of solid and reproducible laboratory and therapeutic procedures.

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IMMUNOHISTOCHEMICAL STUDY OF GHRELIN, APELIN AND THEIR RECEPTORS IN THE DOG OVARIES

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Recent studies have shown that molecules, such as leptin, orexins and cannabinoids, are in general spread ubiquitously in the body very often involving anatomical structures not functionally related to appetite control [1, 2, 3]. The female genital tract is very often implicated, and this has led to the hypothesis that the same molecules are involved in its functional control representing a point of contact between the animal's nutritional status and its functionality. In this context two proteic hormones, ghrelin and apelin, have been already highlighted in the ovary of some animal species [4, 5, 6] suggesting involvement in its functional control. The aim of the present work was to highlight the presence and the localization of ghrelin, apelin and their receptors in the ovary of non-pregnant bitches, with the purpose of providing data that may be useful in fully understanding the functionality of this organ.

The experiment was conducted using six mixed-breed dogs, admitted to the day-hospital service at the Veterinary Teaching Hospital of the University of Perugia and regularly subjected to the ovario-hysterectomy by spaying. The ovaries were removed and immediately fixed in 4% formaldehyde solution and subsequently processed for embedding in paraffin. The immunohistochemical reactions were visualized on 5 µm serial sections, using anti-Apelin (ab59469), anti-APJ receptor (ab140508), anti-Ghrelin (ab129383) and anti-Ghrelin receptor (ab188986) polyclonal antibodies, avidin-biotin-complex and DAB as the chromogen. All the sections were counter-stained with haematoxylin. Sections in which the primary antibodies were omitted, were used as controls for unspecific staining.

The immunohistochemical study showed a strong positivity for apelin, ghrelin and their receptors in some of the ovarian structures. In particular, a positive immuno-reaction for apelin and its receptor and for the ghrelin receptor seemed to be evident in the corpora lutea with a peculiar localization in some of the luteal cells. On the contrary, regarding ghrelin, a positive reaction for this molecule was evident within the wall of small arteries

localized both inside the corpora lutea and in the connectival tissue. In general, the immuno-positive reaction seemed to affect only the cellular cytoplasm and was not observed in other ovarian structures or in the sections utilized as negative controls. Due to the presence of these molecules and their receptors in some of the luteal cells and within the wall of small arteries in corpora lutea and in connectival tissue, we can hypothesize that apelin and ghrelin might influence the functionality of ovarian structures where they are localized, suggesting the existence of autocrine/paracrine mechanisms of regulation.

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THE EFFECT OF AMINOPURVALANOL A ON BOAR SPERM CAPACITATION

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After ejaculation, mammalian spermatozoa are unable to fertilize the oocyte. To become fully fertile, they must undergo a complex process of biochemical maturation within the female genital tract, the so called capacitation. This process is controlled by a delicate balance between activating and inhibiting factors belonging to different cellular pathways (1) and involves virtually the whole sperm proteome (2).

Aim of this work was to study the molecular events involved in capacitation using a predictive computational modelisation (3) based on biological networks theory (mammalian spermatozoa interactome, MSI), whose results will be confirmed and validated by an in vitro experimental approach.

Surprisingly, the analysis of network representing MSI showed that cyclins have an important biological role, as demonstrated by their topological proprieties; indeed, their removal causes the collapse of the network. In the next phase, in vitro, we investigated the role played by cyclins during sperm capacitation.

For this reason, we incubated boar spermatozoa in capacitating condition, by using an already validated experimental model (4), in absence (CTR) or in the presence of 2 μM, 10 μM or 20μM aminopurvalanol A (AA), a cell-permeable purine analog that acts as a reversible ATP-competitive inhibitor of Cdks/cyclins activity. We have analyzed the effect of AA on spermatozoa in terms of acrosome integrity using PSA staining, of fertilizing ability by IVF and of actin polymerization through phalloidin staining. As a result, we found that AA causes the acrosome loss and this event seems to be concentration and time dependent. The analysis of fertilizing rate confirmed that this damage is not random but involved the capacitated spermatozoa. Indeed, we observed low fertilizing rates (44%) at medium doses (10M) of AA after 3 hours of incubation compared to the control (66%).

Therefore, assessing the levels of F-actin on sperm head, we observed that in control samples actin polymerization increases during the time, while the AA treatment inhibits this event in a time and dose-dependent manner. By the results obtained so far, we could infer that the AA affects the sperm capacitation inhibiting actin cytoskeleton rearrangement through the inhibition of actin polymerization. Since during capacitation the F-actin acts avoiding the premature fusion of sperm plasma membrane and outer acrosome membrane, its insufficient polymerization could cause the loss of acrosomes in capacitating spermatozoa.

In conclusion, it is possible to hypothesize that, as in other cell types, in spermatozoa cyclins could be involved in control of cytoskeleton dynamics. We are carrying out further molecular studies to confirm this hypothesis.

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OCHRATOXIN A RESIDUES IN HUNTED WILD BOAR (*SUS SCROFA*) FROM TUSCANY

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Ochratoxin A (OTA) is a secondary toxic metabolite synthesized by Aspergillus or Penicillium species, which can contaminate various crops. The International Agency for Research on Cancer classified OTA as a group 2B possible human carcinogen. OTA is nephrotoxic, mutagenic, teratogenic and immunosuppressive. OTA can also be present in meat of animals where its presence comes as a result of animal feeding with contaminated grain and feed mixtures. The Italian Ministry of Health Circular No 10, dated 9 June 1999, establishes, as a guideline, a maximum value of 1 µg/kg OTA for swine meat and meat products. The significant increase in the wild boar population has resulted in an increased prevalence of wild boar meat, offal and ready-made products in the food industry. The aim of the present study was to determine OTA concentrations in muscle, kidney and liver of wild boar hunted in Tuscany region. A total of twenty wild boars (male n=11 female n=9) were collected in the Province of Pisa from November 2014 to April 2015, animals have been slaughtered and the carcass weight were determined (from a min. of 14.9 kg and a max. of 72.0 kg). Samples of kidney, liver and muscles from each wild boar were collected and analyzed with an enzymatic digestion clean-up and high-pressure liquid chromatography with fluorescence detection method (1). The highest levels of OTA were found in the kidneys of the twenty wild boar analyzed (0.07-2.01 µg/kg, mean 0.58±0.63 µg/kg). The levels found in the liver ranged between 0.08-1.93 µg/kg, (mean 0.53±0.60). The lowest concentrations were found in muscle (0.04-0.77 µg/kg, mean 0.24±0.24). In eight samples of the tissue samples examined in this study (4 kidney and corresponding 4 liver), the levels of OTA were higher than the guideline level (1 µg/kg) established by the Italian Ministry of Health. The present results are in agreement with a previous study conducted in Calabria in wild boars (2). Swine are particularly sensitive to OTA, kidneys showed the highest accumulation of the latter

toxin, followed by liver and muscle tissue, finally the lowest accumulation is represented in adipose tissue. The present results showed the same type of accumulation in wild boar. Traditionally in Tuscany, as in other regions, wild boar meats are used to produce niche products, especially coppa and salami. In agreement with the research of Monaci et al. (3), dried wild boar meat may contribute to overall OTA intake by carry-over effects into processed meats. Monitoring the quality of meat destined for transformation is a priority in order to decrease the possibility of toxin carry-over to humans. The present study confirms that contamination of meat products by OTA represents a potential emerging source of OTA for distinct segments of the Italian population, who are significant consumers of locally-produced wild boar specialties.

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SPARTIUM JUNCEUM L. POISONING IN SHEEP

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Spartium junceum L. is a plant that contain sparteine and cystisine, two alkaloids that can reduce the sensitivity and conductivity of cardiac muscle and produce a direct depression or paralysis of the respiratory center in the brain (1, 2). An outbreak of neurological troubles occurred in winter 2015 in a flock of 10 sheep kept in a rural farm in Civitella Roveto (AQ). All of the animals showed tonic-clonic convulsions followed by muscle paralysis associated to dilated pupils, tremor, tachycardia and tachypnea and in some cases also diarrhea. Two sheep died after worsening of symptoms.

Complete necropsies were performed on two animals. Samples of brain, cerebellum, lung, kidneys and liver were collected and immersed in 10% buffered formalin (pH 7.4) for histopathological examination. Grossly both animals presented the same morphological alterations showing abdominal distension (meteorism) and poor nutritional status; there was diffuse moderate bilateral congestion in the lungs; the liver was slightly enlarged and petechial subdural hemorrhages were found at the inspection of central nervous system. At light microscopy we observed hemorrhages and moderate congestion of blood vessels both in cerebrum and cerebellum, and mild neuronal degeneration and gliosis. In liver and kidneys we observed severe congestion of blood vessels and, in lungs, there was a moderate to severe pulmonary edema associated with multifocal lobular emphysema.

Serological, bacteriological and virological examination excluded any infectious disease. The exposure of animals to toxic substances used in agriculture or other toxic plants was excluded by clinical examination, anatomopathological findings and environmental anamnesis. The presence of bundles of dry broom (*Spartium junceum* L.) that were eaten by the animals led us to support the hypothesis of *Spartium junceum* L. intoxication. After removal of bundles of brooms and dietary change, a complete remission of clinical

signs in whole flock was observed. There is very little documentation of *Spartium junceum* L. intoxication in both human and animals and no cases are reported in sheep. In areas where *Spartium junceum* L. is abundant, familiarity with its appearance and early identification of symptoms in sheep should help prevent future outbreaks of neurotoxicity. Additional research into its toxic principles may help explain the sporadic toxicity of *Spartium junceum* L.

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III Convegno

RNIV

COMPARATIVE EVALUATION OF IMMUNE RESPONSES OF SWINE IN PRRS-STABLE AND UNSTABLE HERDS

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Porcine Reproductive and Respiratory Syndrome (PRRS) is an elusive model of host/virus relationship in which disease is determined by virus pathogenicity, pig breed susceptibility and phenotype, microbial infectious pressure and environmental conditions. Successful disease control corresponds to “stability”, i.e. a condition with no clinical signs of PRRS in the breeding-herd population and no viremia in weaning-age pigs. The aim of this work was to compare the profile and time-course of humoral and cell-mediated immunity of replacement gilts in one stable and one unstable herd, respectively. In particular, we investigated PRRS virus (PRRSV) in serum and group oral fluid samples by Real-time RT-PCR, PRRSV-specific IgA and IgG in oral fluids, serum IgG antibody and the cell-mediated response (PRRSV-specific release of interferon-gamma) in whole blood samples. These parameters were measured in order to identify possible discrepancies in the development and kinetics of the immune response against PRRSV. Gilts got regularly infected around 7-9 weeks after entering the stable farm, and at the very beginning in the unstable one. Four main results must be highlighted: A) the precocity of the Ab response in group oral fluids was similar to that seen in sera; B) circulation of PRRSV was consistently detected in the unstable herd, as opposed to the stable one; C) a balanced IgA and IgG response in oral fluids was only observed in the stable herd; D) an IFN-gamma response was regularly observed in the stable herd, whereas gilts of the unstable one were partly positive at arrival day, only (transfer of maternal immunity). The above findings indicate that a peculiar profile of immune response to PRRSV underlies herd stability. Therefore, the outlined immune parameters can represent a useful readout system to evaluate successful adaptation to PRRSV based on acclimatization of breeding animals and management of pig flow. In this respect, failure of disease control measures could be traced back to farm management and/or

peculiar virus “immunotypes”, affecting the immune response of PRRSV-infected animals.

RESPONSE OF PORCINE MONOCYTE DERIVED MACROPHAGE SUBSETS AND DENDRITIC CELLS TO ASFV STRAINS OF DIFFERENT VIRULENCE

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African swine fever (ASF) is a severe disease of domestic pigs and wild boar, which is currently present in Africa and some European countries [1, 2]. There is no vaccine available and the only control measures are stamping out and movement control [3]. The aetiological agent is the ASF virus (ASFV), a large enveloped DNA virus which primarily infects cells of the myeloid lineage [4, 5]. An improved characterization of the interactions between these immune cells with ASFV strains of different virulence may help underpin vaccine development efforts. Blood derived monocytes were differentiated in vitro into macrophages (moMΦ) or dendritic cells (moDC). moMΦ were left untreated or were classically (moM1) or alternatively (moM2) activated, whereas, moDC were left untreated or matured with TNF-alpha and IFN-alpha. Cells were infected with an attenuated strain (BA71V) and a virulent Sardinian isolate (22653/14) of ASFV, alongside mock-infected controls. Virus-cells interaction was investigated using flow cytometry, ELISA and confocal microcopy. 22653/14 presented a greater ability to infect moM1 compared to the avirulent strain and higher expression of early (p30) compared to late (p72) proteins was observed in BA71V-infected moM1. Infected macrophages displayed a lower expression of CD16 compared to un-infected bystander cells and no differences were observed in the expression of CD163 between infected and bystander cells. The levels of MHC I were similar between mock and 22653/14-infected macrophages, instead BA71V-infected moMΦ and moM2 presented lower percentages of MHC I compared to the mock-infected control. Differences in cytokine responses were observed, with higher levels of IL-18, IL1-alpha and IL-1beta release by moM1 in response to BA71V compared to 22653/14. Both isolates are able to infect moDC and 22653/14 presented greater ability to infect matured moDC compared to BA71V. Higher resistance of matured moDC to

BA71V infection is due to inhibition of late protein synthesis. As macrophages, ASFV-infected moDC displayed lower expression of CD16. Our results revealed significant differences in the response of macrophage subsets and moDC with these ASFV isolates: the attenuated BA71V strain presented higher susceptibility to type I and type II IFN antiviral activity and promoted a higher release of cytokines which might influence the development of protective immunity.

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PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF PORCINE MONOCYTE-DERIVED MACROPHAGES PRODUCED WITH DIFFERENT METHODS

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Macrophages maintain tissue homeostasis and are key players in the immune response to pathogens [1]. Porcine macrophages can be generated in vitro from monocytes differentiated through incubation in media supplemented with porcine serum-plasma [2, 3, 4] or with recombinant human macrophage-colony stimulator factor (hM-CSF) [5], but currently there is no standardized protocol. In this study blood derived monocytes were differentiated into macrophages using six different culture conditions (10-20-30% (v/v) of autologous plasma or 50-100-200 ng/ml of hM-CSF), then differentiation was assessed using light and confocal microscopy, flow cytometry and ELISA. Monocytes cultured with either plasma or hM-CSF increased in dimension (forward scatter; FSC) and granularity (side scatter; SSC), but SSC was higher in macrophages differentiated with porcine plasma and these cells displayed an increased number of elongated projections protruding from cell surfaces. CD163, MHC II DR and CD203a expression were up-regulated following monocytes differentiation into macrophages in all conditions, but CD163 was slightly lower in macrophages differentiated using 30% autologous plasma.
Macrophages differentiated with hMCSF and high percentages of porcine plasma showed an ability to proliferate in vitro. Macrophages differentiated with all the methods displayed higher susceptibility to ASFV infection than monocytes and increased release of TNF-alpha in response to lipopolysaccharide (LPS) stimulation compared to monocytes. Macrophages cultured in autologous plasma showed a higher basal release of IL-1RA compared to those cultured with hM-CSF and displayed a lower ability to release

IL-1 and TNF-alpha in response to classical activation. In addition, using porcine plasma great variability among animals was observed. Data generated in this study suggest that all the protocols are suitable to differentiate porcine monocytes into macrophages, although the use of high percentages of porcine plasma lead to formation of more elongated cells, with lower expression of CD163 and less able to release pro-inflammatory cytokines in response to classical activation stimuli. Moreover with hM-CSF a higher reproducibility between experiments can be obtained. We hope that information generated from this study will facilitate in vitro studies with porcine macrophages.

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DEVELOPMENT OF AN ALTERNATIVE ANIMAL-FRIENDLY HOUSING SYSTEM FOR RABBIT DOES AND KITS

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In Europe an increased interest in the “ethical quality” of animal husbandry is leading to study breeding systems that allow a higher welfare level, without impairment of environmental sustainability, economy and food security. Italy is still the second world rabbit meat producer, while the other traditional producers (France and Spain) significantly reduced their production. The aim of this study was to evaluate the impact of an alternative animal- friendly housing system for rabbit does and kits on animal welfare.

The experiment was managed from February to April 2015, using prototypes colony cages. These cages were characterized by interior spaces divided by removable partitions. Four nests were positioned at the two ends of the cage and equipped with a sliding door to allow controlled lactation. Sixteen nulliparous White New Zealand does were artificially inseminated and divided in two groups:

Control group (C) reared in standard colony cages; eperimental group reared in colony cages with septa (S). Five days before the kindling septa were closed and removed a week later.

Rabbits, for three consecutive reproductive cycles, had been checked for the following reproductive parameters: sexual receptivity, fertility, number of born and weaned rabbits, milk production in the first sixteen days of lactation, kits' weight at birth and at weaning. Moreover, blood samples were collected to evaluate oxidative status and innate immunity at the following intervals:

T0 - a week before the release in colony cages;

T1 - a week after going into colony cages;

T2 - a week after delivery, corresponding to the removing of the partitions in the S group;

T3 - at the weaning of kits.

Free Radicals and Anti-Oxidant activity were performed using enzymatic kits (Diacron, Italy); Serum lysozyme was assessed by the lyso-plate assay (Osserman and Lawlor, 1966); total hemolytic complement was evaluated following the procedure described by Seyfarth, (1976); serum bactericidal activity (SBA) was performed with a microtitre format assay (Amadori et al., 1997) and haptoglobin was measured by using a commercial kit (Tridelta Development Ltd, Kildare, Ireland). Data were analyzed using a linear STATA model (2015). The level of statistical significance was set at $P<0.05$.

Regarding to production performance, the colony prototype (S) showed better results than the classic colony (C) in terms of overall productivity.

Regarding the innate immunity, it was observed that lysozyme is strongly affected by the rearing system. In this test, higher values were observed in the postpartum period in rabbits of C group, indicating a state of suffering of the animals as a result of continuous nest invasions by other rabbits. The complement activity was significantly lower in does of group C7 days after delivery, suggesting a predisposition of these animals to infections. Bactericidal activity and haptoglobin were influenced only by the physiological condition, while the oxidative activity was affected by both the farming system and the physiological status. This study demonstrates that the innate immunity parameters are useful tools to evaluate an animal friendly housing system.

SALMONELLA SEROVAR INTERACTION WITH JEJUNAL EPITHELIAL CELLS

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Salmonella spp infections are an important source of foodborne illnesses and therefore a major public health concern (1). Important studies highlighted the molecular basis of pathogenesis of S. Typhimurium infection, while scanty data are available about other environmental serotypes, often isolated in cases of foodborne disease but not included in pathogenicity studies. Owing to the above, the aim of our work was to verify invasiveness in the IPEC-J2 swine jejunal cell line and the modulation of intestinal innate immunity by six different environmental Salmonella strains. In our study, overnight cultures of 7 different *Salmonella enterica* strains: *S.* Coeln, *S.* Ablogame, *S. enterica* sub-specie diarizone (Strain 1), *S.* Veneziana, *S. enterica* sub-specie diarizone (strain 2), *S. Thyphimurium* and *S.* Thompson isolated from wild boar livers were sub-cultured for 2 h at 37 °C in BHI medium. Each bacterial strain was re-suspended at 1×108 CFU/ml in DMEM/F12 medium (2) and used to infect IPEC-J2 cells; untreated cells were employed as negative control. Bacterial penetration and innate immune responses were evaluated as previously described (2, 3). Differences between data sets were checked for significant differences by Kruskal-Wallis test, followed by a Dunn's post-test. The significance threshold was set at $P<0.05$. All the strains were able to penetrate inside IPEC-J2 cells.
In particular, our results demonstrated greater penetration of S. Coeln ($P<0.0001$) and *S.* Thompson ($P=0.0059$) compared with *S. Typhimurium* (control strain). *S. Diarizonae* 1 ($P=0.0408$) showed lesser penetration with respect to the control strain. Concerning innate immunity, our results showed different abilities to modulate gene expression by the strains under study. In particular, in accordance with another study (2), *S. Typhimurium* infection determined a pro-inflammatory effect characterized by up-regulation of IL-8 ($P=0.022$), TNF-α ($P=0.0003$), IL-1β ($P<0.0001$), p38 MAPK ($P=0.0027$) and IL-18 ($P=0.041$) and an increase of antimicrobial peptide gene expression: bD1

(P=0.001), bD2 (P=0.002), bD4 (P=0.0006). At the same time we observed down-regulation of IL-4 (P=0.03) and MD2 (P=0.0018). On the contrary, *S.* Coeln caused a significant decrease of p38 MAPK and CD14 (P=0.0157, P=0.0431) gene expression and no modulation of antimicrobial peptides. *S.* Thompson caused a significant increase of JNK1 (P=0.0196), NFK-bp65 (P=0.0046) gene expression. *S.* Ablogame down-regulated p38 MAPK (P=0.03), TLR4 (P< 0.05) and TLR5(P< 0.05). Treatment with *S. Diarizonae* strain 1 caused a significant decrease of p38 MAPK (P=0.0412), MD2 (P=0.0044) and bD4 (P=0.0344) gene expression. The adopted cell line had been shown to give valuable information about pathogenicity of *Salmonella* spp. (4). Our data suggest a potential pathogenic role of all the strains under study and different interactions with the host. In particular, our findings about *S.* Coeln and *S.* Thomponson are in agreement with an EFSA report (1).

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2) Schmidt et al 2008.
3) Razzuoli et al., 2013. J Interferon Cytokine Res.
4) Brosnahan and Brown. 2012. Vet Microbiol.

GUT INNATE IMMUNE RESPONSE TO CADMIUM EXPOSURE

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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 for humans and animals and the gastrointestinal tract is the first target of interaction. The toxicity of Cd is related to its ability to modulate the activity of cellular enzymes, to initiate oxidative stress, to suppress mitochondrial functions and disrupt calcium homeostasis. However, little is known about Cd in-teraction with the intestinal tract (1). Owing to the above, the aim of our study was to investigate the effects of Cd on innate immunity using swine jejunal IPEC-J2 cells (2). Cells were seeded into 12-well tissue culture plates (2 mL per well, 2×105cells/mL) and incubated at 37°C in 5% CO2 until confluence. Cells were then treated for 3 hours with 1 μM and 10 μM Cd solutions at 37°C in 5% CO2. We tested five wells for each concentration and untreated wells were used as negative control. Total RNA was extracted and following the reverse transcription step (2) the change in mRNA expression profiles of porcine cytokines IL-1β, IL-6, IL-8, Nk-fb1, Nkfb-p65, MYD88, IL-18, IFN-β, P38, β2-M, TLR4, TLR5, MD2, CD14, TNF-α, bD1, bD2, bD3, bD4, JNK, STAT3 and SOCS1 was investigated using primer sets described in previous studies (2). HPRT1 and GAPDH were used as housekeeping control genes (3). In each sample of IPEC-J2 cells, the relative expression of the selected genes was calculated using the formula ΔCt = Ct (target gene) – Ct (housekeeping). The average intensity of expression (Δct sample - ΔCt negative control) of the genes under study was com-pared among treatment groups by one-way analysis of variance (ANOVA). The threshold for significance was set at $P<0.05$. In cells treated with 1 μM Cd we showed a significant increase ($P<0.05$) of IL-6, IL-8, NFkb1 and NFkb-p65 gene ex-pression and down-regulation of TNF-α expression ($P=0.002$). These data are in agreement with previous studies (1) and highlight a pro-inflammatory effect of low concentrations of Cd. Concerning the treatment with 10 μM Cd we observed up-regulation ($P<0.05$) of BD1, BD2, BD3, IFN-β, IL-18, TNF-α and β2-M and down-regulation of IL-8, NFkb1 and STA3

gene expression. These results suggest activation of the Type I IFNs system; in particular we observed an IFN-β response after treat-ment with 10 μM of Cd. Moreover, we also observed up-regulation of β2-M, indicat-ed as an in vivo marker of Cd exposure in previous studies (4). In conclusion, our results support the hypothesis that Cd exposure may modify the basal level of cytokine expression; specifically, different concentrations of this heavy metal seem to influence different compartments of the innate immune response. These data confirm the ability of non-infectious stressors to modulate innate im-munity; hence, they might cause an alteration of gut interaction with bacteria.

1)Ninkov M., et al. Toxicol Lett. 2015; 237:89-99.
2)Razzuoli E., et al. J Interferon Cytokine Res. 2013; 33 597–605.
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RUMEN FLUID, A NEW DIAGNOSTIC MATRIX IN DAIRY CATTLE FARMS?

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Production diseases of dairy cows include several pathologies and are considered man-made problems caused by the inability of cows to achieve a feed energy intake matching their high production requirements (1). A correct management of production diseases demands early diagnostic and prognostic parameters, in order to implement the necessary adjustments in the management system and reduce the prevalence of clinical cases (2). A previous study of our group showed that forestomachs walls express immune receptors and cytokines, and the rumen liquor contains leukocytes able to produce IFN-γ (3), suggesting an integrated system including receptors, signaling molecules, cytokines and infiltrating leukocytes. Forestomach immune response could react to "dangers" arising within the forestomach environment, but also act as reporter system of disease conditions arising elsewhere in the body. Our working hypothesis implied that ruminal fluids could be an important source of diagnostic information for the identification of herds at risk for production diseases, in addition to the traditional blood and faecal analysis. We first demonstrated that the diet can influence the immune response in forestomachs. Diverse leukocyte populations at very low concentrations and IFN-γ were revealed in some samples of rumen fluids, with a clear inhibition of the response observed in all the animals fed the maize-supplemented diet, compare to a normal and a soy-supplemented diet. We better characterized the leukocytes subpopulations in the rumen liquor, isolating B cells, monocytes, and γδT cells. We also compared the leukocyte composition in ruminocentesis versus nasal probe sampling, and some

differences seem to occur, probably due the fact the samples come from different areas of the rumen, however no significant statistical difference between samples collection techniques was found. Finally we performed a field survey (146 cows from 13 farms) in order to find correlation among the immune profile of the rumen liquor (FACS and molecular analysis), blood, and faecal parameters. Clinically healthy animals showed a farm specific immunologic pattern of the rumen liquor: low CD45 mRNA expression, low or absent IFN-γ, few or absent B-cells. Whereas farms at risk for general wellness presented high levels of CD45 and IFN-γ, increased numbers of B-cells and other leukocyte populations, such as myeloid cells. This immunological pattern of the rumen liquor seems to be associated to inflammatory markers of acute phase response in blood. We can conclude that the epithelial cells of ruminant forestomachs can react to disturbances of the fermentation processes due to improper diets, and the inflammatory response can be sustained by infiltrating leukocytes, able to release cytokines in the rumen liquor. Our data points into the idea that dairy farms could be ranked according to a risk score using the inflammatory markers in rumen fluids (leukocyte populations, CD45 expression). These markers could integrate the usual, consolidated information (e.g. rumen pH and VFA, milk cell counts, blood/faecal analysis).

1)Mulligan et al., 2007.
2) Ingvartsen et al., 2003.
3) Trevisi et al., 2014.

PIGLETS FED SEED-BASED ORAL VACCINE AGAINST VEROCYTOTOXIC *ESCHERICHIA COLI* – IN VIVO STUDY

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Verocytotoxic *Escherichia coli* (VTEC) is responsible of severe enterotoxiaemia in swine. VTEC pathogenicity is strictly related to VT2e toxin and F18 adhesive fimbriae. Nowadays no vaccines are available and an outbreak of the disease requires antibiotic medication, therefore novel strategies, alternative to antibiotics, are required. Plant-based oral vaccines offer an innovative approach to vaccination with the main advantages of avoidance of injections and ability to induce specific antibodies in the mucosa, where the major pathogens gain access to the body.

The aim of this study was to evaluate the efficacy of tobacco seeds-based oral vaccines directed against VTEC infection (ethical authorization: 102/2015-PR).

A competitive indirect ELISA was developed to measure respectively the F18 adhesive fimbriae and the B subunit of verocytotoxin, antigens expressed in Nicotiana tabacum seeds previously produced (Rossi et al. 2013). 36 weaned piglets were divided randomly into 4 experimental groups (CC, challenged control; CT, challenged treated; UC, unchallenged control; UT, unchallenged treated). Treated piglets were fed five times, on day 0, 1, 2, 7 and 14, with 20 g of engineered milled tobacco seeds (expressing 6.6-7.4 µg of F18 plus 34-37 µg of VT2eB) mixed with 20 g of milk powder. Controls received 20 g of wild type of tobacco seeds. The animals were challenged at day 20 with 10E10 CFU of O138 VTEC *E. coli*. During the entire experimental period body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion (FC) were registered individually. From day 20 to 30, animals were evaluated for the general health status and scored daily for specific clinical signs (respiratory, palpebral oedema, epiphora, vitality, faecal consistency, and rectal temperature) with a point-score scale described by Rossi et al.

(2014). The oral delivery strategy guaranteed the total consumption of the treated feed. The uninfected piglets did not show any VTEC-related clinical sign. In the first post-challenge period (days 21-25) CT showed a reduction of ADG and FI lower than CC. CT showed an average total score (from day 1 to day 9 post-challenge) significantly lower than CC for oedema, epiphora, vitality and depression. Death, respiratory and neurologic signs were not observed. These results show that piglets fed tobacco seeds expressing VTEC antigens have overall a better clinical status. This oral delivery strategy appeared effective in reducing the development of clinical signs after challenge with O138 VTEC *E. coli* strain.

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EVALUATION OF THE IMMUNE RESPONSE IN PIGLETS FED SEED-BASED ORAL VACCINE AGAINST VEROCYTOTOXIC *ESCHERICHIA COLI*

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The gut mucosal surface is considered the largest immunologic organ bearing specialized components of the immune system that protect the host against pathogens such as verocytotoxic *Escherichia coli* (VTEC), control responses to food components, and maintain tolerance to harmless external antigens. Oral vaccines can selectively elicit mucosal secretory immunoglobulins A, that play a pivotal role in the mucosal immunity, and a variety of cell-mediated immune responses, including the expression of antigen presenting molecules (major histocompatibility complex, MHC), Toll-like receptors (TLRs) and soluble factors (cytokines and chemokines). Transgenic plants are a valuable platform to produce genetically modified oral vaccines for a large number of human and animal diseases. This study focuses on the immune response elicited in piglets after administration of tobacco seeds-based oral vaccine expressing F18 adhesive fimbriae and the B subunit of verocytotoxin genes from VTEC (Rossi et al. 2014a,b).

36 weaned piglets were divided randomly into 4 experimental groups (CC, challenged control; CT, challenged treated; UC, unchallenged control; UT, unchallenged treated). Treated piglets were fed five times, on day 0, 1, 2, 7 and 14, with 20 g of engineered milled tobacco seeds (expressing 6.6-7.4 µg of F18 plus 34-37 µg of VT2eB) mixed with 20 g of milk powder. Controls received 20 g of wild type of tobacco seeds. The animals were challenged at day 20 with 10E10 CFU of O138 VTEC *E. coli*. Blood samples and small intestinal scrapes were collected from all sacrificed piglets and processed for ELISA determination of serum IgA and intestinal mucosa IgA, TNF-α, IL-8 and CXCL9 (MIG). Samples of jejunum and mesenteric lymph nodes were collected and processed for gene expression study. RNA was extracted, reverse transcribed and assayed with specific primer pairs by Real-Time PCR to quantify the gene expression of the innate immunity

receptors TLRs 2 and 4 and the proinflammatory cytokines IFN-γ and IL-1β in jejunum, and the expression of the antigen presenting molecules MHC type I and II in mesenteric lymph nodes. The challenge significantly increased the expression of MHC-I, which is the molecule responsible for antigen presentation to CD8+ T lymphocytes (CC had the highest expression, followed by CT). UC group showed the lowest expression of MHC-I, demonstrating that the upregulation of this gene expression occurs only in the presence of antigen, either vaccine or pathogen. No statistically significant differences were found for MHC-II and IFN-γ. TLR2, TLR4, IL-1β, TNF-α and CXCL9 were maximally expressed in UT group and minimally expressed in UC group. The proinflammatory cytokine IL-1β resulted highly expressed in CT group in jejunum by PCR. Overall the immune parameters measured showed the highest values in the vaccinated animals versus controls; these results indicate that vaccination with both antigens stimulates the IgA and cytokine production more than the infectious agents.

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EFFECT OF THE HEALTH STATUS IN LATE PREGNANCY ON THE CONCENTRATION OF THE IMMUNOGLOBULIN IN THE COLOSTRUM OF DAIRY COWS

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The success of growth of calves is influenced by the quality of colostrum administered after calving and in particular by its content of immunoglobulins (IgG). The quality of the colostrum depends on many factors, some of these are known, but others have been poorly studied, such as the health conditions in the late gestation. The presence of an infectious-inflammatory condition is able to alter the level of IgG in the blood and, likely, also their transfer in the colostrum. The aim of the work was to study the consequence of an inflammation before the calving, recognized on the basis of the increase of plasma acute phase proteins (APP), on the content of the IgG in the colostrum. Thirty-two Italian Friesian cows were monitored with weekly blood samples between -28 and 28 days after calving on which was performed a large metabolic and inflammatory profile. A homogeneous sample of colostrum was collected at the first milking, for the determination of the chemical-physical characteristics and of the content of IgG. No clinical problem has been detected during the dry period, while some cows showed increased concentrations of positive APP (ceruloplasmin = cucp, and haptoglobin = Hp), which indicated the presence of inflammatory phenomena. Thus, cows were divided retrospectively into two groups, according to the concentrations in cucp, determined 3 days before calving: cucp higher than 2.25 mcmol/L (10 cows; HCP), and cucp lower than 2.25 mcmol/L (22 cows; LCP). HCP vs LCP group showed: higher levels of Hp at -14 days post calving (0.46 vs 0.15 g/L $P<0.1$) and the day of calving (0.74 vs 0.30 g/L, $P<0.05$); higher levels of reactive oxygen metabolites (15.6 vs 12.5 mg/100 mL H2O2, $P<0.01$) and higher levels of bilirubin at 3 days before (6.19 vs 2.93 mcmol/L, $P<0.05$) and 3 days post calving (10.14 vs 7.95 mcmol/L, $P<0.01$). These data indicate that HCP cows suffered of an oxidative stress condition and a liver difficulty to dispose of bile acids, likely because the liver is involved in the acute phase response, and then engaged

in the production of other proteins (i.e. positive APP). The colostrum of HCP cows had a lower content of IgG compared with LCP (61.7 vs 72.3 g/L, $P<0.05$) and a higher fat content (8.55 vs 5.79 g/L, $P<0.01$). The average body weight of calves resulted lower in HCP than of LCP cows (44.5 vs 47.3 kg, $P<0.1$), while the average body weight increase in the first month of life was similar (0.40 kg/d). These data support that the presence of subclinical inflammatory phenomena in the pre calving period could modify the biology of the synthesis of colostrum, reducing the production or the transfer of IgG. Thus, inflammatory events in the pre-partum can contribute to reduce IgG in the colostrum and can impair the immunity of the calf. The higher milk fat content in HCP cows seems likely due to the increase in the blood precursors coming from a more intense body fat mobilization, associated to infective and inflammatory conditions, despite plasma NEFA were not different. Results suggest that a check-up of the metabolic and inflammatory status of cows in late pregnancy provides useful information to improve the care of calves.

COMPARISON OF SKIN TEST AND IFN- γ ASSAY FOR DIAGNOSIS OF BOVINE TUBERCULOSIS IN BLACK NEBRODI PIGS

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Pigs, sheep, goats, buffalo and a variety of wildlife and farmed species are susceptible to *Mycobacterium bovis*. Intra-vitam tests which are officially used to detect bovine tuberculosis in cattle are skin test and IFN-γ assay. The need of a repeated immobilization, the difficulties in the reading and interpretation and the black color of the bristles complicate the routine use of the skin test in black Nebrodi pigs (especially if they are feral pigs, wild boars or crossbreed) (Pesciaroli et al., 2012). The authors compare the use of both tests for the detection of *Mycobacterium bovis* infection in pigs evaluating the possibility to use the IFN-γ assay as alternative for the in vivo diagnosis of the infection in this species. 124 pigs were submitted to in vivo e post- mortem investigations for bovine tuberculosis. The skin test was carried out on the external surface of the ear canal, before the inoculation of bovine PPD a blood sample for the INF-γ assay was collected. On the same animals an anatomo-pathological examination was conducted. Tissue samples were collected and processed for bacteriological investigations. Statistical analysis about concordance of skin test and IFN-γ assay was performed using classical concordance tests (Cohen's Kappa index and McNemar's test). Forty- five resulted positive to skin test while 48 to IFN- γ assay. Moreover comparison between skin test and IFN-γ assay of the 124 subjects showed a concordance of 89.5% and a Cohen's Kappa index of 0.786. During the abattoir inspection twenty nine carcasses showed tuberculous-like lesions while *Mycobacterium* spp. was isolated in 44 animals. The McNemar's test between the two in vivo tests of the latter showed a value of x2=1.8 with a p-value of 0.1797. This low x2 value indicates a non-significant difference. The correlation was of 88.6% and the Cohen's kappa index was 0.77. This means that in

our case the two tests tend to have overlapping performances. We, also, considered the 20 subjects positive for *Mycobacterium bovis*. In this case 18 subjects resulted positive while 2 were negative to both tests. Therefore the correlation was 100% and K=1.00. Overall, we can hypothesize that the results obtained by the two tests are equivalent, making possible the use of IFN-γ assay as alternative to skin test in situations where the control of bovine tuberculosis in pigs is required.

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Scienze Cliniche:

Clinica Medica

ECHOCARDIOGRAPHIC EVALUATION OF RIGHT VENTRICULAR END-DIASTOLIC AREA INDEX IN DOGS WITH PULMONARY HYPERTENSION

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Pulmonary hypertension (PH) can lead to right ventricular remodeling and failure. Right ventricular enlargement is of clinical importance since it reflects RV pressure and volume overload and is associated with prognosis. The aim was to evaluate reliability of the right ventricular end-diastolic area (RVEDA) index to characterize right ventricular size and PH severity in dogs.

The study design was a prospective, multicenter, observational study. We included 99 client owned dogs of different breeds: 59 healthy dogs and 40 dogs with PH. Pulmonary hypertension was classified according to tricuspid regurgitation pressure gradient (TRPG) in mild (TRPG: 36–50 mmHg; n=11 dogs), moderate (TRPG: 51–75 mmHg; n=8 dogs) and severe (TRPG: >75 mmHg; n=21 dogs). Seven dogs with PH had right-sided congestive heart failure (CHF). Echocardiographic view of the RV was obtain from the left apical 4-chamber view optimized for the right heart. Right ventricular end-diastolic area was measured by planimetry at the end of ventricular diastole, tracing from the lateral aspect of the tricuspid annulus to the septal aspect, excluding the area of the annulus and trabecular structures, following the right ventricular endocardium. RVEDA index was calculated as end-diastolic right ventricular area divided by body surface area. Dogs with congenital heart disease, cardiomyopathies and athletic dogs were excluded.

Right ventricular end-diastolic area showed a strong positive linear correlation with body surface area in healthy dogs ($r=0.88$; $P<0.0001$). RVEDA index was significantly higher ($P<0.05$) in dogs with moderate (11.3±3.3 cm2/m2) and severe PH (12.3±3.0 cm2/m2) in comparison to mild PH (7.8±2.0 cm2/m2) and healthy dogs (8.4±1.8 cm2/m2). No differences in RVEDA index were found between dogs with moderate and severe PH and

between healthy and mild PH. A weak positive correlation was found between RVEDA index and TRPG ($r=0.47$; $P<0.05$) and the TRPG was not different between dogs with right-sided CHF (90 mmHg, range 52-134) or without right-sided CHF (77 mmHg, range 36-130). Conversely, RVEDA index was significantly higher ($P<0.0001$) in dogs with right-sided CHF (14.0±2.9 cm2/m2) in comparison to dogs without right-sided CHF (10.2±3.1 cm2/m2). The most accurate cut-off value in the prediction of right-sided CHF was >12.2 cm2/m2 (sensitivity 71%; specificity 73%). Intra- and inter-observer measurement variability was clinically acceptable (coefficient of variation <15%).

The study showed that RVEDA index increases in dogs with PH and is particularly high in dogs with right-sided CHF. This index will likely provide beneficial complementary information in the assessment of PH severity in dogs, especially when TRPG does not correlate with hemodynamic consequence of PH. Further studies are needed to evaluate RVEDA index as a prognostic indicator in dogs with PH.

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DIAGNOSIS AND TREATMENT OF A VEGETABLE FOREIGN BODY IN THE LUNG OF A HORSE

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Inhaled vegetable foreign bodies are rare in the horse, and the only reported cases are of twigs or thorny branches, while retrieving cereal spikes or spikelets from the bronchi of dogs is more common, especially in our area.
Aim of the study is to describe diagnosis and treatment of a vegetable spike from the lung of a 3 year old Standardbred male.
A 3 year-old Standardbred male was admitted at the Veterinary Teaching Hospital of Perugia for coughing and nasal discharge lasting at least 10 months. At admission, the horse showed monolateral nasal discharge (right nostril) and, after exercise, spontaneous coughing. Lung auscultation identified respiratory sounds increased throughout the thorax, except for one region, located caudally and dorsally on the right side, where they were dulled. Thoracic ultrasonography and radiography showed areas of reduced air content, on the right caudal lobes. Bronchoscopic evaluation allowed to identify and retrieve a vegetable foreign body located in the bronchus 1.15. After therapy with antibiotics (Cefquinome, 1mg/kg q24hrs and Marbofloxacin, 2mg/kg q24hrs), endoscopy and thoracoscopy were performed, to exclude migrating foreign bodies. The horse was discharged and resumed training, with good results. Tracheobronchial foreign bodies are rare in the horse, and to the authors' knowledge this is the first description of the removal of an awn from the lung of a horse using a transendoscopic biopsy forceps. The history is similar in all the reports, with sudden onset of coughing as the main presenting complaint. The diagnostic protocol in these cases should include thoracic radiographs and ultrasonography and a thorough bronchoscopic examination, that allows the complete exploration of the lungs. To exclude migrating foreign bodies in the thoracic cavity a thoracoscopic examination is advised. Both endoscopic techniques are mini-invasive and allow for a complete visualization of both the lower respiratory tract and the thoracic cavity. After removal, broad spectrum antibiotics, with or without mucolytic therapy, should be started, to help clear the airways from any residual mucus.

A follow up broncoscopic examination should be performed after 7 days to check for other foreign bodies that could be hidden in the mucus present in the airways during the first exam. In our case, the removal of the spike using transendoscopic biopsy forceps and antibiotic therapy brought a complete remission and a return of the horse to the previous level of fitness.

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EFFECT OF PHYSICAL EXERCISE ON OCULAR RESISTIVE INDEX IN DOG

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Numerous studies concerning acute dynamic exercise, continuous strenuous exercise, isometric exercise, and even brisk walking have been shown a modification of ocular parameters during physical exercise (1-3). Resistive index (RI) is an indirect measurement of blood flow resistance that can be used to evaluate vascular damage in ophthalmologic disease (4).

The purpose of this study was to evaluate variations of resistive index of long posterior ciliary artery (LPCA) during moderate treadmill exercise in dogs.

Thirty-four healthy dogs (16 male, 14 female, median age 2.9 y.o) of different small breeds were underwent an exercise test on a motorized treadmill (professional canine treadmill, ® Grillo, Modena, Italy) for 45 min at 2.5 km/h for 15 minutes, 5.0 km/h for 20 and 2.5 km/h for 10. Resistive index (RI) of long posterior ciliary artery (LPCA) was evaluated before (T0) and immediately after exercise (T1). Color Doppler has been used to determine the location of LPCA. Pulsate Doppler was performed to evaluate peak systolic velocity (PSV), end diastolic velocity (EDV), mean velocity of blood (MVB), and resistive index (RI). Measurement of blood pressure (BP) was performed after physical exercise (T0) and during the rest (T1). Statistical analysis was carried out in order to compare BP and RI values in the LPCA before and after exercise. Data were analyzed using a two-way ANOVA using SPSS Statistics. All results were expressed as means ± standard deviation (SD). $P<0.001$ was considered highly significant. Spearman test was used to correlate the variables.

The mean value of RI before the exercise was 0.727± 0.021, after 0.640±0.015 ($P<0.001$). The mean values of systolic pressure (SYS) before the exercise were 151.11±18.17, after 156±18.73; mean arterial pressure (MAP) was before 102.94±11.12, after 104.11±12.49; diastolic pressure (DAP) was 77.117±11.345 before and 76.411±11.303 after. No

significant differences about blood pressure were been observed. Spaerman's rank correlation evidenced a negative and highly significant correlation between SYS and RI (-.430) and a positive and highly significant correlations between SYS and MAP values (.583).

Orbital and ocular blood velocity parameters were reported in veterinary medicine in dogs (5). Our results showed a highly significant reduction of resistive index of LPCA immediately after exercise. These suggest a peripheral vasodilator effect induced by cathecolamines during aerobic exercise (6). Cathecolamines would act on β_2-adrenoreceptor of sphincter muscle of iris. In conclusion, moderate treadmill exercise in dogs causes significant changes in RI, presumably related to compensative neuro-hormonal mechanisms.

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COMPARISON OF TWO OMEPRAZOLE FORMULATIONS IN THE TREATMENT OF GASTRIC ULCERS IN HORSES

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Gastric ulcers are a common disease of the horse, with prevalence as high as 100% in racehorses in active training. Omeprazole is the drug of choice for the treatment of this disease, and the only active substance of registered products for horses currently available in Italy.

Aim of the study is to evaluate the effect of two different omeprazole formulations (paste and enteric coated granules) on the healing of gastric ulcers in the horse.

The study was conducted, with the approval of the Ethical Committee of the University of Perugia and the Ministry of Health, during the drug development phase of a new omeprazole product, using the same active substance in two different formulations. In a double blinded placebo trial, 32 horses, positive for gastric ulcers, were tested, divided in 4 groups of 8 horses each. 2 groups were given the two formulations of omeprazole (4mg/kg), while two received a placebo product as paste or granules. Clinical examination and gastric endoscopies were performed at inclusion and every 7 days for 4 weeks. The presence and severity of gastric ulcers was graded according to the grading system proposed by MacAllister et al. (1997): the results were recorded and analysed by a repeated measures ANOVA.

During the drug development phase of a new product, the choice of the formulation should be based on the effectiveness, but also on the ease of handling and delivering to the animal. According to our results both formulations were effective in the treatment of the disease, with a reduction of the number ($P \leq 0.02$ for both) and the severity ($P \leq 0.03$ for the paste and $P \leq 0.04$ for the enteric coated granules) of the lesions. Comparing the two groups treated with the placebo and the two with the omeprazole, a reduction of the number and severity of the lesions in the omeprazole group was noted, not statistically different between the animals treated with the enteric coated granules and the oral paste. During the four weeks of observation, a reduction of the number of ulcers was seen after

14 days, and of the severity after 7, more significant in the granules group. In conclusion, both formulations are effective in the treatment of equine gastric ulcers, but enteric coated granules of omeprazole appear to have better results, be more easily handled and more manageable than the paste formulation, making it the formulation of choice for the new marketed veterinary medicinal product.

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APPLICATION OF THORACIC ULTRASONOGRAPHY FOR THE DIAGNOSIS OF BOVINE RESPIRATORY DISEASE IN PIEDMONTESE CALVES

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Bovine respiratory disease (BRD) is one of the main health issues in calves. Thoracic ultrasonography (TUS) was found to be a promising tool in detecting BRD (1). The aim of this prospective study was to describe findings of TUS, clinical examination and bacteriology in post-weaned Piedmontese calves. Animals were examined at 3 experimental times: T0 (day of inclusion), T1 (7 days), T2 (21 days). Data about clinical examination and TUS findings (2-5 MHz convex probe) were recorded for each calf at T0, T1 and T2. TUS was performed on 4 standardized areas of both hemithoraxes: cranio-dorsal (CrD), cranio-ventral (CrV), caudo-dorsal (CaD), caudo-ventral (CaV). TUS findings were classified by mean of a scoring system (USc- 0: no lesions; 1: comet-tail artifacts; 2: lobular consolidation; 3: lobar consolidation) (1). At T0 nasal swabs (NS) and trans-tracheal aspiration (TTA) samples were collected for bacterial culture. Data analysis [Wilcoxon signed rank test, Sensitivity (Se), Specificity (Sp)] was performed with statistical software R, v. 3.2.3. Data were reported as median (min-max), statistical significance was set at $P<0.05$. Twenty calves, aged between 5 and 14 months, from 5 herds, were included. At T0, 14/20 (70%) animals were classified as BRD affected according to TUS findings. Only 9/20 (45%) had concomitant clinical signs. Lesions were found on both hemithoraxes in 8/14 (57%) calves and involved the CrV area in the 78% of cases. Four calves without abnormal TUS findings at T0 have developed lesions at T1 (n=3) and T2 (n=1). Median USc was 3 (0-12) at T0, 4 (0-10) at T1 and 4.5 (0-11) at T2. Statistical difference in USc was found between T0 and T2 ($p=0.03$). Overall, pathogenic bacteria of BRD [*Mycoplasma* spp. (56%), *P. multocida* (28%), *M. haemolytica* (11%) and *T. pyogenes* (5%)] were found in 14 TTA samples. Of these, 11 came from calves with TUS

lesions at T0, while 3 were from calves that developed TUS lesions at T1 and T2. Bacterial culture of NS led to the identification of BRD pathogens in 57% (8/14) of positive TTA samples. Clinical examination showed low sensitivity compared to both TUS (Se: 64%, Sp: 100%) and bacterial culture of TTA samples (Se: 50%, Sp: 67%). TUS had 79% of Se and 50% of Sp compared to TTA culture.

Results of the present study suggest TUS as a promising tool for the diagnosis of BRD in post-weaned Piedmontese calves. This technique can be a practical screening method in field condition, allowing the characterization of lung lesions. According to studies in dairy calves (1, 2), TUS had a higher sensitivity for BRD diagnosis when compared to clinical examination. Moreover, TTA samples seems to be more appropriate than NS for bacteria detection. Our investigation led to a higher Mycoplasma spp. detection compared to other pathogens, showing its important role in the pathogenesis of BRD.

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VETERINARY FORENSIC MEDICINE - FROM VETERINARY LEGAL MEDICINE TO NEW CHALLENGES

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Veterinary Legal medicine is a complex subject of study, which nowadays goes beyond the traditional knowledge of the laws and regulations relating to animal sales, food security, animal health and welfare, professional ethics. In particular, research in the field of mistreatment, killing and other crimes and offenses against animals as well as where fraud in the food industry is concerned have benefited from new operational concepts. They include the application of forensic techniques and the new investigation and assessment tools, which play a useful role against the illegality that jeopardises animal health and food security. Forensic veterinary medicine therefore represents a new area of increasing interest of veterinary legal medicine, which opens up new perspectives in research and on criminal justice. It is the application of a broad spectrum of sciences to answer questions of interest to a court of law.

Veterinarians have an array of duties within veterinary forensic science. In the prevention and suppression of the illicit activities (including in particular food fraud), there is ever an increasing number of cases when the official veterinarian shall be responsible for the functions of the judicial police, thus serving the judicial Authority, and also of cases in which freelance veterinarians must be able to play the role of technical adviser.

This entails the need that the veterinarians become more aware of the accountability systems in which they operate; in fact, these systems become more complex and more stringent precisely because of the new technical tools available, and also because of the different aspects of the law regarding them, that create specific responsibility for the issue under consideration. The ultimate objective is to make full use of its potential in carrying out inspections and in providing support in investigations, and in court. Accordingly, also the need to increase knowledge, in the field of procedures (technical-scientific methods and criteria; Criminal Procedure paths) and regarding the relations with the judicial authority, becomes compelling.

It is therefore essential to promote and disseminate disclosure of information and awareness relating to this emerging matter in the broad sense, including also the existing legal framework of interest (as forensic medicine requires specific skills to be applied in accordance with the various legal frameworks) and trying to clarify the role of the competent judiciary body.

Veterinary forensic medicine is an interdisciplinary field. In this perspective, a unique combination of scientific, forensic and legal knowledge is required. And since all veterinary specialisms are involved, the mutual cooperation of experts in different branches has to be applied to become fruitful in serving the administration of justice.

In the near future, the melding of human and veterinary forensic science will ultimately benefit society, protecting people and animals alike.

The Authors reflect about how the future of the veterinary legal medicine will be written by enlarging its multidisciplinary mission to the forensic medicine as a crucial complementary science related to justice.

RELATIONSHIP BETWEEN DUODENAL ENTEROCHROMAFFIN CELLS DISTRIBUTION AND INFLAMMATORY BOWEL DISEASE SEVERITY IN THE DOG

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Inflammatory Bowel Disease (IBD), since more than twenty years, is the major topic of discussion and research in canine gastroenterology. Several studies about ethio pathology and treatment have been performed, but little attention has been paid to the dysfunction of the regulation of the enteric nervous system that characterizes this syndrome. Nervous control of gastrointestinal motility is a complex process, in which serotonin (5-HT) plays an important role (1). 5-HT, mostly released from enterochromaffin cells (EC) included in gut epithelium, initiates peristaltic, secretory, vasodilatory, and nociceptive reflexes, and it is removed by serotonin-selective reuptake transporter (SERT), located on enterocytes and platelets. Altered serotonergic metabolism has been described in human gastrointestinal diseases (2, 3). A recent report evidenced a higher mucosal concentration of serotonin and chromogranin-A (CgA), an EC marker, in dogs with IBD respect to healthy dogs (4). Following this suggestion, we have proposed to evaluate, in dogs with IBD, an eventual relationship between duodenal EC distribution and clinical condition, endoscopic appearance and duodenal histology. 21 client-owned dogs (7 crossbreed and 14 purebreed), 4 females (2 spayed) and 17 males (4 castrated), mean age of 5.8±3.2 years, were included. Standard diagnostic protocol for IBD was followed and a clinical score index (CCECAI) (5), an endoscopic activity score index (EASI) (6) and an histopathological index of duodenal biopsies (HIDB) (7) were calculated. Furthermore, an immunohistochemical analysis was performed on paraffin-embedded duodenal biopsies to evidence CgA positive epithelial cells (CgA+EC). In particular, five fields per slide were examined, and the percentage of CgA+EC with respect to enterocytes was calculated. A linear regression (preceded by a D'Agostino-Pearson test for normality) was performed between percentage of CgA+EC and CCECAI, EASI and HIDB, respectively. Median (95% confidence index) for CCECAI was 8 (6-10), for EASI was 1.5

(1-3.8), for HIDB was 10.5 (8.4-13.2), and mean and SD of CgA+EC percentage was 1.44% (±0.72). In all linear regressions a significant correlation was not detected. Our results indicated no relationship between disease severity and duodenal CgA+EC concentration. The results cannot be compared with literature because Bailey et al. (2016) (4) indicated exclusively a difference between healthy dogs and dogs with chronic enteropathy without seeking a relationship with respect to the severity of illness. Further research will evaluate if SERT activity is related with IBD severity, and therefore if the decreasing in serotonin reuptake is linked to nociception and clinical signs in these patients.

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ZVL EPIDEMIOLOGYCAL SURVEY IN A PARTICULAR ITALIAN SCENARIO: LAMPEDUSA ISLAND

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Zoonotic visceral leishmaniasis is a sand fly-borne disease caused by *Leishmania infantum* and is widely distributed in temperate and subtropical regions, of both Old and New Worlds. Mediterranean basin is historically considered endemic for ZVL; however strong differences may exist in terms of prevalence among different areas. Lampedusa, is a small island (20.2 km^2) of the Italian Pelagie Islands, the southernmost of Italy and the nearest to North Africa.

This study represents the first investigation on the prevalence of ZVL infection in dogs population and is part of a big study on the presence of ZVL on Lampedusa island.

Sampling on 242 dogs of different age, sex and breed was performed from November 2015 through February 2016. Each dog was submitted to clinical examination by filling a clinical form and to blood sample to detect *Leishmania infantum* infection by using a commercial rapid test. Diagnosis was always confirmed by IFAT; dogs showing clinical signs were submitted to RT- PCR (on blood and/or lymph node). Detection of canine anti-*Leishmania* IgG antibodies was performed by an in-house IFAT following the protocol recommended by the World Organization for Animal Health (OIE) (Gradoni and Gramiccia, 2000). The cut-off dilution was set at the 1:160 titer for detecting established infections; lower titer (1:40-1:80) were also considered as a marker for *Leishmania* exposure (Paltrinieri et al., 2010). The quantitative PCR was targeted on 117 bp fragment inner the constant region in the mini circle kinetoplast DNA (kDNA) according to Reale et al., 2008.

At the end of the study, 70/242 dogs (28.9%) resulted negative to all diagnostic tests. The remaining 172 (71.1%) showed positivity at least to one of the test. In detail 5/172 (2.9%) showed a positive rapid test alone; 148/172 resulted positive to IFAT and 19/172 (11.0%) were positive to RT-PCR. IFAT positive dogs showed antibodies titer ranging

between 1/40 and 1/80 (77/148 - 52.0%) or equal/higher to 1:160 (71/148 - 47.9%). Interestingly no dogs showed at the same time a positive rapid test, IFAT and RT-PCR. Only 23/172 (13.4%) positive dogs showed clinical signs of *Leishmania infantum* infection.

The very high proportion of seropositive dogs demonstrates that the parasite Leishmania infantum abundantly circulates on the island and could constitute a "social alarm" for the human population, also due to the small territory of the island. Restrictive sanitary measures should be considered, particularly by considering the use of large scale application of sand flies repellents.

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KIDNEY INJURY IN DOGS WITH ACUTE PANCREATITIS

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Acute pancreatitis (AP) is a common disease in dogs characterized by a wide spectrum of clinical signs, as anorexia, vomiting, diarrhoea and abdominal pain. AP can lead to kidney injury via hypovolemia, cytokine-induced ischemia, inflammation and oxidative stress. The aim of the study was to evaluate the prevalence of kidney injury in dogs with AP. The study enrolled 65 dogs with positivity to SNAP cPL® test and clinical and laboratory signs suggestive of AP. Dogs with non-pancreatic acute abdominal disease were excluded. WBC, neutrophil count, serum C-reactive protein (CRP), serum creatinine (SrCr) and urea and urinalysis were evaluated at time of diagnosis. The magnitude of AP was assessed using the clinical severity index (CSI) as described by Mansfield (2008). The patients were divided into two groups: survivors and non-survivors. Non-survivors included dogs, which died within seven days from admission. Data were statistically analysed using GraphPad Prism® for Mac. The study population was composed by intact (n=30) and spayed females (n=13), and intact (n=34) and neutered males (n=2). Patients showed median age of 8.8 (0.4–14.6) years, BCS of 5/9 (2/9-7/9) and body weight of 16 (2.5-64) kg. Overall seven-day survival was 67.7% (44/65 dogs). CSI≥5 was associated with poor outcome (p=0.047) and elevated CRP (p=0.014). Dogs with CRP three-fold higher than upper reference range, showed a significantly poorer outcome (p=0.0003). SrCr>1.5 mg/dL and urea>55 mg/dL above the reference range were significantly associated with increased risk of death ($p<0.0001$ and $p<0.0009$ respectively). In this study, overall mortality rate was 32.3%, and 37% in dogs with CSI≥4. However, in this cohort of dogs median CSI was 4 and 66% of dogs were in CSI≥4 group. For this reason, median CSI was used to divide dogs into two groups and dogs with CSI≥5 (n=32) have been associated with increased risk of death (13/32, 40%). Previous study reported an overall mortality rate of 23% for all dogs and 53% for dogs with CSI score≥4. Previous studies have failed to find a correlation between CRP and outcome or CSI. In our cohort of AP patients, CRP showed a low sensibility and it was associated with CSI≥5. Patients

with 3xCRP showed a significantly higher risk of death; comorbility or multi organic dysfunction syndrome could be more frequent in these patients. In canine AP elevated SrCr has been reported as prognostic marker. Previous study found that dogs with renal damage score 2 (anuria or azotemia≥1.5-fold increase in serum urea and SrCr) had a higher mortality rate than dogs with renal damage score 0 or 1. However, renal damage score was a part of a multiple organ CSI, making the role of azotemia unclear. In a more recent study, 55% of dogs with AP showed elevated SrCr, but it was not prognostic. In this cohort of dogs, elevation in serum urea or SrCr have been associated with poor outcome.

In conclusion, CSI, CRP and azotemia may be used to predict outcome in canine AP.

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RETROSPECTIVE OBSERVATION OF 64 DOGS WITH RENAL FAILURE MANAGED BY HEMODIALYSIS

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Acute Kidney Injury (AKI) is a severe disease associated with a sudden onset of renal parenchymal injury most typically characterized by generalized failure. AKI may to be severe and culminated with the requirement for renal replacement therapy (RRT) or death. The aim of this study was to evaluate potential prognostic factors (clinical and laboratory parameters), intra/inter dialysis complications and mortality rate in a cohort of dogs with AKI and AKI/CKD managed by hemodialysis (HD) at Department of Veterinary Science of University of Pisa between 2012 and 2015. We included 64 dogs with anamnestic, clinical, imaging and laboratory findings of AKI or acute on chronic kidney disease (AKI/CKD) managed by hemodialysis. All Dogs were also divided into two groups: survivors and non-survivors. Survivors were defined as patients remaining not dependent by dialysis for at least 30 days after discharge from the hospital. Data were statistically analyzed with GraphPad Prism® for Mac. In our cohort 43/64 were males and 21/64 were females. Mean age and body weight were 5.5 ± SD 3.3 years and 26.7 kg±1SD 1.6 kg respectively. At presentation 26/64 dogs were anuric (<0.5 ml/kg/h), 15/64 were oliguric (<1ml/kg/h) and 23/64 were non oliguric (>1ml/kg/h). 29/64 dogs were in AKI stage 5 (SrCr>10mg/dl), 29/64 were in AKI 4 (SrCr 5-10 mg/dL), 2/64 were in AKI 3 (SrCr 2.6-5mg/dL), 1/64 were in AKI 2 (SrCr 1.6-2.5) and 3/64 were in AKI 1 (SrCr<1.6mg/dL). Most of etiology of AKI in this dogs were: *Leptospira* infection (14/64), *Leishmania* infection (5/64), toxicity (8/64), ethylene glycol (3/64), pancreatitis (4/64), heart stroke (2/64), snake bite (2/64), uretheral obstruction (2/64), pyometra (3/64), unknown (17/64) and other causes (4/64). Non-survivors dogs were 37/6, while survivors were 27/64. T-Test unpaired showed significant difference at presentation in serum creatinine (p=0.047), phosphorus (p=0.0468), and ionic calcium (p=0.042) between survivors and non-survivors. No significant difference was found in serum urea, albumin, C-reactive protein (CRP) and potassium between survivors and non-survivors. In our cohort the overall survival rate was 42.2% and it showed similar to previously data

reported. Serum creatinine, phosphorous and ionized calcium seemed to have a significant prognostic relevance, while serum urea, CRP and potassium did not seem to affect prognosis significantly.

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DESMOPLASTIC EPITHELIOID OLFACTORY NEUROBLASTOMA IN A DISTEMPER VIRUS INFECTED DOG

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A 5 years old male Swiss Shepherd dog, regularly vaccinated for Canine Distemper Virus (CDV), Canine Adenovirus, Canine Parvovirus type 2 and Leptospirosis till 4 years old, was referred for seizures, epistaxis of the right nostril, and ipsilateral eye lachrymation. The patient demonstrated ataxia and behavioural alterations, even aggressiveness against owners. Blood analysis did not show significant changing, but Quick test (Whitness®) was positive for CDV. Cerebrospinal fluid cytology revealed moderate mononuclear pleiocytosis, and a RT-PCR analysis identified the presence of CDV genome. No magnetic resonance (MRI) was performed (not accepted by owners). Feline recombinant Ω-interferon (VirbagenOmega®) was used at 2.5 UM/kg IV for 3 consecutive days, twice in one month, to treat CVD infection (1). The follow-up period of 3 months highlighted a progressive worsening of the patient's conditions, leading to euthanasia. Necropsy revealed a stringy exudate in the right frontal sinus and an irregular, brown-white firm neoformation in the right frontal lobe, compressing the contiguous cerebral parenchyma. Microscopically, the neoformation was infiltrative and composed by round-to-oval cells supported by hypocellular zones of delicate fibrillary stroma. Immunohistochemistry (IHC) revealed multifocal, isolated or organized in small clusters, immunopositivity for cytokeratins, neuron enolase (NSE) and neurofilaments (NF) in the tumour cells; moderate to severe immunopositivity for vimentin, glial fibrillary acidic protein (GFAP) and S100 in the stroma. Moderate and disseminated vacuolization of the white matter associated with digestion chambers, glial cells and small vessels proliferation, and a mild non suppurative inflammation were also detected in the cerebral cortex, mesencephalon and pons, suggesting demyelination compatible with CDV lesions. IHC for CDV was negative. The gross and microscopic features were consistent with the

diagnosis of the concomitant presence of an olphactory neuroblastoma (ONB) associated with a non suppurative demyelinating encephalitis likely due to pre-existing CDV infection. The negative results obtained by IHC for CVD could be due to the previous treatment with feline recombinant Ω-interferon: a high antiviral activity of this specific interferon was described by Wang et al. (1) on CDV infected culture cells. In veterinary medicine, previous studies reported a positive staining of ONBs for NSE (2), whereas GFAP, NF and cytokeratins were most frequently immunonegative (3). A strong cytokeratin expression was reported in canine olfactory neuroepithelioma (4) and a limited immunopositivity in both tumour and supporting stroma was observed in canine ONB (5). To the best of the authors' knowledge, this is the first description of a simultaneous presence of an ONB and CDV brain infection in a dog, but it is impossible to establish a correlation between these two pathologies.

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CLINICAL USE OF MEDETOMIDINE-BUTORPHANOL SEDATION PROTOCOL FOR CATS UNDERGOING BLOOD DONATION

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Feline donor programs routinely require sedation of the cats during the blood collection. Sedation protocols should be effective and safe, allowing for the rapid return to normal function.

The aim of this study was to investigate the effect of blood donation (BD) using medetomidine and butorphanol sedation protocol on selected clinical variables in cats. Thirty cats were enrolled in the study (21 males and 9 females) as blood donors. All measurements were performed as standard monitoring of BD. Mean±SD body weight of cats was 5.9±1.0 kg (4.6-8.6 kg), age was 4.6±2.0 years (1-8 years). Each cat was sedated with a combination of medetomidine (20 μg/kg) and butorphanol (0.4 mg/kg) administered intramuscularly. When sedated, cats received 150 ml of saline solution subcutaneously. Blood (10 ml/kg body weight, maximum 60 ml/cat) was collected from the jugular vein. The clinical parameters evaluated before and after BD included: rectal temperature (RT), heart rate (HR) and systolic arterial pressure (SAP) measured at the radial artery of the right forelimb using a Doppler machine (Minidop Es 100vx, Hadeco; Welchallyn blood pressure cuff 9.1-5.4 cm). RT was measured before and after BD. SAP and HR were measured in unsedated cat (SAP and HR), in the sedated cat before (SAP II and HR II) and after (SAP III and HR III) BD. To detect change in RT was used t-test. Analysis of variance (ANOVA) was used to detect changes in the SAP and HR over time. Significance was set at $P<0.05$. All variables studied were normally distributed at the pre- and post- BD time points except for SAP III and HR III. The mean±SD values for pre- and post- BD RT were 38.4±0.4 (37.7-39°C) and 37.8±0.4 (37.2-38.7°C), respectively. The RT mean change was -0.6±0.4°C. Significant decreases in RT ($P<0.0001$) occurred after BD. The mean±SD values for pre- and post- BD for SAP, SAP II, SAP III were 136±16 (100-170 mmHg), 133±19 (100-180 mmHg), 95.5±20 (70-150 mmHg), respectively; for HR, HR II and HR III were 200±17 (176-244 bpm), 121±6 (108-134 bpm), and 132±7 (120-153 bpm), respectively. Significant decreases ($P<0.0001$) in SAP at every point of

measurement occurred after BD except between SAP and SAP II (P=0.57); HR and HR II showed a decrease (P<0.0001), while HR II and HR III an increase (P<0.0001). BD lasted 32±7 minutes (20-48 minutes). None of the cats had evidence of adverse effects. Atipamezole (40 μg/kg) was given to reverse sedation. Although medetomidine-butorphanol protocol showed to decrease HR, SAP and RT in feline healthy-donors after BD, it appears to be tolerated and it allows a rapid reverse sedation using atipamezole.

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SERUM PROTEIN ELECTROPHORESIS IN THE CAT. REFERENCE INTERVALS USING AGAROSE OR ACETATE CELLULOSE GELS AND THEIR CLINICAL APPLICATION

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Serum protein electrophoresis (SPE) uses different gels to separate proteins. It is mainly indicated to study modifications in protidemia, albuminemia, and globulinemia. Literature suggests variability in the reference intervals (RI) in the different fraction values, using various SPE gels (1, 2). This study was aimed to calculate the RI of SPE using agarose (AGE) and acetate cellulose (ACE) techniques, to compare the two RIs obtained, and to investigate the causes of hyper-gamma-globulinemia in some samples. Five hundred and fifteen blood samples from European short or long-haired cats (>1 year old and <10 years old), from both genders, were collected; 291 samples were tested on ACE (2010-2013; MICROTECH 648 ISO, Interlab®, Rome, Italy) and 224 on AGE (2013-2014; Pretty, Interlab®, Rome, Italy). One hundred and seventy-nine runs (ACE, 105; AGE, 74) were selected based on the following criteria: total protein, albumin and globulin values in RIs; no alterations both in CBC and serum biochemical profile; no alteration at visual inspection of the SPE run. RIs (median and 90% CI of absolute and relative values) were calculated using non-parametric Robust method (Medcalc® software). The Mann-Whitney test was used for the comparison between the two RIs. Finally, the SPE on AGE in 28 runs with hyper-γ-globulinemia were studied using the clinical records. The following SPE fractions were observed in ACE and AGE: albumin, α1, α2, β1, β2, and γ globulins. RIs for ACE were (values: absolute g/dL; relative %): albumin (3.0-3.9; 43-57), globulins (2.7-4.3; 43-57), α1 (0.1-0.4; 1-5), α2 (0.4-1.5; 6-20), β1 (0.3-0.9; 4-14), β2 (0.4-0.7; 5-10) and γ (0.4-1.9; 7-27). RIs for AGE were: albumin (2.9-3.9; 42-57), globulins (2.8-4.6; 43-58), α1 (0.04-0.4; 1-5), α2 (0.8-1.7; 13-22), β1 (0.2-0.7; 2-9), β2 (0.1-0.8; 3-10), and γ (0.6-2; 9-27). Some sub-fractions were observed (# runs): ACE α2A and α2B (12), γ1 and γ2 (13); AGE α2A and α2B (62), γ1 and γ2 (38). For all fractions (except albumin and γ-globulins) statistically significant differences were observed between ACE and AGE ($P<0.05$). Cats with hyper-γ-globulinemia (n=28) were

diagnosed with: Renal failure (17) staged by IRIS; Feline Infectious Peritonitis (1); Squamous cell carcinoma (3); FIV Positive (2); FeLV Positive (1); Mandibular abscess (1); Multicentric and Gastrointestinal lymphoma (2) and one suspected of *Toxoplasma* spp infection. Literature about feline RIs in SPE is inadequate, regardless the type of gel employed. In AGE runs we detected more frequently α2 and γ-globulin sub-fractions that should be further investigated for their clinical significance. Statistically differences between ACE and AGE in European cats were observed and AGE showed a better electrophoretic resolution. The SPE is a semi-quantitative method for protein fractions differentiation, but it can provide a diagnostic orientation.

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INVESTIGATION OF CRP AND OTHER HEMATIC INFLAMMATION MARKERS IN DOGS

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The systemic reaction to acute inflammation, also known as acute phase response, induces some hemato-biochemical changes, which can be evidenced in laboratory findings. The C Reactive Protein (CRP) is a main acute phase response protein elective in dogs to detect inflammatory disease. Other blood parameters have been described as useful inflammation markers i.e. Fibrinogen and Leukocytes (1, 2, 3).

The aim of this retrospective study was to investigate the CRP values in comparison to Fibrinogen (Fib), Albumin (Alb), and Iron (Fe) values, total White Blood Cell (WBC), Segmented Neutrophil (NeuSeg) and Band Neutrophil (Band) counts, and the occurrence of Toxic Neutrophils (Neu TOX), Activated Monocytes (Mon ATT), and Reactive Lymphocytes (Linf REA) in blood smears.

For this purpose, data of 1,837 blood samples was collected over a three-year period (2012-2015). Data collected for each sample included: Fib, Alb, Fe, WBC, NeuSeg, Band, Neu TOX, Mon ATT, Linf REA and CRP, as well as information regarding dog's age, breed, and gender.

Blood samples were divided into 2 groups: "inflammatory"; CRP ≥0.30 mg/dL (#1080) and non-inflammatory; CRP ≤0.29 mg/dL (#757). The 2 groups were compared using: Chi squared for sex, breed, and age; Relative risk (RR) for age; Spearman Rank correlation test (SRct) for all parameters studied; Multiple regression (MR) to assess the relationship between CRP and other inflammation markers; Receiver Operating Characteristic (ROC) curves for diagnostic accuracy of each parameter in comparison to CRP (MedCalc®, 14.8). Dogs belonging to inflammatory group were significantly older (>7 years old) than those of non-inflammatory group ($P<0.05$), (RR, 1.38). Low yet significant ($p<0.01$) correlations between CRP and the other markers were noted using the SRct (R): CRP/Fib, +0.26; CRP/NeuSeg, +0.26; CRP/WBC, +0.24; CRP/Alb, -0.21; CRP/Band,

+0.14; CRP/Fe, -0.08; CRP/NeuTOX, +0.23; MonATT, +0.22. On the contrary, the MR analysis did not show any relationship between CRP and other markers (R2: 0.05 for CRP ≥ 0.30 mg/dL; 0.02 for CRP ≤ 0.29 mg/dL). ROC analysis of the parameters yielded the following results: NeuSeg is a moderately accurate inflammation marker with Area Under the Curve (AUC) of 0.71. The other parameters are less accurate markers of inflammation (AUC) compared to CRP: WBC, 0.70; Fib, 0.67; Alb, 0.64; Fe, 0.64; Band, 0.59. The markers with the best combination of Sensitivity (SS) and Specificity (SP) were: Fib (SS, 52.7; SP, 77.5 for 400 mg/dL cut-off) and Band (SS, 17.6; SP, 98.0 for 0.3 K/µL cut-off). The correlation between CRP and all the parameters studied, except Linf REA, is significant but low because they are affected by many conditions aside from inflammation. None of them is able to predict CRP values. The diagnostic accuracy of each single inflammatory marker is lower in comparison to CRP. In order to increase the diagnostic accuracy of inflammation markers, an evaluation of several parameters simultaneously is warranted, particularly in the absence of CRP measurement.

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CLINICAL, CLINIC-PATHOLOGIC AND RADIOGRAPHIC FEATURES OF THIRTY-FOUR CATS AFFECTED BY LUNGWORM INFECTIONS

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Feline lungworms have recently gained the scientific interest of researchers and practitioners due to the clinical severity of the condition they cause, the frequent presence of mixed infection, the challenges posed in their diagnosis and treatment, and their spread throughout many European countries (1). The aim of this study was to describe clinical, clinical pathology and radiographic features of cats affected by single and mixed lungworm infections. This retrospective study, conducted at the Veterinary Teaching Hospital of University of Teramo from 2013 to 2016, included 34 cats for which complete data from anamnesis, physical examination, thoracic radiography, haematobiochemical analysis and FIV/FeLV serology were available. Diagnosis was obtained by floatation and Baermann methods and parasite identification was confirmed by PCR (1). All cats lived outdoor or were allowed to roam. The animals (16 males and 18 females) aged from 2 months to 11 years and 24 of them were less than 1 year old. Infections by *Aelurostrongylus abstrusus* (21/34), *Troglostrongylus brevior* (4/34) and *Capillaria aerophila* (1/34) and co-infections by *T. brevior/A. abstrusus* (7/34) and *T. brevior/C. aerophila* (1/34) were diagnosed. Twenty-six cats showed respiratory signs, while 8 were asymptomatic. The most frequent signs recorded were: coughing (13/34 cats), dyspnoea (9/34), tachypnoea (6/34), oculo-nasal discharge (6/34), abdominal breathing (5/34) and sneezing (4/34). The lung auscultation showed an increased vesicular sound in 10 cats, and others adventitious sounds, such as wheezing (3/34) and crackles (1/34). Others findings were lethargy (6/34), weight loss (4/34), anorexia/dysorexia (3/34), and hyperthermia (1/34). The most common (8 cats) laboratory abnormality was a normocytic/normochromic anemia. Three cats showed a mild neutrophilia, and one of them also monocytosis. Either eosinophia (2/34) and

eosinopenia (2/34) were detected. Thirty-three cats had radiographic patterns: interstitial in 30 (25 recorded as reticular and 5 as nodular), bronchial in 26, alveolar in 10 and vascular in 2 cats. No abnormalities were observed in a 8-months cat affected by troglostrongylosis. All cats were allowed to roam, confirming the lifestyle of animals as a potential risk factor. Young animals are considered more exposed for their high preying instinct. Moreover, 10 out of 12 cats harboring *T. brevior* had less than 4 months, confirming that this parasite mostly occurs in kittens (1, 2). Cats infected by lungworms share the same clinical and radiographic signs observed to those occurring in other feline respiratory diseases. Furthermore laboratory findings are often unremarkable. Copromicroscopic examinations should be considered as a first step in the diagnostic work-up for feline respiratory distresses. As radiographic changes may be evident before the onset of clinical signs, radiographic examinations are advisable if lungworms are suspected.

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THE EFFECTS OF A CONSTANT RATE INFUSIONS OF HYDROXYETHYL STARCH (130/0.4) ON PLASMA COLLOID OSMOTIC PRESSURE IN HYPOALBUMINEMIC DOGS

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Hydroxyethyl starch (HES) is the parent name of a group of synthetic polymers that are the main plasma expanders in human and veterinary medicine. The primary use of HES solutions was to increase intravascular volume during hypovolemic shock and to bolster intravascular colloid osmotic pressure (COP). The rationale for HES use in small animals[1] has been extrapolated from human medical studies and guidelines. The aim of study was to evaluate the effects of a constant rate infusion (CRI) of HES 130/0.4 on plasma COP in hypoalbuminemic dogs. Dogs with hypoalbuminemia (albumin <2 gr/dl) were included if needed of fluid therapy with crystalloid and colloid solution to restore the hydration status. To support the COP a CRI of 1 ml/kg/hr of HES was administered, for at least 24 hours. Before infusion (T0), and serially at 6, 12 and 24 hours after (T6, T12, T24) a sample of blood was collected to perform CBC, creatinine, urea, albumin, total solids, plasma COP, and venous blood gas analysis. To compare the four samplings (T0, T6, T12; T24) the ANOVA or the Friedman test were used. When needed the Bonferroni adjustement was applied. The level of significance was set at $p<0.05$. Sixteen dogs were enrolled, 8 males and 6 female (1 spayed), with a mean age of 6.5 years, and body weight was on average 49.8 lb (min9–max97). No statistically significant differences were found among plasma COP, CBC, creatinine, urea, albumin, total solids and venous blood gas analysis in the different time of evaluation ($p>0.05$). Four dogs had a peripheral oedema before treatment: in two of them the clinical sign was resolved, in one dog there was an improvement and in the last dog the oedema was unchanged, after 24 hours of infusion. In this study, infusion of HES 130/0.4 at dose of 1 ml/kg/hr per 24 hours in hypoalbuminemic dogs didn't change the plasma COP. So far we know that the Starling's equation overestimate the effect of interstitial fluid COP on fluid exchange between

intravascular and interstitial space and that filtered fluid return to the intravascular compartment via the lymphatic vessels, since no absorption occurs by the capillaries.[2] Considering the revised Starling equation, even if in our study the COP didn't increase during the CRI, the colloid infusion may have helped to preserve it and decrease the transendothelial flow. Regarding the four dogs having a peripheral oedema before treatment, in two dogs the clinical sign was resolved, in one dog there was an improvement and in the last dog the oedema was unchanged. This result may indicate low volume of filtration from plasma to interstice, allowing the return of fluid to the circulation by the lymphatic vessels. The present study is the first evaluating, in vivo, the effects of a CRI of HES 130/0.4 on plasmatic COP in dogs.

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ADVIA120 IS A RELIABLE TOOL FOR CLASSIFYING CANINE EFFUSIONS

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Laboratory analysis of effusions is a crucial step in the diagnostic process for emergency patients and rapid and accurate results can make the difference for treatment and prognosis. Standard laboratory analysis (LAB) is time consuming and requires specific skills, particularly as cytologists. An automated analysis of the cellular component of effusions would be very useful in a clinical context.

Aim of the work was to test the usefulness of the ADVIA120 hematology analyzer in the characterization of canine effusions compared to LAB.

129 effusions (64 peritoneal, 51 pleuric, 11 pericardic, 3 not specified) from 129 dogs were included. Samples were classified as hemorragic, neoplastic (NPL), inflammatory (INF) and transudate according both to LAB (aspect, total proteins, cellularity and cytomorphology) and ADVIA120 (hematocrit, cellularity and scattergrams evaluation (1)). NPL and INF effusions were subclassified with both methods in lymphoma (LSA), carcinoma, mesothelioma, histiocytic tumor, doubtful and in neutrophilic, lymphocytic, macrophagic, eosinophilic, mixed and septic or non septic, respectively. Concordance between the two methods and accuracy of ADVIA120 compared to LAB to detect NPL effusions among non hemorragic samples, INF effusions among non hemorragic and non NPL samples and septic effusions among INF samples were determined. As previously reported in humans (2) moderate to good correlation between methods was detected for neutrophil, lymphocyte and eosinophil counts. Overall concordance in classifying hemorragic, NPL and nonNPL effusions was excellent (k=0,83). Sensitivity, specificity, positive and negative predictive value were 73%, 97%, 93% and 87%, respectively, for detecting NPL samples; 77%, 100%, 100% and 41% for INF samples and 75%, 100%, 100% and 95% for septic samples. In particular, false negative neopalstic results were reported for 8 carcinomas and 2 LSAs while 2 nonNPL cases were wrongly described as mesothelioma and LSA; two histiocytic neoplasms were misclassified as mesothelial and

epithelial. ADVIA 120 incorrectly classified 14 INF effusions as transudates and didn't recognized 2 septic samples. The failure to recognize carcinomas was due to the presence of a notable neutrophilic component while the two LSAs were not detected because of a low cellularity and the presence of small-sized NPL lymphocytes, respectively. The 2 false positive NPL results were due to the presence of numerous mesothelial reactive cells and activated lymphocytes, respectively. Misclassification of INF samples as transudates was the consequence of a low cellularity while false negative septic results were due to the presence of only slightly degenerated neutrophils.

ADVIA120 analysis is a reliable tool for a rapid discrimination between NPL and INF canine effusions and has a very high specificity in detecting septic samples. Total protein measurement and smear evaluation remain mandatory to identify modified transudates and neoplasms in case of low cellularity or significant neutrophilic infiltrate, respectively.

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FELINE LEISHMANIOSIS: CLINICAL SIGNS AND COURSE IN 14 FOLLOWED UP CASES

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Feline leishmaniosis (FeL) is sporadically reported in cats living in endemic areas and it is still not well known[1]. The aim of this study is to raise awareness of FeL providing information on clinical features, treatment and survival time after diagnosis. Fourteen FeL cases were evaluated retrospectively according to the following criteria: diagnosis confirmed at least by IFAT and qPCR[2, 3]; available information about signalment, history, physical examination, clinicopathological abnormalities, retroviral (FIV and FeLV) coinfections, follow up until death or for at least 12 months.

No cat was a pure breed; 8 were males and 6 females; age ranged between 4 and 14 years (median 10 years). Eight cats were antibody positive against FIV and 1 of them tested also positive for FeLV. Main findings were lymphadenia or weight loss (64%), unifocal or multifocal skin lesions (57%): ulcers (5 cats), hemorrhagic blisters (2 cats), nodules (1 cat). Distribution of lesions included the face (4 cats) and head (2) but also carpal region (3) and, in 2 cats, mucocutaneous junctions (eyelids, lips) were involved. In one case skin Leishmania infection was associated with squamous cell carcinoma (SCC). Oral lesions were detected in 6 cats and 2 suffered respectively from uveitis, thyroidal nodules or conjunctivitis. Single cases had pleural effusion, liver enlargement, jaundice. Cell blood count abnormalities were found in 10 cats and non-regenerative anemia was the most common finding. Hyperglobulinemia and chronic kidney disease (CKD) were diagnosed in 8 individuals with IRIS stage of 1 (3 cats), 2 (4 cats) or 4 (1 cat). Seven cats did not receive any treatment because of lack of compliance. Six cases were treated with Allopurinol (10-25 mg/kg SID) and a clinical improvement was obtained in all but 3 of them: one of the latter was then treated with meglumine antimoniate (20 mg/kg SID for 20 days) but the cat worsened because of concurrent SCC; the other two had to stop

therapy after few weeks because of acute kidney injury of unknown origin. One cat was changed after one month administration from fluconazole to itraconazole and then to metronidazole & spiramycin because of lack of efficacy. Thirteen cats died or were euthanized 1-72 months after diagnosis (median: 3 months) and post diagnosis survival time did not differ between treated (median 5) and not treated (median 1) or FIV+ (median 2.5) and FIV– (median 5) cats. Cats (no. 6) with CKD at the time of diagnosis died before (median: 3 months) those (no. 7) not suffering from CKD (median: 8.5) but the difference was not significant.

In conclusion, in *L. infantum* endemic areas FeL should be considered in cats affected by lymph node enlargement, weight loss, ulcerative or nodular dermatitis, chronic stomatitis, hyperglobulinemia, non regenerative anemia, CKD.

Cats can be empirically treated with allopurinol but should be carefully monitored for any adverse event and concurrent disease. Prognosis is extremely variable irrespective from retroviral coinfection or therapy.

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XVI Convegno

S.I.C.V.

TRAUMATIC INJURIES IN FOALS: A 45 CASE RETROSPECTIVE STUDY

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Traumatic injuries are common in equine practice (1, 2, 3) but there is no comprehensive study in foals.

The aim of this study was to assess prevalence and short-term prognosis of foals admitted to the Veterinary Teaching Hospital for traumatic injuries between 2005-2015.

Inclusion criteria were: age <180 days and complete clinical records. Data obtained from records included age, sex, breed, cause of admission, history and definitive diagnosis. According to the definitive diagnosis, foals were divided into groups: trauma (T), colic (C), localized infection (LI), systemic infection (SI), neonatal syndromes (NS), elective procedure (E) and other (O). Short-term prognosis (discharged vs dead/euthanasia), sex (male vs female), breed (Warmblood, Thoroughbred, Standardbred, Arabian, other) and age (0-14 days, 14-30 days, 30-180 days) were tested among groups using Chi-square or Fisher test, as appropriate.

Three hundred and sixty-eight foals were included in the study; 45 (12.2%) were referred for trauma, 9 of which (20%) were injured by the mare. Within group T, mean age of foals was 55 days (range: 12 hours-180 days) and no sex predilection was noted. Short-term prognosis did not differ significantly between T and C, SI, NS as 34/45 (75.5%) foals were discharged. Thoroughbred foals had less chance to be injured compared with other breeds. Arabian foals instead, had increased likelihood to be traumatized by the mare as reported in literature (4). Foals in T, had more chance to be referred at 30-180 days of age compared with NS and DI. Foals injured by the mare had more chance to be referred at 14-30 days of age compared with foals affected by traumatic injuries for other reasons. Trauma was a significant cause of referral, representing the cause of admission in more than 10% of the foals. The overall survival rate is good, nevertheless, on an individual basis, location and severity of the injuries should be carefully considered by the clinician when formulating a prognosis.

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SYMPATHETIC INNERVATION AND ADRENERGIC RECEPTORS IN THE EQUINE DEEP DIGITAL FLEXOR TENDINOPATHY

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Progresses in the field of diagnostic imaging show that the deep digital flexor tendon (DDFT) is one of the most common injured structures in horses with chronic distal limb lameness (1,2). The aim of this study was to verify the presence of significant changes in the sympathetic innervation pattern of equine DDFT in the digit affected by different tendinopathic lesions, investigated with immunohistochemistry (IHC) for tyrosine hydroxylase (TH) and alpha-1 adrenergic receptor (α1-AR).

Fifteen distal limbs selected from 12 horses euthanized for reasons not related to tendinopathies were included in this study. Post-mortem radiographic, ultrasonographic and gross examinations were performed on the suprasesamoidean area of DDFT. Longitudinal sections were collected and processed as in the literature (1, 3). Based on diagnostic imaging, gross and histological findings, lesions were classified as core lesions, dorsal border lesions, oblique splits and diffuse degenerative lesions (2). IHC was performed using 1B5 mouse anti-human TH monoclonal antibodies (Ab) and rabbit anti-human α1-AR polyclonal Ab, validated for equine tissues using western blotting (3). The grade of immunoreactivity was compared with that of healthy horses presented elsewhere (3).

The age of horses ranged from 10 to 27 years. The animals were 8 females and 5 geldings of different breeds. Seven core lesions, 4 dorsal border lesions, 1 parasagittal split and 3 cases with diffuse degenerative changes were identified. Compared to healthy samples significant changes in the expression of sympathetic innervation were not observed, except for the core lesions. They consisted in increased α1-AR-immunoreactivity and reduced TH-immunoreactivity. The α1-AR increasing expression versus TH decreasing expression observed in the core lesions is suggestive of a compensatory new imbalance between the sympathetic mediator (TH) and the sympathetic receptors (α1-AR) as a cause (blood flow) or effect (see fibrosis) of the structural damage (4). Therefore, an excessive

stimulation of tenocytes by α1-AR, along with other mechanical factors, might produce a modified cell activity resulting in an increased extracellular matrix remodelling and in a qualitatively loss of tendon strength (4). Therefore, the excess of adrenoreceptors in the core lesions seems to justify a worse prognosis reported for this type of lesion. The functional relevance and the prognostic perspective of these changes remain to be defined. Further studies are necessary to assess the therapeutic application of these results in athletic horses.

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A PECTIN-HONEY HYDROGEL PREVENTS POSTOPERATIVE INTRAPERITONEAL ADHESIONS IN A RAT MODEL

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Postoperative intra-abdominal adhesion formation has long been considered an inevitable consequence of laparotomy, and the incidence in abdominal surgery is ranging between 67 and 93% [1-3]. Liquid honey has been used intraperitoneally to reduce the incidence of these adhesions [4-6]. However, solid barriers are considered more effective than liquids in decreasing postoperative intra-abdominal adhesion formation; therefore, a new pectin-honey hydrogel (PHH) was produced and its effectiveness was evaluated in a rat cecal abrasion model. Standardized cecal/peritoneal abrasion was performed through laparotomy in 48 adult Sprague-Dawley rats to induce peritoneal adhesion formation. Rats were randomly assigned to a control (C) and treatment (T) group. In group T, PHHs were placed between the injured peritoneum and cecum. Animals were euthanized on day 15 after surgery. Adhesions were evaluated macroscopically and adhesion scores were recorded and compared between the two groups. Inflammation, fibrosis, and neovascularization were histologically graded and compared between the groups.

In group C, 17 of 24 (70.8%) animals developed adhesions between the cecum and peritoneum, while in group T only 5 of 24 (20.8%) did (p=0.0012). In group C, one rat had an adhesion score of 3, sixteen had scores of 2, and seven rats had scores of 0. In group T, four rats had adhesion scores of 2 and one rat had an adhesion score of 1. Significantly lower grades of inflammation, fibrosis, and neovascularization were seen in group T (p=0.007, p=0.001, p=0.002, respectively). PHH is a novel absorbable barrier that is effective in preventing intra-abdominal adhesions in a cecal abrasion model in rats.

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A PRESSURE-SENSITIVE GLOVE FOR STANDARDISATION OF THE FORCE APPLIED DURING DISTAL FORELIMB FLEXION TESTS IN HORSES

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Lameness evaluations with flexion tests are a routine procedure for equine practitioners and are commonly used in both lame and sound horses [1-6]. The force applied by the surgeon, during a flexion test, has a strong influence on the outcome of this test [2, 3, 5]. The objective of this study was to verify if a commercially available pressure-sensitive glove could be employed to standardise the force applied in the equine distal forelimb flexion test. Three experienced veterinary surgeons and three final-year students performed bilateral distal forelimb flexion tests on cadaver limbs and on live horses with a pressure-sensitive glove. All participants were asked to apply a constant force for 60 s using the indicator on the glove display while a camera recorded the value on the glove display. The videos were reviewed and the percentage of time for which the correct force was applied was measured.

No significant differences were found between the percentages of time of application of the standard force between experienced and non-experienced operators (P=0.802). No statistical difference was found between experienced and inexperienced operator either in live horses (P=0.591) and in the cadaver model (P=0.797). The difference in the overall percentage between flexion tests performed in the cadaver or live horse model was significant (P=0.0032).

In conclusion, the pressure-sensitive glove was effective in standardising the force applied during the distal forelimb flexion tests and could become an essential and affordable tool for the equine practitioner, allowing standardisation of the test and an objective assessment during equine lameness investigation.

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EFFICACY OF TOPICAL APPLICATION OF KLOX BIOPHOTONIC SYSTEM IN CANINE AND FELINE ACUTE SIMPLE SURGICAL AND CHRONIC WOUNDS. PRELIMINARY RESULTS

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Cutaneous lesions, whether of an ulcerative or dehiscent character, often need a complex clinical management, not just for the difficult and slow healing, but also for managing the possible secondary complications that can result in infection, physical pain, emotional distress to the patient and lengthy hospitalization. In recent years, KLOX Technologies Inc. has developed an innovative technology platform, the KLOX Biophotonic System ("KLOX"), which consists of a primary multi-LED light device and a topical photo-converter gel, with applications in dermatology and wound treatment. The aim of this research is to investigate whether the KLOX treatment promotes and accelerates the healing process and reduces bacterial contamination in canine and feline acute surgical and chronic wounds. Preliminary results of the first year's research are presented.Two groups of patients are part of this study:

- Dogs and cats having orthopedic surgeries (Group 1). The KLOX treatment was applied every 3 days for 12 days, on the proximal half of the surgical wound. As a control, the distal half of the wound received just saline solution. For patients with bilateral surgical wounds, one was treated with KLOX and the other only with saline. Some patients were subjected to cutaneous and subcutaneous biopsy of the proximal district (KLOX) and the distal district (saline).
- Dogs and cats with delayed healing of surgical wounds, protracted decubital ulcers, burns or traumatic wounds (Group 2).

KLOX was applied every 3 days either on ½ or on all of the lesion (if the patient has more lesions), until complete re-epithelialization. Each application was documented photographically with a millimeter reference scale, and by using Photoshop extended, enabling the degree of tissue regeneration to be measured through the calculation of the

wound area. During the different treatment steps they were performed swabs for bacterial cultures. To date, 25 patients have been treated (12 Group 1 & 13 Group 2); the study has shown a very good regeneration of chronic wounds (Group 2), with a first phase of intense granulation coming from the bottom of the wound (vertical regeneration), followed by a skin growth with wedges to about 6 days from the first treatment (horizontal regeneration). The intense production of granulation tissue has been modulated, in a second time, with a reduction of its volume; therefore, no patient developed exuberant scars or keloids. The regeneration of all the tissue layers involved in the disease process occurred faster versus wounds treated with traditional methods (control). The histological results of biopsies performed on KLOX versus control-treated portions of surgical wounds (Group 1) indicated that the KLOX-treated tissue had better and complete re-epithelialization, minor inflammation of the dermal layer, less neoangiogenesis and the presence of synthesis activities of the connective matrix. Preliminary results showed the efficacy of the KLOX treatment to promote tissue regeneration and inhibit bacterial growth. Further studies will be needed to give strength to this initial research.

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WHICH HEMOSTATIC LIGATURES PRACTICED BY INEXPERIENCED VETERINARY SURGEONS IS MORE EFFECTIVE?

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Veterinary medicine schools always thrive to improve students' skills in order to be ready for the post-graduate practice. Many veterinary schools have moved away from the use of procedures-based methods, toward the use of a skills-based approach for teaching surgery.[1-2] Considering the importance to improve student's skills, and evaluating the patient safety and budgetary constraints, veterinary medicine schools have developed laboratory-based training programs using bench models and simulators to provide surgical skills. [1, 3] It is important to assess the skills acquired by undergraduate students of Veterinary Medicine, to be sure that they will be able to effectively perform basic surgical techniques. One of the major issues is the ability of performing efficient vessel ligatures. To our knowledge, there is no report defining the best haemostatic knots in the hand of a beginner surgeon. The aim of the present study was to evaluate the different types of knots performed by 12 Veterinary Medicine 5th year students. Five different knots (Giant, Slip, Strangle, Surgeon's, Transfixing knots) were showed to students, and for two weeks students self-trained at home. Then they were asked to perform each learned knot four times on an hemostasis simulator that evaluated the end-pressure of the simulated vessel distal to the ligature.[3] Then they were asked to fill a form to express their personal feeling about knots' learning easiness, execution easiness and sealing security. Data were matched in order to evaluate a possible correlation among the confidence of the students concerning the procedure, the suture material, the type of knot and the effectiveness in reducing vessel pressure distal to the ligature. Data were statistically analysed using Friedman test, Fisher's exact test. Students considered the Surgeon's knot the easiest to learn and the Strangle the hardest. They also reported that the easiest knot to perform was the Surgeon's and the hardest the Giant knot. Students felt the most effective knot to seal a vessel was the strangle and the least effective were the Transifixing knot and the Slip knot.

The strangle knot resulted to be the most effective in reducing vessel pressure distal to the ligature compared to other knots.

In conclusion a combination of video tutorial and self training with the aid of an hemostasis simulator confirmed to be useful tools to implement and consolidate students' surgery skills.

The strangle knot is the most effective hemostatic knot in inexperienced hands, although it is considered by students more difficult to learn compared to other, perhaps more commonly teached, knots.

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BLOOD PRESSURE MONITORING UNDER GENERAL ANESTHESIA IN THE DOG: COMPARATIVE STUDY BETWEEN INVASIVE AND NON-INVASIVE METHOD

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Hypotension is a common complication during anaesthesia and it has been hypothesized that monitoring of cardiovascular and respiratory function may reduce anaesthetic related mortality. Perfusion of vital organs is the key function of the cardiovascular system. Aortic pressure measurement is an important indicator of organ perfusion. Peripheral arterial blood pressure is commonly used to assess cardiovascular status in clinical practice, although it differs from the central blood pressure and it is not considered a completely reliable indicator of tissue perfusion. Invasive, direct, arterial pressure monitoring via cannulation of a peripheral artery (IBP) is considered more accurate than non-invasive, indirect, oscillometric method (NIBP) in both awake and anesthetized animals, and allows for continuous measurements instead of intermittent values obtainable with NIBP.

The aim of the study was to compare IBP and NIBP detected simultaneously in dogs during general anaesthesia.

Fifteen ASA I (American Society of Anaesthesiologists) female dogs, aged between 11 months and 4 years, with an average weight of 31.7 kg (range: 19 to 41 kg), 5 mix-breed and 10 pure-breed dogs (among which German and Belgian Shepherd, Labrador and Golden Retriever, Bernese Mountain Dog), undergoing elective laparotomic ovariectomy, were utilized to perform the study. Both IBP and NIBP were measured simultaneously during general anaesthesia, in the same dogs, every 2 minutes for a total of 30 minutes, using multiparametric BeneView 8 anesthetic monitor (Shenzhen Mindray Bio-Medical Elettronics Co., Ltd). IBP measurements were obtained using a catheter placed in the dorsal pedal artery and an electronic pressure transducer. NIBP measurements were obtained using an appropriately sized cuff placed around the contralateral metatarsal region. Systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressures measured in each dog, in each study time, with NIBP and with IBP were compared using a Student t-

test. All data were analysed with statistical software (STATA Software, Version 13.1 SE, College Station, Texas, USA).

All dogs completed the study. The mean±sd SAP obtained using NIBP (98.65±21.70 mmHg) and IBP (101.21±17.90 mmHg) were almost superimposable [P>0.05], while mean±sd DAP and MAP obtained using NIBP (DAP: 58.55±18.17; MAP: 68.55±19.58 mmHg) and IBP (MAP: 66.81±15.89; DAP: 75.07±15.20 mmHg) presented no statistically significant discrepancies [P>0.05]. The trend of NIBP values was lower than that of the IBP values providing interesting ideas for further study.

The results obtained in this study suggest that both methods are accurate and reliable in dogs during general anaesthesia. Non-invasive method seems to be almost as valid as the invasive method for the assessment of the cardiovascular system, in relation to the characteristics of the monitor used.

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GUAIFENESIN-KETAMINE-XYLAZINE INFUSION TO MAINTAIN ANESTHESIA IN MULES UNDERGOING FIELD CASTRATION

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Many minor and surgical procedures can be performed in the field under sedation or general anaesthesia. Numerous drug combinations have been used for sedation, induction and maintenance. The purpose of this study was to determine if the combination of guaifenesin, ketamine and xylazine, commonly referred to as “triple drip”, produce safe and satisfactory total intravenous anaesthesia in mules undergoing field castration, premedicated with xylazine and induced with thiopental. Eight healthy adult intact male mules, aged 4 to 6 years and weighing 380 to 490, were anesthetized to performe field castration. Before anaesthesia a 14-gauge, 13–cm catheter was placed percutaneously in the external jugular vein. Mules were premedicated with 1.3 mg/kg xylazine IV and anaesthesia was then inducted with 6 mg/kg IV thiopental within 10 min after premedication, when the animals were at least moderately sedated. Additional xylazine was administered when the mules were inadequately sedated. Sedation was considered good when lowering of the head, drooping of the lower lip and drooping of the ears were present using a 4-point sedation score. Once the mules were recumbent, the infusion of guaifenesin (50 mg/ml) - ketamine (20 mg/ml) - xylazine (0.5 mg/ml) (GKX) was started to maintain general anaesthesia, approximately 1ml/kg/hr (based on monitoring eye signs, muscle relaxation of the neck, respiratory rate and pattern, and the responses to surgical stimulation. The spermatic cord of each testis was infiltrated with 5 ml of lidocaine to achieve local anaesthesia before the scrotum skin incision. The open technique of castration was applied to all mules for postoperative drainage. During anaesthesia heart rate (HR), respiratory rate RR), rectal temperature (RT) and hemoglobin saturation with oxygen (SpO2) were measured every 5 minutes. Times to sternal recumbency, lateral recumbency and standing were recorded. The data recorded were statistically analysed using simple one-way analysis of variance (ANOVA) and a p-

value>0.05 was considered significant. The qualities of anaesthesia were evaluated using induction, maintenance and recovery scores. The resultes suggest that the premedication using 1.3 mg/kg IV xylazine for mules undergoing thiopental anaesthesia was satisfactory and only one animal needed a supplemental dose of xylazine (0.3 mg/kg IV) to induce better sedation. The total IV amount of thiopental for induction was sufficient to achieve lateral recumbency in all animals. Furthermore, GKX provided adequate surgical plane of general anaesthesia to performe castration in all mules, without responses to the manuality or significant modification of HH, RR, RT, and SpO2 in comparison with the basal values and to maintain a satisfactory muscle relaxation. Recovery from anaesthesia was uneventful, smooth and clinically acceptable in all mules.

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ABOUT TWO CASES OF EQUINE SQUAMOUS CELL CARCINOMA

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Squamous cell carcinoma (SCC) is one of the most common equine mucocutaneous tumors and represent a prognostic and therapeutic challenge to practitioners. It is characterized by local invasiveness, aggressivity and slow tendency to metastatise. The virus of Equine caballus papillomavirus-2 has been linked and involved in SCC development. Risk factors may include also pigmentation, chronic skin irritation and the exposition to ultraviolet light. This paper describes the history, clinical findings, diagnostic imaging (including MR), gross pathology and histopathology of 2 equine cases of squamous cell carcinoma in the eye and in the pharyngeal and guttural pouches epithelium complicated by pulmonary metastases. The size and histopathological features of the primary tumor are the most important factors to prognosis. The malignancy is not a common feature of the cutaneous forms, while it is much more likely in the stomach and at the level of the mouth, pharynx and nasal cavity. Early identification of tumors and prompt intervention are fundamental for a positive therapeutic results and limiting side effects. Further prospective clinical studies will be needed to better evaluate the efficacy of different therapies. Diagnostic imaging, genetic and biochemical insights of the disease, combined with new therapeutic modalities represent the potential development areas in the diagnosis and treatment of this disease. An adequate number of clinical cases and long-term follow up are needed, to limit complications and better understand the mechanisms of origin and spread of the disease.

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ULTRASONOGRAPHIC FINDINGS IN THE EQUINE SACROLIAC REGION OF 24 HORSES

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The normal ultrasonographic appearance of soft tissues and bony structures of the sacroiliac region in horses was studied in order to establish clinically relevant reference parameters.

24 horses, divided into two groups: 1) 12 adult subjects (4 males and 8 females), aged between 7 and 21 years, engaged in sports activities; 2) 12 subjects, aged between 7 and 18 years, all female and pregnant, were examined using a transrectal approach to outline the lumbosacral joint, the sacroiliac joint and its adjacent structures. The history did not report clinical evidence of back pain or hindlimb lameness. All horses were examined following the same protocol. Transrectal examination of the sacroiliac joint consists of evaluation of the bony surfaces of the sacrum and ilium in comparison with the contralateral side. Variation in the ultrasonographic appearance of the lumbosacral joint was also identified, including hyperechogenic regions within the lumbosacral disc, and mild or moderate irregularity of the opposing surfaces of the last lumbar and the first sacral vertebral bodies (mostly mineralization of the intervertebral disc and osteophytes). Ultrasonography provides diagnostic information about soft tissue, articular cartilage and bone surfaces and may help to improve the diagnosis of sacroiliac diseases. The knowledge of the ultrasonographic anatomy is necessary to understand the possible pathological changes in sacroiliac diseases.

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SURGICAL BLEEDING CONTROL USING THE "BLIN DEVICE". PRELIMINARY RESULTS IN ANIMAL MODEL

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Bleeding is a major issue during surgical interventions as well as during accidental vascular lesions. While surgeons usually have access to many different tools to face bleedings, emergency physicians can find themselves quite helpless in different situations.

This study represent a preliminary feasibility test, in animal model, of "Blin device", a medical design developed and patent to control most bleedings when efficient surgical means are inaccessible, using vacuum technique. Animal testing was achieved according to regulatory conditions set by the «Guide for the care and use of Laboratory animals» according to the European directive 86/609/CEE. All experiments were achieved following the recommendations of the University Ethics Committee.

73 wounds were performed on 23 sheep. Different generations of vacuum devices were tested for hemorrhagic lesions on six different cardiovascular structures: left and right ventricles, left and right atria, aorta and pulmonary artery. The hemostasis device described used a suction cup with vacuum applied on the bleeding origin. Device efficiency was analyzed based on the model used, its characteristics and the site of placement. The main objective was bleeding control, the secondary objectives were success at first try and persistent hemostasis at device removal. Very few depression systems as a mean for haemostasis can be found in the literature. The main objective of this system was to obtain an immediate hemostasis, temporary but giving the surgeon and anesthetist enough time for the preparation of a permanent hemostasis. This immediate hemostasis resulted from a mechanical action of the device over the wound. The animal's coagulation did not intervene in this process. Only the immediate results concerning the device have been analyzed as a criteria to achieve the main objective.

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THE FRANKENSTEIN HORSES. CLINICAL AND DIAGNOSTIC IMAGING FINDINGS IN HORSES WITH SUTURE LINE PERIOSTITIS

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Swellings of the equine frontal area can be caused by inflammation of the craniofacial sutures. Suture line periostitis (colloquial term “suturitis”) results in a firm, usually non-painful swelling in the nasofrontal, maxillary and zygomatic region accompanying epiphora. Instability of the craniofacial suture lines, facial trauma and surgical sinusotomy could be predisposing factors. A definitive diagnosis can be reached with radiography and computed tomography (CT). This study describes clinical, CT and radiographic findings of craniofacial suture lines periostitis in two horses with facial swelling. Two horses developed craniofacial suturitis and were presented with a moderate painful facial swelling, epiphora and mild hyperthermia. A 10 year old, Italian saddle horse, gelding developed clinical manifestation after sinuscopy and positioning of a Foley catheter in the conco-frontal sinus for local treatment of a micotic sinusitis. A 16 year old, Wielkpolska, stallion, developed symptom after frontal sinusotomy for the removal of a cystic mass in the left maxillary sinus. A latero-lateral radiographic view of the head in the first horse allowed to recognize bony proliferation, sclerosis and periosteal new bone formation on both sides of the nasofrontal suture. Computed tomography findings in both horses consisted in an intense, irregular periostal thickened bony wall that affected the frontal, lacrimal, zygomatic and maxillary bone. In one case, there was a necrotic bone sequestrum upon the nasofrontal suture line, not detectable on radiographic views. In the second case, the reaction was more intense near the cerclage wires used to fix the nasofrontal flap associated with osteolysis. In both cases the diagnosis was suture lines periostitis. Horses were treated with surgical removal of the necrotic sequestrum in the first case and the removal of the cerclage wires of the nasofrontal flap in the second one. Sample materials were submitted in both cases for a microbiologial testing and resulted sterile. Horses were administered anti-inflammatory drugs for one week with

improvement of the clinical signs. At the 30 weeks follow-up, the owners reported that the nasofrontal swelling and epiphora were no longer detectable. In conclusion, suture line periostitis should be included in the differential diagnosis in case of facial swelling expecially in horses underwent to sinus surgery or after a head trauma. In our cases, CT allowed a more accurate assessment of the bony structure and identification of the underlying causes of inflammation.

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ACEPROMAZINE, DETOMIDINE AND MORPHINE: "THE WOODEN HORSE" EVALUATION OF STANDING SEDATION PROTOCOLS IN THE EQUINE PATIENTS UNDERGOING BONE SCINTIGRAPHY

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In equine practice standing sedation has became increasingly popular. Many protocols have been investigated permitting to restrain patients avoiding general anaesthesia and the risk associated by increasing the threshold to all external stimuli and partially providing analgesia (Muir W. 1981; Dodman N. 1980). The target of standing sedation protocols during diagnostic imaging procedures is to reduce all the reaction of the patients to external stimuli, and to diminish physiological movements. No studies have been published establishing a standard protocol, and the decision of which protocol use is based on anaesthetist preferences and not on evidence-based medicine.The aim of the study is to evaluate two sedative protocols, focusing not only on the lack of response to stimuli and the reduction of physiological movements but also on the immobility of the patient that is mandatory for diagnostic imaging procedures.Thirteen horses referred to perform bone scintigraphy were enrolled in the study. Patients were randomly divided in two groups; both groups received same dose of acepromazine (0.003 mg/kg) and detomidine (10 μ/kg), MOR group received morphine (0.25 mg/kg), the BTF group received butorphanol (0.01 mg/kg). During the procedure to evaluate the horse sedation a simple descriptive scale (Taylor P. et al. 2014) was used; respiratory and hearth rate were recorded and if needed adjunctive boluses of detomidine were administrated. To evaluate the reduction of voluntary and involuntary movements the parameter chosen was the number of retake necessary to obtain an image with excellent diagnostic quality. This parameter was evaluated each time by the same radiologist that was unaware of which protocol was administered. Statistical analysis with T-Test was performed.Heart rate resulted not statistically different (MOR=27.1±2,4;BTF 26.8±3.7); respiratory rate in the MOR group resulted statistically diminished (MOR=9.9±2.3; BTF 13.4±3.1). The sedation score was statistically higher in the MOR group (MOR= 1.6±0.5; BTF 1.0±0.3)

and moreover in the total body examinations. The total dose of detomidine used in the two groups resulted non statistically different (MOR=23.7; BTF 23.2). The total number of retake did not result statistically significant even if the clinical difference was relevant (MOR=7.1; BTF=16.2), but the number of retake for each region investigated resulted statistically diminished in the MOR group (MOR=0.4±0.5; BTF 0.8±0.8). The results of this study demonstrate the supremacy of the MOR sedation protocol to perform bone scintigraphy in horses. Further studies are recommended to evaluate the administration of detomidine constant rate infusion to maintain a required sedation degree. The "Wooden Horse" could by applied in other diagnostic imaging techniques such as CT or MRI and also for various standing surgeries. Other parameters that could be considered are the duration of the exams and also the number of urinations of the patient during the exam to evaluate operators exposure to radiations.

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PREVALENCE AND OCCURRENCE OF FRACTURES IN STRAY CATS OF THE NEAPOLITAN URBAN AREA

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In the stray cats, the most frequent orthopaedic diseases are traumatic fractures as a result of car accidents (1). The aim of the study was to assess the prevalence and occurence of fractures in stray cats of the Neapolitan urban area. The records of the cats admitted to Veterinary Hospital Unit of the local public health NA1, from January 2013 to December 2015, were retrospectively revised. All cats with traumatic fractures, clinically and radiographically diagnosed, were included. Gender, age and date of admission, were recorded. The age was classified into four classes: juvenile (<1 year), young adult (1-3 year), mature adult (3-10 year) and geriatric (>10 year). Data recorded were analysed for gender, age classes and seasons prevalence by using a x2-test. Significant level was set at $p<0.05$. On 4,346 feline referred, there were 207 fractured cats (102 males, 94 females, 7 spayed females and 4 neutered males), for a total of 263 fractures. The axial fractures were 20.5% (54/263) whereas the appendicular fractures 79.5% (209/263). Among the axial fractures, skull fractures were 27.8% (15/54) whereas fractures of the spine 72.3% (39/54). The appendicular fractures involved mostly hind limbs [90.4% (189/209)] than fore limbs [9.6% (20/209)]. Considering the hind limb, pelvic bone fractures had the highest prevalence [52.38%, (99/209)] followed by the femur fractures [24.33% (46/209)]. In forelimbs, humerus fractures had the highest prevalence [45% (9/20)]. The prevalence of males in the sample was significantly higher than females ($p=0.017$). Juvenile class showed the highest prevalence [55.5% (115/207)]. Considering the seasons of admission, there were no significant differences with overall population.

The distribution of fractures in our sample agrees with previous studies all but the pelvic fractures that had the highest prevalence whereas in literature is reported the femur

fractures (1). The females in rare cases moving from one colony whereas males will move where are receptive females (2). This particular behavior may explain the higher prevalence of male subjects and agree with previous study (3). Moreover, young subjects are inexperienced and more exposed to the dangers of urban environments (4).

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EFFECTIVENESS OF LIDOCAINE PERITONEAL LAVAGE FOR POST-OPERATIVE PAIN CONTROL IN DOGS UNDERGOING LAPAROTOMY: PRELIMINAR STUDY

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Lidocaine is an antiarrhythmic agent and a local anaesthetic. Overdose of this drug can cause cardiac and nervous toxicities (Plumb, 2002). Lidocaine could also be used off-label in patients undergoing abdominal surgery to provide post-operative analgesia (Willis & Hunt, 2000; Carpenter et al., 2004).

The aim of the study was to evaluate the clinical efficacy of peritoneal lavage with lidocaine for providing post-operative pain relief in dogs undergoing laparotomy. For this procedure lidocaine was administered off-label.

Sixteen client-owned dogs of different breed, gender, age (1–12 years) and weight (4–37.4 kg) scheduled for surgical laparotomy were enrolled. Inclusion criteria were ASA status classification ≤III based on hematologic and physical examination and absence of cardiovascular diseases. Patients were randomly divided in two groups: SAL group (no.=8), peritoneal lavage with saline and drying, and IPL group (no.=8), peritoneal lavage with lidocaine solution and drying.

All subjects received a common premedication: intramuscular (IM) methadone 0.2 mg/kg and dexmedetomidine 5 µg/kg; induction: intravenous propofol to effect and maintenance: isoflurane in 100% oxygen to effect.

Immediately before closure of the abdominal wall, a peritoneal lavage was performed. In SAL group, irrigation of the abdominal cavity was achieved with 500 mL of saline 0.9%, followed by aspiration of the liquid and abdominal wall closure. In IPL group, a peritoneal lavage with local anaesthetic solution, including 200 mg of lidocaine 2% dissolved in 500 mL of saline 0.9% was performed. For dogs within 10 kg, volumes of saline or lidocaine solution were of 50 mL/kg. The lavage remained in the abdominal cavity for three minutes; it was then aspirated before abdominal wall suture. Pain level of patients was

evaluated in the following six hours by a single operator through the "Glasgow Composite Measure Pain Scale – Short Form (GCMPS – SF)" (Reid et al., 2007); scale scores between 0 and 24 were considered. Scores≥8 were selected as cut-off for administration of "rescue" analgesia, representing treatment failure. Statistical analysis was performed with Mann-Whitney U test. Already in the first 45 minutes of evaluation SAL group showed a percentage of treatment failure of 100%. In IPL group there was only a treatment failure at 180 minutes, representing a percentage of 12.5% that remained constant until the end of the observation period. During the entire study no adverse effects were detected. Peritoneal lavage with diluted lidocaine solution is very effective in immediate post-operative pain relief (6 hours) in dogs undergoing laparotomy. However pharmacokinetic and clinical studies, are necessary to describe lidocaine absorption after peritoneal lavage, compare short- and long-acting local anaesthetics (i.e. bupivacaine and ropivacaine) in order to increase the research data.

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CASTRATION OF DONKEYS UNDER TOTAL INTRAVENOUS ANESTHESIA: ANALGESIC EFFECT OF BUTORPHANOL

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Experimental and clinical studies on butorphanol in horses documented evidence of the analgesia and synergistic effect with sedatives and anesthetic agents (1).

Aim of the study was to determine cardiovascular response to butorphanol during orchiectomy in TIVA anesthetized donkeys and identify the most suitable short term anesthetic technique under field conditions. A population of healthy donkeys referred for castration was investigated. All donkeys were premedicated with acepromazine 30 mg Kg-1 IM and randomly divided into 2 groups:

- group1 (G1): donkeys receiving detomidine 10 μg kg-1 and butorphanol 50 μg kg-1 for sedation
- group2 (G2): donkeys receiving detomidine 10 μg kg-1 for sedation.

Anesthesia was induced with ketamine 2,2 mg kg-1 and diazepam 20 μg kg-1 and maintained with guaifenesin 80 mg ml-1, ketamine 2 mg ml-1 and detomidine 20 μg ml-1 (GKD) at dose rate of 1ml kg-1 h-1 . Monitoring included recording of heart rate (HR), respiratory rate (RR), invasive arterial blood pressure (IABP), clinical signs of anesthetic depht, and intraoperative administered rescue drugs every 2 min and at the time of skin incision, each spermatic cord traction, ligation and transection. Anesthesia, surgery and recovery time and quality were also recorded. All animals were castrated using a closed castration technique. Data were analyzed for normal distribution and a Mann Whitney U test was used to identify differences between means. Level of significancy was setted at $P<0.05$.

Twelve donkeys was enrolled in the study, 8 in G1 and 4 in G2. Groups were homogeneous for age and weight (respectively 21.5±7.5/22±10.5 months and 157.8±59.4/157.2±72.6 kg). Anesthesia (50±8.1/46.3±12.3 min) and surgery time (34.2±6.4/40.3±7.6 min) did not show any significative difference.

Recovery quality was excellent in all procedures but recovery time was significantly longer in G1 (115.9± 74.73 min vs 47±27.18 min), MAP (mean arterial pressure) was significantly higher in G2 at the time of ligation of both spermatic cords. No other significativity was observed although mean HR in G1 was lower tha G2 (41±7.3 vs 46±6.7 bpm).

Although no significancy was detected in intraoperative spontaneous movements, they were observed only in G2.

The use of safe intravenous anesthesia in practice is both desiderable and advantageous (2). It has been reported that TIVA anesthesia is associated with lower incidence of perioperative cardiovascular complications (3).

Since donkeys differs from horses behaviourally, physiologically and pharmacologically (4) the anesthesist should expect to see subtle differences which may affect their anesthetic management.

According to our preliminary results we can assume the use of butorphanol (50 μg kg-1) can safely be used for short term TIVA anesthesia in donkeys castration also under field condition. A greater sample size is required to obtain more solid results.

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ASSESSMENT OF THE EFFICACY OF A RAPID SINGLE BOLUS OF DEXMEDETOMIDINE IN THE TREATMENT OF EMERGENCE AGITATION IN DOG

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Emergence agitation (EA) is a complex multifactorial phenomenon; in human medicine it is defined as a disturbance in the patient awareness of and attention to the environment with disorientation and perceptual alterations, including hypersensitivity to stimuli and hyperactive motor behaviour in the immediate post-anaesthetic period (Kanaya 2015). In paediatric anaesthesia is considered a major issue, leading to physical harm to the patient as well as to the site of surgery, requiring sedation or physical restraint (Voepel-Lewis et al 2003). In veterinary medicine, a sudden awake from anaesthesia with disorientation, paddle, vocalization is reported (Bednarski 2007), but there is a lack of data concerning its prevention and treatment. The aim of this study is to assess the efficacy of a rapid single bolus of dexmedetomidine in the treatment of EA in comparison to titrate-to-effect administration of propofol in dogs. A modified version of the Cole scale (Cole et al 2002) was used to assess recovery from anaesthesia; 15 dogs overcoming the established threshold of 3/5 were enrolled to the study and randomly assigned to the treatment; a dog was excluded from statistical analysis since after 3 administrations of propofol kept on recovering showing EA and was treated lastly with dexemdetomidine. GroupDEX (6 dogs) received a rapid IV bolus of dexmedetomidine (1g/kg); GroupPPF (8 dogs) received a first rapid IV bolus of propofol (1 mg/kg in 30 sec) followed by titrate-to-effect administration. Sedation, recovery/sternal recumbency and standing times were recorded, as well as heart rate (HR), respiratory rate (RR), non-invasive blood pressure (NIPB) and temperature (T°) starting from the IV administration of the drug (T0) and every five minutes till sternal recumbency. Dogs receiving dexmedetomidine were treated with atipamezole 30 min after T0. Both groups were comparable for age, weight, body temperature, pre-treatment HR, RR, NIBP and EA grade; GroupDEX showed a statistically significant shorter sedation time compared to groupPPF (64.27.9 sec vs

124.374.1 sec), which is crucial for the patient and the operator safety in managing EA; no apnoea was detected in groupPPF but the patient management was more demanding. Recovery/sternal recumbency time was longer in groupDEX than in groupPPF without being statistically significative (28.7±6.7 min vs 21.9±11.2 min), despite standing time was shorter (35.5±6.2 min vs 31.9±10.6 min). RR decreased in both groups as an effect of sedation; HR decreased in a statistically significative way in groupDEX while systolic NIBP increased in groupDEX and decreased in groupPPF, accordingly with the known cardiovascular effect of the drugs. In conclusion, a single rapid IV bolus of dexmedetomidine appears to be more effective in managing post-anaesthetic EA, leading to smooth recovery with and without antagonization with atipamezole.

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ULTRASONOGRAPHIC ASSESSMENT OF NORMAL JUGULAR VEINS IN STANDARDBRED HORSES

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Ultrasonography is the most common imaging technique to evaluate jugular vein anatomy and flow and to detect thrombophlebitis. However, definitive information about the normal jugular ultrasonographic measurements in horses are not reported until now. Therefore, this study aimed: a) to establish a reference range of diameters and wall thickness of the jugular vein in Standardbred horses b) to relate diameters and wall thickness to the animal size, sex and age.

Thirty-nine Standardbred horses, 4 males and 35 females, aged 12.3 ±2.8(3-22) years, that had no i.v. injections in the last 6 months, were included. Weight, neck length, height at withers were measured. Diameters and wall thickness of both jugular veins were measured at cranial (CrS), mid (MdS) and caudal (CaS) sites of the neck after 10 seconds of digital compression at its base. Mean ± SE, median, minimum and maximum values for each measurement were calculated. In order to evaluate the influence of age, sex, laterality and size, a multivariate analysis of variance (MANOVA) was applied($P<0.05$).

In transverse scan, the diameters were: long-axis (LA) 2.28±0.24, 2.24±0.27, 2.08±0.37cm; short axis (SA) 1.58±0.22, 1.55±0.33, 1.48±0.3cm; thickness were: superficial (SWT) 0.065±0.01, 0.065±0.02 and 0.067±0.014cm; deep (DWT) 0.071±0.011, 0.072±0.016, 0.064±0.011 cm respectively in CrS, Mds and CaS. In longitudinal scan: LA were 1.56±0.28, 1.46±0.22, 1.38±0.3cm respectively; SWT were 0,08±0.011, 0.08±0.013, 0.08±0.014cm; DWT were 0.08±0.008, 0.08±0.012, 0.08±0.011cm respectively in CrS, MdS and CaS.

SWT was significantly higher in MdS in males, whereas parameters affected by age were: LA in CrS ($P=0.0362$), in MdS ($P=0.0078$) and in CaS ($P=0.0006$), and SA in transverse scan in CdS ($P=0.0043$); SWT in CrS ($P=0.0047$) and in CdS ($P=0.0143$);DWT in CdS

(P=0.0002); in longitudinal scan diameters in CrS(P=0.0163), in MdS (P=0.0008) and in CdS (P=0.0128) and DWT in CdS (P=0.0262). Laterality and size were not related to the veins' measures.

The range of jugular vein diameters and wall thickness varied considerably and were not related to the body size. Differences observed in sex may be related to the intravenous injections received by horses during their race career, that is normally longer in the males. Ageing of the vascular walls is largely studied in human medicine, but no information are available about the influence of the age on the structure of the veins in the horse. Limits of the study were the low number of male horses and the difficulty to include sound horses that did not receive any iv injection during their life.

In conclusion, the determination of reliable reference values for jugular vein measurements at rest in horses might be useful both for clinical procedures and diagnosis of pathological conditions, especially in asymptomatic or paucisymptomatic thrombophlebitis. A routine ultrasound screening of the jugular vein can provide an early diagnosis of wall venous change, preventing the onset of a further occlusive thrombophlebitis.

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PROGNOSTIC FACTORS IN SURGICAL EXCISED SINGLE CUTANEOUS MAST CELL TUMOR IN 110 DOGS: A MONOISTITUTIONAL STUDY

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Cutaneous mast cell tumor (cMCT) is a common skin cancer in dog. In the last 7 years several studies have updated prognostic role of some clinical and pathological variables. Based on these updates we want to analyze the impact of clinical and pathological variables in a monoinstitutional cases series of single excised cMCT in dogs. Single cMCT underwent wide excision were retrospectively included in this study if these variable were all available: signalment, weight, tumor dimension and ulceration, first presentation or local recurrence, WHO clinical stage and substage, Patnaik and Kiupel histological grading, status of surgical margins, and type of adjuvant therapies (if administered). The endpoint was relapse of tumor (local and/or distant). The prognostic effect of each variable was evaluated by Cox model. For covariates measured on a continuous scale the potential non-linear relationship between covariate values and logarithm of hazard (or sub-distribution hazard) was investigated considering regression splines. The Wald test was used to evaluate the statistical significance. Model results were reported as estimated hazard ratios (HR) and 95% confidence intervals (95% CI). Progression free survival curves for each variable were traced by the Kaplan–Meier method.

A total of 110 dogs were included. Most commonly breeds were: mix breeds (27.7%), boxer (15.45%), retriever (13.64). Male and female were equally represented. At diagnosis median age was 8.5 years (1-15 years) and median weight was 25.27 kg (2-47 kg). Tumors were located on trunk in 66 cases, distal limb in 18 cases, head and neck in 17 cases and on perineal region in 9 cases. Tumor median dimension was 2 cm (0.3-17.5 cm); the 91% was at first presentation and 9% a local recurrence; 28% were ulcerated; clinical stage I, II and III were 68.2%, 5.4% and 26.4%, respectively. Regarding

histopatologic results: Patnaik grade I, II and III were respectively 14.55%, 79.09% and 6.37% of cases; Kiupel low grade was 84.55% and high grade was 15.45% of cases; surgical margins was clean, infiltrated and clean but close in 69.09, 29.09% and 1.82% of cases, respectively. Relapse was observed in 16 cases (local relapse in 14 and distant metastasis in 2) with a median time to relapse of 114 days (13-815 days). Overall median progression free survival was not reached (13-2500 days). The variable statistically associated with relapse were: weight (14-25 kg; P=0,04), tumor dimension (>2.5 cm; $p<0.01$) recurrence ($p<0.001$), ulceration ($p<0.001$), clinical stage ($p<0.01$), Patnaik grade III ($p<0.001$), Kiupel high grade ($p<0.001$), margin at risk ($p<0.001$). The results confirmed the prognostic role of several clinical and pathological variables previously documented and in particular clinical stage, Kiupel grading system and status of surgical margins.

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ADRENAL GLAND ULTRASONOGRAPHY IN NEONATAL FOALS: INTEROBSERVER AGREEMENT

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Abnormal adrenal activity is supposed to be involved in several neonatal diseases (1). Recently an easy and not invasive adrenal glands ultrasonographic approach has been described in foals (2). The aim of this study is to assess the interobservers agreement in the evaluation and interpretation of the adrenal glands' ultrasound images. Ultrasonographic images (one image for each adrenal gland) obtained from 18 foals have been reviewed by three different observers: two observers expert in abdominal ultrasound evaluation and one PhD-student ultrasonographer in-training. All observers were unaware of the clinical condition of the foals: healthy, septicemic, with systemic illness, premature and maladjusted. For each image, the observers were asked to describe: echogenicity of the cortex (hypoechogenic vs echogenic), echogenicity of the medulla (echogenic vs hyperechogenic), the size of the adrenal gland (smaller or bigger than normal), and the thickness of the cortex (thin vs thick). Two panels of four reference images, one for each adrenal gland, have been provided. Interobservers agreement for each ultrasonographic finding was determined separately for each adrenal gland using the κ statistic (3). The ultrasonographic images were 36 in total. The interobservers agreements for the right adrenal gland were overall poor for echogenicity of the cortex (from poor to fair [12% to 27%]), echogenicity of the medulla (from poor to fair [12% to 43%]) and thickness of the cortex (from fair to moderate [32% to 56%]), and fair for the size of the adrenal gland (from moderate to excellent [41% to 100%]. The interobservers agreements for the left adrenal gland were overall fair for the echogenicity of the cortex (from fair to good [38% to 72%]) and for the thickness of the cortex (from poor to very good [7% to 85%]), moderate for the size of the adrenal gland (from fair to moderate [24%

to 55%]), and good for the echogenicity of the medulla (from poor to moderate [3% to 53%]). All observers in most of the cases have reported an easier visualization of the left adrenal gland. In this study the interobservers agreement seems to be greater for the left adrenal gland, probably due to the different types of landmarks. The overall mild agreement could be linked to the small number of images reviewed in the study and to the uncommon application of the adrenal glands' ultrasonography in equine neonatal medicine routine diagnostic work-up. The κ-value could have been affected by the prevalence of the different features evaluated: for example the prevalence of the feature 'echogenic cortex' is low in the study, while there are a lot of cases in which the feature 'hypoechogenic cortex' is represented. Imbalance in the distribution of the different features can produce paradoxical κ-values.

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COMPARISON OF INDUCTION, RECOVERY AND CARDIOVASCULAR PROFILE OF ISOFLURANE AND SEVOFLURANE IN HEALTHY SHEEP AT MINIMUM ALVEOLAR CONCENTRATION (MAC).

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Objective: To determine induction, recovery and Cardiovascular profile of sevoflurane (S) and isoflurane (I) at minimum alveolar concentration (MAC) in sheep.

Experimental protocol: Crossover randomized study. After Ethical Committee approval (CIBASA n. 22859/3/07/2012), fourteen adult sheep were anesthetized twice with 40 days of wash out. With the sheep in lateral recumbency, anesthesia was induced with sevoflurane (8%) or isoflurane (5%) delivered in O2 (3 L/min) via face mask. After tracheal intubation the sheep were mechanically ventilated.

Body temperature (T°) respiratory rate (RR), heart rate (HR) and mean arterial pressure (MAP) were recorded at baseline prior to induction (T0). T°, HR, MAP, end tidal agents (ETS and ETI) were recorded at the seven time points: 5' after the induction of anesthesia (T1), at the end of 30' of equilibration period (T2); during the first MAC determination (T3, T4), during the second MAC determination (T5, T6) and at the end of the procedure (T7). Time Induction (IND), Time Extubation (EXT) and Time Standing (STAN) were also recorded.

All data are expressed as arithmetic means±SD or median with IQR. Paired t-test or Wilcoxon-rank were utilized for statistical analysis with $p< 0.05$ statistically significant.

IND was 187 (178-200 for S and 310 (180-240) for I ($p<0.02$), EXT was 424±146 and 458±110 ($p<0.006$) respectively, STAN was 821±321 and 980±347 ($p<0.04$). The MACs values were 2.75±0.54 for S and 1.2±0.32 for I. PAM significantly decreases from baseline at T1 and T2 $p<0.003$ and $p<0.004$ for S and $p<0.00001$ in both points for I. .HR significantly increases with $p<0.001$ and $p<0.02$ in every point recorded for I and only at T1 for S.

There were no significant differences for T° and RR in every Tpoint recorded.

Rapid recovery from anaesthesia is necessary in sheep to avoid the risk of aspiration pneumonia. Although both anaesthetics appear to be suitable for anaesthesia, sevoflurane seems to be more appropriate with regard to induction, recovery and cardiovascular profile after induction e during the equilibration period.

ULTRASONOGRAPHIC EVALUATION AND MEASUREMENT OF UMBILICAL STRUCTURES IN CALVES, HEALTHY AND AFFECTED FROM UMBILICAL DISORDERS: PRELIMINARY DATA

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Due to the high incidence and the considerable economic impact of the umbilical diseases, the practitioner should carefully assess each suspected case, following an accurate diagnostic approach and carrying out an early therapeutic plan. Ultrasonographic examination represents an ideal diagnostic aid in depicting the external and the internal umbilical structures and could help to determine appropriate treatment, representing a simple, cheep and accurate diagnostic tool, available for the practitioner. The aim of this study was to determine, by means of ultrasonography, the size of the umbilical structures in healthy newborn calves and in newborn affected by umbilical diseases, and to evaluate their involution during the first four weeks of life. The present study was carried out on Holstein Friesian calves. The ultrasonographic examination has been performed by the same operator using a portable scanner (LOGIQ Book XP, GE) and a linear multifrequency probe 7-10 MHz, without a standoff pad. Initial scans on each calf were performed within 24 hours of parturition and subsequent scans at weekly intervals, until calves were 4 weeks old. The standard examination was performed with the calf in left lateral recumbency, without sedation but manual restraint, starting with the evaluation of the two umbilical arteries, followed by the evaluation of the extra-abdominal umbilical structures and the left umbilical vein.
20 calves were considered that after a careful clinical evaluation were divided into two different groups, of 10 calves each: group H, including clinically healthy newborns, and group P, including newborns affected by umbilical diseases. The range values of umbilical structures in healthy calves in our experimental study are in line with the literature (1-2). Medium values of umbilical structures in calves affected, at 1, 7, 14, 21, 28 days were :

- umbilical left artery: 0.74 x 0.82, 0.80 x 0.98, 0.71 x 0.88, 0.85 x 0.92 and 0.65 x 0.74 mm (external diameter); 56.33, 72.20, 64.62, 69.52 and 39.74 mm² (area)
- umbilical right artery: 0.76 x 0.85, 0.79 x 0.94, 0.87 x 1.00, 0.83 x 0.90 and 0.70 x 0.78 mm (external diameter); 56.68, 68.11, 77.47, 66.60 and 50.72 mm² (area)
- umbilical veins into the stump: 27.20, 34.39, 27.34, 33.65 and 25.36 mm² (right vein area); 29.12, 36.26, 51.65, 37.27 and 19.26 mm² (left vein area)
- umbilical left vein (SEGMENT A): 127.51, 96.62, 93.00, 51.14 and 32.13 mm² (area)
- umbilical left vein (SEGMENT B): 81.86, 48.31, 55.73, 24.86 and 12.15 mm² (area)
- umbilical left vein (SEGMENT C): 56.76, 35.79, 49.37, 12.16 and 8.58 mm² (area)
- umbilical left vein (SEGMENT D): 56.76, 24.54, 56.29, 12.05 and 11.96 mm² (area)

Using a standardized ultrasonographic technique, it was possible to establish a range of normal and pathological values for the measurement of the umbilical stump, umbilical arteries, umbilical veins and urachus, that may be used as a reference for assessing newborns with suspected umbilical diseases.

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EVALUATION OF PARENTERAL NUTRITION IN MALNOURISHED DOGS

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Total parenteral nutrition (TPN) is defined as the provision of all nutritional requirements by means of these substrates. The clinical indications related to TPN considered are when dogs or cat can't orally or enteral fed. The purpose of this study is to evaluate the efficacy and safety of total TPN in malnourished dogs. From medical records of all dogs receiving TPN signalment, reasons for use, duration of administration, time of hospitalisation and complications of TPN were collected. Fourteen dogs were included in the study, the most common underlying diseases were non-specific gastrointestinal disease (GI) (n=6); other pathologies detected in dog were: hepatic disease (n=1), portal vein thrombosis (n=1), intestinal atony (n=1), post-nephrectomy hospitalisation (n=1), post-enterectomy hospitalisation (n=1), idiopathic megaesophagus (n=1), chylothorax (n=2). The TNP efficacy was evaluated considering albumin blood values and body weight change during the nutritional therapy, moreover all patients were evaluate for metabolic, mechanical and septic complication.

Median time of hospitalisation was 7.07 days (range 1–20 days). Median duration of intravenous nutrition administration was 3.3 days (range, 1–7 days). In the 14 animals receiving TPN no complications occurred. Non-specific GI was the most frequent primary problem in dogs treated with TNP, infact severe GI disease frequently compromises the ability to ingest, transport, digest and absorb nutrients, putting these patients at increased risk of malnutrition. In our study no one of the animal treated after PN had GI disease, no vomit and no diarrhoea. Serum albumin concentration is frequently used as one of the biochemical indicators of nutritional status in humans. All subjects that were judged to be in a poor nutritional state did have subnormal serum albumin concentrations. The presence of hypoalbuminemia in an anorexic dog should prompt immediate consideration for nutritional support therapy. The absence of

hypoalbuminemia should not eliminate the patient from consideration for nutritional support if historical or physical indications are present. Body weight gains in animals receiving TPN for short periods may have reflected a gain in extracellular water rather than an increase in tissue mass. Body weight monitoring is a crude measurement of the efficacy of TPN. Catheter-related sepsis is a reported complication of TPN administration in human medicine. It's most purely defined as an episode of clinical sepsis in a patient receiving TPN for which no other septic focus can be identified, which resolves upon catheter removal, and which is confirmed by catheter tip and blood culture yielding the identical organism.

Clinical sepsis didn't occurred in animals receiving TPN in our study. Only one catheter tip collected from an animal without clinical signs of sepsis was positive Staphylococcus epidermidis suggesting colonization. TPN seems to be a relatively safe method of providing nutritional support and we could consider parenteral nutrition as a bridge from the anorexic patients to the enteral or oral nutrition.

VACUUM ASSISTED CLOSURE SYSTEM IN THE COMPLEX PLEURAL EMPYEMA: A CASE REPORT

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Bite wounds of the chest wall in small dogs can produce piothorax and can be associated with severe damage to chest wall muscles, ribs, and lungs. We report a 13-year-old dog male Poddle who had undergone surgery after a wound dog bite. Chest x-rays showed multiple costal damage while during the wound skin curettage a very small empyema and an extensive necrosis of the biaxial thoracolumbar muscles were detected. This lesions was a putrid contused lacerated wound of approximately 20 cm of length associated with typical signs of loco regional inflammation without fever. Thoracotomy, tissue debridment and resection of the 10th to the 12th ribs were followed by implantation of the vacuum-assisted closure (VAC) system due the extensive tissue necrosis. Definitive citopathologic workup showed a purulent effusion. A bacteriological swab of the thoracic cavity confirmed Pseudomonas Aeruginosa and Clostridiums spp. and specific antibiotic therapy was enhanced. The second look on the first postoperative day consisted of surgical debridement and repetitive lavages of the thoracic cavity with sterile saline solution. Afterwards the VAC device was directed toward the mediastinal structures and the whole thoracic cavity was loosely filled with sponges. To minimize contact between the system and the lung parenchima a surgical cotton sponge was interposed. Negative continuous pressure was set at 100 mm Hg, and administered since the day of surgery. The VAC system sponge was first changed on the 3rd day after implantation. The process was repeated until a macroscopic clinical resolution of the mediastinitis was reached. As the dog patient general condition improved, by means of laboratory analysis and clinical appearance, the further dressing changes were set individually, whereby only the clinical measurements were taken into consideration. However, the VAC system was never left in situ for more than 3 days and the vacuum

was always set at 100 mm Hg. Five dressing changes, always in the operating room and under general anesthesia, were required until definitive wound closure.

Bacteriological smears were taken regularly to evaluate bacterial colonization of the pleural cavity.

The thoracic cavity was definitively closed on 18th postoperative day with moderate mobilization of the muscle layers and the subcutaneous tissue with direct closure of each layer.

The repeated changes of the surgical dressing showed uncomplicated wound healing.

The further course was uneventful and the dog patient was discharged on 22nd postoperative day.

CLINICAL ANATOMY OF THE CELIAC TRUNK IN THE DOG: APPLICATION FOR ELECTIVE AND EMERGENCY SURGERY

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The abdominal aorta has different branches that supply blood to the parietal and visceral structures. Some of the visceral branches are paired and others unpaired; the unpaired visceral branches are the celiac, cranial and caudal mesenteric arteries. The celiac artery is the first branch that vascularizes spleen, stomach, liver, pancreas and the first half of the duodenum [Evans, Lahunta 2013].

The aim of this work is to identify a precise anatomic and topographic framework that locates the origin of the celiac trunk safely and quickly and that could be useful during elective surgical procedures or surgical emergency and give more details on the celiac trunk and its.

The surgical anatomy study was conducted on 20 adults dogs and was focused to find the most effective and rapid laparotomy access to the trunk, basing on the identification of defined and constant anatomical landmarks. In the first 4 animals we proceeded with a standard xifo-pubic laparotomy. In the remaining 16 dogs we decided to proceed with a median xifo-umbilical laparotomy extended to a left paracostal access following the caudal edge of last rib. The celiac trunk arose from the ventral surface abdominal aorta in all the examined animals. Its average length was 16 mm, its average diameter of 5.33 mm. The presence of the "true" tripod was observed in 50% of subjects (#2, 3, 6, 7, 9, 13, 16, 17, 19, 20), while the false tripod, with splenogastric trunk and common hepatic artery as the first branch, in 45% of subjects (#1, 4, 5, 8, 10, 11, 12, 14, 18).

One subject (#15) showed a variant consisting of a celiac trunk bifurcated into common hepatic and left gastric arteries, and splenic artery arising from the cranial mesenteric artery. In 20% of subjects (#8, 11, 16, 17) the origin of the celiac trunk was very close to the aortic hiatus on the thoracic side of the diaphragm, while dividing in its main branches into the abdominal cavity.

The xipho-umbilical + left paracostal access to the celiac trunk we proposed is not without risks but among the different surgical approach surveyed it was the safest and practical to implement. It allows to act at the level of the celiac trunk origin, and

eventually cranial mesenteric artery, considering their extreme closeness, exploiting the presence of a landmark easily identifiable and constant. In conclusion our study let to a better understand of the vascular anatomy of the celiac trunk in case of either elective or urgent surgery direct to these vessels or the abdominal organs related.

EVALUTATION OF THE CARDIORESPIRATORY EFFECTS OF CPAP (CONTINUOUS POSITIVE AIRWAY PRESSURE) IN DOGS UNDER GENERAL ANESTHESIA

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Continuous positive airways pressure (CPAP) implies application of a pre-set positive pressure throughout the respiratory cycle in a spontaneously breathing patient. CPAP is usually applied in a non-invasive mode in humans patients and dogs[1] through the use of face mask or helmet. When CPAP is administered to the patients by means an andotracheal tube it is defined as invasive CPAP[1]. The aim of the study was to assess the cardiovascular and respiratory effects of invasive CPAP administered in healthy dogs under general anesthesia as intraoperative ventialtory support.

After the written owner consent, 20 healthy female dogs (ASA1) were included in the study and were divided in two groups, GROUP CPAP and GROUP CONTR. In all patients was used the same anesthetic protocol with acepromazine maleate (20 µg/kg IM) and morphine (0.3 mg/kg IM), propofol (5 mg/kg IV) and after tracheal catheterization a mixture of Isoflurane (Et ISO 1.3%) and O2. All patients were kept in spontaneous breathing. Physiological parameters were monitored at 5 minutes intervalls throughout anesthesia. Ten minutes after the induction of general anesthesia in 10 subjects (GROUP CPAP) 5 cmH_2O of CPAP were applied and were mantained until the end of anesthesia. The remaining patients (GROUP CONTR) did not receive CPAP.

In order to evaluate the hemodynamic and respiratory effects of the invasive CPAP arterial blood gas (PaO2, PaCO2, SaO2, PaO2/FiO2, P(A-a)O2 and Fshunt), and echocardiographic determination of the Ejection Fraction (EF) and cardiac index (CI).were performed at the followint times: 20 minutes after premedication (Time A); 10 minutes after intubation (before the application of CPAP in the CPAP group; Time B); 30 minutes after the application of CPAP in the CPAP group and 40 minutes after induction in the CONTR group (Time C); 30 minutes after extubation (Time D). Data were compared

between groups and among groups with the ANOVA test or the Friedman test based on the nature of the data (parametric or not parametric respectively).

Intrapulmonary shunt increased in both groups during anesthesia but it decreased significantly in patients receiving CPAP. The same happened for the P(A-a)O2. The PaO2 and PaO2/FiO2 were higher during CPAP compare to the control. The EF was higher during the administration of CPAP compared to the control.

The results of this study indicate that general anesthesia, practiced with the drugs and doses used in this study, causes a significant alteration of the respiratory and cardiovascular function and the administration of 5 cmH_2O of CPAP improves the respiratory and cardiovascular performance during anesthesia and also in the recovery phase.

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USE OF THE L.O.A.D. (LIVERPOOL OSTEOARTHRITIS IN DOGS) SCALE IN THE MANAGEMENT OF OSTEOARTHRITIS IN DOGS

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The Liverpool osteoarthritis in dogs (LOAD) survey is a tool for the evaluation of the mobility in dogs affected by osteoarthritis (OA) through the opinion of the owner (1). The aim of the study was to evaluate the feasibility of the LOAD for the management of conventional therapeutic protocols of OA in dogs. Dogs affected by OA were recruited for the study. All patients underwent an accurate clinical examination, which allowed to identify the characteristics of the lameness. Joints were also scored based on the radiographic appearance. Considering the anatomical area of interest patients were divided in 4 groups: hip, elbow, knee and multiple localization. At the moment of the first evaluation owners completed the LOAD survey and based on the score obtained each subject was assigned to a different group: mild (0–12); moderate (11–20); severe (21–30); extreme (31–52). Based on the physical examination, LOAD score and radiographic appearance all patients were assigned to a different stage of osteoarthritis: mild, moderate and severe. Dogs were treated based on the degree of osteoarthritis. All patients with a mild/moderate OA were treated with robenacoxib (1 mg/kg daily) for 30 days associated with weight control and physical activity. In patients affected by severe OA in addition to robenacoxib, tramadol (4 mg/kg per OS q8) and/or amantadine (5 mg/kg OS q12) based on the judgment of the physician. All patients were re-evaluated 30 days later. The data obtained at the beginning of the therapy (T0) and after 30 days (T30) were compared for the entire population and within the single groups with the Wilcoxon test (significant at $P<0.05$).

Twenty-four patients were enrolled in the study. Based on the protocol of the study AO was severe in 14 patients (58.3%) moderate in 8 patients (33.3%) and mild in 2 patients (8.3%). All patients were treated with robenacoxib moreover 7 of them (29.1%) received also amantadine based on the protocol. The median value of the LOAD of the entire population at T0 was 21.5 (range 30–9) while at T30 it was significantly lower (12, range

29–5). The LOAD score at T0 was 25 (range 30–10) in the hip group, 18.5 (range 30–9) in the knee group, 20.5 (range 30–15) in the elbow group and 24 (range 30–12) in the group of multiple joints. At T30 the LOAD scores were 13,5 (range 25–9), 10.5 (range 23–6), 14.5 (range 29–5) and 13 (range 25–8) respectively. The LOAD score at T30 was significantly lower compare to T0 in the hip, knee and multiple joints groups.

The results of this study demonstrate that the LOAD score can be integrated in a system of grading OA, objectifying to effect of the treatment on the pathology, considering also the subjective response of the patient. The LOAD can be used to monitor the effect of the therapy on the quality of life and mobility of the patient independently from the radiographic and anatomical alterations.

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XIII Convegno
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FORENSIC INVESTIGATION ON A DOG FOUND DECAPITATED. AN UNEXPECTED RESULT

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Trauma caused by the impact of a train is typically severe and instantly fatal. Nevertheless, in the absence of witnesses, determining the manner of death can be difficult. In human forensic medicine the most common dilemma is to clarify whether the death was due to accident, suicide or murder [1]. In May 2015 a Czechoslovakian wolfdog was found dead on the tracks, with the head completely detached from the body. The carcass, in advanced state of decomposition, was sent to IZSLT for necropsy and toxicological analysis. Due to the position of the remains, local authorities suspected that the animal was decapitated with an edged weapon, and that only later were the remains placed on the tracks to simulate having been run over by a train. In January 2016, since no new elements had emerged that could clarify the origin of the injuries, the remains were re-examined at the Referral Center for Veterinary Forensic Medicine to determine the cause of death and decapitation. A forensic necropsy was undertaken, including complete skinning of the carcass and photographic documentation with metric reference. Necropsy showed extensive splancno and neurocranial fractures and fractures of left scapula and tibia. Skinning revealed hemorrhages at the fracture sites and only on the left side of the skull. The neck was separated from the trunk at C7-T1; skin showed irregular margins. Protruding 10 cm from the distal surface of the neck were the stumps of the trachea and esophagus; the latter was longer than the tracheal stump. Stereomicroscopic examination showed thick, curled edges on the distal edge of the esophagus. The trachea was torn away due to tearing of muscle tissue between two cartilaginous rings at the level of the bronchial carina. Black oily material was observed on the left side of the body. Toxicological analysis of liver samples (HPLC, GC-MS) was negative.

Based on the suffusion of blood, visible only after skinning, it can be stated that the traumatic lesions were produced intravitam. It was excluded that the dog was already dead when hit by the train. It was excluded that the head was removed by a sharp

instrument since stab wounds were absent on skin and underlying tissues. Furthermore, trachea and esophagus were both excised by traction, having given way at the point of least resistance. In both cases the lacerated ends were located within the chest, thus in a position that cannot be reached by a cutting instrument. In cases of accidents involving people lying on the rails, impact of a train with speed >100 km/h may result in partial dismemberment; in the case of impact with a crouching person, the most severe lesions are produced on the skull [2]. The trauma described here is compatible with the collision of a high-speed train that hit the dog from back to front and on the left side. Cause of death was due to skull fractures that probably rendered the animal unconscious before being immediately followed by decapitation. The findings obtained from forensic necropsy allowed us to establish that decapitation occurred intravitam due to lethal blunt trauma associated with violent traction on the head.

[1] Nikolic et al 2013
[2] Driever et al 2002

A CASE OF A HUNTING DOG ALLEGEDLY KILLED AND CONSUMED BY WOLVES: VETERINARY FORENSICS FINDS THE REAL CULPRIT

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In the winter 2015-16 several cases of predation and consumption on domestic dogs were reported in Central Italy from local press and social media. The dogs were all used for wild boar hunting. The owners reported that dogs, unleashed in the woods to track the boars,were found within hours almost completely consumed. These cases caused uproar in hunters and dog owners, and the wolf was singled out as the responsible for the killings.

The remains of one dog allegedly killed and eaten by wolves were submitted to 'Centro di Referenza Nazionale per la Medicina Forense Veterinaria' for necroscopic examination, in order to ascertain the identity of the predator. The examined dog was an adult female Maremma hound. The carcass was devoid of the skin and of superficial and deep muscles. The skin was only preserved at the head and distal extremity of left rear limb. Lesions consistent with carnivore bite marks were not identified. The margins of the skin showed a clean edge and were not interested by hemorrhages. The left femur was exposed, with signs of deep furrowing on the distal bone surface. Abdominal and thoracic viscera were not affected by consumption. The appearance of the skin edges, unaffected by hemorrhages, was consistent with the defleshing having occurred postmortem. Lesions consistent with intravitam or postmortem carnivore bite marks were not identified. On the exposed bone surfaces furrowing had occurred, while no punctures or pits were identified that are usually present in cases of carnivore scavenging [1]. Therefore from necropsy findings the signs of consumption on the carcass were not consistent with the action of a carnivore. The carcass appeared to be consumed 'by layers', probably by an animal whose teeth do not allow wide and deep bites. Salivary swab samples were collected from the most apparent lesions produced by scavenging, such as furrows on the bones or torn muscles. The swabs were submitted to DNA extraction, and two panels of 15 and 20 nuclear loci (Short Tandem Repeats, STRs)

specific to *Sus scrofa* and *Canis lupus*, respectively, were applied [2, 3]. No wolf alleles were amplified from any sample, while 13 out of 15 STRs of *Sus scrofa* were successfully amplified. Genetic analysis at STR loci revealed that at least 2 wild boars fed on the dog. The use of forensic pathology and genetics allowed us to identify wild boar as the responsible for this case of consumption on a hunting dog. Other similar cases reported by the press have been likely attributed erroneously to the wolf, despite the absence of scientific proof and to the detriment of the actions undertaken for the conservation of the species. The wolf population has expanded in recent years, causing a resurgence of conflicts with human activities [4] and retaliation killings (with traps, poison or firearms). Poaching is still one of the main threats to the conservation of the wolf [5], thus it is essential not only to carry out appropriate measures for conflicts management, but also to correctly identify the predator in cases of attacks to domestic animals [6].

[1] Young et al 2015
[2] Lorenzini 2005
[3] Lorenzini et al 2014
[4] Lovari et al 2007
[5] Dondina et al 2014
[6] Fico et al 2005

THE EFFECT OF TEMPERATURE AND POST-MORTEM INTERVAL ON THE TRANSPARENCY OF THE EYE LENS IN DOGS

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The postmortem processes and the factors that affect them are very important in the estimation of the postmortem interval (PMI). The time of death and survival period may be used to determine criminal charges in animal cruelty cases. Several postmortem changes can provide valuable information in death investigations and most of these are affected by environmental conditions (1). Many post-mortem modifications have been studied in human as well as in animals, but, to our knowledge, no one considered the modifications of eye lens. The lens is a transparent, avascular organ (2) composed by a high concentration of protein (approximately 300 mg/ml) (3). The maintenance of transparency depend on the function of epithelial cells (2), on the interaction among lens proteins (protein-protein interaction) (3) and on intercellular communication (gap junction) that allows intercellular passage of molecules (up to 1 kDa) such as antioxidants (2). The opacification of the crystalline lens is also possible in the postmortem period because of the low temperatures of the body during the Algor Mortis (4, 5, 6). The aim of the study was to evaluate the modifications of the lens transparency of dead dogs over the time and the opacification of the lenses at low temperatures. We studied the lenses of twenty-five adult dogs at different time of death and at different storage temperature to assess variations of the optical density of the lens using a DOTTIE II transmission densitometer. We created a light source stabilized to calibrate the densitometer to 0.00. We observed and registered the opacification of the lens and the value of the transmitted light in the frozen dogs lens and over time postmortem. In frozen dogs at -18°C for 7 days, macroscopically, we observed opacification of the lenses and increase of optical density. Instead, the removed lenses, stored at room temperature (24°-26°C) showed increase of optical density starting after 8 days postmortem. These results will be useful in forensic veterinary medicine to better evaluate the period of death and temperature of storage of the cadavers. The understanding of the phenomena

underlying the postmortem opacification will also be useful to better understand the pathogenesis of in-vivo ocular diseases such as the cataract in humans and animals.

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ANGIOINVASIVE SUBCUTANEOUS LYMPHOMA IN THE CAT

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Angioinvasive lymphoma (AL), also called lymphomatoid granulomatosis, is a rare angiocentric/angiodestructive lymphohistiocytic proliferative disease that primarily involves the lung. Histologically, AL is characterized by a mixed mononuclear infiltrate containing large and small lymphoid cells, plasma cells and histiocytes with vascular infiltration. Although the pathogenesis of human AL is still unclear, cytotoxic T-cells activation likely play an important role in the development of the disease (2). The clonal B cells proliferation can be associated with Epstein Barr virus (EBV) infection (1). Few cases are reported in dogs (4) and cats (5) affecting mainly the lung. Only one study reported the skin and subcutis as the primary sites of AL in a cat (3). The present report describes the histopathological and immunohistochemical findings of recurrent subcutaneous nodules in an adult cat. A 12 years-old male domestic shorthair FIV FeLV negative cat was presented with single subcutaneous nodule in the right shoulder. The cat did not receive any injection for at least three years. A fine needle aspiration was consistent with a malignant round cell neoplasia. The cat did not show pulmonary radiographic involvement, lymph nodes enlargement and visceral ultrasound detectable lesions. The mass was subsequently removed and, on histology, a diffuse infiltration of atypical polymorphic lymphoid cells, admixed with moderate number of epithelioid macrophages was observed in the subcutis. Angiocentric cuffs of neoplastic lymphocytes, with mural invasion and confluent foci of coagulative necrosis were prominent. By immunohistochemistry, diffuse B cells (CD20+, CD79+) and multifocal angiocentric T cells (CD3+) were observed. Multifocal myeloid/histiocyte antigen positivity was also detected. The morphological and immunophenotypic features were consistent with a T cell-rich B cell angioinvasive lymphoma. One month later a fine needle aspiration of a new nodule in the neck showed the same features. As described in human medicine, the T-cells lymphoid angioinvasion is considered a peculiar feature to distinguish AL among other mixed lymphoid processes. Considering the relationship between AL and Herpesvirus in humans, a viral antigenic stimulation should be investigated also in

domestic animals. To the authors' knowledge, this is the first report of a primary cutaneous AL in the cat, without evidence of visceral involvement.

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VEGFA AND VEGFRS EXPRESSION IN CANINE APPENDICULAR OSTEOSARCOMA

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Osteosarcoma is the most common primary malignant tumour of bones in dogs, occurring most frequently in the axial skeleton (75%), it is locally aggressive with a high angiogenetic and metastatic potential. VEGFRs (VEGFR1, VEGFR2 and VEGFR3) are the most important receptors regulating tumoral angiogenesis following the interaction with VEGF factor.

The aim of this study was to investigate VEGFR-1, VEGFR-2 and VEGFR-3 and VEGFA expression in canine OSA tissues and cell lines to evaluate its prognostic value in relation to clinical outcome of the canine patients.

Thirty-one dogs diagnosed with canine appendicular were enrolled in the study. All the animals underwent a complete clinical staging and were treated with surgery and then they were followed until the recurrence of the neoplasm or death. All the samples were histologically evaluated and immunohistochemically tested for VEGFA, VEGFR-1, VEGFR-2 and VEGFR-3. Histological and immunohistochemical results were evaluated in relation to clinic-pathological data. Total RNA was extracted from 8 canine osteosarcoma cell lines and expression of VEGFR1, VEGFR2 and VEGFR3 canine gene were evaluated by qPCR.

VEGF was present in all analyzed cases and particularly was widely expressed in 33% of cases, moderately expressed in 46% of cases and poorly expressed in 20% of OSA analyzed. Regarding the expression of the receptors we found that the 64.52% of canine OSA were positive for VEGFR-1, 70.97% were positive for VEGFR-2, while 74.19% cases per positive for VEGFR-3. The positivity for VEGFR-1 was statistically associated with the positivity for VEGF ($P<0.05$) and VEGFR-3 ($P<0.05$). Statistical analyses comparing the immunohistochemical results with all clinical-pathological data revealed no statistical associations. Molecular data showed that only D22 cell lines over-expressed all three VEGFRs while VEGFR2 was expressed only by Wall and D22 cell lines and VEGFR3 by D22, Pedro, Lord and Wall cell lines if compared to osteoblastic cell line.

This is the first study investigating VEGFRs and VEGFA expression in canine OSA and cell lines demonstrating that those receptors are widely expressed in this tumor. No statistical association has been found between VEGFRs expression and clinical and histopathological features while a significant association between VEGFR1 and VEGFA has been found. In human osteosarcoma the autocrine loop VEGFR-1/VEGFA is correlated to the malignant progression while VEGFR2 and VEGFR3 seem not to be involved.

These preliminary data suggest that also in canine OSA this autocrine loop can be relevant in the progression of canine osteosarcoma as demonstrated in canine mammary tumors and that be considered a suitable target for innovative targeted therapies.

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EXPRESSION OF CD44, CD10, P63, VIMENTIN, TAZ/YAP, E-CADHERIN, AND BETA-CATENIN IN HUMAN, CANINE, AND FELINE MAMMARY TUMORS

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Mammary cancer is one of the most common type of cancer in women and female dog and cats. In cats it is an aggressive lethal (80-90%) tumor whereas in dogs it is a very heterogeneous tumor both in term of morphological subtypes and co-existing subpopulations. The aim of the study was to investigate different markers in a subset of human, canine, and feline mammary tumors with regards to stem cells and undifferentiated cells phenotype. Particularly, we investigated the expression of CD44, CD10, p63, Vimentin, TAZ/YAP, E-Cadherin and beta-Catenin in both single and dual immunohistochemical staining. Twenty-one canine mammary carcinoma, 15 feline mammary carcinoma, and 5 triple negative human breast cancers were included. Positivity was calculated in a semi-quantitative manner and also precise location of expression was monitored in the normal mammary gland counterpart of each sample. CD44, CD10 and p63 were expressed in the basal compartment of simple canine carcinomas despite not perfectly overlapping in term of percentages of expression. Solid pleomorphic canine carcinomas were predominately negative to CD44, whereas triple negative HBC were diffusely positive indicating only in the latter the predominance of undifferentiated precursors. CD44 was also very evident in the luminal canine compartment suggesting that this molecule is probably more widely expressed in the canine gland and tumors. CD10 was not observed in feline carcinomas and p63 was limited to residual basal/myoepithelial cells delimitation. Curiously, CD44 was variably expressed in feline tumors, also in discordance with a few published data. Vimentin and TAZ/YAP were often co-expressed in canine solid carcinomas showing also a negative correlation with the expression of membranous E-cadherin and beta-Catenin. Similarly, even if with less percentages triple-negative cancers were co-expressing Vimentin and TAZ/YAP. Feline carcinomas were diffusely a highly expressing and co-expressing

Vimentin and TAZ/YAP indicating an aggressive proliferative phenotype. E-cadherin and beta-Catenin were also diffusely decreased on the cellular membrane in aggressive grade III feline tumors. In conclusion, triple negative breast cancer, feline carcinomas, and canine solid pleomorphic carcinomas suggested an epithelial-to-mesenchymal transition phenotype with activation of YAP/TAZ pathway. CD44 was not observed as precisely marking undifferentiated cells since it was more diffusely distributed then expected also in the normal gland. This study would help elucidating the role and the distribution of these markers in mammary carcinomas to better understand the composition of cell subtypes in different tumors of different species.

EXPERIMENTAL INDUCED HYPOFERTILITY IN SARDA BREED RAMS BY BLUETONGUE VIRUS SEROTYPE 1 INFECTION.

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Bluetongue virus (BTV) infection can be clinically unapparent or provoke a fatal clinical manifestation, which includes fever, prostration, stiffness, dispnea, nasal discharge, and anorexia. Among the several symptoms, male infertility has also been described, either in natural illness or after using attenuate BTV strains for vaccination. In these cases BTV infection clinically resulted with alteration of the morphology, decreasing of the number and reduced viability of the spermatozoa or complete azospermia. However, the pathogenesis of such effects has never been investigated. Herein, we experimentally studied the pathogenesis of BTV serotype 1 infertility in Sarda breed rams by using a field isolate. Nine 2-year-old rams were inoculated intradermally and subcutaneously with 3 and 10 ml, respectively, of total BTV serotype 1 infected blood originated from a clinically affected Sarda breed ram. After, 3 rams were serially euthanized at 5, 7 and 15 days post-inoculum (p.i.), respectively. At necropsy a wide sampling of tissues was collected for viral RNA quantification by Real-Time PCR, histopathology as well as for viral VP7 and NS2 proteins immunohistochemical detection (IHC).

No clinical signs were observed in the animals sacrificed at 5 days p.i.. While, in the 3 rams sacrificed at 7 and 15 days p.i., the infection was clinically apparent and merely characterized by the involvement of the genital tract, with severe hyperthermia and edema of the scrotum at 7 days p.i.. BTV RNA was detected in the animals sacrificed at 5, 7 and 15 days p.i., in blood, lymphoid tissues and testicles. Histologically, no lesions were found in the testicles of the rams at 5 and 7 days p.i., while edema and inter-tubular vasculitis with consequent severe degeneration of the tubular germinative epithelium were found in the testicles of the rams sacrificed at 15 days p.i.. By IHC, BTV

was found in the endothelial cells of the testicular, epididymal and scrotal skin capillaries only at 5 and 7 days p.i.. Hypofertility has been reported in rams vaccinated with BTV serotype 2 live modified vaccine and naturally affected by BTV serotype 8. Interestingly, BTV serotype 1 and BTV serotype 8 field strains have not been considered responsible for any lesion in the reproductive tract of rams after experimental infection. In this study, we observed that rams experimentally infected with 2013 Sardinian BTV serotype 1 field strain displayed severe degeneration of spermatic epithelial cells. The IHC results coupled with the histopathological findings clearly indicate that in the testicle the degeneration of the germinative epithelium during BTV infection is ascribed to the endothelial damage of the intertubular capillaries.

E5 ONCOPROTEIN OF BOVINE DELTAPAPILLOMAVIRUSES IS EXPRESSED IN CONGENITAL CARCINOSARCOMATOSIS OF LAMBS

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Papillomaviruses are oncogenic, double stranded DNA viruses responsible for epithelial and mesenchymal tumors in humans as well as in domestic and wild animals.

Bovine Deltapapillomaviruses (δPVs) are known to be the only Papillomavirus causing a cross-species infection. In sheep, the association of Papillomavirus infection with tumors and other disorders has been poorly investigated; indeed, Papillomavirus-associated lesions were described only in adult animals.

To our knowledge, congenital carcinosarcomatosis (CCS) in lambs has not previously been reported. The present study deals with the pathological findings observed in Sarda breed lambs affected by severe CCS at gengiva, palate and muzzle skin level.

Proliferative lesions were macroscopically observed just few days after birth in lambs, which died at about one month of age as they were not able to feed.

Tissues from two lambs were collected for molecular, histopatological and ultrastructural investigations.

Histologically, a mixture of epithelial (keratinocytes) and mesenchymal cells was seen. Numerous mitoses, many of which atypical, were observed in both cell types. In all samples the oncoprotein E5 was detected immunohistochemically. Ultrastructurally, neoplastic cells showed abnormal nucleoli-containing nuclei, the morphology of which was characterized by the presence of deep meandering invaginations giving them a bizarre and lobulated appearance. Atypical nuclei such as micronuclei were also seen. Intranuclear electron-dense particles, 40 nm in diameter, consistent with virus particles were shown.

Herein we documented that E5 oncoprotein of bovine δPV is expressed in sheep and appears to be involved in a malignant lesion of lambs. Our study shows, for the first time, a possible interspecies infection between sheep and cattle. Bovine δPVs are known to be responsible for vertical transmission in cattle as it has been shown that they can infect trophoblasts in vivo. It is conceivable to think that they could be responsible for transplacental infection also in sheep offspring.

Neverthless, the role, if any, of bovine δPVs in carcinogenetic and reproductive disorders of sheep warrants further studies in order to improve our knowledge about molecular pathways leading to neoplastic and no-neoplastic events.

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POXVIRUS INFECTION ("TATTOO SKIN DISEASE") IN TWO STRIPED DOLPHINS (*STENELLA COERULEOALBA*) STRANDED ALONG THE ITALIAN COASTLINE

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Tattoo skin disease (TSD) is a Poxvirus-induced cetacean disease characterized by typical skin lesions. Few pathological descriptions and a limited number of TSD reports are available worldwide. We describe herein the histological, biomolecular and virological identification of TSD in two striped dolphins (*Stenella coeruleoalba*) stranded along the Latium and Tuscany coasts of Italy in 2015 and 2016, respectively. A full necropsy was performed on the two male, juvenile and well-preserved dolphins under study, followed by detailed histopathological and transmission electron microscope (TEM) investigations. DNA extraction from skin lesion samples and PCR amplification of Poxvirus DNA polymerase were also carried out. The first striped dolphin showed wide, coalescing, lightly gray skin lesions with dark edges (tattoos) on the head, while the second one had a single, 2 cm-wide, round, yellowish lesion with a slightly dark edge, affecting the mandibular region. Numerous Poxvirus-like particles were observed in both animals' skin samples by means of TEM. In their skin, a multifocal, severe, hydropic degeneration of the keratinocytes of stratum spinosum was also apparent, with numerous round, 5-10 μm in diameter, eosinophilic, glassy structures (intracytoplasmic eosinophilic inclusion bodies), compatible with type-B poxviral inclusions ("Guarnieri bodies") being additionally found. The overlying stratum corneum was mildly hyperplastic (1.5 times over normal), with heavily hyperpigmented keratinocytes, occasionally hosting Guarnieri bodies. Viral DNA polymerase PCR allowed to confirm the presence of Poxvirus in the skin from both dolphins. To the best of our knowledge, this should be the first report of TSD in cetaceans stranded along the Italian coastline. It has been suggested that anthropogenic factors may play a major role in the emergence of skin diseases, with special emphasis on immunodeficiency originating from exposure to high levels of immunotoxic pollutants, which can be directly linked to TSD occurrence. Further studies are underway, in order to assess the tissue loads of immunotoxic contaminants in these

two dolphins, as well as in order to investigate the potential relationships between the level of exposure to the aforementioned pollutants, on one side, and Poxvirus infection's development, on the other.

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MULTIVISCERAL TETRATHYRIDIOSIS WITH GENITAL INVOLVEMENT IN AN EUROPEAN CAT

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Tetrathyridium bailleti is the second larval stage of *Mesocestoides lineatus*. Human, dog, cat and other carnivores are definitive hosts. *Tetrathyridium* can pierce bowel wall of dog or cat to reach body cavity producing peritoneal larval cestodiasis also known as tetrathyridiosis (1). Aim of this work is to describe the first report of multivisceral tetrathyridiosis with genital involvement in an european cat, highlighting the salient injuries and discriminative framework of infection as well as the important role played by molecular investigation to diagnose the parasite species involved. To the author's knowledge no report describes a parasitic oophoritis and metritis caused by *T. bailleti* and takies into account the rarity as well as the distinctive characteristics of this disease in cat rather than in dog (2, 3). The domestic cat lived in a garden with other conspecifics and it has not been vaccinated or treated against infectious agents and parasites. Physical examination and x-ray evaluation showed moderate abdominal swelling, cough, dyspnea and several pulmonary nodules that at first oriented clinicians towards a diagnosis of cancer. Necropsy carried after the owner request, showed the parasitic etiology of disease. Tissue samples collected during necropsy were routinely processed for histopathological examination. Adult tapeworms and larvae were stored in 70% alcohol, then placed in Petri dishes and observed with stereomicroscope (Zeiss Discovery V12), while flatworms belonging to the larval stage were processed for the observation with the electron microscope Cambridge Stereoscan 240 (SEM). Adult (n=1) and larval (n=1) tapeworms stored in 90% ethanol were sent to confirm morphological identification by means of PCR amplification and sequencing. Necropsy and histopathology showed

multivisceral parasitosis, with free and encysted worms in both body cavities, on serosal surface of the abdominal wall. Several multifocal granulomas were detected in spleen, lungs, uterus and ovary. The framework of pulmonary edema, granulomatous inflammation and emphysema led the cat to death. Morphological and molecular investigation confirm the diagnosis of Tetrathyridiosis. The features of oophoritis and metritis due to tetrathyridia could be interesting for clinicians, since despite the lack of reproductive history, on the basis of the observed lesions, it is possible to hypothesize reproductive function disorders, like oestrus disorders or persistent anoestrus, infertility or primary uterine inertia due to the injuries observed.

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EXPLANTS FROM BOVINE AIRWAYS: A SUITABLE TOOL TO INVESTIGATE THE EARLY PATHOGENESIS OF CONTAGIOUS BOVINE PLEUROPNEUMONIA?

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Contagious bovine pleuropneumonia (CBPP) is a relevant disease caused by *Mycoplasma mycoides* subsp. *mycoides* (Small Colony type, MmmSC). CBPP has been eradicated from most of Europe, while it is endemic in many Sub-Saharan Countries. The pathogenesis of CBPP is poorly understood and mainly investigated in cattle, infected by endotracheal intubation or by contact with infected/diseased animals (Scacchia et al., 2011).

The present study aims at evaluating in vitro models, which could be suitable to investigate the early stages of MmmSC infection and CBPP pathogenesis. The respiratory tracts (trachea, bronchi and lungs) from apparently healthy and regularly slaughtered cattle (n=7) were collected and studied. All samples were tested for *Mycoplasma* spp. by polymerase chain reaction (van Kuppeveld et al., 1994) and proved to be negative.

Two MmmSC strains, both alive and inactivated, were used: "57/13" (Italy, 1991) and "Caprivi" (Namibia, 2003). MmmSC inactivation was obtained by heat-treating (10' at 100°C) or by 10% formalin (50%, v/v). According to van Riel et al. (2007), the adhesion of MmmSC to the host cells was first evaluated on formalin-fixed tissue samples, routinely processed for histopathology. Tissue sections were then submitted to a "double-step" immunohistochemistry (IHC), by sequential incubations with MmmSC and with a murine anti-MmmSC primary antibody.

In addition, MmmSC "colonization" of the respiratory tract was investigated in living explants, which were incubated with both MmmSC strains (1 h at 37°C) and then formalin-fixed, processed for histopathology and submitted to IHC using a murine primary antibody anti-MmmSC.

To better detail the localization of MmmSC, laser scanning confocal microscopy (LSCM) tests were carried out using primary antibodies anti-MmmSC, cytokeratins, von

Willebrand factor and lysozyme. Negative and positive controls were included in each IHC and CLSM run.

MmmSC showed a marked tropism for the lower respiratory tract, while it spared the tracheal and bronchial tissues. IHC and CLSM detected MmmSC attached on and/or inside the epithelial cells of bronchioles and alveoli, the macrophages and the endothelial cells lining the blood and lymphatic vessels, these results closely resembling the IHC pattern observed in naturally infected cattle (Bashiruddin et al., 1999). Inactivated MmmSC strains were able to "colonize" different cell types residing within the cattle airways, thus suggesting that MmmSC could also "passively" adhere to and penetrate inside the host cells.

Our data suggest the suitability of in vitro models - complementary if not alternative to the use of experimental animals which sound useful to investigate the early stages of infection. Further studies are currently ongoing to evaluate the kinetics of MmmSC infection, as well as the host reactions, by means of medium-to-long term organotypic cultures.

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POLYAMINES ACTIVITY IN CANINE INFLAMMATORY COLORECTAL POLYPS BEFORE AND AFTER A PROBIOTIC BACTERIA TREATMENT

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Recent researches demonstrate a correlation between polyamine intake or intestinal exposure, to risk of colorectal neoplasia (Vargas et al., 2012). Furthermore, the role of polyamines, spermidine (SPD) and spermine (SPM), and their precursor putrescine (PUT), regulated in their cellular levels by ornithine decarboxylase (ODC), in cell growth and proliferation is very well recognized. Increased polyamine levels are observed also in patients with IBD with their corresponding inflammatory index revealed that increased concentrations of polyamines found in the most severe inflamed mucosal areas. Some probiotics seem to have anti-inflammatory and tumor inhibitory properties, but few studies have investigated their actions on mucosal polyamine levels. Recently, a demonstration that dysbiosis is associated with canine inflammatory colorectal polyps (ICRPs) development, and that this may represents a potential therapeutic target, was published (Igarashi et al, 2016). In this study, the effects of probiotic mixture on colonic polyamine biosynthesis in dogs with colonic polyposis (CP) were investigated. Histological sections of dogs with a long-time diagnosis of colonic polyposis (n=5) were analyzed. These dogs had received between 112 and 225 billion (112 to 225 x 109) of lyophilized bacteria daily for 60 days, and samples were obtained at baseline (T0) and 30 days after the end of treatment (T1; i.e. 90 days after T0). Histology scores, the expression of PUT, SPM, ODC and DAO positive cells, and the clinical activity index (CIBDAI) were compared at T0 and T1 using paired t-tests or Wilcoxon matched pairs tests, where appropriate. Additionally, levels of cellular proliferation (Ki-67 expression), and apoptosis (Caspase 3 protein expression) in the polyp were also evaluated. After probiotic treatment, significant decreases were observed for CIBDAI ($p=0.006$) and histology scores ($p<0.001$). In

contrast, SPM, PUT, and ODC expression increased ($p<0.01$) after probiotic treatment. Specifically, a significant decrease in colonic polyamine levels, ODC activity and Ki-67 was noted at T1 to T0. In contrast, a significant increase in caspase-3 positive cells and DAO expression ($p=0.005$) was also observed. Polyamines levels suggest a potential anti-proliferative effect of probiotics in hyperplastic mucosa, but also an anti-inflammatory effect associated with a reduction of mucosal infiltration. These effects could be related to increase in some bacteria genera such *Faecalibacterium* after probiotic treatment. Interestingly *Faecalibacterium* catalyzes the irreversible transfer of a propylamine group from the amino donor S-adenosylmethioninamine (decarboxy-AdoMet) to putrescine (1,4-diaminobutane) to yield spermidine, increasing PUT and SPD levels (van Vliet MJ, 2010). In conclusion, this study provides data about the ability of a cocktail of probiotics, administered for 8 weeks, to regulate polyamine levels, by enhancing polyamine biosynthesis and degradation in canine inflamed polypoid colonic mucosa, and to reduce cell proliferation in hyperplastic/neoplastic areas.

LYSOSOMAL STORAGE DISEASE (LSD): MORPHOLOGICAL ASPECTS OF GANGLIOSIDOSIS (GM) IN A FAMILY OF BOARS

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Lysosomal storage diseases (LSD) are heterogeneous group of progressive, lethal, multisystemic diseases. Generally, they have autosomal recessive inheritance and are gene-dose dependent. Inherited LSD show deficient enzymatic function of specific enzymes due to a genetic defect. These pathologies can be classified by the underlying molecular defect or according to the accumulated product. Gangliosides are membrane constituents of neurons; genetic defects in catabolism of these glycosphingolipids cause a neuronal LSD defined Gangliosidosis (GM). The aim of this work is to describe morphological features of GM in all boars of the same brood. 3 littermate boards, from a free ranging farm, presented neurological signs at 9 month of age. Due to the worsening conditions, they were euthanized at 1 year of age and submitted for necropsy. Tissue samples were submitted for viral and bacteriological analysis, routine histology (formalin-fixed and paraffin-embedded) and for TEM (glutaraldehyde-fixed). Also tissue samples were frozen. Tissue were negative for bacteria, CSF and Aujeszky virus. Paraffin sections (brain, spinal cord, peripheral ganglia and retinal ganglion cells) showed enlarged foamy neurons, with finely diffusely vacuolated cytoplasm. Nucleus moved from to the center to the plasma membrane and Nissl substance was effaced. Diffusely hepatocytes were characterized by the same cytoplasmic vacuolization that affects neurons, as well as Kupffer cells. TEM analysis revealed numerous swollen and degenerated neurons. Their cytoplasm was enlarged by the presence of numerous perinuclear membrane bound vesicles and lysosomes were severely filled by membranous material arranged in concentric lamellae and whorls (membranous cytoplasmic bodies). Some neurons appeared completely filled by the pathological lysosomes, that peripheralized or efface the nucleus. These aspects of lysosomes are referred to LSD, particularly ultrastructure

aspects of the content can be ascribed to GM. GM in swine has been described only in 1978 in purebred Yorkshire swine (*Sus scrofa domestica*) and has never been described in boars (*Sus scrofa*). Biochemical investigations are in progress.

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NEUROFIBROMATOSIS TYPE 1 GENE AFFECTS THE TUMORIGENESIS EVENTS IN *CARASSIUS AURATUS*

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Neurofibromatosis type 1 (NF1) is a common dominantly inherited genetic disorder that results from various mutations in the neurofibromin 1 (NF1) gene. The mutational events generates abnormalities in neural-crest-derived tissues that include hyperpigmented skin lesions, glioblastoma, schwannoma and malignant peripheral nerve sheath tumors. The nf1 gene encodes the large protein neurofibromin, which contains a GTPase-activating protein-related domain (GRD) capable of inactivating the RAS proto-oncogene (1). Furthermore, loss of nf1 in a p53-deficient background results in highly penetrant malignant formation (2). Zebrafish is currently the most suitable animal model for studying NF1. Homozygous loss of both alleles in combination generates larval phenotypes that resemble aspects of human diseases and results in larval lethality in 10 days (3). In our studies goldfish (*Carassius auratus*) was used and breeded at the CISS facilities, of the University of Messina (Italy). Little is actually known on the genome of *C. auratus* that up to date has not been entirely sequenced. We selected wild types as a control and diseased fish. Fish showed obvious damages as non-pigmented areas with raised or lost scales and dermal thickening. DNA was extracted from both the tumour site as well as the normal tissue. Since the nf1 gene has never been described in goldfish, we employed heterologous primers based on zebrafish nf1 gene in order to obtain an amplicon. Zebrafish nf1 gene has been properly described, whereas no literature has been shown related to goldfish homologue DNA. Firstly we identified the NF1 gene in goldfish and compare it with the zebrafish homologues, correlating among mutations and characteristics of neurofibromatosis. Later, the sequences with mutations within the goldfish gene were compared with the normal genotypes in order to identify nucleotide changes not linked to specific species-related markers through goldfish and zebrafish. The comparison of protein sequences of the goldfish gene on GeneBank revealed amino

acid changes following nucleotide substitutions in the positions 2279, 2289, 2293 and 2294. This work suggests that mutations of nf1 gene may be involved in the loss of function of neurofibromin protein. Because the gene plays a negative regulation on cell proliferation by inactivating Ras proto-oncogene it could be hypothesized the same molecular mechanism in goldfish. Gene expression test targeted to Ras in *C. auratus* with mutated nf1, could confirm the oncogenetic mechanism. Actually, we conclude that the founded mutations for nf1 genes could give the same models demonstrated for zebrafish.

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NON-HUMAN PRIMATE MODEL OF TYPE 2 DIABETES MELLITUS: CEREBRAL EXPRESSION OF GLUCOSE TRANSPORTERS GLUT1 AND GLUT3 AND ALTERATIONS ASSOCIATED WITH INSULIN RESISTANCE AND COGNITIVE IMPAIRMENT

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There is a large body of evidence documenting the cognitive impairment associated with type two diabetes mellitus (T2DM). These impairments range from mild amnestic effects to almost Alzheimer's Disease-like dementia, but the underlying mechanisms are unknown. Hence, the Insulin Resistance Hypothesis, which theorizes that there is a significant role for insulin in normal cognitive functioning and that T2DM-related insulin dysregulation may contribute to cognitive impairment. For the present study, 15 aged female Rhesus macaques were investigated to evaluate aspects of the insulin resistance hypothesis using a three-pronged approach: confirm body composition changes with physiological testing; gauge cognitive capability using a Novel Object Recognition (NOR) task; and measure expression levels of two glucose transporters, GLUT1 and GLUT3, in the brain regions associated with NOR-related learning and memory.

Body composition and physiological changes consistent with insulin resistance, characterized by hyperinsulinemia and euglycemia were confirmed. In particular, a strong correlation has been observed between abdominal fat, body weight and insulin resistance. More specifically, omental fat weight correlated positively with Intravenous Glucose Tolerance Test (IVGTT) mean insulin levels, indicating that animals with more omental fat have higher insulin levels.

Many physiological parameters correlated positively with NOR task performance such that degree of disease progression predicted poorer cognition in primates with altered glucose metabolism, in particular pronounced insulin resistance. We found both positive and negative correlations among physiological parameters and the expression levels of the glucose transporters. Clear associations are between GLUT1 and GLUT3 immunohistochemical expression levels in brain regions of interest (hippocampus and

entorhinal cortex) and increased body fat and insulin resistance. However, NOR performance did not correlate significantly with the expression of glucose transporters. Overall, our results provide some support for the insulin resistance hypothesis of T2DM-mediated cognitive deficits, although deficits do not appear to be mediated through glucose transporter-regulated mechanisms.

NEUROAXONAL LEUKODYSTROPHY IN THREE CHIHUAHUAS

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Neuroaxonal dystrophy (NAD) is a neurodegenerative disorder characterized by severe degeneration of neuronal cells and their processes. The predominant neuropathological feature is the presence of a large number of spheroids in the CNS.

The aim of this study is to describe the neuropathological findings in 3 cases of NAD in Chihuahua puppies. Two 2-month-old male Chihuahuas from the same litter and a presumably not related 2-month-old female Chihuahua were presented with neurological signs including severe depression, tetraparesis, ataxia, absence of menace response and bilateral strabismus. Post-mortem examination revealed lesions restricted to the CNS in all cases. Grossly there was moderate to severe dilation of lateral ventricles accompanied by atrophy of the cerebral cortex and flattening of the cerebral convolution, as well as cavitation of the subcortical white matter, thinning of the corpus callosum and rupture of the septum pellucidum. Coronal samples of fixed brains were routinely processed for histology and sections were stained with H&E, Luxol Fast Blue, CNPase, neurofilaments and GFAP. Histopathological examination revealed marked and widespread axonal swelling with formation of round to irregularly shaped spheroids, accompanied by gliosis and severe myelin loss. The lesions primarily affected the white matter in the cerebrum and cerebellum, and both white and gray matter in the medulla oblongata, pons and spinal cord. Spheroids were numerous and large in the white matter of the cerebrum, cerebellar medulla, and several nuclei of the brain stem including lateral cuneatus, spinal tract of trigeminal nerve, olivary, solitary tract, lateral lemniscus, cochlear, trapezoid body, and lateral and medial geniculate. The presence of spheroids was moderate in the pontine nuclei, transverse and longitudinal pontine fibers, caudal colliculi and periaqueductal grey matter. A moderate number of spheroids was found in the cerebellar nuclei and in the nucleus of vagus. Spheroid of smaller caliber were found in the cerebral and cerebellar cortices. Scattered spheroids were evident in the reticular substance of the medulla oblongata and pons. Segmental loss of Purkinje cells was observed in all cases, accompanied by cytoplasmic vacuolation in one case.

The lesions observed in our cases were consistent with a form of NAD. In canine NAD, dystrophic axons are mainly found in the grey matter of the sensory brainstem nuclei, accompanied by cerebellar atrophy. In Chihuahuas and Papillons spheroids are also described in the white matter and in the cerebral and cerebellar cortices. Our cases differed from previous reports showing severe cerebral atrophy and high involvement of cerebral and cerebellar white matter with spheroids accumulation, while cerebellar atrophy was limited to mild loss of Purkinje cells. Findings in our dogs resembled Hereditary Diffuse Leukoencephalopathy with Spheroids described in adult humans.

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RISK FACTORS OF GASTROINTESTINAL PARASITES LUNGWORMS TICKS AND LICE IN DONKEYS IN THE ASINARA NATIONAL PARK (SARDINIA - ITALY)

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From June to November 2015 a total of 113 Asinara donkeys (41 albino, 72 coloured) were sampled (91 fecal samples from 36 albino and 55 grey donkeys). All donkeys were surveyed for ticks and lice. Sedimentation, Baermann and modified McMaster methods were performed for endoparasites. The EPG/OPG were calculated. Larval cultures were performed and L3 were recovered by a Baermann technique. Ectoparasites were morphologically identified. The infection's ticks level were recorded defining 3 categories: no infestation, low (1-10 ticks) and high infestation (>10 ticks). Three land cover types were defined to estimate the risk: sparse vegetation; mediterranean shrubland; grassland. Statistical analysis were performed through GLM with a ordinal logistic regression (SPSS 20.0, Chicago, IL). Ninety out of ninety-one donkeys were infected by intestinal strongyles (98.9%), *Strongyloides* (6.6%), *Parascaris equorum* (15.4%), *Oxyuris equi* (2.2%) and *Eimeria leukarti* (2.2%). No eggs of cestodes and trematodes were found. *Dictyocaulus arnfieldi* L1 were found in 46.1% of samples. Fecal pools were positive for *Cyathostominae* (61%), large strongyles (30%) and *Trichostrongylus axei* (9%) L3. Strongyles showed the highest egg excretion (mean abundance=1176.4 EPG; min-max=0-4575 EPG). Significant risk factors associated to strongyle infection (EPG) were: season; geographical distribution of herds and the land cover types. Egg shedding was 10.887 times higher in autumn than in summer and 2.865 times higher in donkeys from the North than those in the rest of the island. Donkeys from spare vegetation areas shed more eggs than other animals (OR=2.507). Albino and young donkeys were more at risk for *P. equorum* than coloured and old donkeys (OR=4.289 and OR=0.978 respectively). *D. arnfieldi* larvae shedding was higher in autumn than in summer (OR=5.577). *Haemaphysalis punctata* (46.2%), *Hyalomma marginatum* (10.7%) and *Rhipicephalus bursa* (43.1%) were found. A total of 58.4% (66/113) of donkeys were infested by ticks

(28.3% albino; 30.1% coloured). The prevalence was 78% (32/41) and 47% (34/72) respectively in albino and coloured donkeys. Albino donkeys group had the highest percentage with high infestation (39% vs 15%; OR=2.865; P=0.021). The highest percentage of donkeys with no ticks (57.77%) were from land with "sparse vegetation" and had a low number of ticks (OR=0.185; P=0,001) than donkeys from other areas. *Haematopinus asini* were found on nine donkeys (8%), 8 albino and 1 coloured (OR=17.212, 95% CI 2.067-143,321, P= 0.009). Significant risks to tick infestation were associated to the colour of coat and the types of land cover. Albino donkeys show a 3.120 times higher risk than coloured donkeys to be infected by ticks. Donkeys from areas with sparse vegetation cover showed a lower risk to be infected by ticks (OR=0.227).

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HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES OF PARASITE ASSOCIATED GRANULOMA DEVELOPMENT IN VISCERAL ORGANS OF GREY MULLETS (*OSTEICHTHYES*: *MUGILIDAE*) FROM SARDINIAN LAGOONS

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Grey mullets (*Osteichytes: Mugilidae*) are cosmopolitan fish that inhabit estuaries and lagoons and are particularly important in the fisheries and economy in particular Sardinian areas. Parasitic diseases have been found to be the main health problem in these populations (1). Fish immune response against these parasites is mainly represented by chronic granuloma development (2). The aim of this work was to describe the structure of parasitic granulomas and their temporal progression in visceral organs of grey mullets by histological and immunohistochemical techniques.

A total of 239 grey mullets were collected from four different Sardinian lagoons (western Mediterranean Sea) in two seasonal samplings. Fish were euthanized (Tricaine Methanesulfonate) and a complete necropsy was performed. Samples of visceral organs, where macroscopic granulomas were detected, were processed for histological examination, stained with Hematoxylin-eosin (HE), Masson's Trichrome (MT) and investigated by immunohistochemical techniques using anti-cytokeratin AE1/AE3 and anti-Vimentin antibodies. Quantitative assessment of epithelioid cells, fibroblasts and collagen component of granulomas was performed with a semiquantitative grading score system, whereas rodlet cells (RCs) and eosinophilic granular cells (EGCs) were quantified with an image analysis software (Rasband, W.S., ImageJ). Microscopical features of the lesions were analysed using Stata 11.2 software (StataCorp LP).

Histopathological examination revealed two groups of different granuloma categories according to the aetiological agent (digenean trematodes or *Myxosporea*). Granulomas associated to metacercariae of digenean parasites revealed a higher number of EGCs

(ρ=0.5197, P<0.05), whereas granulomas due to spore of *Myxobolus* sp. were significantly associated with a higher number of RCs (ρ=0.4296 , P<0.05).

Three developmental stages were identified during the evolution of the granulomas on the basis of common histopathologic features in both parasitic groups. Early stage granulomas were characterised by an intact parasite and minimal inflammatory response. In the intermediate stage granulomas, epithelioid cells (CK AE1/AE3 positive) were evident and represented the most characteristic cells. In late stage, fibroblasts (Vimentin positive) were noticed in large numbers in the outer portion (capsule) of granulomas. At this stage, collagen fiber development showed a significant correlation with the presence of EGCs (ρ=0.4707, P<0.05).

The immunitary response of *Mugilidae* to different classes of metazoan parasites seems to display a low specificity but, even if characterised by a common encapsulation mechanism, some differences were identified in the cell composition and associated inflammation development.

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MOLECULAR EXPRESSION OF GENES RELATED TO WNT/BETA-CATENIN AND HIPPO PATHWAYS IN CANINE AND FELINE MAMMARY TUMORS

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Mammary tumor is the most common cancer and the third most frequent cancer in dogs and cats, respectively. To date is well known that several molecular pathways, such as Wnt/beta-Catenin and Hippo, involved in cell proliferation, tumor progression and metastasis were altered in human breast cancer. On the contrary, there is only few information concerning alterations of these pathways in canine and feline mammary tumors. The aim of the present study was to investigate the expression of several genes directly or indirectly associated with these pathways, such as beta-Catenin, TAZ, YAP, CTGF, ANKRD1, and CYCLIND1 in canine mammary tumor and corresponding non-tumor tissues. Specifically, RNA from 17 canine mammary tumors and healthy tissues was extracted and subsequently, reverse transcribed into cDNA. A semi-quantitative PCR was performed in order to assess the expression level of beta-Catenin, TAZ, YAP, and CTGF. Expression level of these genes was quantified by densitometric analysis using Image J. The expression of beta-Catenin, TAZ, YAP, and CTGF were significantly ($P<0.05$) higher in canine mammary tumors than in non-neoplastic tissues. These results indicates for the first time neoplastic deregulation of the beta-Catenin and Hippo pathways in canine mammary tumors as in human breast cancer. Because feline mammary carcinomas are, in more than 80% of cases, malignant, aggressive, and associated with rapid development of metastasis we extended the study also in 6 feline mammary tumors and 6 healthy tissues. The obtained results were in agreement with those obtained in canine mammary tumors, with a greater difference in the expression level of beta-Catenin between tumor tissues and healthy tissues, probably due to the greater aggressiveness of feline mammary tumors. If this represents a scientifically valid study for dogs and cats, with direct implication in veterinary medicine, it also has implications in comparative oncology with a potential social and economic beneficial impact. Domestic animals represent an interesting model for human cancer.

DIAGNOSTIC RESULTS ON THE POPULATION OF ROCK-PARTRIDGE (*ALECTORIS GRAECA WITHAKERI*) DURING THREE YEARS LIFE PROJECT IN WESTERN SICILY.

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Sicily (Italy) hosts a "relict", endemic population of *Alectoris (graeca) whitakeri*, commonly known as Sicilian Rock partridge (Lucchini & Randi, 1998;). Today, *A. graeca* is included in the IUCN Red List rating of vulnerable species (IUCN, 2012). Recently, in order to improve the residual population, an EU funded Life Natura 2000 project (2011-2012; SICALECONS: "Azioni urgenti per la conservazione della coturnice di Sicilia") has been founded, involving IZS of Sicily for welfare and veterinary aspects. Fifteen Sicilian Rock Partridge (*A. (g) whitakeri*) carcasses were collected. All birds were identified by presumed age and sex. Necropsy was performed for each carcass for laboratory investigations. Additionally, fecal samples collected from wild birds were also screened to identify pathogens carriage as a risk for this specie. For every sample were performed an oropharyngeal, cloacal and oculo-conjunctival swabs. Tissues of suspected lesions were collected from bird carcass and fixed in 10% buffered formalin for standard histological investigations; for putative fungal infection some sections were also stained by PASM (Periodic Schiff-Methenamine). Samples from suspected lesions were also subcultured by standard procedures. The presence of internal parasites was investigated microscopically by direct mount examination through preparation of smears taken either from fecal samples and from 3 portion of the intestine (duodenum, jejunum and caecum). External and internal parasites were also collected and fixed in 70% (v/v) alcohol for future identification. Prevalence rates and intensities value of each parasite species found was calculated for every sample, then for each specimen. Necropsy involved no. 9 females and no. 6 males. Almost all birds showed ematiation and ruffled feathers, and in 4 cases *Goniodes colchici* was found. Mucosal swabs showed in one single case a pathogen strain

of *E. coli* related to granulocytic lesions in liver. Another case of death was due to nodular lung lesions caused by infiltration of *Aspergillus fumigatus* hyphae. The other oropharyngeal, cloacal and oculo-conjunctival swabs were negative for pathogenic bacteria. The evidence of internal parasites was the most relevant finding, showing different types of infestation by nematoda as *Ascaridia compar*, cestoda as *Railletina tetragona* and 4 species of coccidia as *Eimeria legionensis*, *E. caucasica, E. kofoidi* and *Isospora* spp.. Furthermore in 60% of these cases a concomitant pluri-parassitosis was found. Weak body condition have been associated only in two cases to chronic and lethal bacterial or fungal infections. This study represents the first veterinary report on this rare species and underlines the importance to monitor the health status of wild species in the Italian environment in order to preserve local biodiversity.

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EFFECTS OF 17B-ESTRADIOL AND OXYTOCIN RECEPTOR ANTAGONIST IN MURINE SKELETAL MUSCLE

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Although estrogens (E) are considered to participate in the development of the skeletal muscle phenotype, their role is still poorly understood (1). Recently, the oxytocin role (OXT) in muscle regeneration was demonstrated (2). An unexpected induction of oxytocin precursor gene (OXT) in bovine skeletal muscle of estrogen treated animals has been previously described (3-4). A potential cross-talk between skeletal muscle and adipose tissue through adipomyokines activity has been proposed (5).

Aim of the work was to evaluate the possible hypertrophic effect of 17β-estradiol (E) on murine skeletal muscle and OXT role in muscle and adipose growth. In order to evaluate the OXT role in skeletal muscle the competitive oxytocin receptor antagonist (Atosiban, OTA) was employed.

C57BL male mice were randomly divided into groups and orchidectomized except for 5 animals that were sham operated (placebo surgery). Animals were treated with subcutaneous 21-day release pellets as following: group S: sham+placebo pellet (n=5); group K: control with placebo (n=5); group A: 2mg E pellet (n=6); group B: 2mg E + 1.2mg OTA pellet (n=6); group C: 1.2mg OTA pellet (n=6). Procedures were authorized by D.M. 182/2010.

At euthanasia blood sample was collected to perform ELISA test for OXT on plasma. Vastus lateralis (VL) muscle and perirenal white adipose tissue (pWAT) were collected for morphometric analysis, for gene expression studies of OXT, OXTR, myogenic regulatory factors, myosin heavy chain 1 and 2 (MhC1 and 2), atrogin1, insulin-like growth factor 1 (IGF1), adipokines genes and peroxisome proliferator-activated receptor γ (PPAR γ) by quantitative PCR.

No differences were highlighted regarding body weight or plasma OXT concentration between groups. Morphometric analysis of VL muscle did not reveal any fiber size change. Gene expression data on VL muscle showed the up-regulation of OXTR (6.7±3.3 fold increase) in group A ($p<0.05$) compared with K and of MhC1 and fatty acid binding

protein 3 in group B (2.1±0.9 and 2.2±1.1 fold increase respectively, $p<0.05$). Gene expression data on pWAT described a significant decrease of adiponectin, leptin and PPAR γ expression in all treated groups compared with K ($p<0.0001$ in A and B, $p<0.001$ in group C). Contrary to the cattle, E does not induce OXT serum increase in mice, but still increases mRNA OXTR expression in muscle. The results confirm that E exerts effects on several adipokines and in particular represses adipogenic differentiation in pWAT (6, 7) and promotes fatty acids metabolism. Those effects are more intense in group B and are still present in group C supporting that OTA is an antagonist still able to activate some pathways of OXT metabolism (8).

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DIFFERENT APO A-IV TISSUE PROTEIN EXPRESSION PATTERNS IN APOA4 GENE INDUCIBLE AND NOT INDUCIBLE SPECIES AND ITS IMPLICATIONS IN LIPID METABOLISM

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Apolipoprotein (apo) A-IV is a protein known to participate in the regulation of various metabolic pathways, with special regards to lipid absorption, transport and metabolism. Only rodents can synthesizes apo A-IV by both liver and small intestine, whereas its synthesis is restricted to the enterocytes in the other species, human included (Wu, 1979). A study demonstrates apo A-IV role in mouse liver in enhancing triglyceride secretion and reducing hepatic lipid content by promoting very low density lipoprotein particle expansion (VerHague, 2013). In a previous study we demonstrate an inverse proportion between hepatic lipid content and apo A-IV liver expression in *Felis catus*, *Felis silvestris* and *Rattus norvegicus*, highlighting differences between cat and rat lipid metabolism (Piccinini, 2015).

In this study, the apo A-IV protein expression and tissue distribution patterns in domestic, wild cat and rat (n=5) were analyzed. Anamnestic data were collected to exclude any interfering pathology. Histological sections of intestine, liver and adipose tissue were analyzed by immunohistochemistry to assess apo A-IV tissue expression levels and protein cytolocalization.

Significant differences in apo A-IV intestinal levels between cat and rat samples were observed. Specifically, feline enterocytes showed low cytoplasmic apo A-IV protein expression, in contrast to the rat group. Rat liver revealed a marked cytoplasmic immunopositivity, with weak nuclear signal. In contrast, felids hepatocellular signal was weak in cytoplasm and marked in the nucleus membrane. Nuclear apo A-IV signal was also observed in adipocyte from all species samples.

Feline apo A-IV intestinal and cytoplasmic liver low levels are strictly related to the previously observed high hepatic lipid content. In addition, apo A-IV hepatocytes nuclear signals are related with the gluconeogenesis suppression mediated by nuclear receptors. In conclusion, these results confirm apo A-IV "key role" in lipid metabolism, suggesting new interesting hypothesis on the physiopathology of feline hepatic lipidosis and new possible therapeutic strategies in lipid disorders, also in other species, human included.

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MUTATIONS AND POLYMORPHISM IN ALBUMIN GENE OF BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*): FIRST IDENTIFICATION OF MUTATIONS RESPONSIBLE OF INHERITED BISALBUMINEMIA

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Hereditary bisalbuminemia is an asymptomatic and heterozygous condition, characterized by the presence of 2 different albumin fractions with different electrophoretic mobility that leads to typical electrophoretic pattern with double albumin peak (1). In human medicine, is usually revealed by chance, is not been clearly associated with a specific disease and the causative genetic alteration is a point mutation of human serum albumin gene inherited in an autosomal codominant pattern (2). Previously, bisalbuminemia was diagnosed by capillary zone electrophoresis in two Bottlenose dolphin's families (3), but mutations responsible of this condition and the inherited pattern were not identified. The aims of this work are: 1) to investigate polymorphism of Bottlenose dolphins' albumin gene and to identify possible mutations responsible of bisalbuminemia, 2) to identify the inherited pattern of this condition in two Bottlenose dolphin's families. Coding regions of albumin gene were screened for mutations in 15 Bottlenose dolphins kept under human care (9 from family 1, 4 of them bisalbuminemic; 6 from family 2, 3 of them bisalbiminemic) using PCR on DNA extracted from peripheral blood and tissue, and the sequence were compared to the reference sequence to identify DNA alterations. In order to identify mutations able to cause bisalbuminemia, for each non-synonymous variation identified, we studied the genotype-phenotype correlation within the two families. Eighteen albumin mutations (3 synonymous and 15 non-synonymous) were identified in the 2 families. The non-synonymous variations were identified in exon 4, 5, 6, 7, 8, 9, 10, 12 and 13. Based on the genotype-phenotype analysis we identified two heterozygous non-synonymous

variations that co-segregate with bisalbuminemic phenotype: p.Phe146Leu in exon 4 and p.Tyr163His in exon 5. The amino acid change in mutation in exon 5 seems to cause a variation into the secondary and /or tertiary structure of the albumin protein and it's already describe as causative mutation of bisalbuminemia in human beings (4). Our study confirmed that, as human albumin gene, Bottlenose dolphins' albumin possessed a significant degree of polymorphism and we identified 2 mutations potentially responsible of bisalbuminemia. Moreover, we confirm the autosomal codominant trait of this condition also in these animals.

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HEART VALVE PATHOLOGY IN WILD-CAUGHT SWORDFISH (*XIPHIAS GLADIUS*)

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The heart of marine teleosts can be affected by several infectious and parasitic diseases. Little is known about cardiac pathology and, in detail, investigations on heart valve diseases of Swordfish (*Xiphias gladius*) have never been reported.

Aim of this work is to evaluate gross and histopathological lesions of heart valves of wild-caught swordfish hearmarked for human consumption.

Thirty-sevenhearts of swordfish, aged between 3 and 13 years, caught in the Ligurian Sea (Italy), were referred to the Department of Veterinary Sciences of the University of Turin. Gender of the animals was determined by macroscopic observation of the gonads. The age was estimated by counting the bands of skeletal growth on the anal fin spines. Serial sections 1.0 mm thick of the condyle base were obtained, dried for 24 hrs, observed with a stereomicroscope, and the number of rings was counted to assign an estimated age. Heart samples were collected directly on boats and stored in 4% buffered formalin for gross and histopathologic investigations, stained with Haematoxylin-Eosin, Weigert-Van Gieson, Periodic acid-Schiff, Toluidine blue, and Alcian blue PAS.

Gross evaluation of the hearts showed in 34 out of 37 animals (91.9%) chronic pericarditis and in 15 (40.5%) and 8 (21.6%) cases a valvular thickening respectively of the atrio-ventricular and bulbo-ventricular valves. Histological examination of the atrio-ventricular leaflets revealed in 27/37 (73%) Lambl's excrescences; in 20/37 (54%) fibrosis grading 1/3 and 2/3 in 10 subjects; in 5/37 (13.5%) endocardiosis grading 1/3 and 2/3 respectively in 2 and 3 animals. Ichthyophoniasis (n=1; 2.7%), endocarditis (n=1; 2.7%) and lymphocytic infiltration (n=1; 2.7%) were also detected.

The bulbo-ventricular leaflets showed Lambl's excrescences in 14 out of 37 animals (37.8%); fibrosis in 10/37 animals (27%; n=2 grading 1/3; n=8 grading 2/3), and endocardiosis in one case (2.7%).

Gross and histopathological findings in atrio-ventricular and bulbo-ventricular valves of swordfish demonstrated that microscopic evaluation is necessary to identify lesions not always macroscopically visible. Moreover the atrio-venticular valves revealed a higher number of lesions compared to the bulbo-ventricular ones.

Swordfish heart valve pathology shows interesting findings which should be considered for comparative pathology.

Special attention shall be addressed to identify environmental factors possibly responsible for the onset of cardiac pathology in fish.

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XIV Convegno

S.I.R.A.

LH RECEPTOR (LHR), STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR) AND MITOCHONDRIAL MEMBRANE POTENTIAL IN BOVINE GRANULOSA CELLS ARE RELATED TO FOLLICLE SIZE AND ATRESIA GRADE

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Granulosa cells (GC) play a key role in creating a suitable environment for the growth and the maturation of the oocyte as well as in determining the proper endocrine conditions for breeding and fertilization. However, only the GC of the ovulatory follicle have the opportunity to fully provide these tasks. Cumulus-oocyte complexes (COCs) used for in vitro embryo production (IVEP) are collected from follicles whose GC have not fully acquired or have lost these functions. This may be a reason of low IVEP efficiency. LH receptor (LHR), Steroidogenic Acute Regulatory protein (StAR) and Mitochondrial Membrane Potential (MMP) are direct or indirect markers of endocrine functions and putative candidates for evaluation of follicle quality.

This study is aimed at evaluating the quality of GC of bovine ovarian follicles, classified according to their size and atresia grade, in order to provide new information to clarify the poor IVEP success.

Bovine ovaries were collected from abattoir and transported to the lab at 4°C. Follicles were dissected, measured and classified according to their atresia grade (Kruip and Dieleman, 1982). The collected COCs were morphologically classified according to criteria related to follicular atresia (Boni et al., 2002). GC were obtained by scraping the follicular wall and filtered on a 50 µm nylon mesh. For each follicle, a part of GC was fixed with 2% paraformaldehyde for 1h. The remaining part was incubated with 5µM JC1 for 30 min followed by washing and reading with a spectrofluorometer (ex. 490 nm, em. 510 to 650 nm). Negative control samples were treated with 2 µM CCCP for 1 h before reading. The MMP values were expressed as the ratio between the fluorescence peaks at ~595 and ~525 nm. In fixed cells, immunofluorescence was carried out after treatment with blocking solution (20% Sea Block blocking buffer in PBS) with either anti-LH receptor

antibodies (K-15) or anti-StAR antibodies (K-20) at 1:200 dilution for 90 min. After washing twice with TPBS (PBS + 0.05% Tween), the samples were incubated with secondary FITC-conjugated anti-goat antibodies. The samples were read at fluorescence microscope and the fluorescence intensity analyzed by ImageJ. Statistical analysis was carried out by ANOVA (Systat 11.0).

Follicle size negatively affected ($P< 0.01$) the MMP as well as the expression of both LHR and StAR. Also the atresia grade, when evaluated on the basis of COC morphology, negatively influenced ($P< 0.01$) the expression of both LHR and StAR but positively influenced ($P< 0.01$) MMP. The evaluation of atresia grade on the basis of follicle morphology did not show significant effects on both LHR and StAR expression. These results highlight a discrepancy between the morphological characteristics of the follicle/COC and functionality of the GC, as previously demonstrated between COC quality and IVEP efficiency (Boni et al., 2002). Whereas the evaluated parameters represent markers of the steroidogenic activity, it is likely that the mechanisms of follicular regression pass through an upregulation of the GC metabolic activity.

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CADMIUM-INDUCED OXIDATIVE STRESS IN SHEEP HEALTY-APPEARING MATURED CUMULUS-OOCYTE COMPLEXES. A NON-INVASIVE TEST OF OOCYTE TOXICITY

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Several chemicals affect oocyte maturation (MR) and fertilization rates (FR) [1], particularly at low doses. To date, no tests are available to distinguish, by a non-invasive approach, between healthy and toxic matured oocytes to use for assisted fertilization. The aim of this study was to analyze the oxidative status of cumulus-oocyte complexes (COC) exposed during in vitro maturation (IVM) to Cadmium (Cd), a heavy metal of concern for human and animal health, in order to establish a rapid, non-invasive oocyte toxicity test related to its competence. Preliminarily, slaughterhouse-derived ovaries from adult (a, 2-8 years) and juvenile (j, <6 months) sheep, were used for assessing Cd exposure levels in ovarian stroma by mineralization and inductively coupled plasma/mass spectrometry and to collect COCs. Significantly higher Cd levels were found in a- versus j-tissues (27.8±4.9 vs 1.7±1.7 ng/g; $P<0.0001$), indicating age-related ovarian bioaccumulation in the range of nanomolar concentrations. COCs were exposed during IVM to 1 or 100nM CdCl2. Non-exposed COCs were used as controls (CTRL). Partially decumulated COCs, with the 1st polar body, were destined to 1) in vitro fertilization (IVF) or 2) oxidative status assessment by laser scanning confocal microscopy [2]. Intracellular reactive oxygen species (ROS) were analyzed on oocytes (OOs) and cumulus cells (CCs) after staining with 2',7' dichlorodihydrofluorescein diacetate [2]. Data from 3 replicates with a-COCs and 7 replicates with j-COCs were analyzed and are expressed for 1nM, 100nM and CTRL, respectively. Cadmium exposure

did not affect oocyte MR, neither in a-OOs (31/44, 70%, 20/44, 45% and 32/49, 65%: Chi-square test: P>0.05) nor in j-OOs (65/118, 55%, 66/124, 53% and 69/114, 60%: P>0.05) but it reduced FR both in a-OOs (2/26, 8%, 2/21, 9% and 10/27, 37%: P<0.05 at both doses) and j-OOs (17/102, 17%, 17/106, 16% and 29/93, 31%: P<0.05 at both doses). In both a-OOs (n=27, 7-10 oocytes/group) and j-OOs (n=68: 20-26 oocytes/group), Cd exposure increased ROS levels, expressed as arbitrary densitometric units (ADU). In fact, a-OOs displayed 403.3±52.9, 652.4±85 vs 281.1±45.7 ROS values (One-way ANOVA with Dunnett's post-hoc test: P<0.05 at both doses) and j-OOs showed 651.9±203.5, 730.6±300.3 vs 473.2±199 ROS values (P<0.05 and P<0.01 at 1 and 100nM, respectively). As well, in both a-CCs (n=12-18 fields of 50 CCs/each per group) and j-CCs (n=212: 64-79 fields of 50 CCs/each per group), ROS levels increased upon Cd exposure and were 163±69.7 and 231.3±41.4 vs 125.5±34 in a-CCs (P<0.001 at 100nM) and 334±210.6 and 300.9±199.9 vs 168.5±87.8 in j-CCs (P<0.001 at both doses). In conclusion, in vitro Cd exposure at naturally occurring levels induces oxidative stress in matured healthy-appearing a/j COCs and this effect may underlie their reduced FR. Confocal imaging of this oxidative stress biomarker in CCs of matured oocytes can be proposed as a predictive non-invasive test of oocyte toxicity.

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SECRETOMA DERIVED FROM AMNIOTIC PROGENITOR CELLS OR ENDOMETRIAL CELLS IN BOVINE IN VITRO EMBRYO PRODUCTION

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It is known that in vitro produced embryos are of poorer quality when compared to in vivo ones due to several stresses of the in vitro conditions that they do not experience in vivo. A direct result of in vitro stress is the impairment of embryo structure, gene expression and development (Gardner and Lane, 2005). Co-culture systems are largely employed in in vitro embryo production (IVP) to obtain embryos with a higher number of blastomeres and a better implantation rate. This suggests that paracrine mechanisms of communication exist between "helper" cells and embryos. It is reported that use of conditioned media in toto (CM), secreted by cells during their culture, brings to results similar to co-culture systems. CM is composed of microvesicles (MVs) and soluble factors that are present in the supernatant (SN) after removal of MVs. The effect of different components of secretoma has not yet been tested before in IVP. Aim of this study was to understand the role of CM, MVs and SN secreted by bovine endometrial and amnion derived cells on IVP embryo rate and quality.

Embryos were produced from 2.820 oocytes. Presumptive zygotes were randomly transferred in synthetic oviductal fluid with aminoacids (SOFaa)and used as control (CTR) or cultivated, by adding at day 5 post fertilization, 10% of endometrial or amniotic CM or SN or 100x106 MVs/ml. The embryo quality was evaluated at day 7 (blastocyst stage), counting the number of viable blastomeres and the number of cells of the inner cell mass (ICM) by Hoechst 3342 and propidium iodide staining. Uptake of MVs, labeled with PKH-26, and quality of embryos were analyzed by fluorescence microscopy.

Results show that MVs are internalized in the blastomeres. The blastocyst rate is 35.45±2.53% in CTR. Amniotic CM and MVs provide 34.17±3.29% and 32.82±3.26% blastocysts, without statistical difference between them and CTR. The rate obtained by amniotic SN is 25.80±2.83%, statistically lower ($P<0.05$) than the other groups. All the

results obtained by endometrial CM, SN or MVs are lower than amniotic secretoma and CTR (21.69±1.87, 13.70±2.05 and 27.16±1.92% respectively). Among different components of secretoma in both cell line, MVs provide results equivalent or better than CM in toto.In terms of quality, the ICM number in CTR group is 27.6±1.44. The groups cultured with amniotic CM and MVs have values statistically higher ($P<0.05$; 30.4±1.83 and 29.42±1.27, respectively) compared to CTR and SN (25.33±2.54). The ICM number in endometrial CM, SN and MVs is 25.41±1.03, 23.29±1.11 and 25.98±1.14, respectively, that are statistically lower ($P<0.05$) than ICM values of CTR and amniotic secretoma. In addition, the ratio ICM/trophoblast is enhanced with amniotic CM and their MVs compared to CTR and the other groups. Our data show that amniotic CM and MVs result in a blastocyst rate comparable to the CTR, but with a better embryo quality. The hypothesis is that mainly amniotic MVs' cargo might provide a more resourceful environment for the embryos compared to the endometrial one.

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EVALUATION OF TESTES INTERSTITIAL CELL TUMORS USING CONTRAST-ENHANCED ULTRASOUND (CEUS) IN CONSCIOUS DOGS – PRELIMINARY RESULTS

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Testicular tumors represent more than 90% of all canine male genital tumors (1). According to the World Health Organization classification, the main three types reported in dogs are Interstitial Cell Tumor (ICT), Seminomas (SEMs) and Sertoli Cell Tumor (SCT)(2). In a previous study on 232 tumor-affected dogs ICT was the most common tumour identified(3). Examination of the testes with B-mode and Doppler ultrasound allows to explore their parenchyma morphology and provides up-to-date information also about possible lesions or vascularity. Contrast-Enhanced Ultrasound (CEUS) allows to characterize perfusion of testicular lesion and hyperenhancement could be an important feature in the diagnosis of malignancy(4). Since the previous data derived from sedated dogs, the aim of this work is to evaluate testicular ICT testes with CEUS in four conscious dogs. B-mode, Doppler and CEUS were performed. The device was a Mylab 40 Vet (Esaote, Genova Italy), contrast agent (Sonovue®, Bracco, Milan, Italy) at dosage of 0,03/0,04 ml/kg was injected in a cephalic catheter of 20 G followed by a saline flush of 5 ml. A clip of 2 minutes was recorded and the enhancement patterns of testes were described. Focal lesion was classified in hyperenhancing, isoenhancing or hypoenhancing compared to the surrounding testes tissue. Presence of heterogeneity, rim enhancement or prominent inner vessels was also evaluated. Quantitative analysis was performed with a dedicated software (Qontrast®, Bracco, Milan, Italy) to evaluate Peak Intensity (PI %) and Regional Blood Flow (RBF). ROIs of same dimension and depth were drawn in focal lesion and surrounding tissue. After orchiectomy, testes were submitted to histological evaluation. The four conscious dogs presented similar features in CEUS: hyperenhancing of lesion (heterogeneity, rim enhancement and inner vessels), which corresponded to histological diagnosis of ICT. Quantitative analysis provides higher PI and RBF in lesions than surrounding tissue. Mean PI% in lesions was 14.55 ±

5.5 (range 7.4 – 19.0); in non-pathologic tissue was 9.1 ± 4.4 (range 4.2 – 14.7). Mean RBF in lesions was 15.3 ± 7.3 (range 7.7 – 22.4) and 8.7 ± 4.8 (range 3.5 -14.1) in surrounding tissue. In human medicine, CEUS helps to differentiate ICT from other testicular tumors(5). This is the first study using CEUS in conscious dogs. Hyperenhancing and heterogeneous lesions were observed both in ICT as well as in SCT (4). With regard to the influence of anaesthesia on perfusion parameters, our results on non-sedated dogs show similar values to those reported in sedated dogs although the number of cases is still too low to confirm our hypothesis.

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POSSIBLE PRESENCE OF AN AMH-IMMUNO-LIKE PROTEIN IN THE INTERSTITIAL CELLS OF EUTOPIC SCROTAL TESTIS OF PREPUBERTAL UNILATERAL CRYPTORCHID SWINE

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The anti-Müllerian hormone (AMH), a homodimeric cytokine of the transforming growth factor-β superfamily, is synthesized in the gonads of all vertebrate species examined to date (1). AMH plays crucial roles in sexual differentiation and gonadal functions of both sexes. In mammal males, AMH is expressed by the testis Sertoli cells, triggers the regression of the Müllerian ducts during the early fetal life, modulates the gonadal function during the postnatal life up to puberty, and has extra-gonadal effects on the hypothalamic-pituitary-gonadal axis (2). In mammals, circulating AMH appears to be entirely derived from the gonads and its concentrations have been used to assess several testicular pathologies (3) as in hemi-castrated unilateral cryptorchid horses (4). Since the possible role of AMH as an endocrine marker of cryptorchidism in boars is unexplored, the aim of this study was to investigate the gene and protein expression of AMH in testes of neonatal and prepubertal unilateral cryptorchid swine at different ages.

Cryptorchid and contro-lateral normally descended testes of neonatal (8 days, n = 4) and prepubertal unilateral cryptorchid swine of different age (2, 3, and 5 months, n = 12) were used. Immunohistochemical investigation was performed using two different commercial polyclonal anti-AMH swine specific primary antibodies. Western blotting was performed using the same immunohistochemistry primary antibodies. AMH gene expression was evaluated by RT-PCR using primers designed on the specific mRNA sequence.

Independently of the antibody used for immunohistochemistry, the Sertoli cells of all neonatal and prepubertal cryptorchid testes showed strong positive signals for AMH. By converse, positive signals progressively decreased in the Sertoli cells of contro-lateral, normally descended testes from neonatal to late prepubertal phase. Surprisingly, the

interstitial cells of descended prepubertal testes showed an age-related increase of positive immunosignals for AMH. Western blotting data confirmed the specificity of the two antisera used for immunohistochemistry. AMH gene expression was observed in all testes. Even if our data need to be confirmed, we think that it is important to present such preliminary findings that suggest the possible presence of an AMH-immuno-like protein in the Leydig cells of normally descended testis of prepubertal unilateral cryptorchid swine.

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OUTBREAK OF INFECTIOUS BOVINE RHINOTRACHEITIS WITH HIGH CLINICAL SCORE IN RAGUSA (SICILY, ITALY)

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Infectious bovine rhinotracheitis (IBR) is a widespread disease in Italy, resulting from bovine herpesvirus 1. Some Italian Regions started compulsory/voluntary eradication programmes. Sicily has not adopted any programme yet, although the disease is present at a herd prevalence of around 60% (1). Today, the disease is multisymptomatic but most infections run a mild or subclinical course (2); the classical symptoms of IBR (upper respiratory tract symptoms and abortion) are observed in few individuals in an infected herd. A new outbreak of IBR with high clinical score is reported in a cattle herd (243 animals) in the area of Ragusa (Sicily, Italy). The herd was characterized by high reproductive indexes, and was free of brucellosis, enzootic bovine leucosis, tuberculosis and bovine virus diarrhoea. With regard to IBR, the animals were seronegative and unvaccinated. Biosafety measures were strictly applied. Starting from the half of August 2015, 138 cows, 8 heifers, 4 calves, 1 bull (62% of the herd) showed progressive respiratory signs (nasal discharge, conjunctivitis, cough and dyspnoea) and general signs (fever, drop of milk yield and food intake, reduced rumination). Abortion at 4-8 months of pregnancy was observed in 22% of pregnant cows, premature calving in 4% and stillbirth in 9%. The outbreak was dealt with using isolation and symptomatic therapy, but 4% of the animals died. At the end, 19% unproductive cows were slaughtered. A serological screening was performed 7 days after the onset of clinical signs which showed an IBR virus glycoprotein gB ELISA positive reaction, while IBR virus glycoprotein gE was negative. After 20 days a seroconversion of protein gE was finally observed, confirming a major infection of wild strain IBR virus. A vaccination schedule was applied using a live marker and conventional vaccine respectively in young and adult animals. IBR outbreak with high clinical score (abortion, mortality) is a severe risk when IBR-free and unvaccinated herd are close to infected herds. The widespread use of the indirect assay of gE glycoprotein to screen herds has to take in consideration the delayed antibody answer and the less immunogenicity of the protein (3). A double sampling spaced of at

least 21-35 days and a 4-week quarantine period are proposed for evaluation of IBR status of animals, especially before and after movement or participation to animal shows.

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ULTRASOUND MONITORING OF FEMALE REPRODUCTIVE CYCLE IN CAPTIVE ROYAL PYTHONS (Python regius): PRELIMINARY OBSERVATIONS

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The Royal python is one of the reptile species most commonly bred in captivity. For a successful breeding an accurate monitoring of the reproductive activity is necessary. Ultrasonography has been widely used to evaluate reproduction in reptiles. Because of interspecies variability it is useful to investigate the characteristics of a single species.
The aim of this study was to monitor the reproductive cycle of female Royal python by ultrasonography.
A total of 50 adult female were examined at one week intervals between January 2013 and January 2015. All the animals were captive born and fed a diet of commercially raised rats. Pythons were individually housed in racks maintained at temperature of 28°C under a 12:12 h L/D cycle. We performed brief scans on non-anaesthetized P. Regius using a portable ultrasound system and a 7.5-MHz linear array transducer (Esaote MyLab™ClassC®). A layer of conductive gel was applied to the snake's body and a series of ventral and lateral scans of the lower third of each individual body was carried out. Position, ultrasound features, dimension and echogenicity of the ovarian follicles were determined.
Follicles were located laterally on both sides of the body, but we noticed a better view on the right side for nearly all the females. Follicles in different stages of development, follicles undergoing regression and eggs in varying degrees of calcification were observed. On the basis of the ultrasonographic appearance and follicular size, we have divided the reproductive cycle in four phases: anovulatory phase (follicles less than 5 mm), transition (follicles from 5 to 10 mm), folliculogenesis (follicles from 10 to 30-35 mm) and embryogenesis (embryonic development until deposition). Ovulation occurs during folliculogenesis, thus identify the beginning of this phase is important to understand

when to introduce the male. We also evaluated the average duration of each phase, as well as the average value of follicular growth for each of these stages.

Ultrasound allows the visualization of follicles, embryonic structures and viability. With regard to snakes, there seems to be inter-species variability. The present study suggests that by ultrasound it is possible to precisely identify the different phases of the Royal python female reproductive cycle and thus to highlight the right time to introduce the male. It is also a useful technique to identify the females with follicular regression or producing slugs. In conclusion our study shows that ultrasound can be an excellent technique for accurate monitoring of the female reproductive activity in Royal python.

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FOLLOW-UP EVALUATION IN BITCHES SUBJECTED TO MEDICAL, SURGICAL OR COMBINED TREATMENT FOR PYOMETRA.

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Pyometra is a very common reproductive disorder which affects 25% of bitches over 8 years old (1). Ovariohysterectomy (OHE) is the traditional therapy for this condition, however, in the last decade numerous medical treatments have been proposed to treat both open and closed cervix pyometra (1,2). These medical treatments have proven their effectiveness in solving the acute phase of the disease and in preserving in most cases the future fertility. The aim of the present study was to evaluate the outcome of a medical (aglepristone and antibiotics), a surgical (OHE and antibiotics) and a combined treatment for pyometra in bitches, in terms of remission of clinical symptoms and normalization of hemato-biochemical parameters in the post-treatment follow up. Fifty bitches aged 3-16 years were allocated into three treatment groups: Group I (n=20) medical treatment: aglepristone (10 mg/kg SC on days 1, 2 and 8), combined with at least one broad-spectrum antibiotic; Group II (n=20) Surgical treatment: OHE combined with with at least one broad-spectrum antibiotic; Group III (n=10) Combined treatment: Bitches received the same treatment as in Group I, and after one month post medical management they underwent OHE. Before treatment begun and on days 15, 35, and 90, bitches were evaluated for clinical signs and for hemato-biochemical parameters. The proportion of bitches showing complete remission of clinical signs was in Groups I, II and III 25%, 30% and 30% on day 15, 74%, 55% and 80% on day 45 and 100%, 88% and 100% on day 90, respectively. The proportion of bitches showing complete normalization of hemato-biochemical parameters in Groups I, II and III were 23%, 14% and 20% on day 15, 69%, 39% and 70% on day 45 and 100%, 85% and 100% on day 90, respectively. No statistically significant differences were found. All bitches recovered within 90 days, except a small proportion treated exclusively with surgery. None of the treatments showed statistically significant advantages, however medical treatment before surgery should allow to perform surgery in a safer condition for the bitch (2). In conclusion, medical treatment alone can be considered an effective and safe alternative to the

traditional surgical treatment, although the possibility of having recurrences was not evaluated in this study and can't be excluded. Medical treatment, however, remains the first choice for breeding bitches and for bitches with severe concomitant disease, where the anaesthesiologic risk would be too high.

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RETROSPECTIVE ANALYSIS OF THE INCIDENCE AND OUTCOME OF DYSTOCIA IN 601 STANDARDBRED MARES FOALINGS

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The incidence of dystocia is low in mares, but difficult foaling is life threatening for both dam and foetus, and requires veterinary emergency care (Vandeplassche, 1993). The aim of this study was to retrospectively analyse the incidence and the outcome of dystocia in 601 foalings in Standardbred mares. The study was done in a single breeding station offering the foaling control service and evaluated the years 2008-2013. Near term mares were maintained in paddocks during the day and in foaling boxes during the night, where they were monitored continuously by direct or camera observation. An experienced technician was trained to call the veterinarian in case of any obstacle to the normal prosecution of foaling and to prevent as much as possible excessive mare's expulsive contraction, forcing the mare to walk, until the veterinarian intervention. Dystocias were treated by the common obstetrical practices for controlled vaginal delivery, starting within 60 minutes from the call. In four occasions it was necessary to submit mares to general anaesthesia and in two cases to fetotomy. Dystocia occurred in 16/601 parturition (2.7%) with 9 and 5 cases of longitudinal anterior or posterior presentation, respectively, and 2 cases of sitting dog posture. In anterior presentation, dystocia was due to malposture of foetal extremities (n=7), dorsopubic position (n=1) and weak maternal contractions (n=1). In posterior presentation, dystocia was not complicated by abnormalities of position and/or posture. The incidence of dystocia was not influenced by the year of the study, the age or the parity of mares. Six/16 foals died (37.5%), in all cases before the veterinary intervention. Two/9 (22.2%), 2/5 (40%) and 2/2 (100%) foals died in occasion of anterior, posterior and sitting dog presentations, respectively. One/16 mares died (6.2%) as consequence of a sitting dog posture. Dystocia was reported in 4% (Thoroughbred and Standardbred), 8% (Shetland Ponies) and 10% (Belgian Draft Horses) of cases, with a higher incidence in primaparous than in multiparous mares (Vandeplassche, 1993). An increase in foetal death was reported with a delay of delivery

beyond 40 minutes after the rupture of chorioallantoic membrane (McCue and Ferris, 2012). After fetotomy, a 95.8% of mares survival rate was reported (Carluccio et al., 2007). The incidence of dystocia observed in this study was low, probably because observation was limited to Standarbred mares submitted to a strict control of the peripartum period. In this study, the intervention of the veterinarian within one hour from the call was able to rescue all the foals still alive at his arrival and 15/16 mares.

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THE USE OF A FLIPPED CLASSROOM APPROACH TO TEACH CLINICAL ANIMAL REPRODUCTION

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The classical way of teaching in veterinary medicine follows a Teacher-Centered approach, whereby the teacher speaks and students listen and take notes trying to capture everything the teacher says. However, significant points are often missed, the level of student attention fluctuates during the hour and students are generally unable retain all the material that has been presented unless they start reading and studying it immediately after class. On the other hand, a Flipped Classroom approach, a pedagogical model in which lecture and homework are reversed, is based on a student-centered teaching orientation which shifts the focus of instruction from the teacher to include the student, with the latter promoting greater autonomy, engaging in independent problem solving and focusing on practices that enable lifelong learning (1, 2).

The Flipped Classroom, was used during the 2015-16 academic year for senior year veterinary students taking the class of small animal reproduction at the University of Padova. Students were instructed prior to the beginning of the course about the new teaching methodology with meetings in groups of 8-15 students each. Students received the entire set of lectures at the beginning of the course and additional class material on each topic a few days prior to each lecture. In class they were presented with clinical cases on which they worked in groups of four. Use of textbook, class notes, internet and mobile phone was allowed during flipped classroom sessions. Each group of students had to produce a paper with answers and comments to the issues raised by each clinical case. Answers had to include the clinical approach to the case, explanation of clinical signs, reasons for treatment failures if applicable, complete treatment including the production of a prescription with commercial names of each drug to be administered, drug dosage, volume, route and timing of administration. Level of participation by

students was evaluated and counted towards the final grade. Students were also asked to grade their performance in class.The novelty aroused a great deal of interest among the students and was very well received. Findings revealed: a) excellent attendance and active participation by students; b) clinical skills improved (based on comparison to prior year students), and c) student satisfaction in general was higher than previous years. These finding are strong indicators for further exploration of the Flip Classroom as an effective teaching method at least (but not only) in Clinical fields of Veterinary Medicine. Flipped teaching has gained attention in media, lately in academia, but not in Veterinary Medicine. This is the first report on the use of a Flipped Classroom teaching approach in Veterinary Medical teaching.

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PRODUZIONI ANIMALI E SICUREZZA ALIMENTARE

PREDNISOLONE TREATMENT EFFECTS ON TESTIS AND EPIDIDYMIS IN BEEF CATTLE

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Corticosteroids (CSs) are often illegally used as growth promoters in livestock production. Histological analysis on thymus morphology is routinely used as a screening tool to detect CSs treatments in cattle but it is not able to identify Prednisolone (PDN) treatments (1). In order to identify potential biomarkers of PDN treatment, effects on testis and epididymis were investigated.

Fifteen male Friesian beef cattle were divided into two groups: group P was administered PDN 30 mg/day per os for 35 days, while group K served as a control. The beef were slaughtered 6 days after drug withdrawal. Testicular and epididymal samples were submitted to morphometric and ultrastructural analyses. Ki67 score (immunohistochemistry) and apoptosis (TUNEL assay) were evaluated in testis, as well as STK11 gene expression (qPCR).

Morphological and ultrastructural analyses of testis showed a wide heterogeneity of epithelium appearance in animals belonging to group P. The most striking feature observed in treated group was related to the intraluminal compartment of epididymis, where the spermatozoa concentration decreased significantly up to disappear, and this reduction was accompanied by an increased presence of cellular debris. Morphometric analyses showed a significant increase ($p<0.05$) of the area occupied by the seminiferous tubules in group P. The mean number of Sertoli cells, spermatogonia, spermatocytes, mature and immature spermatids did not vary between the groups, whereas group P showed a greater number of immature spermatozoa ($p<0.05$).

Immunohistochemical staining for Ki67 antigen did not reveal a significant difference between the groups. TUNEL assay did not reveal appreciable amounts of apoptotic cells, nor in group K and in group P. Administration of PDN induced a significant ($p<0.01$) down-regulation of the STK11 gene in testis in group P. The expression of STK11, also known as LKB1, in mammalian testis was demonstrated to be an essential regulator of spermatozoa release during spermiation (2), which is defective in the absence of STK11.

Briefly, morphology and ultrastructural appearance of testicular tissue was not exhaustive in identifying treatments, since the findings were very different from one animal to the other. The higher percentage of the area occupied by the seminiferous tubules, the higher number of immature spermatozoa in testis and the reduction of spermatozoa concentration in epididymis, supports the hypothesis that the PDN treatment does not affect the production of mature spermatids, but it interfere with spermiation, probably involving STK11 pathway. The outcomes are represented by an apparently unaltered morphology of testis, along with a huge decrease of spermatozoa in epididymis. Further studies are necessary in order to verify if this simple and cheap analysis could be used as a screening method to identify animals worthy of investigation with chemical methods.

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APPLICATION OF ABSOLUTE QPCR AS A SCREENING METHOD TO DETECT ILLICIT SEX STEROID HORMONE ADMINISTRATION IN MALE BOVINE

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It has been previously demonstrated that the regucalcin (RGN) gene expression is decreased in the testis of male bovines after sex steroid hormone treatment [1, 2]. In this study a qualitative screening method was optimised to detect sex steroid hormones administration using absolute quantification by qPCR of the RGN gene expression in testis.

In Trial 1 18 Friesian male calves, 6 months old, were divided as follows: gr. A (n=6) treated weekly with 17β-estradiol (βE2) for 6 times for a total of 190 mg/animal; gr. B (n=6) treated weekly with testosterone propionate for 6 times for a total of 1050 mg/animal; gr. K1 (n=6) was untreated. In Trial 2 16 Friesian veal calves, 6 months old, were divided as follows: gr. c (n=8) treated with 150 mg/2 weeks of Nandrosol for 4 weeks and 80 mg/day of ractopamine for 31 days; gr. K2 (n=8) was untreated. In Trial 3 12 Charolaise beef cattle, 17-22 months old, were divided as follows: group D (n=6) treated with 20 mg/animal/week of βE2 for 5 weeks; group K3 (n=6) was the control. In Trial 4 32 Friesian beef cattle, 13-20 months old, were divided as follows: group E (n=8) was administered 200 mg of trenbolone acetate (TBA) and 20 mg of βE2 (Revalor-200) as a subcutaneous implant for 89 days; group F (n=8) was treated with Revalor-200 for 89 days in combination with 0.7 mg/animal/day of dexamethasone (DEX) for 40 days; group G (n=8) was administered 200 mg of TBA (Finaplix-H) as a subcutaneous implant for 89 days; group K4 (n=8) was the control. Samples of the testis were collected from each animal and preserved for further analyses. An external standard curve was developed with TaqMan technology. Based on the in vivo experiments described above, the optimal criterion value, sensitivity and specificity of this screening method were established through ROC analysis. Then, RGN gene expression was evaluated in 54 veal calves and 70 beef cattle intended for human consumption. For all animals tested

suspect via qPCR a immunohistochemistry (IHC) with an anti-RGN antibody was performed to confirm the molecular results.

Eleven (20.4%) out of 54 calves and 5 (7.1%) out of 70 beef cattle tested expressed the RGN gene under their respective cut off and they were classified as being suspected of sex steroid hormones treatment. The IHC of the testis of suspect animals revealed a mild decrease of RGN protein expression compared with control animals.

The decrease of RGN gene expression may well be an intriguing biomarker to discover sex steroid hormones abuse in veal calves and beef cattle. Used as a screening test, the described methodology could significantly improve food safety control programs because significant decrease of RGN gene expression was still detected until six days after the last treatment with sex steroid hormones.

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CHARACTERIZATION OF MICROBIOTA IN DONKEY MILK BY HIGH-THROUGHPUT SEQUENCING

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Donkey milk (DM) is considered an adequate replacement for children with intolerances and food allergies due to its nutritional properties. DM's composition is similar to human milk but contains a considerable higher concentration of lysozyme, even compared with other milks (1,2). Several authors have studied the bacteria present in DM using culture dependent methods (1, 2, 4). Yet differences in the design of the studies and limitations of the methodologies have come up with limited knowledge about its microbiota. The development of culture independent methods has opened a new perspective for the study of milk bacteria. The aim of this work was to explore the microbiota of DM using a high-throughput sequencing (HTS) approach.

We used the MiSeq Illumina platform to sequence the V4 region of the 16s rDNA gene from DNA extracted directly from bulk milk samples of 6 different Piedmont's donkey farms, collected in the spring of 2013 and 2014. Sequences were filtered and analyzed with QIIME to determine the abundance, taxonomy and diversity indexes.

All samples' reads were classified into four main phyla: Proteobacteria, Firmicutes, Bacteroidetes, and Acinetobacteria.

The core microbiota, a subset of the bacterial population shared by at least nine samples, of DM is constituted by 24 genera. It comprises commonly associated milk bacteria, lactic acid bacteria (LAB) and species normally found in soil, water and plants.

As expected, more than half of the reads corresponded to Gram-negative genera; the most abundant were Pseudomonas, Ralstonia, Acinetobacter, Cupriavidus, Citrobacter and Sphingobacterium.

On average, only the 4.4% of the reads (ranging among 0.13 and 17%) corresponded to Gram-positive bacteria, mainly LAB. The percentages are low compared to other HTS studies of cow, buffalo and human milk where more than 40% of the reads corresponded to these bacteria. The LAB genera present in our samples were Streptococcus, Lactococcus, Enterococcus, Leuconostoc, Lactobacillus, and Carnobacterium.

Comparisons between the farms showed that almost all of them had high percentages of Pseudomonas spp reads. This genus has been isolated from hay, air, dust, milking machinery and teat surface in bovine dairies. The only DM's farm with a scarce number of these species' sequences, performs hand milking, extensive farming and milks the jennies only when it's needed; however it had high rates of Ralstonia and Cupriavidus, two genera that are phylogenetically related to Pseudomonas.

Sequences of foodborne pathogens were not detected in the samples, as in previous reports (1, 4). Even if this result is encouraging, further studies are needed to investigate DM's safety, specially considering that the final consumers are susceptible patients like elderly persons and children with food intolerances and allergies. In conclusion the HTS approach allowed to asses the composition of DM, underlining how this microbiota is clearly delimited by lysozyme's selective pressure.

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THE INNOVATIVE APPROACH OF BACTERIOPHAGES AGAINST THE FOODBORNE PATHOGEN CAMPYLOBACTER

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Campylobacteriosis is the most commonly reported zoonosis and the leading cause of human bacterial gastroenteritis in EU [1]. Contaminated poultry meat is the major source of Campylobacter infections in humans and in some countries up to 90% of domestic birds are colonized by the pathogen as part of their normal intestinal flora [1]. Bacteriophages are naturally occurring agents and ubiquitous in nature, which prey and kill bacteria. The therapeutic use of bacteriophages has the potential to specifically target Campylobacter in poultry thus reducing the risk along food chain [2]. A group of bacteriophages against Campylobacter isolated from excreta of broilers was selected in order to study their potential application in the pathogen bio-control. Phages were isolated from fifty-one samples of fresh chicken feces [3]. C. jejuni NCTC 12662 and other eight Campylobacter field strains were used as isolation strains. The hosts were chosen on the bases of their different morphological and serological characteristics, in order to increase the opportunity to isolate heterogeneous phages. Bacteriophages were propagated and plaque purified [4]. Their lytic spectum was assessed and genomic DNAs was analyzed by Pulsed-filed-gel-electrophoresis (PFGE). Fifteen bacteriophages were able to propagate and to produce plaques and three of them possessed the broadest lytic spectrum against the panel of Campylobacter strains tested. PFGE revealed a similar size for all the isolated phages of about 140 Kb. Due to the lytic activity and to the ability of some of these phages to infect the most of Campylobacter strains used in our study we suggest to assess them further with in vitro efficacy tests. The application of these phages in poultry has the potential to reduce the risk and incidence of campylobacteriosis in humans.

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GENOTYPIC CHARACTERIZATION AND BIOFILM FORMATION OF FOOD-RELATED *STAPHYLOCOCCUS AUREUS* STRAINS

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Staphylococcus aureus (*S.aureus*) is one of the well known pathogens that can live in a wide variety of environments. Several genotypes of S.aureus that differ in their pathogenicity were identified. The genotype B (GTB) is known for its high contagiousness and virulence potential compared with other genotype (Hummerjohann, et al., 2014). Biofilm production in diverse *S.aureus* subtypes have been reported (Thiran et al., 2015). The aim of this study was to measure the biofilm formation of different food-related S.aureus genotypes. The experiment was conducted on 10 *S.aureus* food-related strains. Gene amplification of the lukEB, sea and sed and the genotyping by RS-PCR, based on PCR amplification of the 16S–23S rRNA intergenic spacer region, were performed (Graber et al., 2008). Biofilm assays were carried out on polystyrene and stainless steel at 37°C (Di Ciccio et al., 2015).

All results were expressed as "Biofilm Production Index" (BPI). Biofilm was compared with reference strains (*S.aureus* ATCC 35556, *S.aureus* ATCC 12600, and *S.epidermidis* ATCC 12228) for each isolate. All isolates were defined into different categories on the basis of their BPIs values. The cutoff point for the biofilm production was the BPI value obtained by negative control on polystyrene and stainless steel. S.aureus strains showing ability to produce biofilms were classified as weak (BPINC ≤ *S.aureus* BPIs < BPI12600), moderate (BPI12600 ≤ S.aureus BPIs < BPIPC) or strong (S.aureus BPIs ≥ BPIPC). Among *S. aureus* strains, 10/10 were positive for lukEB and 1/10 was positive for sea. All *S. aureus* strains were negative for sed. Eight different genotypes were found.

All *S.aureus* strains were negative for GTB. At 37°C, 9/10 of *S.aureus* strains were biofilm producer in at least one tested surface. A total of 6/10 of S.aureus strains were biofilm producer on polystyrene whereas 9/10 were biofilm producer on stainless steel. Moreover, 6/10 of *S. aureus* strains were biofilm producers on both selected surfaces.

In details, 3/10 strains formed strong biofilms, 2/10 moderate biofilms and 1/10 formed weak biofilms on polystyrene whereas 9/10 strains formed weak biofilms on stainless steel.

Further studies are needed to evaluate the biofilm formation of genetically different subtypes of *S.aureus* strains.

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COMPARATIVE PROTEOMIC ANALYSIS PROVIDES INSIGHT INTO THE PROTEINS INVOLVED IN STAPHYLOCOCCUS AUREUS BIOFILM

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Staphylococcus aureus (*S.aureus*) strains are highly adaptable and have the ability to form structures known as biofilms (Di Ciccio et al.,2015), leading to surface colonization and the creation of a niche where the bacteria appear more resistant to antimicrobials and disinfectants (Costerton et al., 1999; Fratamico et al., 2009). To date, studies on the use of proteomic techniques to understand the species-specific mechanisms of biofilm formation are still lacking. The goal of this study was to compare the proteomic profile of the sessile and planktonic form of different strains of *S.aureus* with different biofilm formation index. The experiment was conducted on six strains: S.a ATCC 35556 (strong biofilm producer), S.a ATCC 12600 (moderate biofilm producer), S.a ATCC 29213 (weak biofilm producer),and three S.a wild isolate (strong, moderate and weak biofilm producer). These isolates are well known for their ability to form stable biofilms. All this strains have been grown both in the planktonic and in the sessile form and analyzed through 2D electrophoresis coupled with MALDI-TOF MS. Differential protein expression of *S. aureus* has been evaluated among groups of sessile and planktonic forms in order to discover the mechanisms of biofilm formation common to all analyzed strains. Results highlighted the differential expression of more then 20 proteins. Among them, Putative universal stress protein (Q7A551), ATP-dependent 6-phosphofructokinase (A6U2G5), Putative antiporter subunit mnhE2 (Q2FJ11) and Phosphoribosylglycinamide formyltrasferase (Q5HH12). Obtained results suggest that there is a common mechanism of biofilm formation that needs further investigation. Understanding the common pathways involved in biofilm formation will help to the development of new methods to counteract biofilm formation and to improve food safety.

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PH AS AN INDIVIDUAL PARAMETER IN HEIFERS, A CASE REPORT.

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Low pH values in the rumen may have direct effects on microbial fermentation leading to a decrease in fiber digestibility, and in worst cases to subclinical or clinical acidosis (pH threshold 5.8 and 5.5). The consequences in ruminant could be DMI depression, laminitis, milk fat depression, diarrhea, increased bacteria endotoxin in the blood and inflammation. Those factors can highly compromise animal performances.

Eight Holstein heifers (336±30 d, BW 346±35 kg) were involved in a trial 12-wk long (4 periods of 3 wk: 2 adaptive and 1 for data collection) designed as a double cross-over. Animals were divided in 2 groups fed *ad libitum*, alternatively, with two diets characterized by the same ingredients (grass hay 41.8%, barley straw 27.4%, sunflower 13.7%, corn meal 16.4%, NaCl 0.7%), but different physical form: pellet (P; ∅=8mm) and TMR. In the P diet forages were chopped at 12-mm theoretical length of cut, then mixed with the other ingredients and pelleted. The physical effectiveness factor (pef, % of fiber retained in a 1.18-mm screen) was 20.1% in P and 66.1 % in TMR diet ($P<0.01$). Dry matter intake (DMI), DMI/BW, water intake, rumination time, rumen temperature and pH (average and time below pH 5.8 and 5.5; bolus by SmaXtech®) were recorded daily. Total tract digestibility of the potential digestible NDF (TTdpdNDF) was evaluated during the experimental wk. The study's goal was to evaluate nutritional and dietetic effects, rumen pH and rumination time of the two treatments. In particular we focused our attention on a heifer (H) that shown a singular behavior compared with the others (Hs) involved in the study. Data were analyzed with the statistical program JMP-12 (SAS Institute Inc., Cary NC) using a LSMean Contrast analyses (significant value: $P<0.01$).

During P administration, H compared to Hs had similar DMI but showed lower rumination time (190 vs 265 min/d; $P<0.01$), rumen pH (5.73 vs 6.21; $P<0.01$) and temperature (38.55 vs 38.87°C; $P<0.01$), and showed longer time below pH<5.8 (1013 vs 31 min/d; $P<0.01$). During the TMR administration H, in comparison with Hs, had higher DMI (10.87 vs 8.77 kg/d; $P<0.01$), lower mean rumen pH and temperature (5.74

vs 6.22; 38.71 vs 38.82°C; P<0.01), longer time below pH<5.8 and 5.5 (1002.0 vs 19.0 min/d; 23.0 vs 1.0 min/d; P<0.01), and similar rumination time. TTdpdNDF was similar between H and Hs within the same treatment.

Data shown that heifers, with different rumen conditions, can have similar performance in term of fiber digestibility. Results of the current study suggest that in heifers with similar characteristic of breed, environment and diet, the rumen pH has to be considered an individual parameter, as shown by Palmonari et al., (2010) in dairy cows.

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INVESTIGATION OF BIOFILM FORMATION AND ITS ASSOCIATION WITH BIOFILM ASSOCIATED FACTORS OF FOOD-RELATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS STRAINS

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Microbial biofilm formed on food contact surfaces can lead to significant hygiene and food safety issues. Both methicillin-sensitive and methicillin-resistant Staphylococcus aureus (MSSA and MRSA) have the ability to form biofilms on various food industry surfaces (Mirani et al., 2013). The aim of this study, was to investigated the relationship between biofilm associated factors and the ability of food-derived MRSA strains to form biofilm. Twentytwo MRSA strains and 3 reference strains (*S.aureus* ATCC 35556, S. aureus ATCC 12600 and *S. epidermidis* ATCC 12228) were tested for the detection of some genetic markers associated to the biofilm production, by PCR (Shopsin et al., 2003; Graber et al., 2009); furthermore, the strains were tested for the biofilm production on polystyrene and stainless steel using a previously described method (Di Ciccio et al., 2015). Majority of MRSA isolates (90%) showed similar distribution of adhesion genes (icaA, icaD, cna, fnbA and fnbB), toxin genes (hla and hlb), and staphylococcal regulators (sarA). Majority of biofilm producers isolates (66.7%) were found to carry agr type III whereas most of (87.5%) of the biofilm negative isolates were found to carry agr type I. All MRSA strains were found icaA, icaD, hla positive and fnbB negative. Biofilm formation was observed in the 27.2% of the isolates, of which two strains formed strong biofilms, two moderate biofilms and two formed weak biofilms. The biofilm formation by MRSA strains occurred preferentially on polystyrene (27.2%) compared to stainless steel (9%). In conclusion, this study underline the ability of some genotypes of food related MRSA strains to form biofilm on surfaces largely used in the food industry and the need of carefully control their spread for preventing food safety concern.

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MULTIDRUG RESISTANT AND METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATED FROM RAW SHEEP'S MILK

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Multidrug resistant (MDR) and Methicillin Resistant *Staphylococcus aureus* (MRSA) contamination in milk and dairy products can origin from animals, farm environment, from human in contact with animals or food handlers. Therefore, milk and dairy products could represent a potential source of antibiotic resistant S. aureus strains that could reach human through the food chain. Most of the reports on the occurrence and characterization of MDR S. aureus and MRSA refer to dairy cows, while little information is currently available on strains isolated from sheep's milk. The aim of the present study was to evaluate the presence of MDR S. aureus and MRSA harboring mecA and mecC genes in raw sheep's milk. Phenotypic resistance to antibiotics and the presence of the genetic determinants were also investigated. Bulk tank milk samples and milking machines filters were collected from 17 Sardinian dairy sheep farms. In addition, 3 filters from one cheese-making plant collecting milk from the investigated farms were sampled. The detection of Coagulase Positive Staphylococci was performed according to ISO 6888-1:1999 and the potential presence of MRSA assessed using ChromID MRSA Smart agar plates. Isolates were submitted to PCR for species identification. Minimum Inhibitory Concentration (MIC) for Ampicillin (AM), Cephalothin (CEF), Cefoxitin (FOX), Erythromycin (E), Oxacillin (OX), Penicillin (P), Tetracycline (TE) and Vancomycin (VA) was determined using broth microdilution method (CLSI M07,M100, 2015). The detection of the genes mecA, mecC, blaZ, ermA-B-C, vanA, tetK-M-S-W encoding antibiotic resistance was performed as previously described (Spanu et al., 2014). In this study, 118 S. aureus strains were collected, 65 strains from 17 positive milk filters, 38 from 7 bulk tank milk samples and 15 from cheese-making plant filters. Twelve strains (10.2%) were resistant at least to one of the β-lactam antibiotics tested and 6 isolates showed multiple resistance against AM, FOX, OX and/or P. Among these, 3 strains were identified as

MRSA with MIC values of 16-32 µg/mL for OX and 64 µg/mL for FOX. Interestingly, 2 out of 3 MRSA were also resistant to E (8 µg/mL), despite only 1 strain carried blaZ, mecA, mecC and ermB-C genes. All the isolates were susceptible to CEF and VA and did not carry the correspondent resistant genes. On the other hand, although resistance to TE was not found, 15 and 7 S. aureus strains carried tetM and tetK genes, respectively. The results of the present study suggest the emergence of MDR *S. aureus* also in small ruminants dairy chain which pose a potential public health hazard for the spreading of MRSA strains.

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IN SILICO PREDICTION OF ANTIMICROBIAL RESISTANCE BASED ON WHOLE GENOME SEQUENCING DATA

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A rapid identification of pathogens and prediction of their AMR phenotypes may significantly boost the control of infectious diseases. Different software are publicly available online for in silico search of antimicrobial resistant (AMR) determinant genes and single nucleotide polymorphisms (SNPs) within Whole Genome Sequencing (WGS) data(1). However few studies have been performed to evaluate the agreement between in silico prediction of AMR and antimicrobial susceptibility phenotypes. In the present study in silico prediction of AMR phenotype based on WGS data was compared to antimicrobial susceptibilities in two strains of Salmonella enterica serovar Typhimurium, two strains of *Escherichia coli* and two strains of Staphylococcus aureus. Multiplexed paired-end libraries of extracted DNA were produced with TrueSeq kit (Illumina, Milan, Italy) and sequenced on the Illumina MiSeq platform, generating 250 bp paired-end reads. De novo assembly of reads was performed using VelvetOptimiser 2.2.5 (2). Contigs were submitted to ResFinder and CARD-RGI, two softwares available on line for in silico AMR prediction(1). Strains were submitted to antimicrobial susceptibility test by disk diffusion method as previously described(3). Twelve antimicrobials belonging to eight different classes were tested. *Escherichia coli* ATCC 25922 was used for quality control. Interpretative criteria followed disk diffusion breakpoints reported in CLSI documents(3,4). Enrofloxacin resistant strains were submitted to Minimum Inhibitory Concentration of enrofloxacin by Etest strips following manufacturer instructions (Biomerieux, Florence, Italy). Regarding results, genomes were sequenced with a mean coverage of 80X. After de novo assembly, the shortest sequence length at 50% of the genome (N50) ranged from 55540 to 157455. Both ResFinder and CARD-RGI genotypes showed a correspondence to AMR phenotypes of 92,1%. Regarding discrepancies, ResFinder discrepancies were identified exclusively within the two S. aureus genomes, whereas CARD-RGI discrepancies were identified for all tested species in relation to tetracycline resistance. The discrepancies of in silico predictions might be due to: 1)

determinant genes or mutations not included in the software databases (false negative results); 2) the identification of inducible genes not expressed during the disk diffusion culture method (false positive results). In conclusion in silico prediction of antimicrobial resistance should be performed with caution and should be preferably based on a combination of software tools with curated and up-to-date databases.

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MICROBIOLOGICAL CHARACTERIZATION OF 'IN-USE' KITCHEN SPONGES

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In the last twenty years, numerous studies indicated that several food-borne diseases are related to home environment (1, 2, 6). Among these, the kitchens were the most frequently incriminate environments (3, 4). The main sources of infection in the domestic kitchen are attributed to people, pests, pets, contaminated food and water (3, 5). In positive conditions, microorganisms are able to survive or multiply on several surfaces, such as in kitchen sponges.

The aim of this study was to investigate on microbiological contamination of 100 'in-use' sponges collected from household kitchens. For each sample the following parameters were made: aerobic mesophilic bacteria (UNI EN ISO 4833:2004); Enterobacteriaceae (UNI EN ISO 4833:2004), coagulase positive staphylococci (UNI EN ISO 6888-2:1999); anaerobic sulfite reducing bacteria (on SPS agar); yeast and molds (UNI EN ISO 21527-2:2008), Salmonella spp. (UNI EN ISO 6579:2002), Listeria monocytogenes (UNI EN ISO 11290-1:2005) and Yersinia enterocolitica (UNI EN ISO 10273:2003). MALDI-TOF technology was used to identify 100 enterobacteria strains isolated.

The charge of aerobic mesophilic bacteria ranged from 5.49 to 10.00 log CFU/g, Enterobacteriaceae from 2.78 to 8.18 log CFU/g, yeasts and mould from 3.08 to 7.40 log CFU/g. Coagulase positive staphylococci and anaerobic sulfite reducing bacteria were found in 11 samples with values ranging from 3.00 to 4.26 log CFU/g and from 1.00 to 3.64 log CFU/g, respectively. Enterobacteria strains were identified as: *Enterobacter cloacae* (44%), *Citrobacter freundii* (22%), *Klebsiella oxytoca* (15%), *Leclercia adenocarboxilata* (10%), *Klebsiella pneumoniae* (4%), *Providencia rettgeri* (2%), *Serratia liquefaciens* (2%) and *Acinetobacter johnsonii* (1%). *Listeria monocytogenes* was found in one sample. The findings of this study highlight the potential role of microbial contamination of kitchen sponges as diffusion means of food-borne diseases agents.

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MICROBIOLOGICAL INVESTIGATION OF BOVINE BULK TANK MILK SAMPLES IN FARMS IN EASTERN SICILY

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In 90 dairy farms located in Ragusa (Sicily) was performed a screening on bulk tank milk.

The main purpose of this study was to analyze the issues related to the management through the set of selected tests for this screening. The critical points of management of dairy cows were monitored:

1. Hygiene of milking and bedding with the numbering of Coliforms (average of the values of all farms 973 UFC/ml) and E. coli (present in 31 farms) research;
2. The presence of contagious bacteria causing mastitis through Streptococcus Agalactiae (present in 5 farms) and Coagulase Positive Staphylococci(present in 42 farms) research;
3. Correct hygiene of milking system with the count of the thermophilic bacteria (average of the values of all farms 1.726 UFC/ml);
4. Functioning of milk storage and cooling system through the numbering of psychrophilic bacteria (average of the values of all farms 289.300 UFC/ml);
5. Mycoplasma bovis like pathogen agent able to resist antibiotics(absent).

The sampling was made on the bulk tank milk, with at least two milkings.

77 farms (85.5%) reported value of psychrophiles higher than the reference range of 10,000-20,000 CFU / ml (National Mastitis Council). The arithmetic average of the psychrophilic in the out farms was 326,612 CFU / ml.

Subdividing 77 farms for the result classes, there were: 16 farms with psychrophiles value between 20,000 to 80,000 CFU / ml, 10 farms between 80,000 to 200,000 CFU / ml, 13 farms between 200,000 to 400,000 CFU / ml and 38 farms have exceeded 400,000 CFU / ml.

In addition the value of the milk temperature indicated by the thermometer in the bulk tank has been, averaging between all farms, 4.97 ° C. While average temperature measured by the team with the thermocouple was 6.11 ° C, then 1, 14 ° C above.

The differences between the temperatures measured by the team and those of the tank thermometer were classified according to the time passed between milking and the time of sampling. It was indicated by T1 time from 0 to 20 minutes, T2 from 30 to 60 minutes, T3 from 90 to 120 minutes, T4 from 150 to 180 minutes.

The temperatures measured by the team on average were higher in (T1) of 1.54 ° C, in (T2) of 1 ° C, in (T3) of 0,89C ° and (T4) of 0.71 ° C.

The discrepancy, between measured temperature and those of the farms, shows that the checks on detection system of milk storage temperature are neglected. This lack adversely affects the value of the count of bacteria and also is particularly important for the area in which it was carried out the study, the province of Ragusa. Here the production of the "Ragusano" DOP, a typical cheese made from raw milk, is at the base of the local economy.

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CHARACTERIZATION OF PRODUCTION PROCESS OF LAMB RENNET PASTE USED FOR SICILIAN PDO CHEESE

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Artisanal lamb rennet paste is used for the production of some Sicilian PDO cheeses (Vastedda del Belice, Pecorino and Ragusano) and, besides its enzymatic activity, it is considered an important source for the typical microflora of cheese (1, 2). The artisanal lamb rennet preparation is generally empiric and depends on the local traditions. The aim of this study was to study the effect of different traditional processing techniques on the microbiological and enzymatic features of rennet. Four production process from different farms of western Sicily were examined. During the process some samples were maintened at temperature between 4 c° and 8 c°, other at room temperature. All the samples were analyzed in four different times: abomasa after slaughtering (T0); after 30 and 60 days of salting (T30 and T60) and the final product after 6 months (T180). Each samples, according to the related official methods, was analyzed in duplicate with regard to: i) count of mesophilic and thermophilic Lactococci; ii) count of mesophilic and thermophillic Lactobacilli; iii) count of Enterobacteriacae iv) count of total Coliforms; v) count of Escherichia coli; vi) count of sulphite-reducing Clostridium; vii) count of yeast and molds; viii) research of Salmonella spp. and Listeria monocytogenes; ix) evaluation of pH and aw.

Finished products were also analyzed for coagulation properties using Formagraph and for the lipolytic activity. In all the samples the value of Enterobacteriaceae, total Coliforms and Escherichia coli were always < 100 CFU/g until T30 (Aw < 0.775; pH <4.6) and, at the end of the process T180 (Aw < 0.757; pH <4.5), were not detected (< 10 CFU/g); Salmonella spp. and Listeria monocytogenes were never isolated. Yeast and molds were counted at T0, T30 and T60, ranging from 380 to 4300 CFU/g, but only in the sample maintained at room temperature. Clostridium spp. was isolated in four samples independently by salting conditions. The load of lactic acid bacteria decreased during the process (T0 to T60) and resulted <10 CFU/g at T180, with the exception of a

finished sample that was added of milk according to some traditional uses. According to previous studies (3), the lipolytic and proteolytic activity was higher in rennet from younger lamb. (L.A.= 1386 U/ml; Milk Clotting Activity: r = 7,15 min, K20 = 4,5 min, a30 = 50,4 mm).

The results showed the good hygienic quality of artisanal rennet but demonstrated, at the same time, that it cannot be considered a natural sources of non-starter lactic acid bacteria as for other kind of rennet (1).

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EXPOSURE TO SPOTTED FEVER GROUP RICKETTSIAE OF DOGS FROM CENTRAL ITALY

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Illness of unknown origin and responsive to tetracycline treatment are frequently observed in dogs from italy and attributed to spotted fever group (sfg) rickettsiae (order *Rickettsiales*, family *rRckettsiaceae*) without any diagnostic evidences. With the exception of *Rickettsia rickettsii* and R. *conorii*, the clinical significance of other sfg rickettsiae in dogs remain unclear. However contact of dogs with other sfg rickettsiae has been demonstrated in seroprevalence studies.

Aim of the present study was to investigate the exposure of dogs living in central Italy to sfg rickettsiae in order to support or not this apparent misconception.

Three hundred fourty four owned dogs admitted to the teaching veterinary hospital of Perugia to be screened as appropriate blood donors for the blood bank were sampled (blood and tick collection) between 2011-2013. All the dogs lived in sub-urban and urban areas of Perugia (central Italy).

Serum samples were analyzed by indirect immunofluorescence assay (IFA), using commercial antigens (mega cor diagnostick) of 2 different sfg rickettsial agents e.g. *R. conorii, R. rickettsii*, setting the cut-off dilution at 1:64. Endpoint titers against each rickettsial agent were determined by testing serial twofold serum dilutions. For molecular analysis ticks and whole bood samples of the dogs were processed by DNA extraction using a commercial kit (qiagen) and tested by using a conventional PCR targeting a fragment of the rickettsial citrate synthase gene (glta) (1). The amplicons were sequenced and compared to sequences in GenBank by using the blast 2.0 program.

Overall 16.3% (56/344) of the serum samples reacted to at least one of the two *Rickettsia* species tested by IFA. Thirty eight seropositive samples reacted against *R. conorii*, 46 against *R. rickettsii* and 28 simultaneously to both the rickettsial species.

Serum end-point titers ranged from 1:128 to 1:2048 for *R. conorii*, 1:64 to 1:256 for *R. rickettsii*. Fourteen (4%) canine sera (10 reacting against only *R. conorii* and 4 showing titers against *R. conorii* at least 4-fold higher than those the other antigen) were considered to have been stimulated by *R. conorii* or a very closely related species. However the other reactivities were considered immune responses stimulated by undefined sfg rickettsiae species. All dogs were PCR negative for rickettsiae. A total of 607 adult ticks (395 *Rhipicephalus sanguineus* and 212 *Ixodes ricinus*) removed from the coats of the dogs were submitted to molecular analysis. The presence of rickettisial DNA was revealed in 39 (18.4%) *I. ricinus* and in 10 (2.5%) *R. sanguineus* specimens. The homology searches for the glta sequenced amplicons showed the presence of 3 sfg rickettsial species e.g. *R. conorii*, detected in 10 *R. sanguineus* specimens, *R. helvetica* and *R. monacensis* recovered in 7 (3.3%) and 32 (15.09%) *I- ricinus* ticks.
The serological and molecular results showed a not negligible exposition of the canine population of the present study to sfg rickettsiae.

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RICKETTSIA SPECIES CHARACTERIZATION IN TICKS COLLECTED FROM HUMANS IN SICILY (ITALY)

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Rickettsiae (family *Rickettsiaceae*; order *Rickettsiales*) are obligated intracellular bacteria transmitted by arthropod vectors. Spotted Fever Group of the *Rickettsia* genus comprehends agents of vector-borne rickettsioses. In the Mediterranean area, *Rickettsia conorii* was traditionally considered as the main etiologic agent of the Mediterranean spotted fever. Molecular characterization of strains allowed to identify other *Rickettsia* species involved in Spotted fever in Mediterranean Area.

The study was aimed to the molecular characterization of *Rickettsia* species in ticks from humans in Sicily (Italy).

A total of 42 ticks collected on humans in the years 2012 and 2013 in Messina district (Northeastern Sicily – Italy) was identified by morphological keys (Manilla, 1998). One of these patients showed clinical manifestations of rickettsioses. All the other patients did not show any symptoms. DNA was extracted from one half of each ticks sectioned longitudinally and analysed by PCR for ompA (Oteo et al., 2006), ompB (Choi et al., 2005) and gltA (Roux et al., 1997) to detect the presence of *Rickettsia* spp. DNA. PCR products from positive samples were sequenced.

The following tick species were identified: *Rhipicephalus turanicus* (13 specimens), *Hyalomma lusitanicum* (11), *Rhipicephalus sanguineus* (7), *Dermacentor marginatus* (4), *Haemaphisalis punctata* (3), *Hyalomma marginatum* (2), *Ixodes ricinus* (1), *Rhipicephalus bursa* (1).

Out of the 42 tick samples, 14 resulted positive to the amplification of at least two molecular markers.

Identified *Rickettsia* species included not only *R. conorii*, but also *Rickettsia aeschlimannii, Rickettsia massiliae* and *Rickettsia slovaca*. The first one was detected in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* and this latest tick was the one collected from the only symptomatic patient. *R. aeschlimannii* was found in *Hyalomma marginatum, Hyalomma lusitanicum, Dermacentor marginatus* and *Ixodes ricinus*. *R. massiliae* was detected in four *R. turanicus* ticks and in *R. sanguineus*, while *R. slovaca* was identified in *D. marginatus* and *R. sanguineus*.

Our results showed a great variety of zoonotic *Rickettsia* species in ticks collected from humans in Sicily, highlighting the importance of molecular characterization of *Rickettsia* species. The risk of transmission to humans in Sicily is further enhanced by the widespread distribution and variety of ticks in the island and obtained results emphasize the role of ticks in the transmission of a big range of Spotted Fever Group *Rickettsia*.

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COMPOSITION AND DISTRIBUTION OF TICK POPULATION IN TWO SICILIAN PROVINCES

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Ticks (*Acari*: *Ixodidae*) are obligatory ectoparasites able to transmit a wide range of pathogens to humans and animals. The diffusion of tick borne disease is related to a large number of factors (environment, ecology, genetics and animal management). Sicily is a typical Mediterranean ecosystem, suitable for entomological studies and Sicilian provinces facing the Mediterranean Sea act as cross border between Italy and Tunisia.

The study aimed to a better understanding of composition and distribution of tick population in Sicilian cross-bordering provinces.

Ticks were collected seasonally for one year directly from animals (cattle and sheep) in Agrigento and Ragusa farms. For one year (summer 2014-spring 2015), 15 bovine and 15 ovine farms were monitored seasonally in each of the two Sicilian provinces. Collected arthropods were identified according to morphological keys (Manilla et al., 1998).

A total of 560 ticks was collected (326 in Agrigento and 234 in Ragusa). Out of these, 291 were found on cattle and 269 on sheep. In Agrigento, 67 ticks were collected from bovine and 259 from ovine farms. In Ragusa, 224 ticks were found on cattle and 10 on sheep.

Overall, six different tick species have been found: Rhipicephalus turanicus 38.8%, *Hyalomma lusitanicum* 37.7%, *Rhipicephalus sanguineus* 8.6%, *Hyalomma marginatum* 7.7%, *Rhipicephalus bursa* 5.2%, *Haemaphlisalis sulcata* 2.1%.

A predominance of *Rhipicephalus* spp. (*R. turanicus* 79.9% and *R. sanguineus* 16.4%) was found in sheep, while *H. lusitanicum* was the most representative species in cattle (69.1%). *H. lusitanicum* was found during the whole year with a peak in summer and a greater abundance in Ragusa province, while *Rhipicephalinae* were predominant in

Agrigento province. The study reports tick abundance and diversity related to climatic characteristics and different host specificity. Identified tick species are involved in the transmission of different pathogens to animals and humans (Torina et al., 2010). Differences in tick populations in the two provinces could be related to the different climatic and environmental conditions, farm management, antiparasitic treatments, resistance to acaricides by the different tick species. A predominance of *Hyalomma* species was observed in Ragusa province. These ticks are particularly resistant to arid climates and their life cycle involves wild animals as Lagomorphs that, in this area, increased considerably in number due to the establishment of several natural reserves. Information about the regional distribution and abundance of ticks is necessary for tick control.

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DETECTION OF PSEUDORABIES VIRUS IN DOGS IN SICILY

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The Aujeszky's disease, is a viral infectious disease caused by *Herpesvirus*. The main host is swine but it could be transmitted to many other species of mammals. The pseudorabies virus is endemic in many parts of the world. Carnivores can become infected by ingestion of viscera and/or raw meat. The authors report two cases of pseudorabies observed in dogs in Sicily. Two adult mixed-breed dogs used for wild boar hunting which showed neurological symptoms were subjected to clinical examination. After death necropsy was performed and samples of organs were collected for further diagnostic investigations. The presence of PRV DNA in the field samples was determined by real-time PCR test as described by Yoon et al. 2005. All specimens were also processed for virus isolation and inoculated onto permissive cell lines RK13 (Rabbit kidney) and PK15 (Porcine kidney). Partially sequencing of the gC gene of two positive samples was performed as described (Moreno et al., 2015). The sequence alignments were performed using the ClustalW W method. The phylogenetic tree was constructed with the neighbor-joining method and the Kimura two-parameter model using MEGA 5.0 (Tamura et al., 2011). The Italian isolates were compared with the sequences of the references and field PRV strains that originated from other countries and are available at GenBank. The animals showed to the clinical examination sialorrhoea, increased respiratory rate, tachycardia, dulling of the sensorium, lateral recumbency with tonic clonic seizures, neuropathic itch of the head, congested mucous membranes, high body temperature (42 °C). Pathological examination revealed in both cases abrasion, hair loss and congestion almost exclusively involved the skin head and mostly attributable to episodes of self-injury due to scratching and cerebral congestion. Two samples (brain) resulted positive to PRV real-time PCR and were named 344427-1 and 344427-2. Only one sample was isolated on cell cultures and was tested by PRV real-time PCR and

immunofluorescence, to confirm the presence of virus. Phylogenetic tree of gC gene showed that the two samples were closely related to other Italian sequences obtained from hunting dogs and wild boar and formed a separate Italian group (Italian clade 1). This clade was clearly distinguishable within Muller's clade A, which included the PRV European feral strains. The first report of pseudorabies in Sicily involved one dog, a cat, a fox and a sheep of the same area (unpublished data, 1996). This new report highlights how the disease can be spread to wildlife and thus its fundamental the development of a monitoring plan in these species.

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A SURVEILLANCE SYSTEM OF DISEASES OF SMALL COMPANION ANIMALS IN THE VENETO REGION (ITALY)

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Experts and international public health organizations stress the lack of surveillance of diseases of companion animals and the necessity of its implementation as a priority of the One Health perspective.

This paper presents a project addressing this relevant public health challenge and describes the features of a system for collection, analysis, interpretation and dissemination of data about the health status of pets in the Veneto Region, Italy.

The system provides the construction of a web-based database containing the diagnoses of transmissible and non-transmissible diseases of dogs and cats made by veterinary practitioners joining the initiative voluntarily. Each diagnosis constitutes a single record containing data on the individual animal identification and on other characteristics of epidemiological relevance. The WHO International Classification of Diseases of humans has been adapted to canine and feline diseases to standardize the diagnostic nomenclature. A software for on-line data entry and data management has been specifically created.

As of 31 March 2016 about the 13% (n=80) of the overall veterinary practices of the Veneto Region have joined the project. At that time 705 (505 dogs and 200 cats) records have been entered the database. Three basic epidemiological reports have been distributed among the network and other stakeholders (veterinary associations, people responsible for human and animal health public services, academic experts). Reports and other interactive resources are freely obtainable from the website of the project.

Expected outcomes are to monitor disease frequencies and their trends in time and space, to identify associated risk factors and to produce disease and risk maps, providing epidemiological knowledge supporting the everyday clinical practice. Further expected outcomes are to assess the risk of transmission to humans and to set off the possible role of pets as early sentinels of emerging health threats and as models for the study of

the effects of the exposure to environmental risk factors. The perspective is to stimulate networking within the veterinary profession and between human and animal health professionals, to promote general awareness of the public health relevance of pets and to provide a useful tool to scientists and health policy makers.

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THE DIAGNOSTIC WEB SYSTEM FOR PRIVATE VETS: "TEST REQUEST"

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Currently during digital age, information systems especially in veterinary area are required to manage almost all the activities and data flows getting increasingly paper-less systems. The information systems are essential to reduce manual entry mistakes that almost always could be source of faults and ensure the quality of the saved data. The rule is that each data has to be typed only once by those who created it and it has to be available to all other systems that need it (interoperability).

We tried to simplify the collecting data process for private customers that is often a critical item like difficult to read the hand-written submission form for samples, lack or misunderstanding of mandatory information. For this reason, IZSAM has made available an application called STUD for private Veterinaries through which they can follow the entire sample cycle beginning with the "TEST REQUEST".

The IZSAM Diagnostic activity for all the public and private customers, can be followed by "STUD", a Web application available on Internet. The purpose is to give the opportunity to the applicants to find out information about samples and test reports, to autonomously extract detailed data and to ask on the web about new activities using "TEST REQUEST' function.

The system gives to the veterinary a list with possible inspections for selected material after entering general information including the owner, details for each sample, species, material and the Diagnostic question choosing them in the value lists. It's possible to add more samples in the same request.

This is a sort of "pre-acceptance", directly managed by the applicants. Only when the samples get materially to one of the Institute facilities, the Laboratory Information Management System (SILAB) (1) "read" what the applicant already entered and will complete it if it will be necessary. So we can ensure the "quality" of the data saved

because directly entered by those who know it without further steps or interpretations. The STUD application is available from the IZSAM website or directly at the address https://attivitadiagnostica.izs.it/stud2izsam/. It's possible to enter, with different professional profiles, and utilize a variety of services ranging from simple consultancy of analytical data to different health data collections which may also be used to make decisions. With the bottom "TEST REQUEST", private Vets can interact with the IZSAM entering themselves the needed data for the acceptance of samples and selecting the right diagnostic choices.

The system produces a unique code that will be reported on the samples and on the submission form and info about the cost of tests. Finally veterinaries will give samples to Reception of one IZSAM facilities in Abruzzo and Molise regions.

The STUD not only involves vets within a mechanism which was previously exclusively inside the Institute itself, but gives them the opportunity to follow the sample from the request until the final test report and also can extract data in Excel. Indeed it gives a well customer service in terms of timing.

1) Laboratory Information Management Systems: Current Requirements and Future Perspectives - Chapter 15, pag. 297-309

A LABORATORY INFORMATION MANAGEMENT SYSTEM (LIMS) FOR ANIMAL HEALTH IN SICILY: EXAMPLE OF COOPERATION AND INTEROPERABILITY AMONG NATIONAL, REGIONAL AND LOCAL SYSTEMS

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The integration and interoperability between application systems are one of the cornerstones of the e-government regulation. Sicily currently is a model of integration among national, regional and Local Veterinary Systems. The strategy of both Sicilian Region and Istituto Zooprofilattico Sperimentale (IZS) of Sicily (IZSSICILIA) has been to utilize information systems made available by the Ministry of Health or other IZS investing on customization and training rather than in replicas of what already exists. The aim of this work is to report the experiences of the IZSSICILIA using the LIMS called SILAB-SICILIA through two examples of strong integration: the first is the integrated management of Brucellosis; the second regards SILAB-SICILIA usage as internal adjustment tool integrated with other management applications. Sicily is the region with the highest prevalence of brucellosis in Italy (1). Brucellosis surveillance plan starts from Local Veterinary Services that use the National Animal Health Information System (SANAN), linked to the National Livestock Data Base (BDN), to retrieve all information related to farms and animals. Samples, identified by SANAN number, arrive at IZSSICILIA where are subject to diagnostic tests. Since 2015, in IZSSICILIA, standardization of laboratory processes and sample tracking has been increased using SILAB-SICILIA developed by IZS Abruzzo&Molise (IZSAM).

Typing in SILAB-SICILIA only the SANAN Sampling Number, the loading of all information about sampling activity and samples is automatically activated. When the test results are entered in SILAB-SICILIA, they also are automatically copied in SANAN.

Monthly, data extracted from SILAB-SICILIA feed the National Brucellosis Information System. Twice a year, the data gathered in SANAN are submitted to European Commission (2) and feed the annual collection of National Zoonoses Information System which updates the European Food Safety Authority database (3). About the second point, the choice of SILAB-SICILIA provided by IZSAM as "Software as a Service" was founded on effectiveness and efficiency too. The use of SILAB-SICILIA was an opportunity to re-establish internal rules and behaviors in order to place at the center customer's needs ensuring uniformity across all facilities for both technical and organizational aspects. The tight integration among local, regional and national information systems has increased the quality of the data collected in each database enabling cross-checks and allowing comprehensive reporting. It also enables to satisfy the information debts towards supranational organizations, provides data for the management and governance of the National and Regional Health Service and facilitates the planning of activities, their periodic verification and the customer service.

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CAPRINE ARTHRITIS ENCEPHALITIS VIRUS: GENETIC EVOLUTION, TISSUE TROPISM AND PATHOGENICITY

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Caprine Arthritis Encephalitis Virus (CAEV) is a RNA-virus in the *Retroviridae* family, Lentivirus genus, closely related to Maedi Visna Virus (MVV). Together, CAEV and MVV are classified as Small Ruminant Lentiviruses (SRLVs) and cause progressive inflammatory disease in goats and sheep worldwide. As the other lentiviruses, the SRLVs, are characterized by a period of greater (years) or lesser (months) duration of latency, followed by a gradual development of clinical signs (Sigurdsson, 1954. Br. Vet. J., 110: 255–270). The target cells of SRLVs are the monocyte-macrophage and dendritic cells, which are exploited for virus silent dissemination toward target organs (Blacklaws, 2011. Comp. Immunol. Microbiol. Infect. Dis., 35: 259–69). Once reached the tissues, the monocyte to macrophage maturation stimulates viral replication. In goats, the infection mainly affects radio-carpal joints, lungs, mammary gland and CNS, leading to different chronic disease syndromes such as degenerative arthritis, interstitial pneumonia, indurative mastitis and encephalitis, most common in kids (Ravazzolo et al., 2006. Virology, 350: 116–127). At present, the viral factors that influence the tissue tropism of SRLV have not been identified and no satisfactory explanation as to how those viruses express different tropism and clinical outcome in different animals was established (Murphy et al., 2010. Virus Res., 151: 177–84). The aim of this study was to examine the role of the U3 region of the long terminal repeats (LTR), encoding the viral promoter, in defining viral tissue tropism. For this purpose, different tissues (blood, central nervous system, synovial tissue, mammary gland and spleen) from 19 naturally SRLV-B1 infected goats, have been collected and analysed in order to characterise and compare 60 regions encoding the CAEV viral promoter. While some subjects were long-time nonprogressors (LTNPs), most of the animals were affected by different clinical conditions, such us arthritis, mastitis, pneumonia and neurologic syndrome. Preliminary data obtained from the phylogenetic analysis failed to show any sequences' clustering depending on tissue

source, rejecting the hypothesis that nucleotide sequence variation within the U3 region can be crucial in defining viral tissue tropism (Murphy et al., 2010. Virus Res., 151: 177–84). On the contrary, in some animals the sequenced promoter regions, isolated from different tissue types, were closely related, with particularly evidence for the most severely affected animals. The presence of highly-conserved motives within the U3 region confirms its importance in viral transcription. On the other hand, the sequence analyses underlined the presence of mixed viral population, reflecting the presence of quasispecies. In conclusion, the mechanism of CAEV tissue tropism remains undetermined, and further host and other virological factors should be investigated.

ALLELIC VARIANTS OF OVINE PRION PROTEIN GENE (PRNP) IN SICILY: STATE OF ART FROM THE BEGINNING OF THE REGIONAL PLAN

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In the sheeps, the genotype of the gene PrP determines the highest and the lowest resistance in the animal to suffer the Scrapie. Over 40 polymorphisms have been reported for the PrP gene, but only amino acid changes at codons 136, 154, and 171 of the host gene appear to be strongly linked to scrapie susceptibility. Recently it has been reported the identification of the novel lysine (K) allele in codon 171. The ARR allele is linked with resistance to scrapie, while VRQ is associated with susceptibility. The surveillance was implemented through large-scale testing of small ruminant TSEs, and several Member States introduced breeding programs in order to increase the frequency of the ARR allele and to reduce and eliminate susceptible VRQ allele in sheep populations. The distribution of scrapie genotypes is highly variable depending on the breed or geographic origin. The Region of Sicily based on the guidelines issued by the Ministry of Health, with Decree 4 January '13, requires the Plan of genetic selection for resistance to spongiform encephalopathies in all farms having a higher livestock to 200 leaders and in all herds enrolled in the studbook or high genetic merit. Recently, the Decree 25 November '15 by the Ministry of Health, has implemented preventive measures for the eradication of classical scrapie in sheep to increasing resistance in the entire national sheep population. The purpose of this work is the dissemination of the data obtained relatively to circulating genotypes in Sicily during the 2013-2015 triennium, about 25,276 animals. In the period 2013-2014, the samples were tested in Real Time PCR for the detection of the main mutations at the codons of the PRNP. In 2015, the samples were subjected to mass spectrometric examination as applied to nucleic acids through the instrumentation MassARRAY based on MaldiTof spectrometry. According to current Decree, the broodstocks intended for breeding are classified into the following classes of resistance: rams and sheeps bearing the ARR allele homozygous; rams and sheeps bearing the ARR allele in heterozygosity and broodstock susceptible which have no ARR allele. Genotype frequencies in Sicily were: ARR/ARR 15.56%, ARR/ARH 1.31%,

ARR/AHQ 3.09%, ARR/ARQ 40.66%, ARQ/ARQ 28.83%, ARQ/AHQ 4.14%, AHQ/AHQ 0.29%, ARQ/ARH 1.93%, ARH/ARH 0.09%, AHQ/ARH 0.12%, VRQ/VRQ 0.18%, VRQ/ARQ 1.92%, VRQ/ARH 0.07%, VRQ/AHQ 0.06%, VRQ/ARR 1.74%. For each flock is attributed a genetic certification in relation to the degree of genetic resistance against scrapie of animals that compose it. In particular the following genetic certification levels are defined:

Level I: flocks composed entirely of animals of the ARR/ARR genotype or having been used for the fitted exclusively rams of the ARR/ARR genotype at least 10 years; level IIa: flocks exclusively employ rams ARR/ARR for at least 6 years; level IIb: flocks exclusively employ rams ARR/ARR for at least 3 years; level III: flocks where exclusively using rams with at least one ARR; level IV: flocks that do not meet the requirements of the higher levels. “Allelic variants of ovine prion protein gene (PRNP) in Oklahoma sheep”

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GOAT'S THORACIC PATHOLOGIES. ULTRASONOGRAPHIC AND MICROBIOLOGICAL OBSERVATIONS

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Respiratory diseases have a great economic impact in small ruminant herds, these disorders are generally highly contagious and may be caused by multiple etiological agents (Mashishi 2007). Department of Agriculture, Republic of South Africa). The clinical features vary from severe with acute development mainly involving bacteria, to chronic diseases that become evident only when the disease process affects a large part of the lung (Caporale et al., 2013. Virology, 338(1): 144-153; Shiferaw et al., 2006. Rev. Sci. Tech. Oie., 25(3): 1153-1163). In this context, it is essential to identify on field diagnostic methods that can provide a valid support for the detection and control of these diseases.

The aim of the study was to establish an on field ultrasonographic protocol to identify the pathological lesions in goats.

According to this purpose, the technique has been preliminarly applied to a group of emaciated CAEV infected goats admitted to the DIMEVET hospital of the University of Bologna. The animals were clinically examined and subsequently, they underwent a complete thoracic ultrasound examination. The obtained results were compared with thoracic radiographic examination and after humane euthanasia anatomopathological and histopathological exams were carried out. Microbiological investigations by means of PCR and RT PCR have been performed to identify the nucleic acids of SRLV, PI-3, BRSV, BHV-1, *Mycoplasma* spp. and JSRV in lung specimens. The protocol was subsequently applied in the field to goats showing signs of respiratory disease as well as to clinically healthy animals.

The results showed that ultrasonography is able to identify mainly the superficial lesions such as those of the pleura as well as interstitial lesions, in both asymptomatic and symptomatic animals, even if the sensitivity was lower than those of the radiography which is considered the golden standard diagnostic technique intra vitam.

Microbiological investigations on lung specimens were positive only for SRLV and *Mycoplasma* spp., and this was also confirmed by histopathological exams. The interstitial pneumonia was the most frequent lesions identified in CAEV affected animals with or without clinical symptoms, confirming the viral tropism for the respiratory tissue. This evidence suggests that echographic images of interstitial syndrome could also be useful for an early identification of infected animals in the flock and may help, with aid of others diagnostic tools, in the selection of animal to be kept for productive life. The method has shown itself to be practical, inexpensive and fast in the field, even if it may require further tuning, due to a lack of technical references in this animal species.

FIRST ISOLATION OF *BRUCELLA OVIS* STRAINS FROM SMALL RUMINANTS IN SICILY

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Historically, all italian farms according to the national brucellosis control programme have been constantly monitored by the Rose Bengal (RBT) and complement fixation tests (CFT). According to Ministerial Ordinance 14.11.2006, all heads found reactive at one of the two tests must be slaughtered and investigated for strain isolation. This issue is very important for all implication of outbreaks traceability. Following these surveillance procedures in the year 2015, some heads, belonging *Brucella* free farms, reacted unexpectedly negative for RBT test and mild to strong positive for CFT. Following clinical investigations in the farm and further sampling of tissues at abbattoire, some positive animals showed *Brucella ovis* strain isolation, furtherly confirmed by specific tests. Additional serological investigation carried out on the same samples confirmed the presence of specific antibodies against this strain. The RBT and CFT were performed according to OIE methods, using the antigens from IZS of Abruzzo & Molise and IZS of Lombardia & Emilia Romagna, respectively. Animals are considered infected if they reacted to the RBT and/or were positive to CFT at ≥ 20 International Units/ml. Samples (milk) and tissues were microbiologically processed by classic isolation procedures (OIE, 2012). *Brucella biovars* were identified by morphological, cultural and biochemical characteristics (Corbel & Morgan, 1984). The strains were also classified according to agglutination with monospecific A and M antiserum (OIE, 2012). All strains were sent to IZS of Abruzzo & Molise for further confirmation. Routine histology (Hematoxylin–Eosin staining, H&E) was performed in order to evaluate basic histomorphological features of the specimens. Out of a total of 61 *Brucella* strains confirmed in IZS in 2015 from sheep and goats (20 outbreaks), 5 samples (all isolated from adult sheep) were identified according to OIE procedures, as *B. ovis* strains. Strains were isolated from material coming from two different Sicilian districts: Palermo and Trapani. *B. ovis* was isolated in 3 different outbreaks, from 6 males/1 female (individual milk). Microscopic examination of reproductive tract of rams showed mild to severe focal epididymitis. Lesions were

characterized by mild to moderate, lymphocytes and plasma cells infiltration sometimes scattered or located perivascularly in the interstitial connective tissue. Focal accumulations of lymphocytes, plasma cells, and neutrophils with necrotic debris were also located in ductal lumina. In Mediterranean areas the most prevalent Brucella species isolated from sheep and goats is *B. melitensis* biovar 3 (Godfroid et al., 2004) whilst *B. ovis* is occasionally reported. By identifying *B. ovis* isolates, this report add some new highlights on a disease which should be investigated also for its implication in hypofertility of Sicilian flocks. In all farms history and clinical visit showed for the first time, the presence of clinical epididymitis and orchitis.

Anonymous. Decree of Italian Ministry of Health 02.07.1992 n. 453, concerning the National Eradication Programme for *Brucellosis* in sheep and goat farms. Gazz. Uff. 23/11/1992, 272, 6-22

PREVALENCE OF INTESTINAL PARASITES IN SHEEP UNDER EXTENSIVE FARMING ON THE MOUNTAIN PASTURES IN CALABRIA REGION (SOUTHERN ITALY)

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In Calabria sheep farming is widespread, the total number of these animals is 281114 and the Region occupies the fifth position on a national scale (July 2015-Database Zootecnic Register of Births instituted by Ministry of Health at CSN Institute "G. Caporale" Teramo). They represent an important economic resource for the local market, even in mountain and foothill areas, with a significant income for the weak agri-food sector. Among the diseases of small ruminants are peculiar the ones caused by parasites, both for their biological cycle and the strategies adopted by parasites to remain in their host. They are a serious problem difficult to eliminate, so it is necessary a continuous and constant control. Considering that studies on the parasitological status in sheep bred in the semi-wild state on the mountain pastures in Calabria are lacking, the aim of this research was to acquire recent data on the prevalence of intestinal parasites in this species. The study was done between Mar/Jul 2015 in 15 Calabrian mountain farms (mean altitude 1.000 mt asl) for a total of 300 sheep, homogeneous for grazing season, not subjected to antiparasitic treatments for 6 months. For copromicroscopic tests we used the FLOTAC dual technique, with 2 EPG/OPG/LPG sensitivity. Our research has revealed the presence of several intestinal parasites in sheep. These parasites are found on farms, with the prevalence related to farms, mean parasite intensity (expressed in eggs, larvae, oocysts per gram of feces) and the prevalence for *Cestoda*: GI-strongyles (100%, 414 EPG), *Nematodirus* spp. (73.3%, 4 EPG), *Trichuris ovis* (46.7%, 4 EPG), *Strongyloides* spp. (40%, 2 EPG), lungworms (40%, 15 LPG), *Eimeria* spp. (100%, 616 OPG) and *Moniezia expansa* (73.3%). This prevalence confirms what has been said in other studies: the animals living in natural conditions are most likely to suffer from multi-pathogen insidious burdens than single infection and emphasises the need to adopt the most effective control strategies in the sheep farms in Calabria Region. In the farms object of our research, anthelmintic treatments are done

once a years without any parasitic diagnosis and the dosing of medicines doesn't always follow a careful evaluation of animal weight. Diagnosis is the crucial point in the parasites control and it is decisive for an effective treatment. In fact, considering that the health of breeding is the base premise to achieve good productive and reproductive performances, only with healthy animals and with the control of parasitosis, after diagnosis and targeted treatments, production and reduced operating costs may be increased.

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TWO YEARS CROSS SECTIONAL SURVEY ON 84 FARMS TO IDENTIFY RISK FACTORS ASSOCIATED TO INFECTIOUS MASTITIS IN SMALL RUMINANTS IN SICILY

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Ruminant livestock in Sicily concerns more than 15,000 farmers delivering high quality dairy products. Endemic diseases such as contagious agalactia (CA), and bacterial mastitis (*Staphilococcus aureus*, Coagulase-negative Staphylococci (CSN) and *Streptococcus* spp. affect this sector. Two years study (2012-2014) screened 84 Sicilian farms in order to determine the prevalence of these diseases and their correlation to risk factors linked to animal welfare, management and traditional farming. The study concerned a questionnaire (no. 22 different variables) associated to representative sampling and laboratory investigations: blood serum (no. 1972) and milk collected from all mammary quarters/udders (no. 4947) from suspected cases or, randomly sampled. Serum samples were screened by an indirect commercial ELISA test for antibody against *Mycoplasma agalactiae*. Isolation procedures have been carried out according to standard international procedures (Nicholas and Baker, 1998, National Mastitis Council, 2004). Two different statistical approaches have been applied: zero inflated Poisson (ZIP) model regression was used to estimate the importance of different factors on number of positive animals showing antibodies against *M. agalactiae* whereas zero truncated Poisson (ZTP) regression was used to investigate the risk of infection in milk due to all most prevalent pathogens of mastitis in small ruminants. *M. agalactiae*, CSN, *Staphilococcus* aureus and *Streptococcus* spp have been considered. ZIP model is mainly utilized for a dataset with an excess of zeros (serum sample) whereas ZTP regression is appropriate for dataset without zero such as in our data resulted from microbiology/isolation of the pathogens. The models were built using the zip command in STATA, version 9, software (SataCorp LP, College Station, Texas). *M. agalactiae* showed 6% of prevalence of individual milks (19% of farms) whereas *S. aureus* showed 8.24% (21% of farms), *Streptococcus* spp. 3.37% (69%), SNC 34.35% (95%) respectively. A prevalence of 8.9% CA antibodies was

determined.

The relative risk factors (RR) estimated for contagious agalactia risk, according to ZIP model (for serum sample screened for CA) were found "clinical history of “Udder infection” and "Vaccination" with RR and 95% confidence interval respectively 1.57 [1.04; 2.37], 1.68 [1.06; 2.67]; an interesting finding was the lower susceptibility observed in “only one specie” farm, where only ovine or caprine animals showed lower risk value 0.49 [0.34; 0.69], compared to mixed farms (sheep and goats together). The RR value estimated from ZTP regression for each pathogen (related to their isolation from milk samples) were “milking period” for *M. a.* with RR=2.97 [1.08; 8.18] and “poor hygiene of bedding” for *S. aureus* with RR=1.149 [4.57; 28.87].

For *S. aureus* risks factor involved significantly, were a total of 8 out of 22: “poor hygiene of bedding” 4.98 [2.27; 10.93], “poor hygiene of milking point” 2.46 [1.17; 5.14], “fleece condition” 1.74 [1.24; 2.44], “use of internal restocking” 1.83 [1.28; 2.73], “feedstuff quality” 5.63 [1.61; 19.77], “common grazing” 5.41 [1.91; 15.31], “common water suppliers” 4.05 [1.94; 8.47].

BRUCELLOSIS IN SICILY: WHICH RESULTS TEN YEARS AFTER MINISTERIAL ORDINANCE 14.11.2006 (ADDITIONAL MEASURES FOR ERADICATION CAMPAIGN) ?

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Italian farms according to the national brucellosis control programme must be constantly monitored by the Rose Bengal (RBT) and complement fixation (CFT) tests. The isolation of *Brucella* organisms from clinical samples has previously been performed only occasionally. A new Italian regulation introduced in 2006 for endemic Italian regions, including Sicily, states that all seropositive animals must be slaughtered and additionally target tissues must be examined by microbiological culture. *Brucella* biovars were identified by morphological, cultural and biochemical characteristics (Corbel & Morgan, 1984, OIE 2012). In order to discriminate between field strains of *B. melitensis* biovar 1 and vaccine strain Rev.1, the following characteristic tests were considered: a) size of colony, b) inhibition of thionin and basic fuchsin (20µg/ml) and c) penicillin or streptomycin susceptibility at 5 UI/ml and 2.5 µg/ml concentration. All strains, when officially confirmed as Rev.1 by phenotypic methods at IZS of Abruzzo & Molise, were also sent to Animal & Plant Health Agency in Weybridge (APHA) UK for further molecular analysis. Real time PCR assays for species/ vaccine determination using defined single nucleotide polymorphisms (SNPs) were run (Whatmore et al., 2006; Gopaul et al., 2008; Gopaul et al., 2010). The last decade 2006-2015 of microbiological investigation reports 75 % of outbreaks in Sicily caused by *B. melitensis* 3, with a total number of 539 outbreaks. In the year 2010 there was the first isolation of vaccine strain B. melitensis Rev.1. Particularly, out of a total of 51 *Brucella* strains confirmed in IZS in 2010 from sheep and goats, 5 samples (belonging one farm) were identified as *B. melitensis* Rev.1 strains. Vaccine strain was also reported in the year 2011 (2 outbreaks) and 2015 (1 single outbreak). All these isolates represent the first findings of vaccine strain in Italy. The strains were isolated from Messina, Caltanissetta and Catania district. In conclusion, this study brings to

light new findings which may complicate the regional eradication programme and highlights the importance of the identification of circulating strains.

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THE GENOMIC AND METAGENOMIC ANALYSIS FOR DIAGNOSIS AT IZSAM

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Ten years ago was born a new technology called Next Generation Sequencing (NGS), and, within a short time it has undergone a rapid evolution in terms of increase in the production of data capacity and lower costs. The NGS applications are various: starting from sequencing of new genomes, analysis of the transcriptome until the microbiome study through a metagenomics approach (1).

In a microbiology lab, the traditional diagnostic path is based on the isolation of bacteria and virus using specific media or cell cultures so as it can be able to identify and characterize the pathogen; that's a long and sometimes difficult process especially when there is an unknown variant about which there are no specific laboratory tests. The metagenomics approach provides a quick turnaround time as it not requires culture isolation and subsequent pathogen typing. Anyway the metagenomics is still a technique for research.

Protocols and workflows streamline and a sudden interpretation of produced data, are making accessible the genomic sequencing of microorganisms and subsequent bioinformatics analysis on the data for routine diagnosis at IZSAM. The Laboratory Information Management system SILAB has been adjusted to trace all WET and DRY analysis on the original sample, to simplify and support several steps needed and to make activity more efficient, controlled and recorded. In most cases, the first step is in charge to the Analyst of "traditional" diagnostic Sections who asks for genomic analysis of bacterial or viral strains. Basically it is "another distribution" but it produces in SILAB a new simplified Acceptance record with a new Submission Number (NRG). It reads from a CSV file, with the samples codes that you want to sequence. Through the

ID of the strain (which recalls the original NRG plus the univocal sample progressive) SILAB provides all metadata (sampling point, reason, material, species, customer, owner etc.) and can show all results of the original sample. For each sample, you can distribute using SILAB, NGS and/ or metagenomic tests.

Bio-informatic staff reads the working sheet from SILAB and pull out it as a CSV file, select sequencing samples, complete sample sheet and use it to start test. When the sequencing and the quality test process are completed, the bio-informatics receive an email with a result file that is read by SILAB for uploading results. The result of NGS test is the location (URL) of the output file: FASTQ trimmed. In addition to the department equipment's, we use a server with a terabyte space for saving raw data.

Genotyping of pathogens is an essential step in the surveillance of infectious diseases and for studying of epidemic outbreaks. SILAB usage, according with sequencing technologies, put in order operational flow, reduced faults, made available data to all authorized users. Above all, it is a support for the analysis and finally for diagnosis as thanks to a button, can get the history of a sample from which the strain comes from.

1) Choice of Next Generation Sequencing pipelines in:"Bacterial Pangenomics" Methods in Molecular Biology, Vol. 1231 11/2014, ISBN: 978-1-4939-1719-8, DOI: 10.1016/j.ttbdis.2016.03.012

CHARACTERIZATION OF BVDV-2 OUTBREAK IN NORTH-WEST ITALY: FROM MILK TO NGS

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Bovine viral diarrhea virus (BVDV) types 1 and 2 are members of the Pestivirus genus of the *Flaviviridae* family. This genus also includes the HoBi-like virus, tentatively classified as BVDV type 3. Infection is sustained by persistently infected (PI) carriers, which acquire the infection with a noncytopathic (NCP) strain early in the fetal stage and remain immunotollerant virus shedder throughout their life. The lifespan of PI animals is usually short, due to early culling or the development of mucosal disease (MD), a fatal outcome arising from the biological behavior of the NPC strain, which mutates to its cytopathic (CP) counterpart. Since the E2 glycoprotein is the most divergent antigen between BVDV type 1, 2 and HoBi-like and is able to elicit neutralizing antibodies, in this study we developed a multiwell antibody ELISA based on the E2 proteins and we evaluated the applicability of it for surveillance purpose, using pooled milk samples. Amino acid sequences, corresponding to the ectodomain of the E2 protein of the three BVDV types were retrieved from the GenBank database. Genes were subcloned into pSecTag2/Hygro plasmids allowing the extracellular sorting and secretion in the medium of transiently transfected mammalian cells (HEK293T). The method was applied to a total of 436 milk pools collected from 179 dairy farms. 33 farms showed only negative pools. A total of 111 pools (63 farms), showed a clear reactivity against BVDV-1. 9 milk pools belonging to 3 farms reacted against BVDV-2. In particular, 3 pools belonging to a single farm showed a clear reactivity in E2 ELISA. Information about animal management revealed that in the remaining two farms a double-strain live vaccine was used. 14 pools showing a moderate reactivity against HoBi-like were detected. The BVDV type could not be ascertained in 177 pools. The farm with clear BVDV-2 reactivity was further investigated. Spot tests on young animals that had never been vaccinated confirmed that most of them were reactive against the type 2 antigen. The identification of PI animals was carried out by antigen ELISA.

Among the PI, two of them (830 and 821) were selected for viral strain characterization. The first animal expired after developing MD during the investigation and samples from spleen and intestinal epithelium were collected. For each sample, the 5'UTR region was amplified to identify the BVDV type. In order to characterize the two strains, a set of primers designed on the NS2-3 region allow the amplification of 2.2 Kbp fragments, submitted to NGS (MiSeq, Illumina). Consensus sequence from 821 was homologous to NCP BVDV-2 reference sequences, whereas 830 showed at least two variants of a divergent viral strain. The variants showed an insertion of two different bovine ubiquitin partial sequences leading to suppose the occurrence of multiple events of genome modification from NCP to CP. In one case, the polyprotein coding frame is respected, but in the second one, at least one stop codon is present along the ubiquitin sequence. Further investigations will be carried out on 821 in order to understand the compartmentalization of BVDV-2 and its evolution from NCP to CP.

INNATE IMMUNE SYSTEM GENE EXPRESSION IN LYMPHOCYTE, MYELOMONOCYTIC AND EPITHELIAL CELLS FROM MASTITIC BOVINE MILK

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Bovine mastitis is a multifactorial disease responsible for serious economic losses worldwide in dairy farms. Variations in presentation of the disease between pathogens may result from differences in the innate immune system competence to mount initial defences (1). These may be linked to factors such as recognition of pathogen derived antigen e.g. by Toll-like receptors (TLR4, TLR2) (2) or the mobilization of bactericidal effector molecules such as the β-defensins (DEFB5) (1), chemokine (CCL2) (3) or antimicrobial peptides (LAP, LTF, LYZ, TAP) (4, 5). The objective of the present study was to investigate the gene expression profile of main factors involved in innate immune system in healthy and mastitic dairy cows. In the three principal types of somatic cells the gene expression level was calculated. In this study, 43 Friesian cows were included on the basis of somatic cell counts (SCC) and bacteriological analysis of the milk: 24 milk samples from clinical cases of bovine mastitis by *S. aureus* and 19 milk samples from healthy dairy cattle (SCC< 200.000 cell/ml) were included. All milk samples were centrifuged and the cell type separation was performed by magnetic labeling. In particular, myelomonocytic cells (monocytes and granulocytes) were obtained through a first positive selection by anti-CD11b. In a second positive selection, lymphocytes were separated by anti-CD45, whereas in the flow-through epithelial cells were selected. These cells were observed and quantified by flow cytometry. Total RNA was extracted from each cell type of cells and the relative amounts of specific target genes, like TLR4, TLR2, CCL2, CCR4, CD14, DEFB5, LAP, LTF, LYZ1, and TAP, were determined by quantitative PCR Moreover, PPIA, GAPDH, and SR5 were tested as housekeeping genes for normalization (comparative Cq method). Mastitis influence on the expression of each gene in different cell types was assessed through GLM univariate analysis (IBM SPSS Statistics v23.0). The result was considered to be significant if $p<0.05$.

S. aureus infection induced a significant up-regulation of TLR4, both in myelomonocytic cells and lymphocytes; TLR2 and TAP were over expressed only in myelomonocytic cells while lymphocytes showed an up-regulation of CCL2 . Epithelial cells from mastitic milk did not show any significant difference of target gene's expression. These results confirm the relevant TLR involvement in immune response. The present study provides relevant information for the identification of candidate genes for the genetic profile related with the resistance to mastitis. In the future ,we intend to study the gene expression profile, of myelomonocytic cells, from healthy cows coming from farms with different mastitis incidences to identify genes involved in the mastitis resistance.

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EVALUATION OF A NEW DIAGNOSTIC TOOL FOR IBR SURVEILLANCE IN DAIRY HERDS: A FIELD STUDY IN CUNEO PROVINCE

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The bulk milk represents a cost effective matrix to monitor the serological status of the infection within dairy herds (Reber et al. 2012). Many infectious diseases, such as Bovine Infectious Rhinotracheitis (IBR), are object of voluntary or compulsory eradication programmes based on the individual serological test of receptive animals (2004/558/CE). When a marker vaccination is in place, the possibility to monitor the seroconversion in milk samples, requires a gE ELISA able to detect specific antibodies with an appropriate sensitivity and specificity (Van Oirschot J.T, 1999). Despite the low level of IgG in bulk milk samples as well as the reduced sensitivity of gE blocking ELISA applied to this matrix (Schroeder et al. 2012), a new diagnostic tool is now available. Eradikit® Bulk Milk Surveillance Kit plus (Eradikit®BMSK plus, In3diagnostic Italy) consisting in a sequential precipitation of IgG and others milk proteins, followed by a gE indirect ELISA, seems a promising tool for IBR surveillance of vaccinated herds. In this study the method was applied in a heterogeneous sample set of dairy herds (297) of Cuneo province, in comparison with the individual serum assay. Individual milk samples were collected from 70,000 lactating cows by the breeders association (APA) and used, before milk parameters determination, to make pooled milk of a sample size up to 40 animals. Each pooled milk was subjected to sequential precipitations according to the user protocol and the final enriched fractions were tested in gE indirect ELISA. In three different test sessions (2200 pools), 140 classified positive farms and 157 recognized gE negative herds were monitored over twelve months, calculating the field sensitivity of the method. All farms classified as gE negative by the official gE

blocking ELISA scored negative to Eradikit® Bulk Milk Surveillance Kit plus (specificity: 100%, 95%CI: 97.7%-100%). In a single herd a recent viral circulation was demonstrated by the gE blocking ELISA in individual serum assay, confirming the bulk milk results . This result support the use of the bulk milk for the early detection of viral circulation.

Among the positive farms enrolled in this study, all but 2 with a herd-prevalence higher than 2.5% were tested as positive, while under the 2.5 % the positive farms remained negative.

The field sensitivity of the method seems adequate for surveillance purposes in gE negative farms and suitable to detect viral circulation at prevalence level higher than 2.5%. Since data about the identification tag of lactating cows are available, this information will be used in order to evaluate the infection prevalence in the milk pool and, consequently, the diagnostic sensitivity of the method.

In case of a seroprevalence lower than 2.5% or in presence of old whole-virus vaccinated cows, Eradikit® BMSK-plus could be used for eradication purposes reducing the pool sizes to detect a weak positive sample diluted in negative milks.

PREVALENCE OF *COXIELLA BURNETII* IN DOMESTIC RUMINANTS IN SICILY, ITALY

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Q fever is a zoonosis with a great economic impact on livestock production and it is caused by *Coxiella burnetii*, an obligate intracellular bacterium, widespread throughout the world. The organism infects principally goats, sheep and cattle, even if also a wide range of mammals can also act as reservoirs (Million and Raoult, 2015). *C. burnetii* causes abortions, endometritis and infertility in cattle and small ruminants. To date, few studies on *Coxiella burnetii* prevalence in domestic ruminants in Sicily are reported (Torina et al., 2007).

This study was aimed to estimate the molecular and serological prevalence of C.burnetii in Sicilian domestic ruminants (cattle and sheep). For one year (summer 2014 - spring 2015), 15 bovine and 15 ovine farms were monitored seasonally in two Sicilian provinces, Ragusa and Agrigento, with a total of 60 farms. *Coxiella burnetii* antibodies were determined by an indirect enzyme immunoassay ELISA (IDVet, Montpellier, France). Presence of pathogen DNA was investigated by PCR through the amplification of a region of the htpB gene (To et al., 1996). Prevalence values were compared seasonally in the different provinces.

The results of the serological survey showed a high prevalence in sheep and cattle farms from the two Sicilian provinces.

Prevalence values were higher in sheep than in cattle from both the provinces and during almost all the seasons of the year.

Many farms resulted positive at the serological survey in all the seasons. Molecular analyses didn't show any *C. burnetii* positive animals.

The results indicate that Sicilian cattle and sheep are commonly exposed to *C.burnetii* infections in Sicily with a high overall seroprevalence in almost all the farms of the two analyzed provinces.

Obtained data can update the available information on pathogen presence and spread in the territory, contributing to infection prevention and control, with a positive impact on animal and human health. These data can be a warning for public health workers at risk of Q fever, especially if they are in contact with dairy ruminants or their products. In addition, animal health workers and livestock managers should be aware of the possibility of *C. burnetii* infection in their animals and be encouraged to introduce active surveillance programs and management methods in order to control infections.

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MYCOPLASMA INFECTIONS AT RISK FOR SICILIAN DAIRY SECTOR: THE CASE OF *MYCOPLASMA BOVIS*

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In a previous bacteriological and pathological survey on slaughtered cattle from Sicily, *M. bovis* was identified in 27,3% (9/33) of pneumonic lesions confirming the importance of this pathogen for bovine respiratory disease (BRD) in Italy (Loria et al., 2004). *M. bovis* is responsible in Europe of other emerging syndromes particularly mastitis (Nicholas and Ayling, 2003; Sachse et al., 2009, Radaelli et al., 2011; Puleio et al., 2014). Aim of the study was to evaluate the role of *M. bovis* in bovine dairy sector in Sicily through bacteriological and molecular investigations. No. 90 farms, characterized by higher somatic cells levels in bulk tank milks, all belonging Ragusa districts, have been screened to identify eventual mycoplasma infection. Bulk milk sampling was carried out in milk refrigerator container with sterile syringe, after vortexing for 5 minutes. Individual milk from each udder was collected by proper disinfection of teat. An aliquot of milk samples (300 µl), were cultured (10% in vv) in 5 ml vials filled with mycoplasma broth (modified Hayflick broth - Oxoid). In addition a direct plating of 25 µl of milk was also performed. After 2-3 days incubation at 37°C in a humidified atmosphere with 5% CO^2, broths were subculture in mycoplasma agar (Oxoid) plating a drop (25 µl). Broths were examined daily for signs of growth or changes of pH indicated by a colour change in the media. Plates were examined every day for one week under 35X magnification for the typical “fired egg” appearance

DNA was extracted from broth cultures containing different mycoplasmas isolates with a PrepMan Ultra Sample Preparation Reagent kit (Applied Biosystems®). PCR was performed using primers specific for the *M. bovis* mb-mp81 gene (Foddai et al.,2005), and the following thermal protocol:

an initial denaturation at 94°C for 3 min, 30 cycles at 94°C for 1 min, 54°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 10 min. Agarose gel electrophoresis was performed to analyse PCR products.

Out of 90 farms, no. 2 were found positive for isolation of *M. bovis* from bulk tank milk. All strains have been confirmed through Molecular Biology methods. Further analysis for identification of singular positive udders/heads showed low prevalence of positive (excreting) cows (1.16 % out of 86 cows). In spite of large number of "at risk" farms due to higher level of somatic cells, recently negative for common endemic mastitic pathogens, we confirm the circulation of *Mycoplasma bovis* in Sicilian dairy production. However this low prevalence doesn't represent a significant risk for our livestock but should stimulate veterinary professionals and laboratory experts to include also this pathogen among cause of bovine mastitis in Sicily.

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THE SLAUGHTERHOUSE TO UPDATE THE PRESENCE OF BOVINE ENDOPARASITES

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Gastrointestinal (GI) nematodes infections of cattle still represent a constrain on the efficient raising of cattle on a global scale. Infections with these parasites are rarely lethal, but can occasion substantial losses in productivity from usually subclinical infections (Charlieret al., 2009). Nevertheless up to date information concerning prevalence and associated determinants (e.g., sex, age, on-farm management and husbandry)is lacking in our country. The present study focused on providing current data on GI nematode parasitic infection prevalence and epidemiology in adult cattle bred in Italy. The survey was performed collecting 427 faecal samples from a bovine slaughterhouse in the province of Bologna (Italy) from 2014 through 2015. Samples obtained from single animals processed, were analyzed by qualitative copromicroscopical examinations. From the same animals 100 abomasa were randomly selected and examined by necropsy technique to assess the presence of worm burdens. With the present study we would determine whether certain host factors can affect GI nematode faecal egg patterns in farmed cattle. At this purpose each animals processed was identified in the National Livestock Identification System (NLIS) to obtain additional information about biological and zootechnical parameters. The surveyed animals were of various age (from 1 to 26 years old) and mainly female (93% of the sample). Those female were both dairy cows (63%) and brood cows (37%). 90% of the total samples were adult animals (>2 years old). The data collected pointed out a very uneven geographic distribution of the facilities, where animals tested were housed. The farms were located in 11 regions and 33 different local districts within Italy. GI nematode eggs were detected in 31% of individual faecal sample examined for the qualitative assessment of worms burdens using floatation technique. Evaluation of abomasa by necropsy technique exhibited 13% prevalence of GI helminthes. The genus detected were *Ostertagia* sp., *Trichosrongylus* sp. and *Cooperia* sp. The descriptive data obtained

were analyzed trough statistical software systems in order to measure the significance of possible associated determinants (age, sex and management conditions) on prevalence of GI helminthes. The faecal output of nematodes eggs was significantly related ($p \leq 0,05$, $x2=0.000046$) with the livestock category: brood cows were found infected more frequently as compared to dairy cows (prevalence rate 74% vs 37%). At herd level the percentage of positive samples was dependent on a significant manner on the stocking density: facilities with 50-100 animals housed showed higher prevalence rate both than larger (>200) and smaller (0-50). Our survey provides evidence that GI parasitism is widespread at present in adult cattle bred in Italy, with relatively high prevalence rates although the significance of the problem seems to be still underestimated by technical experts in the field.

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PRELIMINARY SURVEY ON THE PRESENCE OF *NEOSPORA CANINUM* IN DAIRY AND BEEF CATTLE IN SICILY

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Neospora caninum is a worldwide protozoan parasite. The dog is the definitive host but can also act as an intermediate host as well as cattle, sheep, goats, horses and deer. These hosts become infected through food and water contaminated by dog faeces containing oocysts. In cattle, infection can be vertically transmitted from dam to calf and lactogenically. This protozoan is regarded as a major cause of abortion in cattle. Most abortions occur at 5-6 months of gestation. The diagnosis most used is an enzyme-linked immunosorbent assay (ELISA) technique. In Italy epidemiological studies showed a prevalence ranged from 44.1% in cattle from southern and northern Italy (Otranto et al., 2003), 55% in sardinian cattle farms (Varcasia et al., 2006) to 77.8% in cattle farms located in southern Apennines area (Rinaldi et al., 2005). As it is known cattle with antibodies to *N. caninum* are more at risk to abort than seronegative cows. Therefore the aim of the present study was to investigate on the presence of *N. caninum* in dairy and beef cattle bred in Sicily. The study was carried out in Ragusa province, an area located in the south-east of Sicily. The economy of this province is strongly related to the cattle farming. From January to June 2015, a blood sample from cattle present in several farms were collected. Data on attitude, age and sex were registered. The antibodies to *N. caninum* were detected by a commercially available ELISA-kit (ID Screen® *Neospora caninum* Indirect Multi-species). A total of 2,101 (1,991 females and 110 males) cattle from 34 farms were serologically tested, 323 heads were positive (312 female and 11 male) at the ELISA test with an overall prevalence of 15.37% (14.85% in females and 10.00% in male). The parasite presence was detected in 30 out of 34 sampled farms (88.23%), with a prevalence in the single farm ranging from 0.98% to 57.14%. No significant statistical difference were detected between males and females ($p=0.1455$). The prevalence was significant higher in adult and old animals compared to calves ($p= 0.00432$ and $p=0.0038$ respectively). Also the prevalence was significant

higher in dairy (p=0.0055) than in beef cattle. As underlined from the results herein reported *N. caninum* is widely present in the study area, the 88.23% of farms were positive for *N. caninum* presence. This result matches with a similar study performed in southern Italian Apennines (i.e., 77.8%, Rinaldi et al., 2005). Considering the direct (i.e., abortus) and indirect (i.e., increased lactation time, possible loss of milk yield) economic losses related to the disease, the prevalence of positive farms observed in the present study, you need to improve the monitoring activities and the prevention of neosporosis in cattle in Sicily.

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GASTROINTESTINAL NEMATODE INFECTIONS IN SHEEP: DIAGNOSIS WITH MINI-FLOTAC TECHNIQUE ON FARM

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Gastrointestinal nematode (GIN) infections cause severe diseases and affect welfare and productivity in all classes of sheep. Therefore, a rapid diagnostic method for detection of GIN directly on farm is needed in order to quickly plan and apply appropriate anthelmintic treatments. Diagnosis of GIN in ruminants mainly relies on Faecal Egg Count (FEC) techniques (Roeber et al., 2013). The aim of this study was to evaluate the use of the Mini-FLOTAC technique (Cringoli et al., 2013) on farm using a novel kit composed by Fill-FLOTAC, Mini-FLOTAC and a portable microscope. Individual faecal samples were collected from five flocks in the Salerno province in southern Italy. In each farm, 20 samples were collected from sheep. Parasitological analyses were performed by comparing the Mini-FLOTAC technique on farm and in the lab. In each farm, twenty faecal samples were pooled (Rinaldi et al., 2014) in four composites of five samples each (two grams for each individual samples) for a total of ten grams. Each composite was diluted using a sodium chloride based flotation solution (FS2, specific gravity = 1200) (dilution ratio = 1:1). Each diluted pool was put into the Fill-FLOTAC container and a sodium chloride flotation solution (FS2; gravity = 1200) was added (dilution ratio = 1:10). The suspension was then thoroughly homogenized, filtered through the Fill-FLOTAC and used to fill the two chambers of the Mini-FLOTAC. After 10 minutes, the top part of the flotation chambers was translated and the Mini-FLOTAC was read under a portable microscope. Mini-FLOTAC was used at the analytic sensitivity of 10 eggs per gram (EPG) of faeces. Statistical analysis was performed by SPSS using Spearmann's rank correlation coefficient. GIN EPGs ranged from 518 to 2,467 EPG (mean = 1023 EPG) using the Mini-FLOTAC technique on farm and from 546 to 2,535 (mean = 1070 EPG) using the Mini-FLOTAC in the lab. Statistical analysis showed a significant correlation (rho = 0.9966; $P<0.001$) between results obtained using Mini-FLOTAC on farm and in the lab. These results showed that Mini-FLOTAC on farm is a valid alternative to its use in the lab with some advantages, permitting an easy

diagnosis of GIN directly on farm by vets. However, capillary and accurate trainings by expert parasitologists will be needed before the routine use of the technique on farm in order to promote good practices of parasitological diagnosis.

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DIAGNOSIS OF TOXOPLASMOSIS IN A STRAY DOG BY DIRECT GENOTYPING FROM MUSCLE BIOPSY

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Early diagnosis of clinical toxoplasmosis in dog is a critical point for early treatment and effective recovery. The aim of the study was a rapid in vivo diagnosis of clinical toxoplasmosis in dogs for a rapid treatment and *T. gondii* typing to evaluate the relation between clinical signs and specific genotypes. To confirm the suspect of *Toxoplasma gondii* infection in a stray dog with a muscular atrophy and hyperextension of hind limbs, a muscle biopsy was performed from superficial gluteus and genomic DNA was extracted. *T. gondii* specific fragment was amplified using a high sensitive nested polymerase chain reaction assay [1]. The lineage type of *T. gondii* was determined by PCR-RFLP of the amplified SAG2 gene [2] and by microsatellite markers in a multiplex PCR [3]. Microsatellite analysis was carried with GeneMapper 4.0 (Applied Biosystem). Specific therapy for toxoplasmosis (Clindamycin hydrochloride; 25 mg/kg orally twice daily for 4 weeks) and aquatic physiotherapy were performed to help solve the muscular atrophy.

ELISA rapid tests excluded other causes of paralysis in dog as tick-borne disease (Lyme disease, ehrlichiosis, anaplasmosis and babebiosis), while a positive response for *T. gondii* IgG antibodies was detected. To exclude a *Neospora caninum* infection, the protozoan most closely related to *T. gondii* cause of dermatitis and paralysis in dogs, a 314 bp *T. gondii* specific fragment was amplified from the sample. RFLP analysis revealed that the 3'- and 5'-end amplified fragments of the SAG2 gene was undigested with the corresponding restriction enzymes, recognizing the genotype I. Microsatellite analysis confirmed this result identifying clearly the clonal type I. After the first week of treatment the dog started to move the hind limbs; after two weeks it was able to stay in quadruped station and the ulcerative lesion in tarsal region was healed. In the third week the dog regained partial ambulation and after four week the ambulation returned normal.

Our study reports a severe clinical case of toxoplasmosis in a stray dog and showed that the detection of parasitic DNA in the tissue is a useful diagnostic method in facilitating early treatment of the disease important for a timely clinical recovery. These data confirm the importance of early diagnosis of toxoplasmosis in dog and how clinical signs are often related to specific genotypes.

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SURVEY ON THE PRESENCE OF PoPs AND HEAVY METALS IN LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) STRANDED IN SICILIAN SHORES

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Inorganic contaminants and persistent organic pollutants (POPs) are present in aquatic systems worldwide as a consequence of their widespread usage and long-range transport. The bioaccumulation of these toxic substances has become a matter of concern for its possible transfer to food chain and impact on several wildlife species of the marine environment, including loggerhead sea turtles (*Caretta caretta*). In this work a total of 71 stranded loggerhead sea turtles *Caretta caretta* were analysed for POPs and heavy metals (As, Pb, Cd) detection. All the turtles were found and caught in Sicilian coasts from 2013 to 2014. All the samples were measured and weighed. The muscular and adipose tissues were analysed for toxicological studies. About 5 g of muscle and fat tissue were subjected to an Accelerated Solvent Extraction (ASE) automated system for PCBs analysis by Gas Chromatography equipped with triple quadrupole mass spectrometer. The heavy metal analysis were conducted by an Inductively Coupled Plasma Mass Spectrometry method after microwave digestion of about 1 g of sample. A Limit of Detection (LoD) of 0.003 mg/kg was set up for As and Cd detection while the LoD for Pb was set up at 0.004 mg/kg. The validation of the method for PCBs detection revealed a a LoQ of 1 ng/g of fat. The measuring range for organochlorine analytes was between 100 and 1500 µg/kg. About the 59% of the samples revealed PCBs concentrations under the LoDs. The maximum PCBs concentrations was reached in a turtle from Palermo coasts. PCB 180 and 138 were the more detected PCBs in all the turtle samples. Only two turtles, stranded in Valderice bay, revealed organochlorine pesticides (4.4 DDE) concentrations over the LoD on their adipose tissues. About the 78% of the turtles examined in 2014 detected heavy metals concentrations over the LoD, with maximum values of 4.09 ppm for Cd, 6.9 ppm for Pb and 9.1 for As, while all the turtles examined in 2013 revealed Pb, Cd and As levels under the LoD. The highest

PCBs values were detected in turtles found at Termini Imerese and Palermo, while all the turtles found at Augusta, Avola, Capopassero, Carini, Catania, Cinisi, Lampedusa, Lipari, Riposto, Pozzallo, Santa Flavia, Taormina and Ustica showed PCBs levels under the LoD. Very high mean As levels were found in loggerhead sea turtles stranded in Milazzo, with a maximum of 9.1 mg/kg. Results showed a major presence of heavy metals in muscle tissues, while PoPs were mostly found in adipose tissues suggesting a marked difference due to the chemical properties of the two pollutant. The highest PoPs levels reached in loggerhead sea turtles stranded in Palermo and Termini Imerese can be attributable to disastrous phenomena such as the fire at the Bellolampo landfill in 2013 and 2014, where a large amount of waste was burned uncontrollably. The environmental fate of these xenobiotics may be traced by the analysis of turtle's tissues. Generally, loggerhead turtles exhibited a higher metal load than other turtle species, this could be explained by differences in diet habits being food the main source of exposure.

ISOLATION OF *FUSARIUM SOLANI* AND *FUSARIUM OXISPORUM* IN EGGS FROM LOGGERHEAD SEA TURTLES IN THE MEDITERRANEAN BASIN: PRELIMINARY DATA

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Loggerhead sea turtle (*Caretta caretta*) is a protected species often reported in the Mediterranean Sea and unfortunately often stranded. Several dangers jeopardize their survival e.g. accidental bycatches and infectious diseases. Among the hazard affecting the nest, fungal infections cause huge losses. The aim of this study was to identify the presence of a fungal infection on *C. caretta* eggs collected in a beach of the Mediterranean basin. Four unhatched eggs collected from a *C. caretta* nest in Linosa Island (Sicily, Italy) were sent to the microbiology laboratory of the Istituto Zooprofilattico Sperimentale della Sicilia (Italy). Eggs were deformed, dented and reddish black coloured and containing reabsorpted embryos. Specimens from egg shell, inner content and 10 sand samples obtained from the nest and surrounding areas were used for microbiological analysis. Samples were enriched in peptone water (plus NaCl) and then seeded in blood agar, in two other culture media (Tripton Soia Agar plus NaCl and thiosulfate citrate bile salts sucrose agar) and in Sabouraud dextrose agar (pH 5.6). In Sabouraud agar, colonies microscopically identified as *Fusarium solani* and *F. oxisporum* were found. The last was also confirmed by PCR and sequencing (1). No fungal growth was observed from sand cultures except for *Aspergillus* spp. *Pseudomonas* spp. was isolated in both eggs and sand. This data suggest that the fungal infection is not due to an environmental contamination but was already present in the mother reproductive tract.

F. solani, F. falciforme and *F. keratoplasticum* were already isolated from sea turtle eggs (2–6). In addition, *F. solani* and *F. oxisporum* were previously found in difference sea

turtle species in Australia (7), Cabo Verde (8) and Brazil (9) and phenomena of high mortality rate and unhatched eggs were related to the fungal infection (3–5). Our data confirm the presence of mycetes belonging to *Fusarium* genus and, in accordance with data reported by several authors suggest a role in unhatching of *C. caretta* eggs. To the best of our knowledge, this is the first report of *F. oxisporum* in eggs from loggerhead sea turtle in the Mediterranean basin. These findings suggest, in our opinion, that fungal infections originated from the female and no from the environnement. Therefore, researchers should do not underestimate the several losses related to fungal infections and should investigated the suspicious cases of unhatched eggs in order to identify the causative agent of the reabsorpted embryo.

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APIS MELLIFERA SICILIANA: GENETIC FINGERPRINTING APPLICATION TO STUDY THE GENOME VARIABILITY

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The distribution of *Apis* subspecies can be influenced by beekeeping activities. Large scale migratory beekeeping and trade in queens, coupled with the promiscuous mating system of honeybees, have exposed native European honeybees to increasing introgressive hybridization with non-native subspecies, which may lead to the loss of valuable combinations of traits shaped by natural selection. Consequently, in organic beekeeping the use of local breeds or ecotypes is highly recommended. The subspecies *Apis mellifera siciliana*, whose natural habitat is Sicily, has been threatened with extinction due to hybridization with *A. m. ligustica*, introduced into the island by beekeepers when commercial beekeeping became widespread (1970s). A small population of *A. m. siciliana* survived mainly on the Eolie islands. This work herein assesses a genetic database built on microsatellite profiles to estimate the genetic structure of the Sicilian honeybee population, and determine gene pool through the appraisal of the introgression from *A. m. ligustica*. Twelve microsatellite loci were analysed with multiplex-PCR. This method provides a valuable colony-selecting parameter which allows an effective re-introduction of *Apis sicula* into the main island (Sicily). The results of this work could improve the management of the bee breeding technologies. Furthermore, the project envisages sampling of Western Sicilian bee populations in order to find new lineages to minimize inbreeding on the current Eolian population. Thus, this study involves extensive field sampling and laboratory analyses at nuclear level to identify the frequency of 10 unlinked STRs loci.

For this purpose a multiplex-PCR technique was optimized in order to amplify multiple loci in a single reaction. The polymorphism was detected by amplifying the microsatellite sequences using fluorescent dye primer-labeled complementary to the regions flanking the STRs.

Two different panels were set to assess a fingerprinting tool based on the following locus: A007, A088, AP55, A113, AB124, A29, A8, Ap226, A43, A14, A79, A44. Allele frequencies were analised by Genealex and Structure software packages to search for hybridization. Genetic diversity was assessed based on the allelic database. GenAlEx was employed to estimate the number of alleles (Na) and the frequencies at each locus (Af), the observed and expected heterozygosity (Ho and He), the AMOVA analysis, and the genetic distance among the two populations based on the principal component analysis (PCA).

ATTENUATED VACCINE OF *SALMONELLA TYPHIMURIUM* MONOPHASIC VARIANT IN PIGLETS: EVALUATION OF EFFICACY IN HOMOLOGOUS AND HETEROLOGOUS CHALLENGE INFECTION

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Salmonella Typhimurium Monophasic variant was identified during the second part of nineties and its incidence is increased during the last two decades. As *S. Typhimurium*, is widely spread worldwide and clinically indistinguishable (Echeita et al., 1999). Pigs could be chronically infected and introduce bacteria into slaughterhouse contaminating the food chain process with a high risk for human health (Hauser et al., 2010; EFSA, 2015). The aim of this study was to analyze efficacy of two attenuated vaccines, *S. Typhimurium* ΔznuABC and *S. Typhimurium* Monophasic variant ΔznuABC in homologous and heterologous challenge infections in piglets, because, these two strains are the most common isolated in pig farms and they could be simultaneously present in piggeries.

Twenty-eight animals were divided in 6 groups, two unvaccinated (5 in group E and 5 in group F), two vaccinated with attenuated *Salmonella Typhimurium* ΔznuABC (3 in group A and 5 in group B) and two vaccinated with attenuated *Salmonella Typhimurium* ΔznuABC Monophasic variant (5 in group C and 5 in group D). Three groups were infected with virulent *S. Typhimurium* (A, D, E) and three with S. *Typhimurium* Monophasic variant (B, C, F) at day 36 after vaccination. Clinical investigations were weight and temperature measurement. Fecal samples were collected at day 1, 4, 9, 15, 23, 29 and 35 after vaccination and at day 2, 7, 10, 14 and 20 after infection for microbiological analysis. At day 20 after infection, piglets were euthanized and samples

of tonsils, ileocecal lymph nodes, spleen, ileum, caecum and colon were collected for microbiological and histological investigations.

Results have underlined that weight was not affected by vaccination and infection. The trend of vaccines shedding was similar among groups and particularly, a sharp decline was present in both groups during the first week after vaccination. Vaccination with both attenuated strains was more effective in reducing shedding of mST than shedding of *S. Typhimurium*. Tonsils were the most colonised organs and mSTΔznuABC was more effective in reducing heterologous colonization.

In conclusion, vaccination with mSTΔznuABC seems to be the preferable choice reducing faecal shedding of homologous and heterologous virulent strains significantly. Furthermore, it determines a reduction in organs of immune system, as tonsils and lymph nodes, during heterologous infection with virulent *S. Typhimurium.*

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MONITORING ANTIMICROBIALS CONSUMPTIONS IN PIG FARMS WITH DIFFERENT APPROACHES

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Veterinary medicinal products (VMPs) are widely used in pigs including antimicrobials critically important for human medicine (CIA) [1]. Bacteria may develop antimicrobial resistance (AMR) that affect both animal and human health [2]. Decreasing antimicrobials consumption is pivotal to slow down AMR spread and proper standards to monitor VMPs usages are essential. However, a unique standard for veterinary medicine is still under discussion. Prioritisation of antimicrobial classes for human medicine is also debated and different classifications were proposed [1, 3]. The aim of this study was to test different approaches for monitoring antimicrobials usage in pig farms.

42 fattening farms, with at least 2000 pig produced per year, were selected from herds included in a project implemented by Ministry of Health in collaboration with IZSLER, Lombardy Region and Official Veterinary Services. Data for 2014 were collected retrospectively. Consumptions were calculated using a Defined Daily Dose Animal standard (DDDAit) and a mass-based standard: milligrams of active ingredient (AI) consumed per kilogram of live weight produced (mg/kg meat). DDDAit were used to calculate, for each farm, mean days of therapy per pig per year (average weight at treatment 100 kg) with two different approaches. One considered each used AI independently; the other considered VMPs with a combination of AIs as a single antimicrobial. CIAs were identified according to WHO ranking [1] and further stratified with EMA classification [3].

The 42 farms produced 220,569 pigs (mean 5368, range 2056-15798) and 37,797 tonnes of live weight (mean 920, range 354-2686). Mean consumptions, at farm level, were 196 mg/kg meat (range 20-530), 27.69 days/pig/year for single AIs (range 3.41-

81.52) and 22.71 days/pig/year for combinations (range 3.41-81.52). Days/pig/year differed significantly (p-value<0.0001) between the two standards, mean difference was 18.36% (range 0.00%-48.95%). 24.69% of consumed DDDAit were CIAs. According to EMA classification, 38.90% of these CIAs were category 1 antimicrobials and 61.10% category 2.

Although practical to calculate, mass-based standards do not consider AIs power and should be discontinued. DDD-based approaches can be more accurate but collecting data without an electronic prescription system is time consuming. Widely accepted standards are strongly needed for both consumptions calculation and AIs prioritisation. Particular attention should be posed when monitoring usages of combinations VMPs. Considering AIs separately increases combinations VMPs impact on overall consumptions, and so, it may discourage abuses. Nevertheless, these VMPs can reduce risks of AMR via theirs AIs synergic effects against some bacteria.

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BIOMOLECULAR INVESTIGATION ON PREVALENCE OF AVIAN MALARIA IN COMMON KESTREL (*FALCO TINNUNCULUS*) IN EMILIA ROMAGNA (ITALY)

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The Common Kestrel (*Falco tinnunculus*) is one of the most successful falcons, present throughout the world, with the exception of Antarctica, the tundra and deserts. European populations are stable in many countries but decreasing in others. In Europe the population size is estimated to be decreasing by less than 25% in 16.2 years (three generations) (1). In Italy the breeding population is estimated at 5,000-1,0000 pairs. Aim of this work is to evaluate the prevalence of *Haemoproteus* spp./*Plasmodium* spp. and *Leucocytozoon* spp. in Common Kestrel from Emilia Romagna region (Italy). During a wildlife disease surveillance program, 57 Common Kestrels were collected injured or dead. Samples from spleen (n=52), liver (n=43) and cardiac blood clot (n=52) were collected for biomolecular investigations. Infections were detected during a first screening from blood smears (2) and from DNA extracted from the sampled organs following a nested-PCR protocol (3) DNA sequencing was performed on testing positive organs. Sequences were manually edited and cytochrome b gene haplotypes were identified using the Nucleotide BLAST application of GenBank. Mixed infections were recognized by the presence of double peaks on the electropherograms [4]. Whenever possible, the identities of the parasites involved were assessed by comparing the double peak patterns with previously known sequences of parasite haplotypes obtained from GenBank and MalAvi. Thirty out of 57 (52.6%) animals tested positive by nested-PCR for *Haemoproteus/Plasmodium* spp. and 1/57 (1.7%) tested positive for *Leucocytozoon* spp. In 38 (66.7%) Common Kestrels spleen, liver and cardiac blood clots were collected: 8 (21%), 5 (13.2%) and 9 (23.7%) resulted positive for *Haemoproteus/Plasmodium* spp. respectively in all, in two organs and in one only, whereas 16 (42.1%) animals resulted negative. In 14 out of 57 (24.5%) animals only two

organs were sampled: 2 (14.3%) animals tested positive for both organs, 6 (42.8%) for one organ, and 6 (42.8%) scored negative. In 5 out of 57 (8.8%) animals only cardiac blood clot was sampled: 1 case (20%) resulted positive for *Haemoproteus/Plasmodium* spp., and 4 samples (80%) tested negative. In order to identify which genera and lineages were present in the birds, all organs testing positive were sequenced. Not all the sequences were sufficiently clear to detect the lineage. Sequencing allowed the identification of three different lineages of *Haemoproteus*. All the samples resulted positive for *Haemoproteus*-Lesser Kestrel 03 (H-LK03), a parasite already detected in Lesser Kestrel (*Falco naumanni*) and Common Kestrel, belonging to a clade of closely related lineages that infect chiefly falcons and owls. Moreover in 4 animals a possible mixed infection by *Haemoproteus* lineages differing in 1-2 base pairs was also revealed. Further analysis are necessary to confirm the identification of these possible new *Haemoproteus* lineages.

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DEVELOPMENT OF DIAGNOSTIC TOOLS FOR RODENT HEALTH STATUS EVALUATION

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Despite the continuous development of human and veterinary biotechnology, the use of mice and rats as laboratory animals is still common in several fields of research. Different viruses and other pathogens may infect those animals, affecting experimental conditions and invalidating test results. In this perspective, monitoring mice and rats health conditions is mandatory for research studies and could be also useful to improve animal welfare in experimental facilities, in agreement with 3R policy.

The Laboratory of Cell Cultures of IZSLER, as the Italian Reference Centre for alternative methods to animal testing, developed a panel of diagnostic tools, according to FELASA recommendations for the health monitoring of laboratory animals in breeding and experimental units (Rehbinder et al., 1996).

ELISA and Real-Time PCR have been set up to identify Pneumonia Virus of Mice, Mouse Parvovirus, Theiler's Murine Encephalomyelitis Virus, Murine Hepatitis Virus, Murine Polyomavirus, Ectromelia virus, Reovirus-3, Murine Adenovirus, Murine Norovirus and Murine Cytomegalovirus. Indirect sandwich ELISA were developed using specific immunoglobulins directed against the Minute Virus of Mice (MVM) and Ectromelia virus presented in a solid phase by hyperimmune rabbit sera, previously produced at IZSLER.

Sera and feces were obtained from different strains of mice and rats collected from several experimental units and evaluated with the above-mentioned techniques.

The results showed the massive presence of Parvovirus in apparently pathogen-free murine colonies facilities, according to previous published data. ELISA results were compared to those obtained by commercial kits. These diagnostic tests, based on both serology and molecular biology, proved to be efficacious in identifying virus infection and the correlated immune response. This could be a valid tool in order to prevent introduction and spread of pathogens into experimental facilities.

The diagnostic panel described could be available at IZSLER as diagnostic service to check health status of mice and rats colony for commonly infectious agents in public and private facilities. In order to implement this panel, pathogens such as bacteria and parasites will be enclosed as well as other laboratory animals species.

ECTOPARASITES OF WILD RABBIT (*ORYCTOLAGUS CUNICULUS*) IN SICILY

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The European wild rabbit *Oryctolagus cuniculus* is a widespread wild mammal, and, in regions where it has been widely introduced, it became an invasive species for the environment and ecosystem. The high presence of this lagomorph could represent a threat for the health of humans and sympatric domestic animals. In fact, the wild rabbit may serve as host for ticks and some tick-borne pathogens (i.e. *Francisella tularensis, Coxiella burnetii* and *Rickettsia conorii*). The aim of this study was to improve the knowledge on the ectoparasites of the European rabbit in Sicily.

The survey was conducted on 105 rabbits confiscated by local authorities because illegally hunted in the province of Ragusa between June and July 2011. Each animal was checked for ectoparasite presence and information on sex, weight, morphological measures and age were recorded. The tick load (i.e. frequency and abundance) was assessed on eleven anatomical sites (i.e. face, ears [right and left], neck, forelimbs [right and left], thorax, abdomen, hindlimbs [right and left] and perineal area). Flea presence and abundance were assessed by combing rabbits using a fine teeth comb. The arthropods found were identified at species level using morphometrical keys.

Seventy-nine (75.2%) out of the 105 examined rabbits were positive for ticks whereas only 3 (2.9%) were infested by fleas. The collected ticks were belonging to three species: *Rhipicephalus pusillus* (119 males, 91 females and 31 nymphs), *Rhipicephalus turanicus* (2 females and 1 male) and *Ixodes gibbosus* (1 nymph). Ticks were found in each inspected sites but ears, forelimbs and abdomen were significantly higher infested. The lowest tick load was observed in the perianal region and hind-limbs. All the fleas found (1 male and 2 female) were identified as *Spilopsyllus cuniculi*.

The present investigation represents the first survey on ectoparasites of wild rabbit in Sicily, and contrarily to other similar investigations (Frank et al., 2013), it was

conducted on a large sample size. The flea *S. cuniculi*, typical of rabbit, has been reported in other studies conducted in Central Europe (Frank et al., 2013), and it is involved in the transmission of Poxvirus myxomatosis the agent myxomatosis a serious threat for wild and domestic rabbit. The ticks *R. pusillus* and *R. turanicus* are commonly reported in wild rabbit (Frank et al., 2013) and are involved in the transmission of several pathogens such as *C. burnetii* and *R. conorii*. Interesting, the retrieval of *I. gibbosus*, a tick typically reported in livestock, suggest the existence of sympatry between wild and domestic species and the factual risk of pathogens exchange. In conclusion, the high tick prevalence (75.2%) observed demonstrates that the wild rabbit plays a crucial role in tick fauna maintenance in natural environment and that it could act as source of vectors and pathogens to sympatric domestic animals and humans as well.

Frank R, Kuhn T, Mehlhorn H, Rueckert S, Pham D, Klimpel S. . 2013. Parasites of wild rabbits (*Oryctolagus cuniculus*) from an urban area in Germany, in relation to worldwide results. Parasitol Res. 112(12):4255-66.

A FIVE YEARS STUDY ON PESTICIDES, CARBAMATES, RODENTICIDES AND METALDEHYDE POISONING IN DOGS OF SICILY (SOUTHERN ITALY)

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The poisoning of animals is a growing phenomenon in recent times that has assumed very alarming proportions throughout the national territory. The most common causes of poisoning are related to the control of wildlife management, the real disorder that these animals may cause, until the criminal intimidation. In this work, a number of 533 dead dogs were analysed for toxicological studies asked by from regional and central bodies in order to describe the pattern of poisoning that occurred from 2010 to 2015. All the specimens were inspected at the necropsy room of the Istituto Zooprofilattico Sperimentale della Sicilia. After the autopsy, all the stomach, kidney and liver samples were removed and sent to the Chemistry and Food Technologies Area for toxicological analysis. A high-pressure liquid chromatography (HPLC) method was carried out for the detection of carbamate and rodenticide levels, while a gas chromatography method was performed for pesticides (GC-ECD/ECD) and metaldehyde (GC/MSD) detection. The validation of the methods produced a limit of detection (LoD) of 0.5 mg/kg for carbamates and rodenticides, 0.05 mg/kg for pesticides and 1 mg/kg for metaldehyde. Results obtained revealed pesticides as the primary group of toxicants, with mean values of 4.13±2.39 mg/kg and very high concentrations of endosulfan α and β (max value of 12 mg/kg). The highest pesticides concentrations were reached in stomach samples from 2015. An increasing trend of pesticides concentration overtime was revealed, with highest values during the biennium 2014-2015. Only 43 dogs reached metaldehyde levels over the LoD, with a maximum of 16726 mg/kg in a stomach sample from 2015. Methomyl levels were reached only in 96 stomach samples, with a mean value of 2074.42±1776.02 mg/kg. Only five cases of rodenticides poisoning was verified during the five years, with 3 positive case for bromadiolone, one for warfarin and one for difenacoum (2530 mg/kg in stomach sample). All the results obtained

revealed a constant increase of poisoning events, although this increased number is directly proportional with an increase of interest and awareness to this thematic resulting from the Ordinanza del Ministero del lavoro, della salute e delle politiche sociali 18 dicembre 2008, which describes the rules on the prohibition of the use and possession of poisoned baits. The highest concentrations of toxicants were detected in stomach probably due to their rapid mode of action which mainly affects the central nervous system leading to the death of the animal. The aim of this survey was to determine the incidence and frequency of confirmed intoxications in animals in Sicily and to emphasize its relevance in veterinary practice for animals.

DETECTION OF *TOXOPLASMA GONDII* FROM FAECES OF PET CATS FROM CENTRAL ITALY

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Toxoplasma gondii infection is a worldwide parasitic zoonoses with an high health-risk impact for humans. The key epidemiological role played from felids is related to the oocyst shedding that contaminates water and food sources. Nevertheless misconceptions continue to lead the owners to emphasize the key role of the household cats in human *T. gondii* infection. Aim of the present work was to test a population of pet cats for oocyst shedding in order to better define the role having from cat-ownership and keeping of cats inside houses in the epidemiology of human infection. Seventy eight pet cats were selected in 2014-2015 among the feline patient population entering for the routine analysis at the laboratory of Parasitology of the University of Perugia (Central Italy). The cat enrollment was based on the evidence of exposure to *T. gondii*-associated risk factors (i.e. age <3 years, outdoor access, hunting activities, feeding with raw viscera etc.) deduced from a questionnaire filled by the owners. Faecal specimens were collected from each animal and processed by a centrifugation-flotation technique using Sheather solution (specific gravity 1270), for the detection of *T. gondii*-like oocysts. Faecal samples were also used for DNA extraction and screened by specific nested-PCR assay targeting the B1 gene as described by Lin et al. (2000). Purified products were sequenced for identity confirmation.

2 out of the 78 faecal samples collected (2.6%) were found to be positive for *T. gondii*-like oocysts. Overall 14 faecal specimens (17.95%) resulted positive for *T. gondii* DNA; 4 DNA positive-cats showing to be excretory for *T. gondii*-like (n. 2) and *Cystoisospora felis* (no. 2) oocysts by microscopy.

The biomolecular assay revealed a rate of *T. gondii* presence in stool specimens significantly higher than that expected and reported in worldwide cat populations (range 0.5-6%) investigated by using microscopic method, followed by confirmatory

PCR. However the faecal-PCR positive rate was perfectly in line to results obtained from Mancianti et al., 2010 (16%) in a study conducted on a stray cat population of Central Italy. This finding confirmed that: i) microscopic method is not a robust assay for identification of potentially infective cats in cross-sectional surveys; ii) a considerable rate of the cats living in the studied areas encounters *T. gondii* within the first 3 years of age; iii) the life style is of crucial importance in defining the epidemiological impact played by household cat. However the significance of the faecal-PCR positive findings should be interpreted with caution, because it is of unclear origin and just the oocyst shedding makes cats important from a public health point of view. The speculation that the recovered *T. gondii*-DNA could be attributed to the presence of a low oocystic shedding rate would impose to revisit the dynamic of excretion in the definitive host, as well as the epidemiological role of a cat after primary infection. Further studies should be conducted to better interpret the recovered faecal-PCR positivity.

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SEROTONIN AND SERUM HEAT SHOCK PROTEIN (HSP70) CHANGES IN HORSE DURING DIFFERENT STRESS CONDITION: ACUTE EXERCISE, SLAUGHTER AND TOXOPLASMOSI DISEASE.

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Heat shock proteins (Hsps) are a group of highly preserved proteins present in the cells of several mammalian species. They are highly inducible by a variety of pathological, physiological, and environmental stress. A serotonin synthesis inhibitor reduces Hsps production in the rat following trauma (Sharma et al., 1995). An increase in serum 5-HT concentrations is often related both to acute exercise and common horse's pathologies as laminitis. Unlike other animal species, the Horse is resistant to *Toxoplasma gondii* infection. *Toxoplasma gondii* is a tryptophan autotrophic consumer. We hypothesized that stressors such as acute exercise, time attending slaughter and Toxoplasmosis disease in horse would result in the increase in plasma 5-HT concentrations and in the release of Hsp70 from stressed cells into the blood. Also, Hsp70 gene (mRNA) expression and serotonin precursor enzyme Tph1 (tryptophan hydroxylase) in horse intestine and leukocytes, as well as the Tph2 in brain and leukocytes, has been observed. The study was carried out on 95 clinically healthy horses. All samples were assayed for the *T. gondii* research by Real Time PCR with Taq man probes targeting B1 gene (locus AF146527) and serologically using the "Equine IgG kit" (Fuller Laboratories). Leukocytes pellet were obtained from whole blood by insulation with Lympho-Paque (Genaxxon Bioscence) and total RNA was extracted using "Quiazol" from leukocytes pellet and from organs (brain, intestine) in the slaughter. mRNA was reverse-transcribed into cDNA using the "Quantitect Reverse Transcription kit" (Qiagen) and the target genes were amplified by PCR real time with "Quantinova Sybr Green PCR kit". Data obtained by horses who underwent acute exercise (gallop) showed that exercise induces a significant post-exercise increase of plasma 5-HT and serum Hsp70. Moreover, a significant increase of gene (mRNA) Hsp70

expression and serotonin precursor enzyme Tph1 expression, and a relative decrease of Tph2 in leukocytes, was observed. In the *T. gondii* positive horses the Tph1 expression was more elevated than in the *T. gondii* negative horses. Slaughter horses were assessed for leukocytes and intestines Hsp70 gene expression, showing an increased expression in the first. Furthermore, Tph2 showed greater expression values than Tph1 in intestine and brain. In conclusion, both physical exercise and metabolic stress activate Hsp and serotonin pathway in the horse.

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EPIDEMIOLOGY OF ZOONOTIC DISEASES IN PIEDMONT – NORTHWESTERN ITALY: A RETROSPECTIVE ANALYSIS OF HOSPITAL DISCHARGE RECORDS.

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For health-care professionals and policy makers to implement effective preventive and control strategies against zoonotic diseases, it is fundamental to have detailed epidemiological data. These can be gathered by working within an integrated system that incorporates the experience and the joint activities of physicians, veterinarians and biologists under the basic principles of One Health. Hospital Discharge Records (HDR) are in this context, an important source of information on incidence, spatio-temporal patterns and economic costs. The analysis of the HDR data of all regional health-centers from 1999 to 2012 had as main goal to gather information on presence and incidence of zoonoses across Piedmont, ascertain the existence of more sensitive population groups and in general, to assess the impact of these diseases on public health as far as costs and resource demand. A total of 24,643 cases of zoonotic diseases were included in the analysis. Statistical and geographical analysis were performed with R 3.2 and QGis 2.1 software respectively. The use of the HDR allowed to evaluate the epidemiological situation of major and emerging zoonotic diseases. The most common zoonotic infections confirms the European trend whit *Salmonella* as the most frequent zoonosis, and *Toxoplasma gondii* as second one but with an high mortality rate respect Salmonellosis. Instead discrepancies exists on Echinococcosis which appears to be third and largely underestimated if compared to National data. Despite the useful information emerging from this study, we gave evidence to certain limitations that can arise from the use of HDR as epidemiological data source (i.e. absence of some zoonoses that follow different registration procedures - tuberculosis, obvious discrepancies between field-data and HDR – *Taenia solium* highly reported in HDR while it is no more reported in Italy since decades, and insufficient diagnostic specificity – bacterial zoonosis). This study is proposed as starting point to improve the quality of HDR for

their further use as a tool for a sounded evidence-based medicine with respect to emerging or re-emerging zoonosis.

A PHYSIOLOGICAL EXPLANATION OF EQUINE "RESISTANCE" TO *TOXOPLASMA GONDII*

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Equidae have been defined "resistant" to *Toxoplasma gondii*'s infection (Montoya and Liesenfeld, 2004), and few positive cases have always confirmed this resistance (Tassi, 2007), describing some mechanisms involved into intracellular parasites and factors affecting the invasive and latent form. Lactate has been identified as an inhibitory component of "supernatant" in resistant cells culture to *T. gondii* (Weilhammer et al., 2012), and on this basis, the variables studied by gene expression were: LDHA (lactate dehydrogenase) expressed in heart, skeletal muscle and in leukocytes, and LDHC expressed in testis and in leukocytes, TSPY (testis specific protein), which is usually over-expressed in subject with Toxoplasmosis. Finally was verified the expression of the protein IRGC, belonging to the IRG system (Immunity Related Protein G) and associated with the intracellular destruction of pathogens. Sixty adult horses have been used. Samples were assayed for *T. gondii* research by PCR Real Time with Taq Man probes for B 1 gene (locus AF146527). Leukocytes pellet was obtained from whole blood using the Lympho-Paque and total RNA was extracted from leukocytes pellet and organs (brain, intestine, skeletal muscle, testis, heart and blood) of the slaughtered horses. RNA was reverse transcribed into cDNA using the "High Capacity cDNA Reverse Transcription Kit" and the target genes were amplified by PCR real time with "Sso Advanced Universal Sybr Green Supermix". *Toxoplasma* positive horses showed lower circulating LDHA leukocytes value, LDHC and TSPY expressions higher than negative specimens. IRGC leukocytes expression showed higher values, according to expression observed also in the testicles, myocardium and gut. *Toxoplasma* negative slaughtered horses showed higher LDHA expressions in skeletal muscle than myocardium. Sport horses, compared

to other species, are continually subjected to a turnover of lactate, based on physical activity performed and the degree of training and performance. Lower LDHA expression in positive horses was correlated with untrained or elderly subjects, and the highest LDHC expression with a local self-regulating. The higher IRGC expression in positive horses' leukocytes than negative, could be due to the indirect immune system involvement. It can also not be excluded that the equine "resistance" to *T. gondii* infection due to its physiological "sprinter born" metabolism.

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MOLECULAR EPIDEMIOLOGY OF *STREPTOCOCCUS EQUI* SUBSP. *ZOOEPIDEMICUS* ISOLATED FROM HORSES WITH RESPIRATORY DISEASES

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Streptococcus equi subsp. *zooepidemicus* (SEZ) is one of the most important bacteria associated with inflammatory airway disease in British young horses (1). SEZ has been isolated also from humans with different kinds of diseases, therefore it is suspected of being a zoonotic agent (2). Some subtyping techniques have been described for molecular epidemiological investigations of SEZ isolated from horses (3, 4, 5).

The aim of this study was to investigate the molecular epidemiology of SEZ isolated from horses with a history of respiratory diseases in Italy.

A total of 46 SEZ strains identified by multiplex PCR (6) and isolated from nasal swabs, tracheal washes, guttural pouch lavages, bronchoalveolar lavages and lungs of horses with different respiratory signs were typed by a PCR technique based on the 16S-23S rRNA gene intergenic spacer. The PCR typing method was based on four different reactions (3, 4, 5).

A single expected PCR product was obtained in 26 out of 46 samples, 16 of which corresponded to type A1. Multiple products were obtained from 20 samples. In particular, two PCR products were obtained from 16 samples, 8 of which gave an unexpected PCR product of about 200 bp, three products were observed in 3 samples and 5 products were present in one sample. The unexpected PCR product was sequenced and named A2a.

In all, 16 different SEZ types were isolated. Similarly as reported in British horses, type A1 was the most prevalent intergenic spacer. Furthermore, the new type A2a was found. These results show that SEZ isolated from horses in Italy represent a wide diversity of strain types and confirm that SEZ are heterogeneous bacteria. Further investigation will be carried out in asymptomatic horses. Molecular epidemiological

studies of SEZ infecting horses could be useful for selecting candidate strains to be included in experimental vaccines.

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SEROPREVALENCE OF *DIROFILARIA IMMITIS* IN KENNELS IN THE CAMPANIA REGION, SOUTHERN ITALY

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The cardiopulmonary nematode *Dirofilaria immitis* is increasingly reported in dogs in Italy, as in the rest of Europe (Otranto et al., 2013). Several factors (e.g. global warming, changes in vector seasonal population dynamics and movements of animals) may play a role in this recent rise in reports of infection of *D. immitis* (Genchi et al., 2009; Otranto et al., 2013). The aim of this study was to investigate the seroprevalence of *D. immitis* in dogs from 68 kennels of Campania region (southern Italy). Specifically, 537 blood samples (from 5 to 10 per each kennel) were collected from dogs aged over 2 years. The samples were transported to the laboratory and centrifuged at 3000 rpm for 10 min to obtain sera, then stored at -20°C until testing for seroprevalence of *D. immitis* using DiroCHEK® ELISA (Synbiotics, San Diego, USA), according to the manufacturer's instructions (sensitivity = 85-100% and specificity = 100%) (Courtney et al., 2001). Antigens of *D. immitis* were detected in 24/537 (4.4%; 95% Confidence Interval, CI = 3.0-6.7) dogs in 6 out of the 68 kennels (8.8%; 95% CI= 3.6-18.9). The prevalence of *D. immitis* reported in this study is higher than the previous study conducted in the Vesuvius area (0.6%) (Cringoli et al., 2001) of the same region confirming the spread of this parasite in low endemic areas. Therefore, a regular parasitological surveillance, appropriate diagnostic tools, treatment strategies and a high quality standard of hygiene could be very useful to guarantee the health and welfare of kennels, as recommended by the European Scientific Counsel for Companion Animals Parasites (ESCCAP).

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POSTERS

2P

EFFECT OF DIET ON HAIR CORTISOL AND DHEA CONCENTRATIONS IN MOUSE

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Obesity and MetS (Metabolic Syndrome) are both linked to persistent long-term hormonal and metabolic changes. In most of the studies, cortisol (C) and dehydroepiandrosterone (DHEA) concentrations have been measured in obese and normal-weight subjects, obtaining heterogeneous results. Plasma, saliva and urine, matrices that represent time-point or short-term steroids exposure, were used for these studies. The aim was to study C, DHEA and C/DHEA ratio of mice pups in the hair, matrix capable of providing cumulative hormonal exposure. Sixty C57Bl/6 mice pups (30 males and 30 females) were housed in a temperature-controlled environment (22±2°C) and on a 12h light/dark schedule, under ad-libitum access to food and water for 16 weeks (welfare: Italian Law Decree 116-92 and EC Directive 86-609-EEC). Control and experimental diets were offered immediately after weaning (3 weeks old pups), for 16 weeks. Four experimental checkpoints were established (T1: 4 weeks, T2: 8 weeks, T3: 12 weeks and T4: 16 weeks of diet). T3 and T4 showed the hormonal concentrations of pubertal animals. Twenty-seven pups (13 females and 14 males) were randomly group-housed in cages (6 for T1, 5 for T2, 6 for T3, 10 for T4) and assigned to control diet (CTRL: D12328, Research Diets, New Brunswick, NJ). 33 pups (17 females and 16 males) were randomly group-housed in cages (5 for T1, 6 for T2 and T3, 16 for T4) and assigned to the HFHC diet (HFHC: D12331, Research Diets, New Brunswick, NJ - plus 42g/L fructose/sucrose in drinking water). At each experimental checkpoint, all the animals of one cage for each experimental group were suppressed. Hair strands were carefully cut with scissors as close as possible to the skin from the back of the mice, paying attention to not to wound the animals. Hair was stored in an envelope at RT in a dry room until use. C and DHEA hair concentrations was measured by a solid-phase microtiter RIA assay (Peric et al.,

2016 adapted in the mouse). Only at 8 weeks the HFHC group showed significantly higher C concentrations than the CTRL group (1.56±0.06 vs 1.92±0.130 pg/mg; P<0.05). DHEA concentrations were significantly reduced in the HFHC group than the CTRL group at 4 (114.64±13.93 vs 69.08±5.33 pg/mg; P<0.05), 8 (71.67±7.08 vs 50.38±4.74 pg/mg; P<0.05), 12 (73.27±8.29 vs 41.59±1.60 pg/mg; P<0.01) and 16 weeks (65.26±3.35 vs 55.05±1.84 pg/mg; P<0.05). The C/DHEA ratio was significantly increased in the HFHC than the CTRL group, at 8 (0.023±0.002 vs 0.040±0.005; P<0.01), 12 (0.024±0.003 vs 0.036±0.004; P<0.05) and 16 weeks (0.019±0.001 vs 0.025±0.002; P<0.01). The gender effect was not significant. Taking into account the lag time required for the hair emersion from the skin (about 1 week), the significant stimulation of the C at 8 weeks in the HFHC group refers to its chronic elevation from 5 to 7 weeks of treatment when the animals were not pubertal. Conversely, DHEA shows a chronic reduction in obese mice leading to consider an independent adrenal regulation of C and DHEA, both stimulated by ACTH.

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Peric et al. 2016 J Appl Anim Welf Sci 18:1-8.

4P

IN VITRO TECHNIQUES TO STUDY VALVULAR INTERSTITIAL CELLS OF MITRAL VALVE IN MAMMALS /DOMESTIC ANIMALS. A CELLULAR APPROCH FOR THE INVESTIGATION OF VALVULAR INSUFFICIENCY

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The study of cellular and molecular mechanisms of valvular interstitial cells (VICs) is an emerging research area for veterinary medicine and comparative pathology. Despite many clinical investigations have been done to study canine mitral insufficiency, several doubts remain concerning the underlying etiopathological mechanisms. In case of degenerative disease of the mitral valve, the leaflets accumulate myxomatous tissue in spongiosa and fibrosa layers, characterized by proliferation of VICs with neovascularization, increased matrix, fibrosis and calcification. In order to study these hypotheses, the authors decided to perform assays from bovine mitral valves, collected at the slaughter house, immediately after the animal's death. Valves were conserved in cold and sterile phosphate-buffered saline (PBS). In the lab, the atrial aspect of the mitral leaflets was removed by scraping with a scalpel: when the endocardial surface was opaque, subendocardial material was collected and seeded with complete medium, and routinely incubated. Cell suspension (5×10^5 cells/mL) was seeded on glasses: at 80% confluence, they were washed three times with PBS, rinsed with 4% formalin (10 min) and conserved at 4°C with 0.03% sodium azide solution. Glasses were treated for immunocitochemistry (ICC) for vimentin, factor VIII, and actin smooth muscle antibodies. Other cell aliquots were seeded in 96 well plates to perform a proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Wells were divided in: blank (only medium), control (cells and medium), and treated (cells with 0.05%, 0.1% and 0.5% of hydrogen peroxide - H_2O_2). Plates were routinely developed at

the prefixed time point (2, 24, 48, and 72 hours). Other cells were seeded in Petri dishes and treated using the same H_2O_2 concentrations, fixed and dyed with Diff Quick staining. The ICC showed a strong and diffuse cytoplasmic positivity for vimentin and occasional for actin smooth muscle. All cells were negative for factor VIII. These results demonstrated that this isolation method is able to isolate VICs from bovine mitral valve. Results obtained by proliferation assays demonstrated that high concentrations of H_2O_2 were lethal, but the lowest concentration induced morphological modifications. The data obtained by this preliminary assay demonstrated that: 1) it is possible to isolate and start a primary culture from bovine mitral valve leaflet, 2) ICC was able to confirm point 1), 3) a noxious stimulus mimicking oxidative stress (H_2O_2) is able to induce morphological modification. Next steps should include the ICC on H_2O_2-treated cells in order to monitor the antibody expression, receptor identification, study concerning inhibiting/stimulating drugs acting on VICs differentiation to evaluate new pharmacologic treatment approaches. In the authors' opinion, VICs investigation will give a new understanding of comparative pathology of the mitral valve disease.

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5P

IN VITRO EVALUATION OF NANOPARTICLES EFFECTS IN EQUINE MESENCHYMAL STEM CELLS

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Nanotechnology is nowadays expanding and involves knowledge from physics, chemistry, biology, materials science, health sciences, and engineering with potentially interesting applications in all the fields of science, also in veterinary medicine.

Among nanomaterials, nanoparticles made of iron, cobalt, or nickel oxides offer promising possibilities in many biomedical applications including bioimaging, diagnostic technology, and drug/gene delivery into cells (Gupta and Gupta, 2005).

In regenerative veterinary medicine mesenchymal stem cells derived from the adipose tissue (ASCs) were used in some cell therapeutic applications especially in equine orthopedic field (Marx et al., 2015).

Nanoparticles could influence ASCs proliferation and differentiation with potential genotoxic and cytotoxic effects, as suggested by previous studies in different types of cell cultures (Magaye, 2012).

The aim of this study was to investigate the in vitro effects of iron, cobalt, and nickel nanoparticles on ASCs.

Different experimental protocols were applied to evaluate several parameters such as biological activity, cellular up-take, molecular and genetic effects. The ASCs were cultured in vitro, added with two different concentrations of iron, cobalt and nickel nanoparticles (1,5 µg/mL and 3 µg/mL) (IoLiTec Nanomaterials).

Samples were analyzed for self-renewal (CFU assay by Giemsa stain), cell viability (MTT assay), osteogenic and adipogenic differentiation (Von Kossa and Oil Red O stain, respectively), expression of staminal cell markers by flow cytometry (CD90, CD44, CD 4E1) and Real-Time PCR (ALP, ACRP30, Nestin, TGF-β).

Moreover, evaluation of intracellular localization in stem cells by Transmission Electron Microscopy (TEM) was performed.

Our results demonstrated that treated ASCs showed decreased proliferative capability and cell viability compared to control. Osteogenic and adipogenic differentiation capacity resulted unchanged, suggesting that their differentiation potential after the exposition to nanoparticles may not be influenced.

All three different nanoparticles showed cytoplasmic internalization, except for cobalt that showed nuclear and mitochondrial localization, suggesting a potential genotoxic activity according to MTT test. Flow cytometry analysis demonstrated no significant differences in markers expression, and Real time PCR revealed no alterations of stem cell markers evaluated.

Our preliminary results are promising in order to establish the influence of ASCs physiological activity in terms of genotoxic and cytotoxic effects exerted by nanoparticles.

Currently, available information on their potential toxicity is still poor investigated and controversial data have been reported. Further studies are necessary to understand how these nanomaterials interact with cellular systems and the potential adverse consequences on animal health. In particular, investigation of P53 gene expression and telomerase activity will be performed to evaluate potential cell damages.

6P

AN IMMUNOHISTOCHEMICAL STUDY EVIDENCES GHRELIN AND ITS RECEPTOR IN THE HAIR FOLLICLE OF THE SHEEP

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Ghrelin is a peptide hormone discovered in 1999. It is mainly secreted by the endocrine cells of the gastrointestinal tract and acts by binding to a specific receptor. Ghrelin presents a wide tissue distribution and is involved in numerous central and peripheral actions including hormonal, orexigenic, neurological, cardiovascular, and immunological activities (1).In this work, the expression and localization of ghrelin and its receptor was investigated in the skin of sheep by means of immunohistochemistry.

The immunohistochemical reaction was performed on ovine skin samples collected from the neck ventral region of healthy animals regularly slaughtered at the abattoir. Samples were fixed in formalin and embedded in paraffin. Dewaxed sections were incubated with 3% peroxidase-blocking solution and with normal goat serum to block the endogenous peroxidase activity and non-specific binding respectively. Successively, serial sections were incubated overnight with polyclonal anti-Ghrelin and anti-Ghrelin receptor antibodies (Abcam Cambridge UK). The reaction was visualized using Vectastain ABC kit and DAB (Vector Laboratories, Burlingame, CA, USA).

The analyses performed evidenced a clear and intense immunostaining for both ghrelin and its receptor in the ovine skin. Staining was mainly localized in the hair follicles (HF). Positivity to ghrelin was observed in a short area of the HF at the level of the soprabulbar region; it involved the inner cells of the outer root sheath, including the companion layer, and the cells of the inner root sheath. The receptor, instead, was observed in all cell layers of the outer root sheath and extended more than ghrelin, from the soprabulbar region to the isthmus. Other than HF, ghrelin showed a weak positivity in the soprabasal

layers of the epidermis which nevertheless were negative to the receptor. Finally, the receptor was clearly expressed by the cells of the sweat glands.

At present, the skin is described as an endocrine organ since it is the target of several endocrine signals and, at the same time, it is itself capable of producing substances with hormone-like activity (2). The study of ghrelin and, more in general, of adipokines at the skin level represent an interesting and current topic for domestic animals including sheep. It was shown that some adipokines, such as leptin and adiponectin, are widely involved in the metabolism of the skin, and, more specifically, of the HF in both physiological and pathological conditions (3, 4). Ghrelin is a recently discovered molecule and there are until now a few surveys on ghrelin at the skin level. However the strong immunohistochemical expression of ghrelin and its receptor evidenced in the HF of the sheep let us to suppose that ghrelin may have a role in the HF activity probably acting through a paracrine or autocrine mechanism.

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7P

ANTITUMORAL EFFECT OF A NANOTECHNOLOGY-BASED PHOTODYNAMIC THERAPY

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In the biomedical field, several nano-systems based approach have been developed for diagnosis, drug delivery, and therapeutics applications. The nanotechnology design have the great potential to be a multifunctional platform that can combine with conventional therapeutic. The nano-system proposed in the present study is a core-shell SiC/SiO2 nanowires (NWs), functionalized with porphyrins as photosensitizer, for concurrent photodynamic therapy (PDT) and radiotherapy for cancer treatment.

The aim of the present work is the in vivo evaluation of the antitumoral effect of the activated nano-system by means of radiotherapy. A murine (n=16) malignant heterotopic pleural mesothelioma model was obtained by the subcutaneous inoculation of 1x106 IL-45 cells in the left and right flunks of male adult Fisher 344 rat. The tumor progression was monitored by conventional measurements until the growth reached the mean volume of 500 mm3, when animals were randomly split into 4 grups: Vheicle (V), Radiotherapy (Rx), Control Nanowires (NWs) and Nanowires with Radiotherapy (NWs+Rx). The day after of the NWs intratumor inoculation, the rat belonging to the Rx and NWs+Rx groups were radiotherapy treated at the dose of 4Gy. At the end of experimental time, the tumors were harvested, an half was formalin fixed and the other was cryofixed.

The tumor growth resulted homogeneous in all animals until the treatments, following the NWs inoculation and the radiotherapy a slowing of tumor progression was observed in both Rx and NWs+Rx groups, while no changes of the kinetics were observed for the other groups. The histological analysis of tumor confirmed their sarcomatoid nature, characterized by twisted bundles of spindle fibroblast-like cells in a collagen matrix. The necrotic foci within the tumor are characterised by pyknotic nucleus with obvious thickening and shrinking, and nuclear degranulation typical of karyorrhexis with nuclei fragmented and eventually disappear. Their histomorphometric analysis revealed a greater number of necrotic foci per area unit in the NWs+Rx as compared to the other groups. With regard to the parameter of necrotic area fraction, calculated as the necrotic area compared to the area of the tumor tissue analyzed, a trend of NWs+Rx group to present higher values was detected. Analyzing the size frequency distribution of the necrosis areas a different pattern among groups resulted. It was revealed the prevalence of small foci in the V and NWs groups while a prevalence of larger foci in the Rx and NWs+Rx groups. The apoptotic activation was evaluated by Terminal deoxynucleotidyl transferase dUTP Nick End Labeling assay (TUNEL) and the coexistence of apoptotic and necrotic cell death process were detected within the necrotic foci. The tissue oxidative stress, that is the endpoint of photodynamic therapy, was quantified in the tumors by evaluating the lipid peroxidation, using the TBARS method. The results indicate an increasing trend of tissue peroxidation in both groups Rx and NWs+Rx compared to V and NWs.

8P

MYCOPLASMA LIPOPROTEINS ARE MAJOR DETERMINANTS OF NEUTROPHIL EXTRACELLULAR TRAP FORMATION

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Neutrophil granulocytes are paramount to innate responses as major effectors of acute inflammation. Among the various strategies enacted by neutrophils to eliminate microbes NETosis is a novel distinct antimicrobial activity in which an interlacement of chromatin fibres rich in granule-derived antimicrobial peptides and enzymes is extruded (Neutrophils Extracellular Traps, NETs). NETs contribute to the pathogenesis of acute and chronic inflammatory disorders. The interactions of mycoplasmas and innate immune cells, in particular neutrophil granulocytes, are poorly defined. Here we describe NET formation in vivo in the mammary gland and milk of sheep naturally infected by M. agalactiae. Also, we assess the contribution of liposoluble proteins, the most abundant component of the *Mycoplasma* membrane, in inducing NETosis. We demonstrated that living *M. agalactiae* cultures, liposoluble proteins obtained form different Mycoplasma species, and synthetic lipopeptides induce NET release at levels comparable to what observed with other stimuli, such as lipopolysaccharides and phorbol 12-myristate 13-acetate. Stimulation of neutrophils with synthetic diacylated lipopeptides based on the *M. agalactiae* P48, P80, and MAG_1000 proteins, combined in a mix or used individually, suggests that NETosis is not dependent on the lipopeptide aminoacid sequence. Results provide evidences for the mechanisms underlying NET activation in mycoplasma infections, and on their contribution to pathogenesis of mycoplasmoses.

Carla Cacciotto, Tiziana Cubeddu, Maria F. Addis, Antonio G. Anfossi, Vittorio Tedde, Gessica Tore, Tania Carta, Stefano Rocca, Bernardo Chessa, Marco Pittau and Alberto Alberti. *Mycoplasma* lipoproteins are major determinants of neutrophil extracellular trap formation. Cellular Microbiology 2016 (In Press): DOI: 10.1111/cmi.12613

9P

THE HEALTH STATUS ASSESSMENT OF THE APIARIES IN WESTERN SICILY: OBSERVATION TRIENNIUM 2013-2015

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Depopulation of the apiaries that have invested also Italian beekeepers and the problem related to the presence of the small hive beetle in Calabria and Sicily, have increased the importance of the issue bee - hives. A collection of Sicilian data from 2013 to 2015 by the laboratory of the area Palermo of Istituto Zooprofilattico Sperimentale of Sicily is reported in this study. The area of interest is represented by the provinces of Agrigento, Caltanissetta, Enna, Messina, Palermo, Trapani, with a total of 619 on a total of 1,200 apiaries for the entire Sicilian territory. Following the activation of the surveillance plans for the monitoring of bee colonies loss, a total of 540 samples of bees and honeycomb / brood were analyzed (157 in 2013, 132 in 2014 and 251 in 2015). We focused on the following bee-diseases: American and Europeanfoulbrood, *Varroa*, acariosis, nosemiasis, small hive beetle. The analytical results showed that only the parasite *Varroa destructor* is present in Sicilian apiaries with a positivity of 4.8% in 2013, 7.5% in 2014 and 1.59% in 2015. The monitoring plans made possible to delineate a first picture of the health situation of the apiaries in western Sicily. The observation of the three-year period showed a reduction for diseases such as varroasis that is considered endemic in Sicily. The honeycombs had larvae with a dark gray color mainly in autumn and winter. The microbiological survey detected the presence of microorganisms that are not normally pathogenic for bees as *Pseudomonas* spp., *Bacillus cereus*, *Campylobacter* spp., *Escherichia coli* and *Corynebacterium* spp. Additionally in some cases wax moth was also observed. The impact of the occasionally presence of orientalis Wasp in Sicilian reality

need to be evaluated. Bee populations are in great danger almost worldwide and surveillance and intervention plans on the apiaries are very important to control bee populations. In Sicily bee-farming is considered “a lesser breeding" but actually is very important in the economy of the island since honey productions reached peaks of excellence in culinary tradition (medlar honey etc.). Additionally planned actions for bee health are very important to preserve the black sicula bee maintaining Sicilian biodiversity.

10P

LONG TERM STORAGE AND REPRODUCTIVE EVALUATION OF AN INNOVATIVE BOAR SEMEN EXTENDER CONTAINING SUCROSE AND AN ENZYMATIC AGENT

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No disclosure exists wherein the saccharolytic enzymes are associated to the preparation of formulations for animal semen extenders (International Publication Number WO 2015/193265 A1). In the present study, the Authors developed an innovative boar semen extender (Formula®) for long–term storage at 15°C. The extender formulation is based on the use of a saccharolytic agent (invertase enzyme between 1000 and 30000 U/L) associated to a polysaccharide as energy source precursor, added with two antibiotics (gentamicin sulfate and marbofloxacin). The innovative extender was evaluated in vitro for sperm morphology, spermatozoa genomic (AOT) and acrosome integrity, during the extended doses storage, while the total and progressive sperm motility was assessed and evaluated by a computer assessed sperm analysis system (CASA). The semen was collected and processed from 10 boars of different breeds (Landrace x Large White, hybrids C21 and Goland). The boar sperms, diluted in Formula® maintained a mean high progressive motility (>70%) for 12 days at 15°C of storage. Following the in vitro results, an artificial insemination field trial was performed. A total of 125 sows, belonged to a farm practicing integral swine production were artificially bred, using a post-cervical insemination with 1.5 x 109 sperms in 100 ml volume till three times/oestrus. Pregnancy was detected by ultrasound examination at 21-23 days after the last insemination. The fertility rate was 98.4% (n=123), the farrowing rate was 96.7% (n=119), the mean number of piglets born/sow were 15.28 and the mean of piglets born alive/sow were 14.87. Considering the field trial results, the use of this semen extender is highly advisable and it clearly showed that it is possible to obtain a higher number of piglets in Italy as Denmark, one of the most advanced country in swine industry.

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11P

CULTURE OF CANINE MESENCHYMAL STEM CELLS FROM OVARIAN BURSA ADIPOSE TISSUE, ENDOMETRIUM AND ABDOMINAL ADIPOSE TISSUE: A COMPARATIVE STUDY

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Mesenchymal stem cells (MSC) can be isolated from a variety of adult animal tissues, replicated in vitro and induced to differentiate into different cell lineages. In the dog many districts have been investigated for MSC but never the female genital tract.
In present paper we simultaneously studied the tissue collected from three different sites: ovarian bursa adipose tissue, endometrium and abdominal adipose tissue, used as control. We considered 7 intact bitches with the following inclusion criteria: primipary/nulliparous, ranging in age from 1 to 3 years, clinically healthy, weighing 10-30 kg. Samples of abdominal adipose tissue, uterine horn (middle portion) and ovarian bursa were collected in a sterile environment during surgical procedure for elective ovariohysterectomy. Isolation, expansion and differentiation were carried out according to Bunnell et al (2008). Total RNA was extracted and cDNA were synthesized from 2 µg RNA to analyze the expression of marker typical of MSC: CD44, CD29, CD90, CD13, CD133, CD73, CD105, Oct4, CD45, CD31, CD34, GAPDH. RT-PCR were performed with these cycling condition: annealing 35 cycles at 55°C for 30 sec; denaturation at 94°C for 30 sec; extension at 72°C for 30 sec. Osteocytes, chondrocytes and adipocytes have been isolated from endometrium and abdominal adipose tissue (4 bitches), from ovarian bursa adipose tissue only two lines were tested (5 bitches). All isolated cells were able to adhere to plastic support generating colony forming units, showed similar fibroblastic-like morphology and ability of subculture; MSC isolated from abdominal adipose tissue and endometrium showed plasticity and expressed specific surface antigens. Cells isolated from ovarian bursa adipose tissue did not express specific surface antigens and did not show plasticity. We reported for the first time isolation and characterization of MSC from canine endometrium, a tissue that after ovariohysterectomy is routinely discharged and the rate of success for MSC isolated from this tissue was 100%.

The ease of isolation and multilineage potential make these cells ideal candidates for therapeutic strategies and tissue repair in humans and animals.

In principle, a stem cell for regenerative medical applications should meet the following criteria: should be found in abundant quantities, can be collected by a minimally invasive procedure and can be differentiated along multiple cell lineage pathways.

In conclusion, our data showed that canine endometrial stem cells can be easily isolated and display typical characteristics of stem cells. In regards of the possibility of clinical application, further studies should be performed to investigate whether the endometrial cells in animals with uterine pathologies show the same characteristics observed in healthy bitches, if they have a role in preventing the disease or whether they may contribute to the onset.

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12P

PRENATAL DIAGNOSIS AND NEONATAL TREATMENT OF FOETAL ANASARCA IN ENGLISH BULLDOGS

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Foetal anasarca is a multifactorial disease characterised by accumulation of subcutaneous and visceral fluids. A prenatal diagnosis with ultrasonography is possible considering the increased risk of dystocia (1, 2). Neonatal treatment with diuretics has been proposed (1, 2, 3). Eleven 2-4-year-old English Bulldog bitches were strictly monitored during pregnancy by ultrasound (General Electric Logiq E9 with 8-18 MHz linear probe) and progesterone assay (ELFA, bioMéurieux). Each bitch received a mean of seven ultrasound examination from 17 to 63 days from estimated ovulation (progesterone 5-10 ng/ml). A condition of foetal anasarca in one or more foetuses was diagnosed in 4 bitches after the 50th day of pregnancy. Ultrasound findings were diffuse or localized subcutaneous oedema, sometimes associated with thoracic and abdominal fluids. Foetal cardiac frequency in affected pups did not significantly differ from unaffected pups (220±20 bpm). Progesterone profiles in the monitored bitches were in the normal range, with values always above 15 ng/ml. Estimation of ovulation day, foetal biometry and progesterone drop were used to plan a C-section that was performed at 62±1 days after ovulation. A total of 14 pups affected by foetal anasarca were extracted live but with a low APGAR score (1-3). Weight was 1.5-4 times increased in affected puppies. Furosemide at the dose of 10mg/Kg im was administered and urination was stimulated every 30 minutes. Every 3 hours the weight loss was measured and every 30g of lost weight 1 mEq of potassium chloride was administered per os. The therapy was efficient in 3 cases (21%), which presented modest fluid accumulation in the region of the neck. The other 11 pups died within 36 hours. English bulldog has a breed genetic predisposition to anasarca. Authors discourage the use of breeders with a history of anasarca in previous pregnancies. Prenatal diagnosis of the disease allows an elective C-section and a prompt neonatal treatment. The efficacy of drugs in pups is controversial for the lack of pharmacodynamic studies; consequently, the empirical efficacy of the

proposed treatment1,2 need a wider validation and is probably limited to mild cases. Recovered anasarca pups should not used as breeders.

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13P

SCHMALLENBERG VIRUS IN SARDINIA: A 3-YEARS FOLLOW-UP STUDY AFTER THE 2012 EPIDEMIC EPISODE.

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Schmallenberg virus (SBV) is a novel virus belonging to the genus Orthobunyavirus of the *Bunyaviridae* family. SBV has been the causative agent of congenital malformations in stillborn ruminants in Europe. At the end of 2012, SBV was detected for the first time in Sardinia.

Herein, we report the findings of a long-term follow-up in Sardinian sheep flocks after this SBV epidemic. Firstly, in 5 sheep flocks SBV epidemiological, entomological, serological, virological, and anatomo-pathological data were recorded during 1 year follow-up study. In addition, to evaluate if SBV re-circulated throughout the period 2013-2015 in Sardinia, a serological investigation on other 94 flocks was carried out together with a passive surveillance plan by which the cases of malformed lambs, suspected to be SBV infected, were recorded on the total Sardinian sheep population.

In the 5 flocks, malformations in the stillborn lambs were observed in the earlier lambing period (November to December 2012), while no cases were found in the following winter months which corresponded to the lambing period of the ewe-lambs. The seroprevalence was 23.9% to 73.8%, while the incidence of malformed lambs varied from 19% to 3%. In one flock the incidence of malformations in ewes with twin pregnancy was significantly higher (88%) than in ewes carrying a single pregnancy (4%).

Only 75% of the malformed lambs resulted positive to SBV by RT-PCR, with the brain being more frequently infected than spleen. Sequencing demonstrated that Sardinian SBV isolates had not statistically significant differences in the genome when compared to other European isolates. Serological survey on the flocks found that a rate sheep born after 2012 were SBV positive in the period from 2013 to 2015. By passive surveillance, sporadic cases of malformed stillborn lambs were found after 2012, however virological

examinations did not confirm the presence of SBV. The entomological survey did not find SBV in the examined pools of captured *Culicoides* spp.

The pathological aspects encountered are similar to those that have already been observed in Northern Europe and to those attributed to Akabane virus and other viruses of the same family. The fact that, the deformed lambs were tested RT-PCR positive more in the brain than in the spleen, confirms the specific tropism to the nervous tissues of SBV, and indicates that the brain is the most appropriate organ for diagnostic purpose.

During 2013, in the 5 flocks under study, we did not find cases of SBV in lambs born from ewe-lambs which are commonly mated in Sardinia in the period September-October. Given that the ewe-lambs were probably exposed to the SBV prior to the mating period, this demonstrates that they have acquired an active immunity sufficient to prevent the disease. Interesting, the serological survey suggests virus circulation even after the 2012 epidemic episode. In addition to this finding, the sporadic discovery by passive surveillance of malformed lambs in the 2013-2015 period confirm that SBV is still present in the Sardinian sheep population and an overwintering of this virus could be a possible occurrence.

14P

PREVALENCE OF CHRONIC MASTITIS IN APPARENTLY HEALTHY BUFFALOES: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS

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Buffalo mastitis is a major problem in the dairy industry. It causes considerable economic losses due to the sub-clinical course, which leads to a prolonged presence of the microorganisms in a herd, influencing productivity or causing alterations of the milk. Persistent infections are often associated with an impairment of the immune response, due to factors related to both, the infectious agents and the host. In buffalo, diagnostic criteria are the same as used for cattle, and somatic cells counts (SCC) are the most useful test. The aim of this study was to perform anatomical and histological evaluation of udders obtained from regularly slaughtered buffaloes, in order to identify and characterize subclinical-mastitis. 50 udders were examined by SCC from buffaloes grouped by age and days in milk. Samples of upper, middle, low gland areas and of the whole teat were collected from each quarter and routinely processed for histological examination. Immunohistochemistry and immunofluorescence staining were also performed using monoclonal antibodies against CD4, CD8, CD79 and MHCII, for the typing of infiltrating inflammatory cells. Two out of 50 udders (4%) showed neither anatomical nor histological alterations whereas in other two out of the 50 (4%) udders, anatomical lesions were found. The remaining 46 (92%) udders, in which no anatomical lesions were found, showed histological features characterized by infiltration of lymphocytes and plasma cells, which proliferated diffusely around large and small ducts and alveoli in the middle, low gland and in the teat region. Interstitial lymphocytes were immunoreactive for CD4, CD8 CD79, and MCHII. CD8+T lymphocytes were predominant over CD4+ T lymphocytes and occurred also in close contact with epithelium and between epithelial cells. This feature may confirm also in the buffalo, the hypothetic role of CD8 cytotoxic lymphocytes as cell scavengers, removing old or damaged secretory cells; the presence of those damaged cells could increase the susceptibility of mammary glands to infections (1).

In 22 out of 46 udders affected by chronic mastitis (47.8 %), in the low gland and in the teat region, sparse lymphocytes and plasma cells evolved into aggregates that eventually organized to form follicle-like structures, referable to as tertiary lymphoid organs (TLO). Previously, similar features were also detected in urothelial cancers of buffaloes (2).

The pathophysiological significance of the lymphoid neogenesis is still unclear. TLO formation is now considered a common feature of many chronic inflammatory diseases and might have a role in perpetuating the immune responses against persistent antigens.

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16P

CEREBRAL TOXOPLASMOSIS IN A NEWBORN STRIPED DOLPHIN

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Toxoplasma gondii is a protozoan parasite that commonly affects a wide range of birds and mammals, including humans. This study describes cerebral toxoplasmosis in a newborn striped dolphin found stranded on the Sicilian Sea coast of Italy (Torre Faro, Messina), in September 2013. The carcass of a male *Stenella coeruleoalba* (length = 98 cm, weight = 8 kg), was necropsied and tissue samples were collected from lungs, heart, liver, kidneys, spleen and brain and preserved in 10% buffered formalin. Subsequently, tissues were processed by conventional histological techniques and 5 µm sections were stained with Hematoxylin and Eosin, Periodic Acid-Shiff (PAS) and then examined by light microscopy. Immunohistochemistry (IHC) for *T. gondii* was performed in tissues using a rabbit polyclonal antibody (GTX15170, Genetex). In order to screen for Dolphin Morbillivirus (DMV) antigen in paraffin embedded tissues, immunohistochemistry technique was performed on all tissues. Biomolecular investigations for *T. gondii* were also performed. Microscopically, lungs showed severe sub-acute interstitial pneumonia with mononuclear leucocytes infiltrate and fibrin exudation with degenerated neutrophils and macrophages filling the alveolar lumens. Moderate lymphoid depletion was observed in the spleen. Microscopic examination of H&E-stained, paraffin embedded sections of formalin-fixed tissues revealed the presence of intracytoplasmic oval structures 2–4 µm in diameter in the brain. Multifocal areas of lymphoplasmacytic inflammation were found in the brain. This infiltrate was organized as multifocal, inflammatory nodules, composed predominantly of lymphocytes surrounding moderate numbers of protozoal cyst. Both IHC and RT-PCR detection of a *T. gondii* were positive. Immunohistochemistry of cerebrum revealed tissue cysts that stained positive for *T. gondii*. PAS positive bradyzoites were observed within tissue cysts in hearts and brain. All tissues immunohistochemically analysed for Morbillivirus antigens were negative.

This case of disseminated toxoplasmosis might be resulting from transplacental infection in the neonatal striped dolphin. Evidence to support transplacental transmission is the animal's young age and detection of numerous *T. gondii* tissue cysts in the brain and heart. *T. gondii* oocysts are highly environmentally resistant and could be transported from land to the marine environment. Although *T. gondii* is considered as an opportunistic agent in aquatic mammals, studies suggest that this protozoan might be a primary agent on these species (1).

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17P

ANAPLASMA MARGINALE CHARACTERIZATION IN TWO SICILIAN BOVINE HERDS

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Species of the genus *Anaplasma* are obligate intracellular bacteria, vectored by ticks, responsible of anaplasmosis in human, ruminants and other animals.

In ruminants, *A. marginale* and *A. ovis* invade erythrocytes, removed later from the circulation by reticuloendotelial cells, resulting in mild-to-severe haemolysis and anaemia. Bovine anaplasmosis, one of the most important disease of ruminants worldwide, is endemic in Sicily causing important economic losses.

It has been generally accepted that *A. marginale* infects cattle, while only sheep and goats are susceptible to *A. ovis*. However recent reports provide evidence for possible *A. ovis* infection in cattle. In order to identify the pathogens involved and to estimate the possible relationship between strains and clinical or subclinical disease in cattle, serological, molecular and microscopical analysis of blood samples were carried out during asymptomatic and symptomatic outbreaks of anaplasmosis in two Sicilian bovine herds. After clinical examination, blood samples were collected from all the cattle.

Sera, obtained after blood centrifugation, were serologically analysed using a commercially available cELISA test to detect antibodies against the msp5 protein of *Anaplasma* spp. Freshly heparinized blood smears stained with Giemsa stain have been microscopically examinated in order to identify *Anaplasma* affected cells.

Genomic DNA was extracted from EDTA-blood samples and quality and quantity examined by a spectrophotometric method before amplification. For preliminary evaluation the *Anaplasma* spp. msp4 gene was tested by PCR using primers that amplify both *A. marginale* and *A. ovis* msp4 gene. PCR-positive samples were further tested using two set of primers specific for *A. marginale* or *A. ovis*. *A. marginale* msp4 gene shows a nucleotide deletion at position 120 not traceable in *A. ovis* msp4 gene.

After this molecular analysis positive samples were selected, purified and sent for sequencing (using both strands as template) to confirm the identity of the amplicons for both *A. ovis* and *A. marginale* msp4 and to identify these strains.

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18P

EPIDEMIOLOGY AND GENETIC CHARACTERIZATION OF BORDER DISEASE VIRUS CIRCULATING IN SARDINIA

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Border Disease Virus (BDV), a pestivirus from the *Flaviviridae* family, is an important pathogen of sheep and goats responsible for significant losses in farms around the world. In spite of the relevance of this pathogen there are only a few epidemiological studies on BDV infection and, as a consequence, the economic impact on small ruminant productions is probably under- estimated. The aims of this study are i) to determine the distribution of BDV in small ruminant farms in Sardinia and genetically characterize circulating strains ii) analyze the relation between seroprevalence, Somatic Cells Count (SCC) and milk yield. ELISA was performed using "BVDV/MD/BDV p80 Protein Antibody Test Kit" (IDEXX) on serum of bulk tank milk (BTM) samples collected from 1291 Sardinian sheep flocks and 72 goat herds between spring 2014 and 2015. The number of sampled farms corresponded to 8.5% of all registered farms in Sardinia; sampling was performed according to the distribution of the farms in the Sardinian region. RNA was isolated using Qiamp Viral RNA mini kit from the cellular fraction of each ELISA positive BTV sample and amplified by rt-PCR using complementary primers to a highly conserved region in the untranslated regions (UTRs) of the viral genome. The amplicons were sequenced for phylogenetic analysis. Geographic distribution of collected specimen, seroprevalence and virological positive samples were analyzed via GIS (ESRI ARCGIS 10.3) ELISA screening shows a seroprevalence of 8.3% among goat farms and 10.5% among ovine farms. These values are higher than 8% of prevalence average recorded in Europe but close to the 11% observed in Italy (1, 2, 3). In sheep flocks we highlight that the distribution of the seroprevalence, indicates above as 10.5% in average, is uneven between Sardinian provinces. Ten from the ELISA positive samples were found rt-PCR positive. The sequence analysis indicates that all the amplified samples match with BDV genomes and the phylogenetic analysis revealed that all the viruses clustered in the same

group classified as BDV-7, previously described in Italy from Lazio and Tuscany isolates (4). Despite what reported in the rest of Italy, where also BDV-1, BDV-3 and BDV-5 (5, 6) have been isolated, BDV-7 is the only group isolated in Sardinia so far. According to the first analysis, variations in SCC and milk yield appear to have no significant correlation with seroprevalence and virus positivity in the screened farms. This study represents a snapshot of current distribution of small ruminants Pestiviruses and a genetic characterization of BDV circulating strains in Sardinia. Further studies should be led in order to understand the origin, the evolutionary story and the epidemiology of BDV-7 in Sardinia. The role of BDV as an immunosuppressive agent, exacerbating the pathogenicity of co-infecting microorganisms, should also be considered.

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4. Giammarioli et al, 2011
5. Rosamilia et al., 2014
6. Giammarioli et al., 2015

20P

GASTROINTESTINAL PARASITES IN SHEEP: EXAMINATION OF EGGS AND OOCYSTS DIURNAL RELEASE

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The parasitic infections are a very common health problem especially in grazing animals. The gastrointestinal parasites in sheep often are cause or contributing cause of a decrease in production and sometimes death (1). Several indicators of the degree of gastrointestinal parasites infestation were identified in small ruminants e.g. the FAMACHA system as pathophysiological approach, or the assessment of the physical condition of every single animal (2). However, the most common and practical method to estimate the prevalence and intensity of parasite infestation remains the Egg Counting Technique (3, 4). The aim of this work is to describe the diurnal rhythms of eggs and oocysts expulsion for the main gastrointestinal parasites in an organic Sicilian sheep farm in the province of Palermo, placed at an altitude of 800 meters above sea level. Five adult sheep were selected after coproculture. For this study the feces were collected and analyzed three times a day (morning, afternoon and evening), for 5 consecutive days. The techniques used were the flotation with dense solution of sodium nitrate and glucose having a specific gravity 1350 and the eggs and oocysts counting using the McMaster chamber. On each sample were made two counts and the mean and standard deviation values were calculated on the data obtained from each animal, each class of parasites and each phase of the day (5). To test whether there were significant differences between the average number of eggs and oocysts collected at different times of the day, they were built the confidence intervals with a probability of 95% and α equal to 0.05. The results obtained demonstrated for gastro intestinal nematodes a decreasing ejection in the three moments of the day and for coccidia a maximum ejection in the evening stage. For that's concerning the Nematodes there was a significant difference between the mean number of eggs released in the morning compared to the other two moments of the day, while no significant difference is found between the release in the afternoon and in the evening. As regards coccidia the differences found in the three times of day do not appear to be significant. The study of the dynamics of emission of eggs and oocysts of the main classes of parasite could represent an useful support in the formulation of intervention strategies.

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21P

DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* ISOLATES FROM OVINE MASTITIS

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Antimicrobial therapy is widely used in livestock against infectious diseases causing significant economic losses to the dairy industry. Antimicrobial resistance of clinical *Staphylococcus aureus* isolated from ovine mastitis against drugs is frequently reported and it often causes the clinical failure of the therapy. The in vitro susceptibility testing and the monitoring of drug resistance in the main pathogen involved in mastitis represent an important tool to guide veterinarian in selecting the most effective agents for the effective therapy. Macrolides and lincosamides (erythromycin, tylosin, clindamycin, spiramycin) are referred to the ML group of antibiotics and are closely related functionally. They are commonly used for treatment of mastitis and various bacterial infection such as mycoplasmosis. Resistance to ML group may be either constitutively or inducibly expressed; depending on the mechanism involved, different phenotypes are showed, suggesting an inducible or constitutive type of ML resistance.
In order to investigate the type of resistance phenotype in erythromycin-resistant strains isolates from mastitis, a group of 49 *S. aureus* were selected based on erythromycin resistance (MICs from >2 to >256µg/ml), from 900 clinical isolates. All the 900 *S. aureus* isolates were collected from dairy sheep with clinical mastitis all over Sicily from 2002 to 2012 by diagnostic Laboratories and they were tested for antibiotic susceptibility according to the CLSI method, during a monitoring survey in Sicily (project IZS Si RC03/2009). In this study we performed the double-disk diffusion test (D test) with erythromycin (ER 15µg) and clindamycin (DA 2µg), following the procedure recommended by CLSI. 28 out of 49 strains tested showed MICs >256 µg/ml, with the phenotype ER-R, DA-R, suggesting the constitutive resistance, only 5 exhibited the inducible phenotype ER-R, DA-D+, whereas 16 showing the phenotype ER-R, DA-S. The occurrence of

inducible ML resistance in *S. aureus* isolates from mastitis (5/49) can be related to the extensive use of ML such as tylosin and tylmicosin in dairy sheep in our region. The clinical significance of detecting inducible ML resistance is related to the potential for resistance to develop on therapeutic treatment. Early detection of inducible ML resistance on mastitis causative agents, by the D-test, may help in the use of macrolides to avoid treatment failures.

22P

MAIN FECAL BACTERIAL GROUPS IN DOGS POSITIVE FOR *LEISHMANIA*

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Lamour et al. (2014) recently suggested a link between gut microbiome and *Leishmania* infection in mice. They showed that in two different mouse models of cutaneous leishmaniasis, susceptible or self-healing models, with the progression of the infection also the fecal microbiome changed, with *Clostridia* class higher in the first group and *Gammaproteobacteria* classes higher in the second one, suggesting that the intestinal microbial composition could be linked to a different response to the disease. Aim of the work: In this study we investigated the main fecal bacterial groups in dogs positive for *Leishmania* spp., with the aim of evaluating possible differences between enrolled dogs. Materials and methods: Naturally voided fecal samples were collected from 8 dogs resulted positive (exposed, infected or sick) for leishmaniasis by IFAT or ELISA (dog no. 4). In most cases dogs differed for IFAT titers and for time elapsed between positivity detection and samples collection. Five dogs (2-4, 6, 8) were not presenting any concomitant disease and were not undergoing any treatment. Four dogs (2, 3, 6, 7) were not symptomatic at the time of fecal sampling; only one dog (4) was undergoing therapy for leishmaniosis at the time of fecal sampling, and 4 dogs (2, 6-8) had been previously treated for the disease, while for the remaining three it was a first diagnosis. The samples were frozen briefly after collection. All dogs were fed on commerce diet, with the addiction in dog n.1 of cooked “pasta” and meat. A Real-Time quantitative PCR (qPCR) procedure was used for the quantification of the main bacterial groups of intestinal microbiota by using specific primers as reported by Nasuti et al. (2016). DNA was extracted from dogs’ fecal samples using Stool DNA isolation kit (Norgen, Thorold, Canada). *Bifidobacterium*

spp., *Bacteroides-Prevotella-Porphyromonas* spp., *Clostridium coccoides-Eubacterium rectale* group, *Enterobacteriaceae*, *Lactobacillus* spp. and *Staphylococcus* spp. were quantified using a Mx3000P Real Time PCR based on SYBR Green detection. The more represented group in all dogs *was Clostridium coccoides-Eubacterium rectale* group with a mean value of 3.4x109 CFU/g of feces, while *Lactobacillus* spp. was the less present with a mean value of $3.6x10^3$ CFU/g of feces. The ongoing therapy at the sampling time seemed to affect (not significantly) the log values of *Clostridium coccoides-Eubacterium rectale* group and *Lactobacillus* spp. that both were higher in the treated dog (4) compared to the mean log values of those untreated.
To the authors' knowledge this is the first study on patients positive for leishmaniasis with these selected fecal bacterial groups. Even if it presents some limitations (small number of dogs, great variability within the variables), it would be interesting to deepen the possible correlation between leishmaniasis and the intestinal microbiota in the dogs suffered from this disease.

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23P

CHARACTERIZATION OF *LEISHMANIA INFANTUM* EXTRACELLULAR VESICLES AND *LEISHMANIA*–MACROPHAGE INTERACTIONS

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The *Leishmania* infection caused by the intracellular protozoan *Leishmania* spp., is a wide spread disease in tropical and subtropical areas. In Italy, the prevalence of the visceral form disease is due exclusively to *Leishmania infantum* ZMON1, is growing. The parasites occur in most parts of the world and the infection is also growing in non-endemic areas. *Leishmania* species generate 4 different clinical pictures: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and diffuse cutaneous leishmaniasis (DCL) (World Health Organization, 1991). Leishmaniasis causes significant morbidity and mortality worldwide and it is an important public health problem. In the absence of effective human and canine vaccines, the only feasible way to treat and control leishmaniasis is through the use of affordable medications. Recently has been proposed that exosome secretion as one of the strategies used by the parasite to orchestrate beneficial changes in the host environment ensuring a successful infection. Exosomes (exo) are nanovesicles secreted from different cell types, including infected cells and pathogen directly. In-vitro-isolated exosomes play a crucial role in host-pathogen interactions and are able to induce modifications in non-infected neighboring cells or act as antigen presenters for the immune system.
In this paper that *Leishmania infantum* promastigote and amastigote released exosomes and they are able to modulate immune response of macrophages. Human cell lines used were U937 cells and macrophages derived from U937 after treatment with 25 ng/ml phorbol 12-myristate 13-acetate (PMA). Exosomes were collected by *Leishmania infantum*-conditioned medium by ultracentrifugation. Western blot assay used antibodies to HSP70, HSP83/90. IL10, IFNγ secretion was evaluated by ELISA. We first purified extracellular vesicles shed by *Leishmania infantum* promastigotes and amastigotes on a sucrose gradient and later we characterized these extracellular vesicles for HSP70,

HSP83/90 and acetylcholinesterase. Furthermore, we performed a NanoSight nanoparticle tracking analysis revealed an average of the mode value of 76±5 nm for promastigotes and 94±5 nm for amastigotes exosomes. All these data demonstrated that *Leishmania infantum* released exosomes. The treatment of U937 cells and macrophages with 10 μg/ml of promastigote and amastigote exosomes showed an increase in motility of these cells that can facilitate the progression of infection. We showed also an overproduction of IL10 by macrophages after treatment with exosomes that support parasite persistence and disease establishment, while exosomes limited the production of IFN-γ that block the parasite killing and host protection. In conclusion, we demonstrated that *Leishmania infantum* released exosomes that are able to contribute in the disease establishment and may be an appropriate candidate for a vaccine therapy in prophylaxis and treatment.

24P

IN VITRO ACTIVITY OF TRANS-STILBENE AND TERPHENYL COMPOUNDS IN *LEISHMANIA MAJOR*, *LEISHMANIA AETHIOPICA* AND *LEISHMANIA AMAZONENSIS*

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Leishmaniasis is a disease caused by an intracellular protozoan parasite (*Leishmania*) transmitted by the bite of a sandfly. Most of the antileishmanial modern therapies are not satisfactory due to high toxicity or emergence of resistance and high cost of treatment.

In the last years several natural compounds and their synthetic analogues have been tested against *Leishmania*, and many of them have demonstrated potential as leishmanicidal agents. Stilbenes-based compounds are widely represented in nature, and have become of particular interest to chemists and biologists because of their wide range of biological effects including chemopreventive, antitumor, antioxidant, antimicrobial, anti-inflammatory and antihistaminic activities. Recently, several natural and synthetic stilbenoids have been studied for their leishmanicidal properties, and some of them, including resveratrol (trans-3,4',5-trihydroxystilbene) and piceatannol (trans-3,3',4',5-tetrahydroxystilbene), have shown anti-*Leishmania* activity in vitro.

In light of the above, recently we described a study in which we evaluated the anti-leishmanial activity of the stilbene ST18 and the terphenyl TR4, presented the best activity and safety profiles. These compounds showed a leshmanicidal activity higher than trans-3,4',5-trimethoxy-3'-amino-stilbene (TTAS) and the ability to induce apoptosis selectively in *Leishmania infantum* while sparing macrophages and primary epithelial cells. The aim of this study is to evaluate the anti-leishmanial activity of ST18 and TR4 in *Leishmania major, Leishmania aethiopica* and *Leishmania amazonensis*.

A strain of *L. aethiopica* (MHOM/ES/72/L100), L. major (MHOM/SU/73/5ASKH), *L. amazonensis* (IFLA/Br/67/PH8) collected from Centro di Referenza Nazionale per le Leishmaniosi (C.Re.Na.L.) were treated in phosphate buffered saline (PBS) and cultured

at 25 °C and pH 7.18 in RPMI-PY medium. A number of 4x106/ml promastigotes of *Leishmania* were suspended in flasks with RPMI-PY medium and treated with serial concentrations of each compound. TR4 and ST18 showed anti-*Leishmania* promastigotes activity in a dose-dependent manner and, at 5, 10, 20, 30, 40, and 50 µM causing reduction parasite viability. Inhibitory concentrations 50 (IC 50) for TR4, estimated by GraphPad prism were 10 µM in *L. major*, 27,5 µM *in L. aethiopica* and 20 µM in *L. amazoniensis* respectively; while IC50 for ST18 were 50 µM in *L. major*, 10µM in *L. aethiopica* and 47,5 µM in *L. amazoniensis* respectively. In conclusion with this work we showed that these compounds not only showed a potent toxic action against *L. infantum* species, but both TR4 and ST18 also acts against other species of *Leishmania*. The determination of the mechanism of action of each compound is currently unknown but is the subject of intense investigation in our laboratories. Better understanding of the mechanisms of ST18 and TR4 actions may help in finding new targets for the treatment of *Leishmania* parasites.

25P

"*THYMUS VULGARIS*" EOS IN SKIN INFECTIONS OF DOG

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The indiscriminate use of antibiotics in medicine has contributed to the spread of microbial resistance, for this reason the use of essential oils (EOs) represents a promising alternative in the prevention and treatment of bacterial infections. *Thymus vulgaris* EOs, for its chemical composition in phenolic and terpenic compounds, shows various therapeutic activities (antimicrobial, antifungal, anti-inflammatory, etc.) (Rota et al., 2008). The aim of this study was to assess the effectiveness in vivo of *Thymus vulgaris* EOs against pathogen strains in skin infection of dogs.
The study was carried out on 18 half-breed dogs affected by skin infections, housed in kennel "Milleusi" of Messina (Sicily, Italy). The animals showed clinical signs such as itchiness, alopecia, redness of the skin, presence of pustules or scabs, ulcers and/or erosions. The lesions were observed at the level of abdomen, groin and thigh. General symptoms included slightly raised body temperature, anorexia and depression. The cytological investigation for deep infection was negative.
The bacteria isolated by microbiological test from the skin swabs samples of these dogs, subsequently identified by MALDI-TOF-MS, were: *Staphylococcus pseudointermedius* (7 samples), *Staph. sciuri* (4 samples), ESBL *E. coli* (3 samples) and *Proteus mirabilis* (4 samples). The mycological investigations for dermatophites and the research of mites in all skin samples analyzed, instead, were negative. The susceptibility of the isolated microorganisms to *Thymus vulgaris* EOs was estimated by the in vitro bacteriological test (CLSI 2008), in comparison to some antibiotics used in veterinary medicine (amoxicillin, oxytetracycline and thiamphenicol) and to Cytrus bergamia EOs.
The dogs, divided in two group a random, were treated topically for 7 days with *Thymus vulgaris* EOs (Group 1: n. 10 animals) and *Citrus bergamia* EOs (Group 2: no. 8 animals) respectively in the areas affected by cutaneous lesions. In all animals treated with *Thymus vulgaris* EOs, the clinical signs decreased rapidly within 5 days from

administration, with complete remission after 7 days of the end of treatment. No bacteria were present in skin samples 7 days after administration. None of the treated animals showed local or general side effects. This study documents the effectiveness of *Thymus vulgaris* EOs for topical use in dogs affected of skin infections, caused by Gram-positive and negative bacteria. In addition, this EOs seems to be a possible alternative or additional treatment to antibiotics (Savoia, 2012) in dermatological infections, particularly in cases refractory to conventional therapy.

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26P

DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF CRYPTOCOCCOSIS IN A KITTEN: A CASE REPORT

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A 3-month-old male kitten was referred to veterinarians for repeated seizure: clinical examination was normal, blood samples were aimed to blood cell count, biochemistry, *Toxoplasma gondii* IgG and IgM, and *Cryptococcus neoformans* detection (polymerase chain reaction technique - PCR). A positive value was revealed for *Cryptococcus neoformans*. A specific therapy was established using levetiracetam (dose: 20 mg/kg), and fluconazole (dose: 5 mg/kg). The central nervous system (CNS) was investigated using magnetic resonance imaging (MRI) in presence of a paramagnetic intravenous marker: hyperintensity in olphactory lobes, nostrils and olphactory sinus was highlighted in SeT2 sequences with moderate enhancement after the marker administration. CNS remaining parts were normal. Specific cultures were assessed to isolate *Cryptococcus neoformans* by blood and nasal swab, but they were all negative. Hematological controls were performed every 15 days. After 33 days, seizures began once again, becoming more and more frequent in the further two weeks. This led to the hypothesis that drugs were not efficient and clinicians decided to proceed with a recovery in a veterinary hospital in order to administer amphothericin B, every 48 hours for three consecutive times, at the dose of 1 mg/kg, checking creatinine and blood urea nitrogen (BUN) after every administration. Seizure disappeared. Following hematological controls highlighted increased levels of creatinkinase, BUN, alkaline phosphatase and cholesterol. The clinical examination was good and the PCR repeated two weeks after the treatment was negative for *Cryptococcus neoformans*. Nowadays the cat is 7 months old, and seizures disappeared two months ago. Hematological controls are quite normal, and only the BUN value is slightly beyond the range. It is known that Criptococcosis is one of the most frequent feline mycoses in adult cats worldwide, more in America and Australia. Usually clinical symptoms involve nose, skin, lungs, lymph nodes, CNS, and eyes. Several exams were assessed to diagnosticate this disease, but false negatives are common, except for

PCR and MRI. The treatment of localized diseases is generally successful using azole drugs; nevertheless in most cases the prognosis is poor, with a median survival time of 4 months with fluconazole and 8 months with itraconazole. Only disseminated diseases require an additional treatment with amphotericin B, and some cats require a long-term (>1 year) treatment or an indefinite therapy. In the authors' opinion, this case report should be worthy of attention because of 1) the age of the patient, 2) the limited localization of *Cryptococcus neoformans* that required a therapeutic plan similar to a disseminated disease, 3) the fact only PCR and MRI were positive, 4) usually a long-term drug therapy might be potentially toxic, but not in this case analysis, 5) the rare result of a good prognosis.

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27P

OXIDATIVE STRESS IN CANCER STEM-LIKE CELLS: BIOLOGY AND MODULATION STRATEGIES

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Oxidative stress (OS) is related to several human diseases (e.g. tumors). It was demonstrated that inflammatory and/or infectious stressors may alter the redox status. Regarding cancer, different neoplasms are characterized by OS-adaptive mechanisms that may influence tumor progression. Recent researches suggest that cancer stem-like cells (CSCs) are resistant to high levels of reactive oxygen species (ROS), that conversely are able to cause a cytotoxic damage in other cell types.

The objective of this work was to evaluate the redox status in CSCs isolated from canine and feline mammary tumors. A total of 66 (suspected) mammary neoplasms were collected: 57 from dogs and 9 from cats. For each specimen histological diagnosis (WHO) and immunohistochemistry detection of stem markers (e. g. CD44) were performed. Fifteen fresh tissue samples were in optimal conditions to set up primary cultures. Typical stem properties (sphere formation, differentiation ability, expression of CD44) were verified in all the primary cultures maintained under stem permissive medium (w/o serum, and in the presence of bFGF/EGF) and used in the experiments. With the aim of investigating OS mechanisms, CSC cultures were exposed to a pro-oxidant stimulus for 24-48h: a mixture of peroxide lipids (LOOHs), named K600, at different concentrations (0.1-1% and 0.01-0.1%). K600 derives from a peroxidation treatment of organic extra virgin oil that allows to obtain a LOOH concentration of 600 EqO2/kg oil (K600, International Patent no. PCT/IT 2008/000693). The effects of K600 in CSCs were analyzed by evaluating cell toxicity (MTT assay assessing cell metabolic activity of viable cells and fluorimetric determination of DNA as index of proliferative activity), by measuring levels of OS products (thiobarbituric acid-

reactive substances, TBARs) as well as of RTKN (Rhotekin) protein and by performing a Nitro Blue Tetrazolium (NBT) assay. CSC treatment with K600 induces an increase in the intracellular basal levels of OS: TBAR levels indicated an increase of lipid peroxidation index (malondialdehyde products) after 24h of exposure. NBT test showed that superoxide anion mainly derived from mitochondrial rather than cytosolic compartment. Results from MTT assays indicated that K600 determines cell viability reduction (by 80%) with respect to untreated cells. A significant inhibition of cell proliferation at 0.025% K600 after 48h of treatment was observed and the RTNK protein levels also decreased (by 60%) after 24h of K600 exposure as evaluated by immunoblotting and densitometry. These results show the ability of K600 to induce cytostatic/cytotoxic effects by modulation of the oxidative balance. Moreover, these data may be useful to highlight the possible OS mechanisms involved in tumor processes, to identify new targets for prognosis and innovative therapeutic strategies for both animals and humans.

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28P

GRAZING GOATS FED POLYUNSATURATED FATTY ACID: AN EFFECT ON IMMUNE PROFILE, MILK YIELD AND QUALITY

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Nutritional properties of goat milk are known for its healthy features, referring to fatty acid (FA), protein, and amino acid composition (Silanikove et al., 2010). Fat supplementation is able to interfere with the modulation of immune responses with potential beneficial effects on animals (Caroprese et al., 2015). Extruded linseed was used as supplement in dairy goats increasing the levels of milk FA n-3 (Bernard et al., 2015). The present study aimed to understand the improvement of immune profile, milk yield and quality of grazing dairy goat fed fish oil or linseed. Twenty-four Garganica goats were divided into three groups; fish oil group (FO) that received 1.5 kg/day of concentrate and supplemented with 50 g/day of microencapsulated fish oil, linseed group (LIN) that received 1.35 kg/day of concentrate and supplemented with 150 g/day of whole linseed, and control group (CON) that received 1.5 kg/day of concentrate without fat supplementation. Goats had free access to pasture (9.00 to 17.00), and water was available ad libitum. Goat milk samples were analysed weekly to determine milk chemical composition, fatty acid profile, and somatic cells count. During trial goats' health status was monitored by measuring cell-mediated immune responses, after intradermal injection of phytohemoagglutinin, and humoral immune responses after chicken egg albumin (OVA) immunization. Diet based on linseed supplementation significantly increased milk, fat, protein, and casein yields as compared with CON and FO milk. Fat content increased in LIN milk as compared with CON and FO milk. Linseed modified goat milk fatty acid profile, registering lower SFA and higher PUFA than FO milk. The modified FA composition of LIN milk resulted in a lower atherogenic and thrombogenic indexes than FO and CON milk. Linseed administration reduced the humoral immune responses of goats in terms of anti-OVA antibody production; however, the cellular immune responses were not affected by fat supplementation. Dietary linseed

supplementation in grazing dairy goats may support feeding programs to improve their milk composition and quality, affecting the humoral immune responses.

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29P

EFFECT OF PARATUBERCULOSIS INFECTION ON WELFARE PARAMETERS OF DAIRY COWS: PRELIMINARY RESULTS

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The aim of this study was to evaluate the immunological and metabolic conditions of cows infected by MAP, compared with healthy cows reared in the same herd, to verify the role of MAP infection as predisposing factor for other infectious and metabolic diseases occuring in the peripartum. For this purpose, we adopted a case-control study in which cases were defined as animals testing positive in serological ELISA and/or fecal culture for MAP. The controls were selected among healthy animals of the same herd (test-negative and asymptomatic). Blood samples of 13 positive and 13 negative cows were collected 5 times, at days -30, +3, +10, +30, +100 with respect to calving. A metabolic profile was measured in each sample, including positive and negative indicators of the acute phase response (APP, i.e. haptoglobin, bilirubin, ceruloplasmin, albumin, cholesterol, paraoxonase). The APP values were used to calculate the Liver Functionality Index (LFI), which defines the inflammatory response during the first month of lactation. The cell-mediated immune response to MAP was investigated by an IFN-gamma release assay on whole blood samples. Flow cytometry immunophenotyping was performed on isolated peripheral blood mononuclear cells (PBMC) using a panel of monoclonal antibodies to bovine monocytes and sub-populations of lymphocytes. We could not calculate the LFI of 4 cases out of 13, because they were culled before 30 days post partum. Reasons of early culling were left-displaced abomasum, milk fever and mastitis. The mean LFI was -3.15 and -2.84 for cases and controls, respectively, with no significant differences between the two groups under study; interestingly, lower values of Ca, albumin and creatinine were measured in case animals. The most common abnormalities in the prevalence and fluorescence intensity of surface marker expression were a decreased prevalence of CD8+ T cells and MHCI+ PBMC. Some PBMC samples also showed an abnormal concentration of NKp46+ (NK) cells. No significant differences were observed between MAP-positive and control cattle. However, by including in the

control group only the 5 IFN gamma-negative cows, increased prevalence of B cells and a reduced prevalence of CD45-positive PBMC were only observed in some MAP-positive animals.

The negative LFI values highlight an altered metabolic profile in both groups. This was worse in the MAP-infected group, which also produced less milk. However, drug usage was greater in the control group, because MAP-negative cows were treated for metritis, mastitis and lameness, whereas the positive ones were culled as soon as they showed symptoms of illness or decreased milk production. A greater number of observations are badly needed though to corroborate the initial hypothesis and draw an inference as to a peculiar influence of MAP infection on welfare parameters and homeostasis of bovine PBMC.

30P

KIDNEY INNATE IMMUNE RESPONSE TO CADMIUM EXPOSURE

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Cadmium (Cd) is a pollutant, toxic and carcinogenic heavy metal released into the environment by human activities like agriculture and industries. Humans and animals can be exposed by ingestion of contaminated food and water, this can lead to a chronic or acute intoxication on the basis of Cd intake in diet. Kidney represents the major target in chronic pollution; renal lesion is characterized by glomerular dysfunction due to Cd accumulation in proximal renal tubular. At molecular level the toxicity of Cd is related to its ability to significantly modulate the immune system; currently, few data on the immune-modulatory effects of chronic exposure are available (1). For these reasons, Cd pollution represents a possible risk for animal and public health (2). The aim of our research was to investigate the effects of Cd on innate immunity in kidney's samples of wild boars naturally exposed. Twenty samples were tested for Cd contamination and split in three groups on the basis of concentration: Group 1 (G1: 0-3.5 ppm), Group 2 (G2: 3.6-7.4 ppm) and Group 3 (G3: 7.5-21.6 ppm). After anatomic-pathological test, 30 µg of kidney were homogenized with 600 µl of lysis buffer. Total RNA was extracted and after the reverse transcription step (2), changes in mRNA expression profiles of porcine cytokines IL-1β, IL-6, IL-8, Nk-fb1, Nkfb-p65, MYD88, IL-18, IFN-β, P38, β2-M, TLR4, TLR5, MD2, CD14, TNF-α, bD1, bD2, bD3, bD4, JNK, STAT3 and SOCS1 were investigated using primer sets described in previous studies (3). HPRT1 was used as housekeeping control gene (4). In each specimen the relative expression of the selected genes was calculated (ΔCt=Ct(target gene)–Ct(housekeeping)). The average expression intensity of the genes under study was compared among groups by one-way analysis of variance (ANOVA). The threshold for significance was set at $P<0.05$. Our results showed a significant modulation of IL-1β, IL-6, JNK, STAT3, P38, CD14, NFkb-1 and NFkb-p65 gene expression. In particular, G3 indicated a decrease of IL-6 level and a P38 MAPK and JNK up-regulation. Moreover Cd exposure (G2, G3) induced down-

regulation of CD14 and up-regulation of STAT3, NFkb-1 and NFkb-p65. Low-concentration of Cd (G2) determines a decrease of IL-1β and JNK gene expression. According to in vitro studies (1), these results support the hypothesis that Cd exposure may modify the basal level of cytokine expression; indeed our data suggest that Cd can activate STAT3 and the NF-kb pathway but also inhibit CD14 gene expression. JNK gene expression decreases in G2 and increases in G3; furthermore, IL-6, a pro-inflammatory cytokine, and P38 MAPK are up-regulated by high Cd concentration; IL-1β is conversely down-regulated by low values. Our data suggest that different concentrations of Cd are able to influence various compartments of the innate immune response in kidney.

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31P

COMPARISON OF STANDARD PROTOCOLS WITH AND WITHOUT ZINC SUPPLEMENTATION FOR TREATMENT OF CANINE LEISHMANIASIS IN AN ENDEMIC AREA

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Success of therapy in canine leishmaniasis (CanL) depends on the efficiency of the cellular immune response (1). Zinc deficiency can compromise immune system function, including T cells. In dogs with clinical leishmaniasis have been observed low serum zinc levels. The aim of this work is to evaluate the effect of zinc oral administration during treatment of CanL in order to verify the possibility of a better disease control. Eighteen dogs, of both sexes, were employed in the trial. They were between 1 and 10 years old and they showed clinical-pathological alterations suggestive of leishmaniasis and the presence of the parasites in lymph-nodal smears. Dogs were subdivided in three treatments groups: group A or control group (6 dogs), therapy consisted in: meglumine antimoniate for 30 days in association with allopurinol for 7 consecutive months; group B (5 dogs), therapy consisted in: meglumine antimoniate for 30 days in combination with zinc for 12 months; group C (7 dogs), same treatment of the A group but integrated with oral administration of zinc for a total of 12 months. For each enrolled dog a clinical score was assigned and laboratory monitoring was performed in the following times: T0; T30; T60, T90, T150, T360 during a follow-up period of 12 months. The data set was subjected to the two-way variance analysis. Each treatment protocol was then analyzed separately by applying the Tuckey's test for repeated measures in order to assess the differences between the times of analysis considered. All data were expressed as quadratic means values. The significance was set at $P<0.05$. No statistically significant differences were registered for total clinical score neither between times nor between groups; differently the skin score showed a significant decrease between T0 and T90 in group A and between T0 and T60 in groups B and C. The A/G ratio showed significant increases between T0 and T60 in the C group and between T0 and T150 in group A. Two dogs, one of group A and one of group C, showed clinical and laboratory relapses

respectively at T210 (17%) and T360 (14%). The results of this study show that the supplementation of zinc per os (ZincogenPet, pearls, NBF - Lanes, at dosage recommended by the firm) in the conventional protocol for CanL results in an increase in serum zinc concentration, in a faster response to therapy and in an elongation of the disease free interval time. The positive effects of zinc integration could derive by various biological functions in which zinc is involved, especially immunological, inflammatory and antioxidant processes (2). Results of this study encourage the supplementary oral intake of zinc during conventional treatment for canine leishmaniosis. Anyway further data on a larger population are auspicable to support these statements.

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2. Prasad, 2009

32P

TRANSFUSION OF CANINE BLOOD TO A CAT

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Blood transfusion may be troublesome in cats and when haemoglobin-based oxygen carrier solutions or compatible feline donors are not available, xenotransfusion with canine blood is performed as a life-saving procedure (1, 2, 3). Few cases are reported and more data are needed to evaluate risks of xenotransfusions. The aim of this report is to describe a case of transfusion with canine blood to an anemic cat monitored for short and long term reactions. A 1 year old domestic shorthair neutered male cat was presented for severe lethargy and anorexia. Physical examination findings included flea infestation, hypothermia, very pale and icteric mucous membranes, dehydratation and splenomegaly. Cell blood count (CBC) revealed severe normocytic hypochromic anemia (Hb 2.2 g/dL) and thrombocytopenia (82.000/mm^3). Basic metabolic panel showed increase in total bilirubin (1.07 mg/dL) and creatinine (1.87 mg/dL) values. Rapid serological tests for FIV and FeLV were negative. The cat was treated with doxycycline (10 mg/kg/day PO for 4 weeks) and prednisone (1.5 mg/kg/day PO for 14 days). Due to lack of feline blood, a transfusion with 200 ml fresh whole canine blood was performed. No acute transfusion reactions were noted. CBC was performed after both two days and one week and showed a steady improvement of the anemia (Hb: 5.7 and 5.9 g/dL respectively). The clinical condition improved progressively. Major cross-matching test performed 7 months after transfusion showed severe hemolysis and minor cross-matching test revealed severe agglutination. At that time, the clinical condition of the cat was good and CBC values were in the reference range. According to limited number of cases described in the literature, no severe acute adverse reactions occurred in cats receiving a single transfusion with canine blood (1, 2, 3, 4, 5). However previous studies revealed a high prevalence of natural occurring antibody in cats against dog erythrocytes and showed that antibodies against canine red blood cells are produced within 4-21 days after a transfusion (1, 4, 5, 6). Any repeated transfusion with canine blood later than 6 days after the first one causes severe transfusion reactions which are frequently fatal or

milder reactions that reduce the benefit of xenotransfusions (1, 5). This is confirmed by result of the cross-matching tests performed in this cat 7 months after transfusion. In conclusion, transfusion of cats with canine blood is not "best practice" and must not be considered as a routine procedure. However, we added confirmation that in exceptional emergency situations where other better options are not available it provides a prompt stabilization of critical anemic patients. (2, 4).

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33P

VALIDATION OF HYDROPHOBIC LITTERS USED FOR FELINE URINE COLLECTION FROM LITTER TRAYS

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Voided samples are used for urinalysis when: urine culture is not performed, voided bladder occurs during consultation or serial urinalyses are required. Some hydrophobic litters (HL) are available for feline urine collection from litter trays but we are unaware of published studies which evaluated their use as a pre-analytical variable. The aim of this study was the validation of two different HL when used to collect feline urine samples for urinalysis and quantitative proteinuria (qPr) and creatininuria (qCr) assessment. We compared urinalysis results of 109 feline urine samples tested as such and after contact with HL (52 with Catrine TM (C-HL), Kruuse, Denmark; 57 with Kit4cat TM (K-HL), Coastline Global, CA, USA). Urine samples obtained by cystocentesis or voided bladder were put in 2 tubes, one of them containing granules of one of the HL under study. Urinalysis (Combur 9®, Roche Diagnostics, Switzerland; Reichert VET360, USA) was performed on both aliquots by a same operator 60' after collection and then qPr, qCr and urinary protein:creatinine ratio (UPC) were assessed in 34 pairs of samples for C-HL and in 57 for K-HL (Catalyst™ slide urine P:C ratio, IDEXX laboratories, The Netherlands).

Most of urinalysis (104/109) detected at least 1 urine abnormality. Evaluation of paired results of urinalysis, qPr, qCr and UPC was made and correlation between paired qPr, qCr and UPC values was calculated. Samples placed in contact with C-HL had no difference in color, odor, nitrites, ketones, bilirubin, blood/hemoglobin assessment, but 2 samples were slightly turbid and 9 had slight differences of SG, 7 of pH, 2 of proteinuria, 3 of glucosuria; bacteriuria was observed in 2 samples, lipids in 4 and artifacts in 10. Samples placed in contact with K-HL had no difference in clarity, color, odor, proteinuria, glucosuria, nitrites, ketones, bilirubin, blood/hemoglobin assessment, but a slight difference in SG (8/34) and pH (1/57) were found; in the urinary sediment lipids

were observed in 1 sample. Differences in qPr respectively ranged between 1 and 7 mg in 9/34 C-HL samples and 1 and 19 mg in 30/57 K-HL samples. Discrepancy of creatininuria in 27/34 C-HL samples (range 1-183 mg) and in 31/57 K-HL samples (range 0.3-20 mg) occurred. As a consequence, UPC values differed in 9/34 (26.5%) C-HL samples and in 15/57 (26.3) K-HL samples. However these slight differences did not affect IRIS substaging of proteinuria1. Moreover an excellent correlation ($r2>99$, $p<0.0001$) was found between qPr, qCr, UPC values obtained with paired samples of both HL.

In conclusion, clinical evaluation of urinalysis results is not affected by the use of the HL under study and they can be of valuable help for the collection of voided samples of feline urine. The occurrence of bacteriuria should however be confirmed with appropriate further evaluations.

1. http://www.iris-kidney.com

34P

MAY WE USE EDTA PLASMA OR HEMOLYTIC SAMPLES TO DETECT IN DOGS ANTI-*LEISHMANIA INFANTUM* ANTIBODIES BY IFAT?

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Leishmaniosis is a vector-borne zoonotic disease endemic in South Europe where it is caused by *Leishmania infantum* (1). The dog is the primary domestic reservoir and the infection is transmitted by sand flies of the *Phlebotomus* subgenus (2). The gold standard for the serological diagnosis of canine leishmaniosis is the indirect fluorescence antibody test (IFAT) (3). The assay permit a quantification of specific serum IgG antibodies against whole promastigotes (). No evidence is available about the influence of hemolysis or the use of plasma on anti-*Leishmania* IFAT titers, but in the real life it may happen that this kind of samples are the only available for serological tests. The aim of this study is therefore to assess whether hemolysis or the use of EDTA plasma samples affect the results of a *Leishmania* specific IFAT. Fifty canine blood samples collected in both serum and EDTA tubes were evaluated. Blood serum and plasma samples with no visible hemolysis were selected. Their respective tubes were vigorously shaken to hemolyze the left over samples. Hemolytic EDTA plasma and serum samples were visually selected on the basis of a moderate or severe hemolysis corresponding to an absorbance ≥ ,786 by reading at 450 nm. IFAT was performed using *L. infantum* (strain MHOM/IT/80/IPT1) antigen slides manufactured by the National Reference Centre for Leishmaniosis (C.Re.Na.L., Palermo, Italy) and fluoresceinated rabbit anti-dog IgG (Anti-IgG-FITC, SIGMA) diluted in PBS 1:200. The manufacterer's protocol was followed apart from the cut-off dilution that was established at 1:20 and titer was determined on serial twofold dilutions. Samples of the same dog were evaluated at the same time and on a same antigen slide. Titers ranged from <1:20 to 1:40,960 (not-hemolytic serum and both plasma samples) and 1:81,920 (hemolytic serum). The median titer was the same for all the four samples obtained for each dog (1:20). There were no significant differences between titers obtained with hemolytic and non-hemolytic samples (for both serum and

plasma) and comparing serum and plasma samples. Although 21% of titer discrepancy was observed testing serum samples (both hemolytic and not-hemolytic), 23% testing plasma samples (both hemolytic and not-hemolytic) and 16% testing non hemolytic serum and plasma, in all cases the difference was within one dilution. In conclusion, EDTA plasma and hemolytic EDTA plasma or serum can be used – in case of need - for the detection of anti-*Leishmania* IgG by IFAT.

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35P

ANTIBODY AND MOLECULAR PREVALENCE OF *LEISHMANIA INFANTUM* IN STRAY CATS FROM MESSINA AREA

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The role of cats as reservoir of *Leishmania infantum* is still unclear but many studies detected in endemic areas not negligible antibody and PCR positivity rates (1). Stray cats may serve as sentinel of exposure to *L. infantum*. The aims of this study are: to provide antibody and PCR prevalence rates in colony and stray cats from Messina; to assess associations between clinical data and antibody/PCR positivity.

Between November 2014 and January 2016, 112 stray/colony cats from Messina were evaluated when admitted to the Veterinary Teaching Hospital of the University of Messina for health problems or because included in a trap-neuter-release program. Signalment and physical examination findings were recorded. Blood samples, oral (no. 88) and bilateral conjunctival swabs (no. 91) were collected. Cell blood count (CBC) (no. 105) and quantitative (q) blood PCR (no. 94) were performed on EDTA blood; qPCR was performed from swabs and anti-*L. infantum* IgG antibodies were detected in serum by IFAT (cut off: 1:40). Serological and molecular techniques were carried out at the National Reference Center for Leishmaniosis (C.Re.Na.L, Palermo) as previously described (2, 3).

Forty-four male (4 neutered) and 67 females (1 spayed) cats were evaluated. Forty-five (40%) were about under 1 year of age and 67 were estimated to be older. Most of cats (103/110=94%) were short-haired. Body condition score (BCS) was ideal, overweight or obese (3-5/5) in 97/112 (86.6%) cats and 15 overall cats were thin or emaciated (BCS=1-2/5). Based on history, physical examination and CBC, only 30 cats (26.8%) were clinically healthy. Among other findings, some compatible with feline leishmaniosis (FeL)1 were recorded: lymphadenopaty (n=37), ocular lesions (n=25), skin lesions (n=7) and mucocutaneous lesions (n=2). Anemia was found in 43/105 cats (40.9%) and scored as mild (no. 33), moderate (no. 7) or severe (no. 3). Neutrophilia (no. 29 cats),

leukocytosis (no. 28), monocytosis (no. 26), eosinophilia (no. 14), thrombocytopenia (no. 12), thrombocytosis (no. 9), lymphocitosis (no. 6), eosinopenia (no. 5), neutropenia (no. 2), lymphopenia (no. 2), leukopenia (no. 1) and basophilia (no. 1) were also detected. Univariate analysis of the categorical data was performed using the chi-square test or Fisher's exact test. Seroreactivity to *L. infantum* was found in 40.2% (45/112) of cats, with 1:40 (51.1%), 1:80 (35.5%), 1:160 (6.7%), and 1:640 (6.7%) titers. PCR analysis tested positive (blood: 4 leishmania/ml; conjunctival swabs: 1700 and 980 leishmania/ml) only from an antibody positive cat (titer 1:640). All the oral swabs were negative. No difference in antibody prevalence was found according to sex, age, fur length, BCS, health status, occurrence of clinical signs compatible with FeL, CBC abnormalities.

This study confirms that in endemic areas it is not negligible the percentage of stray cats antibody positive against *L. infantum* (1). Their epidemiological role should be clarified better exploring the infection status and the infectivity of cats.

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36P

FELINE RENAL LYMPHOMA: PATHOLOGICAL FINDINGS, LYMPHOCYTES PHENOTYPING AND FELV GP70 VIRAL ANTIGEN TISSUE EXPRESSION

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Lymphoma, the most common neoplasms in cats, is classified according to the anatomical location into different types. Neoplastic cells may be composed by different types of cells, such as B and T lymphocytes, that are determined by immunophenotyping, using immunohistochemistry (IHC). Cats infected with feline leukemia virus (FeLV, Gammaretrovirus) have a higher incidence of lymphoma than uninfected cats. The proposed mechanisms of tumoral development are insertional mutagenesis or persistent stimulation of host immune cells by viral antigens. The aim of this work was to examine the pathological findings, the FeLV expression and the immunophenotype in feline kidneys affected by malignant lymphomas. Neoplasia of 19 cats with renal lymphoma were buffered formalin fixed and paraffin embedded. Heamatoxylin-eosin stain was performed on 5µm paraffin sections as well IHC for FeLV gp70, CD3 and CD79. Gender was 52.6% (10/19) male and 47.4% (9/19) female. The age ranged from 8 months to 17 years: 26.6% (5/19) young (0-2 years), 47.4% (9/19) adult (2-10 years) and 26.3% (5/19) were aged (>10 years). Renal lymphoma appeared primitive in 5 cases (26%), in 8 cases (42%) appeared secondary to multicentric lymphomas, in 3 cases (15.7%) to mediastinal lymphomas and other 3 cases (15.7%) to gastric and intestinal lymphomas. In the immunophenotyping, 73.,7% (14/19) were CD3 positive and the 27.3% (5/19) were negative; 26.3% (5/19) were CD79 alpha positive and 73.7% (14/19) were CD79 alpha negative; expression of gp70 protein were detected in the 78.9% (15/19) of renal neoplasia, while the 21.1% (4/19) were negative. In this study we detected an elevate incidence of the renal anatomical localization. There was no statistical association (Fisher's exact test, $P<0.0001$) between the renal lymphomas, age and sex but there was an elevate percentage, more than 2/3 of cases, of adult and aged affected cats. The neoplastic population had an elevate expression of CD3, characterizing

the lymphocytes as belonging to the T subset. Moreover, there is an elevate amount of neoplastic cells that express gp70; this allows that neoplastic lymphocytes are infected by FeLV virus, which is even in active replication in neoplastic cells. The CD3 and gp70 markers showed strong statistical correlation (Pearson correlation index); thus the clonal expansion of T lymphocytes was correlated with the presence and active replication of the virus.

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37P

OVARIAN TERATOMA IN AN ADULT FEMALE *ZOOGONETICUS TEQUILA* (WEBB & MILLER, 1998): HISTOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES

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The Mexican Goodeid, *Zoogoneticus tequila*, is considered nearly extinct in the wild and it is maintained in captivity by the nonprofit international "Goodeid Working Group." A pathology survey on the unique Italian colony was previously published by the authors, but no other literature reports regarding pathologies of this species are available. The present case describes an adult female showing a progressive coelomic distension, suggestive of gestation. In fact, *Z. tequila* is a viviparous species and females give birth to free-swimming fry after an intraovarian gestation. However, after a period of anorexia of two days' duration, the fish died and was immediately submitted to necroscopic and histopathologic exams. Macroscopical examination confirmed the abdominal enlargement, associated with the presence of a mucous secretion from the genital pore. A large multilobulated mass (2x1.5x1 cm) occupied most of the coelomic cavity, causing intestinal occlusion by compression of the intestine without infiltration. Samples of the mass were routinely processed for histology. Additional sections were also subjected to immunohistochemistry for pan-cytokeratin, vimentin, glial fibrillary acidic protein (GFAP), and neuron-specific enolase (NSE). Histologically, the mass consisted of a complex variety of tissues derived from the three germ cell layers, suggesting a diagnosis of teratoma. Structures of ectodermal origin included multifocal, keratinizing squamous epithelium, and large areas of nervous system tissue resembling cerebrum. Mesenchymal tissues (mesodermal origin) consisted of skeletal muscle, cartilage, fibrous connective tissue, and adipose tissue. Numerous, simple-to-branching glandular structures lined by intestinal-type epithelium (endodermal origin) were also admixed. All the epithelial components were pancytokeratin-positive. Extensive neuronal and glial differentiation was confirmed by intense and diffuse immunoreactivity for GFAP and, to a lesser extent, for NSE. In addition, multiple, densely-cellular islands and branching trabeculae of

undifferentiated tissue, characterized by mild cellular and nuclear atypia, and sparse mitotic figures, were observed. The presence of sparse residual oocytes in different stages of development indicated an ovarian origin of the tumour. Teratomas are neoplasms containing multiple tissues originating from more than one germ cell layer. They may be classified as benign (mature) or malignant (immature) depending on the degree of anaplasia or the presence of embryonic-like undifferentiated elements. Teratomas are occasionally described in fish, especially in viviparous species, even though they are usually poorly characterized at the histological and immunohistochemical levels. In this case, even though metastatic or implanted masses were not noted, the presence of poorly differentiated elements in addition to mature structures indicate a definitive diagnosis of ovarian immature teratoma.

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38P

INCREASED EXPRESSION OF THE CHEMORESISTANCE MARKERS P-GLYCOPROTEIN (PGP) AND BREAST CANCER RESISTANCE PROTEIN (BCRP) IN A CANINE CUTANEOUS MAST CELL TUMOR TREATED WITH CHEMOTHERAPY AND TYROSINE KINASE INHIBITOR

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The onset of multidrug resistance can be related to PGP and BCRP expression, already described in canine neoplasia (2, 3). Medical therapeutic approach for canine mast cell tumors (MCT) includes conventional chemotherapy and tyrosine kinase inhibitors (TKIs). TKIs are able to block disregulated KIT activity (1), whose immunohistochemical (IHC) pattern has been related to the biological behaviour of MCT (4). A case of a non-responsive to treatment MCT is described below and IHC expression of PGP, BCRP and KIT were examined, before and after treatment. A 7 years old, female spayed, mixed-breed, dog was referred for a cutaneous carpal mass and ipsilateral prescapular lymphadenomegaly. Lesions were removed and the final diagnosis was cutaneous MCT (Patnaik's Grade 2, Kiupel's low grade) with lymph node metastases. The owner refused adjuvant chemotherapy and 6 months later, 3 new MCT were diagnosed in the surgical scar region. A Vinblastine + Prednisone protocol was initiated. After three doses, the disease progressed and tumors were excised: final histologic diagnosis was cutaneous MCT (Patnaik's Grade 2, Kiupel's high grade), infiltrating subcutaneous MCT and MCT lymph node metastasis. Masitinib, a TKIs, was added to the chemotherapeutic protocol. After 2 months, the dog developed new MCTs and a rescue protocol with Lomustine was initiated. Due to the disease progression, the dog was euthanized 285 days after diagnosis. A retrospective IHC exam with PGP, BCRP, KIT was performed and evaluated with already described methods (4, 5, 6). Samples collected before chemotherapy did not show PGP (0%cell/HPF) and BCRP (<10%cell/HPF) expression; KIT's staining expression was pattern I in the cutaneous mass, and pattern III in the node metastasis. After chemotherapy, an increased expression of PGP from null to low (<10%cell/HPF) in the cutaneous metastasis, and from null to intermediate (10-50%cell/HPF) in the subcutaneous and nodal metastasis were detected; BCRP expression was positive in all

the samples (>10%cell/HPF); KIT staining was pattern III (diffuse cytoplasmic) in all the samples. The increase in PGP expression, seen in MCT after the first treatment, is likely to be related with the onset of PGP-mediated chemoresistance, being Vinblastine a substrate of PGP (5). These chemotherapeutic drugs, instead, are not BCRP substrates (7), however, the BCRP expression has been associated with biological aggressiveness (7), present in this case. Pattern III of KIT, seen in all metastases, is related to worse prognosis (4). The increased expression of PGP, BCRP and KIT in this case could be related to a chemoresistant and malignant neoplastic phenotype.

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39P

OVARIAN FIBROTHECOMA IN A SACHSEN WARMBLOOD MARE

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Nowadays in human medicine the term “fibrothecoma or fibrosed thecoma” is used to describe a rare benign ovarian neoplasm with gonadal stromal cell origin belonging to thecoma-fibroma group. (1, 2). The fibrothecoma is formed by collagen producing fibroblasts, in addition to theca cells containing lipids (3). In mare this tumor is rare and to date only three cases have been reported (4, 5, 6). A 18-year-old Sachsen Warmblood mare was presented to the Veterinary Teaching Hospital of Camerino University, with a history of infertility. Rectal palpation and ultrasonography examinations revealed an enlarged right ovary with an anechoic cyst cavity (10-12 cm), surrounded by a thick and hyperechoic wall measuring 1-2-cm, no evidence of follicular activity was observed. An ovarian tumor was suspected and surgical excision of the ovary was performed. Grossly the ovary was totally occupied by a large mass, with whitish, irregular and lobulated surface measuring 21x13x12 cm. On cut surface a solid thick wall with white-yellowish bands circumscribing a large central cystic area was observed. Tissue specimens were submitted for histopathological examination and immunohistochemical analysis using antibodies to vimentin, alpha-smooth muscle actin (α-sma), inhibin, CD99, CD117, MIB1, S100. Histologically the ovary was totally effaced by a densely cellular neoplasm composed of spindle cells with oval or spindle-shaped nuclei, arranged in interlacing bundles on a fibrous stroma. Multifocally neoplastic cells had lipid vacuoles within cytoplasm, a feature consistent with thecal differentiation. Anisocytosis and anisokaryosis were mild. Immunohistochemistry revealed a strong and diffuse staining of neoplastic cells for vimentin and a multifocal positivity for α-sma, as observed in two other studies in mare (4, 5). Multifocally neoplastic cells had strong positive immunolabeling for inhibin, a sex cord-stromal marker not evaluated in the other studies in mare. Negative staining for CD99, CD117 and S100 was noted and ki67 proliferation rate was low. The histopathological and immunohistochemical findings of this rare

ovarian neoplasm are consistent with a fibrothecoma and our results are similar to those found in the few reported cases in mare. The expression of inhibin observed in this case demonstrated that tumor was hormonally productive, a status associated with infertility problems.

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40P

AN UNCOMMON CASE OF CLEAR CELL ODONTOGENIC CARCINOMA (CCOC) IN A DOG: IMMUNOMORPHOLOGICAL CHARACTERIZATION AND LITERATURE REVIEW

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In dogs and cats, the oral cavity cancers are classified as odontogenic tumors (neoplasia arising from tooth-forming tissues), non-odontogenic tumors, or non-neoplastic lesions (WHO, 2010). To understand the classification of odontogenic tumors, generally considered to be rare, it should be remembered that dental organ pre-ameloblasts and basal lamina induce development of mesenchymal cells into odontoblasts, which produce dentin and induce pre-ameloblasts to mature into secretory ameloblasts. These reciprocal sequential inductive interactions between dental epithelium and mesenchyme form the basis for classifying epithelial odontogenic tumours, which comprehend mesenchimal inductive (ameloblastic fibroma, dentinoma, ameloblastic odontoma, complex odontoma, and compound odontoma) and non inductive (ameloblastoma, adenomatoid ameloblastoma, and calcifying epithelial odontogenic tumour). A few clear cells may be present in odontogenic cysts, while odontogenic neoplasms composed predominantly of clear cells are quite rare. They include calcifying epithelial odontogenic tumours, ameloblastoma and odontogenic carcinoma (Iezzi et al., 2002). Our case, belonging to a 8 yrs old Irish Setter unspayed female, showing a ulcerated 5x4x3 cm maxillary gingival mass localized at level of 4° superior premolar (tooth 208), consisted histologically of islands and sheets of moderately PAS-positive clear cells, that were separated by fibrous septa. Corresponding lymph-node was moderately involved. Immunohistochemically, the tumor cells were positive for pan-cytokeratins, epithelial membrane antigen (EMA), but negative for S-100 protein, smooth muscle actin, desmin, human melanosome-specific antigen-5 (HMSA-5), CD3, CD45, and glial fibrillary acidic protein. On the basis of these observations, a diagnosis of clear cell odontogenic carcinoma (CCOC) was made. To date, only 74 well-documented cases (Kalsi et al., 2014) have been reported in human, but no case in veterinary medicine. Based on its

morphologic, histochemical and immunophenotypic features, CCOC was distinguished from other primary and metastatic clear cell tumors of the oral and maxillofacial regions.

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41P

EARLY GENETIC SELECTION: POSSIBLE MEAN TO INCREASE THE EFFICACY OF GENETIC SELECTION PLANS FOR RESISTANCE TO SCRAPIE

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Molecular genetics in livestock has been subject to extensive study during the last two decades. These studies are related to gene-based selection of Mendelian traits (mainly diseases and genetic defects), marker assisted selection and introgression. Furthermore, molecular information is increasingly used to assist breed conservation programmes and to improve understanding of the origin and domestication of livestock. Scrapie, the prion disease of sheep, is the most common natural form of transmissible spongiform encephalopathy, a group of diseases which also include Creutzfeldt-Jakob disease in humans and BSE in cattle. Genetic susceptibility to scrapie is strongly modulated by allelic variations at three different codons in the sheep PrP gene. Breeding for scrapie resistance has, therefore, been considered an attractive option for the control of this disease. This can be done by selecting for the allele that is associated with the greatest degree of resistance to scrapie (the ARR allele). Breeding programmes to eliminate scrapie can pose a threat to rare breeds that have a low frequency of the resistant genotype. The objectives of a conservation programme may include not only ensuring the survival and integrity of the target population, but also improving its reproductive rate and performance while maintaining its specific SCRAPIE resistance features. The purpose of the research project is to evaluate the genetic progress in the share of comeback on rams in order to increase the presence of the resistant genotype unlike the national plans that prescribe the genetic testing only for selection of animals reproductive age. Early genetic selection could keep the costs of management of breeding, because the farmer could take down the leaders susceptible at an early age without having to keep the subject up to the age of reproduction. The sicilian breeders showed a strong interest for the possibility to do the genetic selection before that the rams they get to the age of reproduction. We before planned a selections of sheep farms without brucellosis and other infectious diseases, that acceded to the regional plan of genetic selection for the scrapie eradication.

we before planned a selections of sheep farms without brucellosis and other infectious diseases, that acceded to the regional plan of genetic selection for the scrapie eradication. We implemented a method to the simplified identification and a form to permit the animals registration. 895 ovine were tested in the last two years and they show this genetic trends: ARR/ARR 28.83%, ARR/ARH 0.34%, ARR/AHQ 2.79%, ARR/ARQ 47.82%, ARQ/ARQ 13.97%, ARQ/AHQ 2.23%, AHQ/AHQ 0-11%, ARQ/ARH 0.34%, ARH/ARH 0.00%, AHQ/ARH 0.00%, VRQ/VRQ 0.22%, ARQ/VRQ 0.56%, VRQ/ARH 0.00%, VRQ/AHQ 0.11%, VRQ/ARR 2.12%. The method used was MALDI-TOF mass spectrometry for analysis of polymorphisms in the genome sheep for the detection of susceptibility to scrapie. Mass spectrometry applied to nucleic acids for allelic discrimination.

42P

HEAVY METALS LEVELS IN CHICKEN FROM SICILY AND SURVEY ON A POSSIBLE RELATION WITH PARASITES INFESTATION

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Heavy metals are intrinsic component of the earth crust; however, today soil contamination with heavy metals is an environmental problem on a global scale and it is becoming increasingly important as industrialization increases. Heavy metals have long biological half-lives and have an accumulation potential in different organisms such as chicken (Zhuang Ping et al., 2009). Parasites are attracting increasing interest from ecologists as potential indicators of environmental quality because of their important response to anthropogenic pollution (Sures et al., 1999). Certain parasites can accumulate heavy metals at concentrations that are orders of magnitude higher than those in the host tissues. Heavy metals have been quantified in a small number of parasites of terrestrial hosts, for this reason little was known about the accumulation of toxins within terrestrial parasites. In this work a total of 40 chicken samples (*Gallus gallus domesticus*) were analysed for parasitological and heavy metals levels assessment (cadmium and lead) in order to verify a possible difference in heavy metals bioaccumulation between infested and non-infested chicken. The parasitological studies were conducted by copromicroscopic examination with enrichment by flotation and by visual inspection of intestine samples. All the parasites larvae were identified by microscopy. The heavy metals detection was conducted on muscle samples by an Atomic Absorption Spectrometry method after microwave-assisted digestion. The method verified Limits of Detection of 0.01 mg/kg for Cd and 0.02 mg/kg for Pb. Results from parasitological analysis revealed the presence of *Heterakis gallinarum* in the intestines of ten chicken samples. The copromicroscopic examination verified the presence of Heterakis gallinarum eggs in ten samples and the presence of coccidian oocysts in other

ten samples. The chicken samples resulted negative to parasitological analysis were considered as control group for heavy metals detection. No significant differences were found between heavy metals concentrations of infested and non-infested chickens ($p<0.05$), suggesting the absence of a possible relation between heavy metals bioaccumulation of terrestrial parasite and their hosts. All the muscle samples of chicken reached Pb and Cd levels under the limits imposed by the EC Reg. 1881/2006. About the 42% of the samples revealed Cd and Pb levels under the Limit of Detection confirming the decreases of heavy metals along the soil-plant-insect-chicken food chain.

43P

SICILIAN WILD BIRD MOLECULAR CHARACTERIZATION BASED ON DNA BARCODING

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Sicily is a wild fascinating place in the center of migratory routs with natural parks offering habitats for many bird species. Generating a database DNA barcodes linked to named specimens could provide a new strategic key for identifying species, whose power can increase taxon coverage with faster and cheaper sequencing. The aim of this study was the establishment of a database (the BOLD Identification System for COI sequences from the 5'-region of the mitochondrial Cytochrome c oxidase subunit I gene) to investigate the diversity of bird species present in Sicily. The study was conduct on dead wild birds in the recovery centers. The birds were collected during four years and stored at Istituto Zooprofilattico of Sicily in Palermo. Methodology involved the collection of several wing muscle tissue samples taken by punch biopsy, from birds collected on the Nebrodi mountains. DNA was extracted and amplified by specific gene PCR targeted to Cyt-b accordingly to DNA barcoding technologies. Amplification products were confirmed on 1% agarose gel (stained with gel red) and purified before sequencing step. In this study, the finding of large COI sequence differences among different species confirms the effectiveness of COI barcodes for identification purpose. Since DNA sequence analysis for the identification of bird species is a powerful conservation tool, this type of data can also be useful in criminal investigations, as well as for the design of species-specific and anti-poaching strategies. This work could be important to explain the geographic distribution as well as the phylogenetic relationships of birds. The identification of species using DNA barcoding, which has had considerable development in recent years, is therefore very promising for the high specificity and tolerance to mutation. Furthermore, DNA highly degraded from animal rests can be analysed for this purpose too.

45P

DIAGNOSIS OF TOXOPLASMOSIS IN A STRAY DOG BY DIRECT GENOTYPING FROM MUSCLE BIOPSY

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Early diagnosis of clinical toxoplasmosis in dog is a critical point for early treatment and effective recovery. The aim of the study was a rapid in vivo diagnosis of clinical toxoplasmosis in dogs for a rapid treatment and *T. gondii* typing to evaluate the relation between clinical signs and specific genotypes. To confirm the suspect of *Toxoplasma gondii* infection in a stray dog with a muscular atrophy and hyperextension of hind limbs, a muscle biopsy was performed from superficial gluteus and genomic DNA was extracted. *T. gondii* specific fragment was amplified using a high sensitive nested polymerase chain reaction assay [1]. The lineage type of *T. gondii* was determined by PCR-RFLP of the amplified SAG2 gene [2] and by microsatellite markers in a multiplex PCR [3]. Microsatellite analysis was carried with GeneMapper 4.0 (Applied Biosystem). Specific therapy for toxoplasmosis (Clindamycin hydrochloride; 25 mg/kg orally twice daily for 4 weeks) and aquatic physiotherapy were performed to help solve the muscular atrophy.

ELISA rapid tests excluded other causes of paralysis in dog as tick-borne disease (Lyme disease, ehrlichiosis, anaplasmosis and babebiosis), while a positive response for *T. gondii* IgG antibodies was detected. To exclude a Neospora caninum infection, the protozoan most closely related to *T. gondii* cause of dermatitis and paralysis in dogs, a 314 bp *T. gondii* specific fragment was amplified from the sample. RFLP analysis revealed that the 3'- and 5'-end amplified fragments of the SAG2 gene was undigested with the corresponding restriction enzymes, recognizing the genotype I. Microsatellite analysis confirmed this result identifying clearly the clonal type I.

After the first week of treatment the dog started to move the hind limbs; after two weeks it was able to stay in quadruped station and the ulcerative lesion in tarsal region was healed. In the third week the dog regained partial ambulation and after four week the

ambulation returned normal. Our study reports a severe clinical case of toxoplasmosis in a stray dog and showed that the detection of parasitic DNA in the tissue is a useful diagnostic method in facilitating early treatment of the disease important for a timely clinical recovery. These data confirm the importance of early diagnosis of toxoplasmosis in dog and how clinical signs are often related to specific genotypes.

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46P

LAPAROSCOPIC NEPHROSPLENIC SPACE ABLATION WITH A BARBED SUTURE IN 8 HORSES

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Left dorsal displacement of the large colon is a common condition in horses, with reported recurrence rates ranging from 3.2% to 21% [1]. A number of techniques for laparoscopic ablation of the nephrosplenic space have been described [2], with the most common being ablation with laparoscopic suturing [3]. Specifically, a new type of suture material has been evaluated in both human and veterinary surgery. The use of barbed sutures in horses has been described in both open and laparoscopic procedures [4]. Eight horses (5 geldings and 3 females) were evaluated for laparoscopic closure of the nephrosplenic space following a history of recurrent left dorsal displacement of the large colon (LDDLC). All animals underwent clinical examination and complete blood profile characterization. Transrectal palpation and transabdominal ultrasonography were performed to exclude the presence of organs in the left paralumbar region. A left flank laparoscopic approach in the standing horse was used. A continuous suture was placed in the cranio-caudal direction between the renal and the splenic capsule using unidirectional barbed suture material. This allowed obliteration of the nephrosplenic space without the need for knots to secure the leading and terminal ends of the suture line. In all horses, two months postoperatively, transrectal palpation was performed; at this time, closure of the caudal part of the nephrosplenic space was evident. In two cases, laparoscopic follow-up was performed, and confirmation of complete closure of the nephrosplenic space obtained. Telephone follow-up revealed that symptom recurrence was not noted in any horse. Among the various preventative measures available for LDDLC, laparoscopic nephrosplenic space closure with unidirectional barbed suture material should be considered as an option. The current study describes a change in portal sites, which, compared to the standard technique, yielded –in the authors' opinion– optimal results in terms of the field view and

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47P

SHORT- AND LONG-TERM FOLLOW UP OF UMBILICAL SURGERY WITH EPIDURAL ANAESTHESIA IN 26 PIEDMONTESE CALVES

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Umbilical infections, such as omphalophlebitis, omphaloarteritis and infection of the urachus are common during neonatal period in calves [1]. If medical treatment fails, resection of infected umbilical structures is usually performed [1, 2]. Twenty-six Piedmontese calves referred to the OVU of the University of Turin for persistent umbilical diseases were included in the study. A clinical examination and abdominal ultrasonography was performed on all calves upon admission. The umbilicus was examined by palpation and by ultrasonography using 2-5-Mhz convex probe. Calves were administered high volume epidural anaesthesia with xylazine (0.05 mg/kg) and lidocaine (0.3 mL/kg of 2% solution) and placed in dorsal recumbency. The abdomen was surgically prepared and an elliptical skin incision around the umbilicus was performed. The abdominal cavity was then entered at the cranio- or caudo-lateral aspect of the umbilical mass to avoid accidental damage to infected umbilical remnants. Infected urachal remnants not extending to the bladder, infected umbilical artery remnant, and infected umbilical vein remnants not extending to the liver were dissected, double ligated, and removed. Infected urachus remnants extending to the bladder were similarly isolated, removed and a cistoplasty performed In two calves the infected umbilical vein remnants extending to the liver were marsupialized. Abdominal incisions were closed by a simple continuous patterns on the fascia followed by a modified intradermal continuous suture. Antibiotics and non steroidal anti-inflammatory drugs were administered before surgery. Intra and postoperative complications were recorded as well as short term, one week after, and long term follow up, 6 months after surgery, and survival rates. Twenty-six calves, 11 male and 15 female, mean age 26 days (1-150), mean weight 99 kg (40-300) were included. Out of 26 cases included in the report, 12 had only one structure infected (5 urachus, 5 vein, 2 arteries), 14 had multiple structure infected (3 urachus and vein, 3 urachus and arteries, 6 vein and arteries, 1

panomphalitis). In 8 calves infected urachus remnants extending to the bladder required a cistoplasty for complete resection, and in 2 calves the infected umbilical vein remnants extending to the liver were marsupialized. All calves recovered uneventfully, no intraoperative complications occurred and they were discharged from the clinic the same day of surgery. At short term follow up the only reported postoperative complication was incisional infection (8 cases). At long term follow up obtained 6 months post surgery in 29 calves normal weight gain was reported. One calf that underwent vein marsupialization, was euthanized 9 days postoperatively because of systemic infection. In conclusion, umbilical surgery in calves carries a good success rate when performed in hospital settings under epidural anaesthesia.

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48P

HIGH VOLUME EPIDURAL ANAESTHESIA ALONE IS EFFECTIVE FOR ABDOMINAL SURGICAL PROCEDURES IN PIEDMONTESE CALVES

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Epidural anaesthesia has been described in cattle for various surgical procedures. [1] Combination of intravenous (IV) xylazine and caudal high volume epidural anaesthesia with xylazine and lidocaine has provided satisfactory analgesia for abdominal surgery. [2] This combination has good and prolonged analgesic effect but in young animals IV xylazine may increase cardio-respiratory collateral effects. [3] The aim of the present study was to investigate whether epidural administration of a xylazine-lidocaine combination alone, without IV xylazine, would provide satisfactory analgesia for abdominal surgical procedures in calves. Forty-two calves were referred to the OVU, University of Turin, for various surgical disorders (omphalitis, ventral hernia, intestinal strangulation or atresia) requiring abdominal surgery. The skin over S5-Cc1and Cc1-Cc2 intervertebral spaces was clipped and aseptically prepared. An 18-gauge, 3.75 cm needle was inserted perpendicular or slightly inclined in a cranio-caudal direction to the skin surface and advanced between two adjacent vertebrae (S5-Cc1 or Cc1-Cc2) with the “hanging drop” technique. [1] A syringe was then attached to the needle and the anaesthetic solution of 0.05 mg/kg xylazine and 0.3 mL/kg of 2% lidocaine was slowly injected. Animals lost the muscle tone on the hind limb and reached a sternal position in approximately 2 minutes from injection. Calves were placed in hind limb frog position for 10 minutes to facilitate bilateral distribution of anaesthetic before being moved on the surgical table, placed in dorsal recumbency, and the abdomen surgically prepared. An inverted V bloc of 2% lidocaine to provide additional analgesia to the skin and fascia cranial to the umbilicus was performed. Duration of anaesthesia and duration of surgery were recorded as well as heart rate, respiratory rate and body temperature upon arrival, and every 5 minutes. Time to reach sternal position and time to discharge were recorded. Fourty-one (98%) calves didn’t showed any complications and only 1 calf (2%) presented neurological symptoms and was euthanized immediately after surgery. Mean anesthesia

time was 120 (100-140) and mean time of surgery was 65 min (50-80). Forty-one calves have been discharged from the hospital within 2 hours after surgery. Administration of tiletamine-zolazepam was planned as rescue anesthesia in case of failure of the epidural protocol, but it was not needed in any animal. The results of this report indicated that high volume epidural anaesthesia with a xylazine/lidocaine combination is a valid anaesthetic option and proved to be safe and effective for abdominal surgery in calves.

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49P

REAL TIME EVALUATION OF THE HEMODYNAMIC RESPONSE TO A MINI FLUID CHALLENGE IN HEALTHY DOGS

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A patient is considered a "responder" to a fluid challenge when cardiac output (CO) or stroke volume (SV) increase of at least 10% following a bolus of fluids administered in a short time. The primary aim of the study was to investigate real time variations of CO and SV immediately following a mini fluid challenge in healthy dogs. The secondary aim was to explore variations of heart rate (HR), invasive mean arterial pressure (I-MAP), and end tidal carbon dioxide (EtCO2) as indicators of the response to a fluid challenge and systolic pressure variation (SPV) as a predictor of fluid-responsiveness in dogs under mechanical ventilation. Dogs classified as ASA I or II were premedicated with fentanyl 5µg kg-1 IV, induced with propofol IV to effect and anaesthesia was maintained with isoflurane in oxygen to obtain an end tidal concentration of isoflurane (EtISO) between 1.1 and 1.3%. Volume controlled mechanical ventilation was set to produce a peak inspiratory pressure (PIP) between 9 and 11 cmH2O and respiratory rate (fR) was varied to obtain normocapnia (EtCO2 between 35 and 45 mmHg). An arterial catheter was introduced in the left metatarsal artery and connected to a hemodynamic multiparametric monitor (MostCare®, Vytech Health, Padova, Italy). After stabilization of the patient for 15 minutes, ventilatory variables were kept constant for each dog throughout the study. Baseline variables for I-MAP, HR, CO, SV, SPV and EtCO2 were recorded 3 times at 30 seconds intervals. A mini-fluid challenge of 3 mL/kg of Lactate Ringer's solution at 38°C was injected IV over 1 minute. After the bolus the same variables were recorded at 30 seconds intervals for 150 seconds. Mean values recorded before and after the bolus were compared with a t-Student test for paired data. Post-bolus values were compared with baseline values with a one way ANOVA for repeated data with a post-hoc Dunnet's test. Sensitivity and specificity were

calculated for cut-off values of SPV. Twenty dogs were enrolled. Hemodynamic parameters and EtCO2 post-bolus didn't show any significant difference from baseline values. Eight dogs (40%) proved to be responders (R) with an average increase of CO of 13.97%, while 12 dogs (60%) were classified as non-responders (NR). Only 2 dogs benefited with an increase in MAP >5%. Variations of EtCO2, HR and MAP were poor indicators of the response. A cut-off value of SPV of 5% best performed in discriminating R and NR dogs, with a sensitivity of 0.5% and a specificity of 0.54%, being a poor predictor of fluid-responsiveness. Response to a fluid challenge can't be detected or predicted from indirect parameters and should be directly detected with measurement of CO or SV variations.

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50P

A MODIFIED SEMI-CLOSED CASTRATION TECHNIQUE IN 15 HORSES

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Castration is one of the most common surgical procedures in the horse. Although considered a routinary procedure, surgical complications constitute the most common cause of malpractice claims against equine veterinarians. Surgical removal of the testis can be performed using an open or a closed technique as reported in literature. A modification of the closed technique called semi-closed or half-closed technique allows the surgeon to inspect the vaginal cavity, to place proper ligatures on the vascular bundle without excessive surrounding tissue, and to emasculate the vascular bundle alone. [1-2] The drawback of this technique is that requires significant manipulation of the structures involved and in case of inguino-scrotal hernia, accidental puncture of the herniated bowel may occur. The aim of this study is to present a modification of the semi-closed technique that allows the surgeon to benefit from the closure of the parietal tunic of the closed technique and benefit from the possibility of placing proper ligature on the vascular bundle of the open technique, without risk of accidental damage to other structures.

Fifteen horses were referred to the Department of Veterinary Sciences, University of Turin for elective castration. All animals underwent full clinical examination and complete blood profile characterization. After induction of anaesthesia the animals were placed in dorsal recumbency and the scrotum and the inguinal space prepared routinely for surgery. After skin incision, the common vaginal tunic was bluntly separated from the skin. A longitudinal incision through the tunic was made on the most ventral aspect of the testis. The testis and the funiculum were exteriorized as to perform an open castration. The ligament of the tail of the epididymis was severed and the vascular bundle ligated and or emasculated as proximal as possible. The bundle was then checked for any leakage and repositioned inside the inguinal canal. The

vaginal tunica was then brought distally and emasculated as proximally as possible, without comprising into the jaw of the emasculator also the vascular bundle already transected. The skin was closed with a modified intradermal suture. Intraoperative, postoperative complications were recorded and long term (6 months) follow up obtained by telephone. All fifteen horses recovered uneventfully from anaesthesia, and no complications were recorded either intra- or post-operatively nor at 6 months telephone follow up. The technique resulted subjectively easier to perform and required less tissue manipulation than the standard semi-closed technique. Based on the results of this report the modified semi closed technique is proved to be a safe and effective method for castration in horses and could be used as an alternative to the conventional techniques.

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51P

HISTOPATHOLOGICAL EVALUATION OF GONADAL DISORDERS IN *MUGILIDAE* AS POTENTIAL INDICATORS OF ENDOCRINE DISRUPTING CHEMICALS IN SARDINIAN LAGOONS

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The presence of polluting substances in aquatic environments, such as endocrine disrupting chemicals (EDCs), can cause adverse effects on fish reproduction by interfering with the endocrine system. EDCs, such as plasticizers, pesticides, dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and estrogens can lead to intersex in fish. The role of arsenic (As), as an endocrine disruptor, has been already shown in animal and human cell lines.1 Arsenic appears to suppress the ability of a steroid hormone receptor to respond to its normal signal. The potential correlation of gonadal anomalies in fish to As exposure is still not studied. Intersex disorder identifies a simultaneous occurrence of male and female gonadal tissue. Mugilid fish are considered as sentinels to study the effects of EDCs on reproductive system in wetland environments. 2,3 To our knowledge, there are no previous studies investigating the occurrence of intersex condition and arsenic levels of *Mugilidae* in Sardinian lagoons as biological indicators in assessing aquatic ecosystems health.

In this work, 4 species of *Mugilidae* (*Chelon labrosus, Liza aurata, L. ramada* and *Mugil cephalus*) from 4 Sardinian lagoons (Cabras, Calich, Marceddì and San Teodoro) were analyzed for gross morphology, histology and trace elements from to 2013 to 2015. Complete necropsies of 495 specimens were performed and gonads were 10% buffered formalin fixed, paraffin embedded and stained with hematoxylin-eosin. Dorsal muscles were dissected and homogenized for chemical analyses. Mercury (Hg) was quantified with a Direct Mercury Analyzer, while other trace elements (Al, As, Be, Cd, Ce, Co, Cr, Cu, Fe, La, Mn, Mo, Pb, Ni, Sb, Se, Sn, Tl, V and Zn) by Inductively Coupled Plasma-Mass Spectrometry. Macroscopic and histological examination of the gonads revealed the

presence of 33% normal male, 64% normal female and 3% intersex. In detail, intersex mullets were found in San Teodoro (6.5%), in Marceddì (3.4%) and in Calich (1.7%) lagoons. No gonadic alterations were instead observed in Cabras lagoon. The maximum values for Hg, Cd and Pb in fish muscle were never exceeded in the examined samples. Arsenic level was higher in San Teodoro (1.71±0.69) and Marceddì (1.49±0.74) than in the other lagoons. In particular, the San Teodoro lagoon (where anthropogenic pressure is higher than in the other ones) showed the highest percentage of intersex specimens as well as the highest value of arsenic that is actually considered an endocrine disruptor and may be causing the reproductive damage observed in *Mugilidae*.

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52P

OUTBREAK OF POX GLOSSITIS IN A CANARY FLOCK

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Pox is a common viral disease of domestic birds including canaries, which can occur in two different clinical forms: 1) “cutaneous”, characterized by proliferative nodules on the non-feathered skin; 2) “diphteric”, with fibrino-necrotic and proliferative lesions in the mucous membranes of the upper respiratory and gastrointestinal tract (Tripathy & Reed, 1997; Marruchella & Todisco, 2010). The present study reports the main and peculiar features of a pox outbreak recently observed in a canary flock. The flock consisted of 200 colored canaries and had suffered from respiratory acariasis two months before. Then, about 30% of canaries died after showing large, striped, yellow-to-red, nodular lesion on the tip of the tongue. At necropsy, no significant lesion was further observed. Simultaneously, about 40 canaries showed a mild, periocular alopecia and quickly recovered. Tongue lesions were sampled for pathological and biomolecular diagnostic investigations. Microscopically, the tongue epithelium appeared hyperplastic and embedded large aggregates of bacteria. Clusters of epithelial cells were markedly enlarged and contained eosinophilic A-type cytoplasmic inclusion bodies, which strongly supported the diagnosis of pox. As a consequence, tongue samples were submitted to polymerase chain reaction (PCR) in order to amplify a 581 bp poxvirus-specific DNA sequence (Lee & Lee, 1997). PCR tests proved to be positive, thus further confirming the diagnosis of pox glossitis. Pox still represents a serious concern for canary flocks. The present report indicates that the tongue could represent a major target of infection and that pox should be considered in the differential diagnosis of nodular glossitis in canary birds. Considering the possible genomic divergences of avian poxvirus strains (Manarolla et al., 2010), further analysis are currently ongoing to better define the molecular features and the tissue tropism of this strain.

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53P

FIRST REPORT OF SEVERE GRANULOMATOUS DISEASE IN A BY-CAUGHT LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) FROM THE ADRIATIC COAST OF ABRUZZO (ITALY): A POTENTIAL THREAT FOR AN ENDANGERED SPECIES

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Sea turtles are classified as “endangered” by the International Union for the Conservation of Nature (IUNC). Overfishing, boat/ship collisions, pollution, climate change, eutrophication, alien species introduction and emerging infectious diseases rank among the main threats for sea turtles. Sea turtle strandings and bycatches have increased in recent years along the Adriatic coast of Abruzzo and Molise Regions. Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale” (IZSAM), with a constantly updated marine animals’ monitoring plan, exploits these events for the assessment of the health status of free-ranging sea turtles. An adult female loggerhead sea turtle (Caretta caretta) was accidentally caught alive in March 2016, being promptly hospitalized at Centro Recupero Tartarughe Marine ”L. Cagnolaro”. The health status of the turtle was thoroughly investigated, including also blood and serum analytes. The turtle exhibited, within 36 hours before death, lethargy and acute respiratory distress. It was subsequently subjected to a detailed post mortem examination at IZSAM, with tissue samples being collected during necropsy. Histopathological, microbiological, parasitological and biomolecular investigations for *Chlamydia* spp., along with selected histochemical stains (PAS, Ziehl-Neelsen and Grocott) were performed, coupled with ecotoxicological analyses for heavy metals. Macroscopically, large nodules (3-5 cm in diameter) were diffusely observed in the liver, alongside with calculi and atrophy affecting the right kidney, thus revealing a severe, systemic, chronic pathology.
Histologically, multiple granulomas with a central necrotic area, surrounded by epithelioid and giant cells, were found in liver, brain, stomach and lung. In gastric

granulomas several microorganisms were also seen in haematoxylin-eosin-stained sections, with PAS and Ziehl-Neelsen stains being also negative for fungi and acid-fast microorganisms, respectively. *Aeromonas hydrophila* was isolated from the lung parenchyma.

Although bycatch, followed by drawning, played a pivotal role in this turtle's death, showing a good state of nutrition, the chronic, underlying pathology most likely acted as a weakening condition which did not allow the animal to adequately react to external stimuli. Further diagnostic investigations are needed to better define these unusual pathological findings.

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54P

HEPATIC MYELOLIPOMA IN A FERRET (*MUSTELA PUTORIUS FURO*)

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Myelolipoma is a rare mesenchymal benign tumour primarily composed of mature adipose tissue with scattered hematopoietic elements in various proportions [1]. Several cases of myelolipoma have already been reported in animals, including non-human primates, dogs, cats, wild felids and birds. So far only a splenic myelolipoma has been reported in ferret [2]. Myelipomas are typically non-functioning tumours and therefore often asymptomatic. As in humans, myelolipomas in animals are usually diagnosed incidentally on abdominal imaging or at necropsy, without previous clinical signs of illness related to the tumour. A 6-years-old neutered male white ferret was presented with sudden apatia and anorexia. Abdominal ultrasonographic investigations revealed two hepatic masses, which were surgically removed. Specimens were fixed in 10% phosphate-buffered formalin, routinely processed and embedded in paraffin. Paraffin sections were cut at 3 µm and stained with haematoxylin and eosin. Histologically, the mass showed mature adipocytes associated with hematopoietic elements, represented by granulocytic, erythrocytic and megakaryocytic series at different stages of maturation. These findings were consistent with a diagnosis of myelolipoma.

The pathogenesis of myelolipoma remains still unclear: it is considered to be a hormonally induced metaplasia of the stromal cells or primitive mesenchymal cells [3]. It has also been reported that adipocytes and myeloid cells in human adrenal myelolipoma show the same clonal cytogenetic abnormality, which suggests that adipocytes and myeloid cells may proliferate in a clonal manner [4]. Moreover, nonrandom X-chromosome inactivation was found in both myeloid elements and the adipose tissue of human adrenal myelolipomas, suggesting that the haemopoietic components and the adipose tissue of myelolipomas may clonally proliferate and originate from common, pluripotent stem cells [3]. Another hypothesis is based on embryonic hematopoiesis, which occurs diffusely throughout the peritoneal connective tissue and regresses with

development of other hematopoietic tissues [5]. This phenomenon could result from activation of dormant hematopoietic stem cells in the peritoneum that had been active in embryonic stage [5]. Myelolipomas are also speculated to be derived from bone marrow emboli that lodge in the different organs [6]. Others authors suggest that myelolipoma is a choristoma, arising from normal hematopoietic stem cells, misplaced during embryogenesis [7]. The few case reports in the veterinary literature suggest that myelolipoma is a disease of the geriatric domestic animal [8, 9], with a similar scenario to that reported in humans [10].

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55P

IMPACT OF THERMAL TREATMENT ON ELISA DETECTION OF CASHEW

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It has been described that cashew nut allergy is able to induce more severe reactions than peanut allergy (1); furthermore, different Authors reporting an increase of cashew nut allergy occurrence in children (2, 3). Then it is critical to have suitable methods to detect these allergenic proteins in foods. Thermal exposure, altering protein structure, may influence the performance of methods aimed to identify specific antigens. Our objective was firstly to test the ability of 2 different types of ELISA kits (A: Immunolab GmbH, B: Bio-Check UK) to detect the presence of cashew and, secondly, to examine some sample of market food products, (characterized by declaration in their food label of the absence of this allergen) in order to verify possible events of cross contamination.

With the aim to evaluate the effect of temperature (similarly to the food industrial processes) as factor able to induce structural changing in proteins, cashew specimens were exposed to the follows treatments: 1) boiling, 2) roasting at 80°C 10', 3) roasting at 180°C 10', 4) roasting at 180 °C 30'. Data were elaborated by Shapiro–Wilk test to evaluate the distribution values. For every ELISA assay, differences in terms of cashew detection (mg/kg), after thermal treatment, were analysed by one-way ANOVA (Prism graphPad 5.03). A p-value ≤0.05 was considered statistically significant. To evaluate the hidden cashew protein in food, eight food categories were tested (N=142): cereals and chocolate based products, candies and products showing high levels of sugar, condiments, beverages, meat, milk and fish.

Data indicated a normal distribution. Boiling ($P<0.0001$), roasting at 80°C 10' ($P<0.0001$), 180°C 30' ($P<0.0001$) and 180°C 10' ($P=0.02$) induced a significant decrease of performances in both kits respect to the untreated cashew. The A kit was chosen for the second part of the study because results of the last treatments (180°C 30') were quantifiable (about the Limit of Detection); conversely, the B kit performance showed unquantifiable data. Eight out of 142 samples were positive for the presence of cashew

protein in a range of 2-3.8 mg/kg and with a mean concentration equal to 2.85±0.59 mg/kg. Positive values were observed for hazelnut “cannoli” and wafer, dark, white “gianduiotto” chocolates and hazelnut chocolates, walnut condiment, cheese and pear “confetti”. Hidden cashew allergens in food may be considered a critical problem for cashew allergic patients. In our study the 5.6% of the total investigated samples, not declaring the presence of cashew protein in their label, showed positive results. It is possible evidence the importance to evaluate the most suitable allergen contaminant detection method in food, able to reveal a possible cross contamination occurred during production process. As known, the multipurpose production process of nuts is considered a critical point. For these reasons, the cleaning steps are essential to avoid the cross contamination, in order to ensure the consumer safety.

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56P

OCCURRENCE OF AFLATOXIN M1 (AFM1) IN DONKEY MILK COLLECTED IN THE NORTH OF ITALY

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Mycotoxins are a group of highly toxic compounds produced by fungi or yeast. Aflatoxin M1 (AFM1) is a mycotoxin responsible of many toxic effects. IARC reported that there is sufficient evidence for the carcinogenicity of AFM1 in experimental animals and the toxicity of AFM1 was classified to the IARC's carcinogenicity group 2B. When animals ingest contaminated foodstuffs, mycotoxins are metabolized and transferred to animal products, such as milk or meat, thus becoming a risk to human health. Donkey milk has the most comparable protein composition to human milk among different species and is well tolerated by children with cow milk protein allergy (CMPA). Moreover, it possesses natural protective antimicrobial factors and a specific epidermal growth factor (EGF) that suggest its beneficial impact on gastrointestinal mucosa. It represents a good natural breast milk substitute during early infancy and is well suited to children, elderly, and convalescent who have a reduced immune defense system. Since AFM1 is not destroyed by production processes because is thermostable, it is of great importance to control raw milk. In this study, donkey milk samples were analyzed to assess the presence of AFM1 with the purpose to check that the level of mycotoxin was below the maximum residue limits (MRLs) set by current European Union (EU) legislation: 0.050 µg kg-1 for raw milk, heat-treated milk and milk for the manufacture of milk-based products; 0.025 µg kg-1 for infant formulae and follow-on formulae, including infant milk and follow-on milk. To verify the content of AFM1, monitoring was carried out on 63 donkey samples collected from a farm of the North of Italy. For this aim, an immunoaffinity-based technique was used and a fast and sensitive high performance liquid chromatography with fluorescent detection (HPLC-FD) method was developed and validated. The rate of recovery was 87.7%, LOD and LOQ were respectively 0.0025 µg kg-1 and 0.0125 µg kg-1. One sample showed a level of contamination equal to 0.0044 µg kg-1, far below the limit of 0.025 µg

kg-1 established by the European Union for milk for infants. Our study shows that donkey milk sampled in the North of Italy is a safe food as regards the presence of AFM1 and is not a real risk to human health. These results suggest a special care taken with lactating donkey's feedstuff and official controls carried out to ensure the safety of the consumer.

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57P

USE OF SNAILS (*HELIX ASPERSA*) AS SENTINELS TO EVALUATE ENVIRONMENTAL CONTAMINATION BY POLYCYCLIC HYDROCARBONS AND TRACE ELEMENTS

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The terrestrial gastropods can take and bio-concentrate environmental contaminants as a result of the contact with the ground, ingestion of soil, vegetation, water and by inhaled air. High concentrations were found in their tissues after exposition to these contaminants. Moreover, they are non-migratory and can be able to provide information related to the contamination of a site, representing a valid indicator of environmental quality. This can complete the partial vision coming from contamination of soil or plants that may be due to the use of contaminated irrigation water or sewage sludge, manure or fertilizers or because of illegal waste spill (1, 2). Unfortunately, this phenomenon was revealed in some areas of the Campania region, creating an alarm situation for the environment quality of agricultural and livestock production resulting in a public concern. The risk is that environmental contaminants such as dioxins, PCBs or PAHs, as well as potentially toxic elements, can enter the food chain.

The aim of our project was to use terrestrial gastropods as biosensors of the degree of environmental contamination and then evaluation of the levels of certain environmental contaminants in Campania region.

In the present study, 622 samples of the garden snails (Helix aspersa) were collected from different areas of Campania region to determine levels of toxic elements as lead, cadmium and arsenic. Also elements known as essential but toxic in high amounts as copper, zinc, aluminum, chromium, cobalt, selenium and tin and elements without any biological actions but good indicators of pollution of various origin, i.e uranium, manganese, thallium and strontium, were taken into account. As regards contamination

from organic pollutants, ubiquitous polycyclic aromatic hydrocarbons (PAHs) were determined in all samples.

Before analysis samples were dried at 40°C. Trace element analysis was performed by Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) after a microwave assisted digestion procedure. The determination of the PAHs was carried out by HPLC with fluorimetric detector.

The results obtained allowed to identify significant differences for the concentrations of certain trace elements in different sampling areas, in particular the median of some elements (As, Co, Mn, Pb, U, V) was higher in samples taken in Caserta province that is most affected by phenomena of illegal dumping of industrial or domestic waste in fields or by the roadside. As to PAH, levels were below the limit of quantification for each PAH except the presence in trace in few samples from Avellino, Napoli and Caserta provinces.

In conclusion, our study confirmed that snails can be considered environmental sentinels thus can be used to assess the presence of pollutants and to determine the transfer factors from soil, water and air to the various trophic levels of food chain. The results of this study should be related to the results of the investigation on the environmental matrices.

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58P

ANTIBIOTIC-RESISTANT *SALMONELLA* STRAINS IN LIGURIAN WILDLIFE

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A jointed statement between EFSA and ECDC confirms that the antibiotic resistance is considered a significant risk both for human and animal health. In this context, the *Salmonella* genus bacteria are particularly important, because represent the second cause of foodborne disease in Europe [1]. Furthermore high prevalence values of multi resistance have been detected. The purpose of this work was to evaluate the presence of antibiotic-resistance (AMR) *Salmonella* strains in liver of Ligurian wild boars. In the 2013-2015 hunting seasons a total of 2423 samples were collected, obtained as follows: 580 from Imperia, 602 from Savona, 1033 from Genoa and 208 from La Spezia. Samples were analyzed to detect *Salmonella* spp. by Real Time-PCR; positive specimens were confirmed according to the ISO 6579:2002. The isolated strains were tested, by Kirby-Bauer method [2], to verify sensitivity to different types of molecules: Triple Sulfa (3SU 250), Cefotaxime (CFT 30), Tetracicline (TE 30), Ampicillin (AM 10), Amoxicillin plus clavulanic acid (AMCL 20+10), Cefalotin (CEFA 30), Ceftazidime (CTZ 30), Chloramphenicol (C 30), Colistin (CL 10), Streptomycin (S 100), Ciprofloxacin (CIP 5), Enrofloxacin (ENR 5), Gentamicin (GM 30), Kanamycin (K 30), Nalidixic acid (NA 30), Sulfametox and Trimethoprim (SXT 23.75-1.25). The prevalence of positive results was 9.2% (220/2423) mainly isolated from the Imperia province 13.8%, followed by Genoa 10.1%, Savona 6.2% and La Spezia 1.4%. Typization of the isolated strains showed the presence of only one specie, *S. enterica*, and of the following subspecies: *S. enterica* subsp. *enterica* (59.5%), *S. enterica* subsp. *salamae* (20%), *S. enterica* subsp. *diarizonae* (13.2%), *S. enterica* subsp. *arizonae* (4.1%), *S. enterica* subsp. *houtenae* (3.2%). The study of AMR profiles evidences that 99.1% was resistant to at least one molecule and 80.5% was characterized by multidrug resistance. Other drug resistances are expressed as

follows: 3SU (97%), S (40.86%), TE (39.56%), K (33.04%), SXT (30.46%), CTZ (29.56%), CEFA (28.7%), AM (23.9%), AMCL (19.13%), NA (16.08%), CFT (13.5%), GM (8.26%), ENR (6.95%), CL (4.34%), C (2.61%). Our data are in agreement with previous studies [3-4] and confirm the high spread of antimicrobial resistance in wildlife, especially in the multidrug form [4]. *S. Typhimurium* and *S. Enteritidis* serotypes showed very low levels of resistance to the critically antimicrobials, as Cefotaxime (1 strain of *S. Typhimurium*). Additional studies will be needed to investigate further epidemiological sources and the wildlife's role as potential reservoir of bacterial strains, expressing gene encoding multidrug resistance factors, both for domestic animals and humans.

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59P

SURVEY ON PARASITIC INFECTIONS IN CAPTIVITY AND WILD BIRDS OF PREY

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Italy represents an important bridge for many species of migratory birds of prey. Moreover, several sedentary species remain in the territory throughout the year. In addition to wildlife, it is possible to maintain in captivity birds of prey with C.I.T.E.S. regulation: in parks, in falconry centres, in breeding with release program or in bird-care centres. In spite of their impact on biology and on sanitary status, knowledge on the parasitic infection in birds of prey is scant; particularly for Italian wild and domestic populations, few data are available in literature (Papini et al., 2012).

Since the importance of parasitic diseases is notable and since the possession of raptors in captivity is increasing, the aim of the present work was to investigate on parasitic infections in birds of prey, with special emphasis in those kept in captivity. From July 2015 to January 2016, 72 stool samples and 34 samples of whole blood in EDTA were collected from four centres in northern Italy from 27 *Accipitridae*, 1 *Cathartidae*, 15 *Falconidae* and 25 Strigidae and 4 *Tytonidae*. Individual data and information related the management were collected. Copromicroscopic analyses were performed using FLOTAC® technique (Cringoli et al., 2010) with the flotation solution S8 (Potassium iodomercurate, PS=1440). Blood smears were prepared for Giemsa 5% stain in order to detect haemoparasites. Additionally, plasma samples were tested for antibodies anti-*Toxoplasma gondii* with a commercial direct agglutination test (Toxo-Screen DA; BioMérieux). General linear model was performed in order to evaluate risk

factors associated to the presence of parasitic elements in faeces with SPSS v.20. Thirty faecal samples resulted to be positive for parasitic elements (P=41.7%). Particularly, 30.5% of animals resulted to be infected by nematodes, 18.05% by protozoa, 5.5% by trematodes and 2.8% by cestodes. Ten birds showed co-infection with more than one taxa involved. Concerning risk factor analysis, hybrids resulted to be more at risk of infection (p-value=0.009; OR=100.633) than wild species. Feeding with fresh meat enhances the risk of infection (p-value=0.023; OR= 35.441) than the feeding with frozen meat. Finally the risk of infection rises in adults in comparison to the young animals (p-value=0.020; OR=15.2). Any difference was not recorded between wild birds (5 positive specimens out of 14 examined, P=35.7%) and animals in captivity (26 positive specimens out of 58 examined, P=44.8%). Analysis of blood smears allowed the detection of *Leucocytozoon* in one specimen and *Haemoproteus/Plasmodium* in six birds. Three animals resulted to be infected by *T. gondii*. Measures of preventive medicine should be systematically applied: hygienic measures, control measures to prevent the access of species vectors of parasites to the centers, an annual parasitological screening followed by an adequate parasites control plan (Cooper, 2002).

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60P

OCCURRENCE OF *HYMENOLEPIS NANA* IN CHINCHILLA (*CHINCHILLA LANIGERA*) FROM ITALIAN BREEDING FACILITIES

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The tapeworms *Hymenolepis nana* and *Hymenolepis diminuta* (*Cestoda, Cyclophyllidea*) are enteric parasites of rodents and humans, that can become infective through ingestion of cysticercoid-infected arthropods (the intermediate hosts) or directly through the embryonated eggs. Auto-infections within the definite hosts are also described. *H. nana* infections are observed with higher frequency than those caused by *H. diminuita*. Among captive rodents, chinchillas (*Chinchilla lanigera*) are becoming even more popular companion animals in several countries, including Italy. Although *Hymenolepis* spp. eggs are commonly recovered from veterinarian practitioners in stools of chinchillas, little is known about the epidemiology and prevalence of tapeworm infections in these animal species. Aim of the present study was to investigate the prevalence of Hymenolepis infection in chinchillas (*C. lanigera*) reared in three Italian breeding facilities across Italy. From April to December 2010 three different breedings of *C. lanigera* located in Central-Southern Italy were investigated. Overall 104 faecal pool samples were collected from cages harbouring a number of animals ranging from 1 to 4. Fifty four faecal pools were collected in the facility 1, 28 in the facility 2 and 22 in the facility 3. All animals were asymptomatic at the time of sampling. Each faecal sample was tested by combined sedimentation-flotation method with Sheather solution (specific gravity 1.28). Identification of *Hymenolepis* species was based on morphologic and morphometric characteristics of eggs (Steinmann et al., 2012). A McMaster technique, with a sensitivity of 50 eggs per gram (EPG), was used to quantify the number of eggs in positive samples. An high prevalence rate for *H. nana* infection (39.42%, no. 41 on 104 faecal pools, 95% confidence interval=30-49.5%; mean EPG=16.33; range=1-5500) was detected within the investigated facilities. The prevalence rates observed in the 3 different breedings were quite similar and ranging from 36% to 41%. No *H. diminuta* eggs were recovered.

The results obtained showed that H. nana infection can be very common in chinchillas and underlined the importance of a routine parasitological examination before introducing these animals in domestic settings.

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61P

MICROBIOLOGICAL AND PARASITOLOGICAL FINDINGS IN EXOTIC ANIMALS FROM A CITY PARK IN PALERMO (VILLA D'ORLEANS)

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Exotic species are considered as the new companion animals especially in occidental world. Beside the wild bird of prey found mainly in the countryside's, it may happen to cross *Psittacidae* in urban parks, zoo and protected areas and chances of come into contact with some exotic species increase. The aim of the present study was to individuate and identify potential zoonotic pathogens in specimens collected from exotic animals rescued in Villa d'Orleans, a city park in Palermo. Fecal samples collected from 17 different boxes were analyzed for microbiological and parasitological agents. Samples came from 38 recovered animals grouped in 17 boxes: *Cyrcus aeruginiosus, Falco sparverius, Ciconia ciconia, Corvus corax, Ichthyaetus audouinii, Buteo buteo, Parabuteo unicinctus, Streptopelia turtur, Streptopelia decaocto, Pavo cristatus, Carduelis carduelis x Serinus canaria, Testudo hermanni, Testudo graeca, Cacatua galerita, Nymphicus hollandicus, Amazona aestiva,* and *Macaca sylvanus*. Microbiological tests were performed to detect *Salmonella* spp. according with OIE recommended methods and suspect growing colonies were identified by micromethods, and biomolecular tests. Parasitological analysis were also performed using the traditional flotation technique (solution of sodium nitrate and glucose density 1350) and Ritchie and Ziehl Neelsen modified methods for the detection of oocysts of *Giardia* and *Cryptosporidium*. Two out of 17 specimens (11.7%) from *N. hollandicus* and from *C. carduelis* x *S. canaria* were found positive to *Salmonella* spp. (molecular identification is still ongoing). The overall prevalence of gastrointestinal parasitic infections was 58.8% (10/17) in particular, *Capillaria* spp. (5/17), *Dermanyssus gallinae* (5/17), *Heterakis* spp. (2/17) and coccidia (1/17) eggs were found and also larval stages of nematode in 2 boxes (11.7%). Parasitological analysis were negative in 7 specimens (41.2%), coinfestation were found

among *Capillaria* spp., *Eimeria* spp., and *Heterakis* spp. in *P. cristatus* and between *Heterakis* spp. and *D. gallinae* in *A. aestiva*. In our study, 5 pathogens were identified and 3 of them of zoonotic concern. In fact *Salmonella* spp., *Capillaria* spp. and red mites such *D. gallinae* are recognized as causative agents of emerging zoonotic diseases: salmonellosis (1–3), dermatitis (4) and capillariasis (5, 6) as reported also in Europe (3, 4). Due to the strict contact among animals and humans and because of the sharing of the same habitat, prophylactic measures towards environmental contamination especially zoonotic agents, should be undertaken as well as awareness of the risk factors.

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62P

IMPACT OF THE ACCIDENTAL INGESTION OF PLASTIC DEBRIS ON THE SURVIVAL OF SEA TURTLE: THE CASE OF *CARETTA CARETTA* ALONG THE SICILIAN COASTS

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Plastic pollution has a global distribution and its detection in the sea threats the survival of many species (1, 2). Anthropogenetic activities are responsible of huge mortalities for sea turtles, estimated in the Mediterranean sea. Most cases of death are associated to accidental ingestion of fishing gears (3, 4) or plastic debris (5). The aim of this study was to identify the nature of plastic debris ingested by sea turtles stranded in Sicilian coasts. Since April 2014, 161 stranded loggerhead sea turtles (*Caretta caretta*) were rescued from Sicilian coasts and recovered at the Centro Regionale di Recupero for sea turtles, located at the Istituto Zooprofilattico Sperimentale della Sicilia (Italy). After morphological traits recording, physical and X-rays examinations were carried out to evaluate the health condition and individuate accidental ingestion of radiopaque foreign bodies. Twenty out of 161 do not showed radiopaque foreign bodies or other macroscopical causes of stranding. All of these were treated with medical therapy to promote intestinal peristalsis. In addition, surgery for fishing gears removal showed plastic in the gastrointestinal tract of 14 animals. Animals arrived with evident signs of suffering (ectoparasites, algae and/or emaciation) were not always associated with death. Out of 34 animals included in this study, 22 died due to the poor health condition for the presence of debris in the gastrointestinal tract (no. 15) e.g. stones, rope, wood and fishing gears while 7 for external lesions, obstruction, abscesses. Some of the 22 specimens showed signs of severe gastrointestinal injuries, like intestinal intussusceptions (no. 3) and curling (no. 5), celomitis (no. 6) and lumen obstruction (no.

3). The other 12 out of 34, included 2 in which surgery to remove longlines was done, expulsed correctly the plastic debris and were release into the sea after rehabilitation and when organic functions were restored. Plastic debris found were recognized as bottle cap, entire cuki© bag, sanitary napkin for women, scotch, straws and fragments of hard and soft plastic.

Previously, some authors have sought to develop a diagnostic methodology aimed to highlight radiolucent foreign bodies with questionable results inasmuch the slow gastrointestinal transit caused delayed diagnosis (6). In our study, the presence of plastic in the digestive tract was frequently associated to other foreign bodies suggesting the onset of pica in animals with peristaltic disorders. However, accidental ingestion of foreign bodies is until now an important causes of stranding (2, 7) but plastic debris ingestion could be treated with medical therapy only with good results, despite the lack of non invasive diagnostic tools available.

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63P

A STANDARDIZED ULTRASONOGRAPHIC EXAMINATION OF LAPAROTOMY WOUNDS TO DETECT EARLY SIGNS OF INFECTION IN HORSE: PRELIMINARY RESULTS.

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Infection of the incision site after an exploratory laparotomy is one of the most common post-operative complications of this procedure in horses. The infection can lead to dehiscence of the cutaneous suture or if it goes deep in the muscular layers it can result in an abdominal hernia.

Aim of the study: The purpose of the study is to determine if the use of a standardized ultrasonographic examination of the laparotomy incision at day 5 and day 10 after surgery can be useful to detect early signs of infection that have no apparent clinical manifestation.

Laparotomy incisions underwent an ultrasonographic examination at day 5 and day 10 after surgery; longitudinal images were obtained with a depth of 4cm in a cranio-caudal direction parallel to the incision in both sides with a linear probe (10 MhZ) and ethanol 90% was used to ensure adequate contact with the transducer. The cutaneous, subcutaneous and muscular layers were visualized together with the cutaneous and the muscular sutures by one trained operator and possible signs of infection were recorded. This signs were: edema, fluid within the subcutaneous layer, accumulation of fluid with or without hyperechoic spots around the muscular sutures that can infiltrate deep towards the peritoneal fat.

Together with that a clinical examination of the wound was performed daily from one day after surgery with a through palpation of the incision to detect swelling, pain or spilling of fluid from the sutures.

Both ultrasonographic and clinical examination have been well tolerated by the patients and only in few cases a light sedation was needed to allow the scan of the wound. The time needed to perform the ultrasonographic examination never exceeded 15 minutes

and it was performed without restraining the patient. Pain at palpation, especially if focal, and spilling of sero-purulent material at clinical examination were always associated with the detection of signs of infection at ultrasound while early signs of infection as small amount of fluid and\or hyperechoic spots around the muscular sutures were most of the time not associated with alteration at the clinical examination but , especially if found at day 5, were always associated with the development at some points of the postoperative period of a clinically manifested infection of the wound of various degree.

Systematic ultrasound examination of the laparotomy wound at day 5 and day 10 postoperatively is a relatively easy and not time consuming procedure that helps the clinician to predict an infection at the incision site and possibly establish an early treatment to avoid one of the most common post-operative complications after a laparotomy is performed.

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64P

A NEW, EASY-TO-MAKE, PECTIN–HONEY HYDROGEL ENHANCES WOUND HEALING IN RATS

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The use of honey in wound healing is ancient. It could be used alone or in combination with other compounds and became a topic of interest in several investigation in last decade [1, 2]. Pectin has been recently investigated for various biomedical applications, such as drug delivery, skin protection and as scaffold for cells [3]. Pectin is inexpensive, can be extracted from renewable sources, is not cytotoxic, acts as a gelating agent, and is suitable for many biomedical applications [4]. The aim of the present study was to develop and evaluate a pectin-honey hydrogel (PHH), forming a membrane applicable on the wound, and to compare this dressing to liquid honey for wound healing.

Thirty-six adult male Sprague-Dawley rats were anesthetized and a 2x2 cm full thickness excisional model was used to create the wounds [5]. Animals were randomly assigned to four groups (PHH, LH, Pec and C). Pectin-honey hydrogel was applied under a bandage on the wound (group PHH), liquid Manuka honey was applied under a bandage on the wound (group LH), pectin only hydrogel was applied under a bandage on the wound (group Pec), while in C group only the bandage was applied to the wound. Images of the wound were taken on days 0, 2, 4, 6, 8, 11, 13, 15, 18, 21 and 23 after surgery. The comparison between the area at day 0 and at the time-set days was used to calculate the ratio of the wound reduction and compared between groups.

Wound area reduction rate was faster for PHH, LH and Pec group compared to the control group and among PHH, LH and Pec even significantly faster for the PHH group. Surprisingly Pec group had a faster wound healing than LH, even if was not statistically significant.

This is the first study, to date, to use pectin in combination with honey to produce

biomedical hydrogels for wound treatment. Considering the results obtained in the present study, the use of PHH is effective to promote and accelerate wound healing.

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65P

A DEEP POST-CASTRATION FUNICULITIS RESOLVED WITH MARSUPIALIZATION IN ONE HORSE

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Castration is one of the most commonly performed surgical procedures in horses, and the most common complications encountered are bleeding, evisceration, eventration and septic funiculitis [1]. Septic funiculitis had historically been classified as “scirrhous cord” (infection of the spermatic cord remnant with *Staphylococcus* sp.) or “champignon” (infection of the spermatic cord remnant with Streptococcus sp. with purulent discharge from the scrotal incision) [2]. These conditions may resolve with antibiotic therapy and re-establishment of drainage but sometimes surgical removal is required. Complete removal of the abscess is usually easily performed in most cases when it is limited to the portion of spermatic cord external to the inguinal canal. Aim of this report is to describe the marsupialization in one case of post-castration septic funiculitis that extended deeply in the inguinal canal and its short and long term follow up.

A 4-years-old Standardbred gelding was presented for chronic septic funiculitis after 3 weeks post-castration. Unilateral septic funiculitis with *Streptococcus* sp., *Enterobacter* sp. and *Klebsiella* sp. was diagnosed. Antibiotic therapy was initially provided with procaine benzylpenicillin (8 mg/kg IM) and dihydrostreptomycin (10 mg/kg IM) but, because of the persistence of the problem, surgical removal was elected. The horse was placed in dorsal recumbency under general anaesthesia, and the inguinal space and the scrotum prepared routinely for surgery. The fistulous tract on the scrotum was opened and the abscess was partially drained. Dissection of the abscess was performed till the point that resulted evident that it extended deeply into the inguinal canal, hence the impossibility of totally safely remove it. The most distal part of the fistulous tract was removed and the remaining proximal portion was marsupialized and suture to the skin

over the external inguinal ring. Two-months follow up was obtained by telephone with the referring veterinarian.

The horse recovered uneventfully from anesthesia and in the next 2 weeks the abscess was irrigated twice a day with antimicrobials. Two weeks after discharge the referring veterinarian reported that viscous material was still draining from the wound. Thus ceftiofur-loaded gelatin was inserted in the fistolous tract three times 72 hours apart. At two month follow up no discharge was detected and no complications noted.

To the best of our knowledge this is the first report of marsupialization of a septic funiculum in horse. The technique used proved to be effective.

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66P

INFLUENCE OF NECK POSITION ON MOST COMMON RADIOGRAPHIC MEASUREMENTS PERFORMED IN THE CERVICAL SPINE OF THE HORSE

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The radiographic examination is the most widely used diagnostic technique to investigate the cervical spine of the horse. Several measurements can be performed to investigate different abnormalities involving this vertebral tract. Even if some of this vertebral measurement are not easily repeatable they are largely used in the clinical practice.
The purpose of this prospective study was to determine the influence of horse's neck position on radiographic measurements of intra- and inter-vertebral ratio, of the the sinovial facet joint's lenght, of the angles between two adiacent vertebrae and in the detection of dysplasia between the vertebral head and vertebral fossa.
A cervical radiographic examination was performed in 18 clinical sound horses under sedation. Lateral-lateral radiographs were obtained in the following 3 positions: neutral-position1 (mouth at the level of the shoulder joint); low-position 2: (mouth at the level of the carpal joint) and high-position 3: (mouth at the level of the withers). One trained analist performed measurement of intra and inter vertebral ratio, of the length of the synovial facets joint, of the alignment between two adjacent vertebrae and of the ratio between vertebral head and fossa. Excel program was used to calculate the mean and standard deviation of each measurements. Neck position's influence in every measurement was calculated using Friedman ANOVA and Tukey's test, with significative value at $P < 0.05$. A statistically significant difference has been identified in the measurement of the intra-vertebral sagittal ratio at the level of the C4 in position 2 and at the level of C5 in position 3 compared to position 1. A statistically significant difference was identified at the level of C4-C5 in the measurements of the inter-vertebral ratio with the neck in position 2 compared to position 3. Regarding measurement of facet joints' length a statistically significant difference was identified at the level of C2-C3, C3-C4 and C4-C5 in position 3 compared to 1. Finally, it was identified a statistically significant difference in the measurement of the intervertebral angles at the level of C4-C5 in position 3 compared to position1 and at C5-C6 in position 2 compared to 1.

Considering that an extreme variability exists among different operators performing measurement during cervical radiographic examination, it is extremely important to standardise the correct positioning of the horse during the acquisition of radiograms: the horse must be maintained with the neck in a neutral position with the tip of the noise at the level of the shoulder. To guarantee the absence of movement and the correct position of the horse, the patient should be sedated accordingly and the tip of the chin should be placed on a fixed support.

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67P

DENDRITIC CELLS WITHIN LYMPHOID FOLLICLES: A DIFFICULT SKEIN TO BE UNTANGLED

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Different types of dendritic cells (DCs) are known to exist within the lymph nodes, playing crucial roles for the nodal microenvironment. Among these, follicular dendritic cells (FDCs) reside within the B cell follicles of secondary lymphoid tissues and have the ability to present unprocessed antigen in the form of immune complexes. Unlike "conventional" DCs, FDCs are stromal in origin and lack phagocytic activity and lysozyme. On the contrary, "conventional" DCs are hematopoietic and activate naïve T cells by presentation of processed antigen via MHC molecules (Aguzzi et al., 2014). However, the immunohistochemical (IHC) distribution patterns of FDCs and DCs often overlap, thus raising many questions about the real nature of "dendrites" running throughout the lymphoid follicles.

The present study aims at discriminating between FDCs and DCs, by means of laser scanning confocal microscopy (LSCM) investigations.

The study was carried out on bovine (n=5) and porcine (n=5) lymph nodes, collected from apparently healthy and regularly slaughtered animals. Such tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax by standard methods. Tissue sections (5 µm-thick) were cut and stained with haematoxylin and eosin. Then, CNA.42 and lysozyme were used as IHC markers for FDCs and DCs, respectively. Finally, the distribution of FDCs and DCs was evaluated by double-labeling indirect immunofluorescence technique and LSCM examination. The co-localization was assessed by means of the Image J software (Pearson's coefficient, Rr).

Microscopically, all the lymph nodes under study showed a more or less pronounced follicular hyperplasia. IHC revealed a dense network of both FDCs and DCs within the

germinal center of the lymphoid follicles. Surprisingly, CNA.42 and lysozyme largely co-localized at LSCM examination, with very high Rr values.

Taken together, our results indicate that the distinction between FDCs and DCs by means of morphological techniques is often questionable. Further studies, possibly using additional markers, are needed to ascertain if and how the distribution patterns and the expression profiles of FDCs and DCs are influenced by their differentiation and/or functional status.

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69P

FUNCTIONAL AND MORPHOLOGICAL ADAPTATIONS OF THE DIGESTIVE SYSTEM INDUCED BY DOMESTICATION IN CATS

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Several studies have showed the macroscopic difference in the gastrointestinal tract between the European wildcat (*Felis silvestris silvestris*) and the domestic cat (*Felis silvestris catus*). Digestive system in the wildcat is shorter than in domestic species and this feature is considered distinctive in the taxonomic classification of subjects (Schauenberg et al. 1977). This study is a part of a large investigation regarding the microscopic anatomy of the gastrointestinal tract of European wildcat, associated to the study of intestinal microbiome. Its main purpose was to enhance knowledge about this species, to get a comparison with domestic cat, and to evaluate if and how domestication has influenced the functional and morphological development of this apparatus, also changing the gut's microflora.

To this aim we collected, weighted and measured the gastrointestinal tract of twenty European wildcats. Afterwards, intestinal sections were sampled, treated and observed at the microscope in order to evaluate histological characteristics as the villi height and width, crypts depth and wall thickness. Moreover, we wanted to study the intestinal production of an apolipoprotein that is believed directly related to the development of hepatic steatosis, decreasing the amount of lipids deposited in the liver. For this purpose, liver specimens were collected and treated to study histologically the degree of vacuolar degeneration of hepatocytes. Data were analyzed and compared with those of the domestic cats coming from our database. In attempt to evaluate the microbiome, feces and rectal ampulla were collected and sent to the Texas A&M University for pyrosequencing analysis (data not shown).

Results demonstrated significant differences in intestinal structure between *F. catus* and *F. s. silvestris*. Villi coming from domestic cats were significantly shorter ($p<0.0001$) and wider ($p<0.0142$) than in wildcats that showed crypts deeper ($p<0.0009$).

Domestication has led to significant changes in adaptation regarding both behavior and diet. Several studies showed the correlation between diet changes (protein, carbohydrates, and fiber concentration) and morphological adaptation in the gut of different species (Altmann, 1972; Hampson, 1983; Goodlad et al., 1988; Pluske et al., 1996; Sritiawthai et al., 2013).

Moreover, data from liver study showed that domestic cat has higher levels of apolipoprotein compared to the wild cat and that the percentage of lipids in the liver was lower in *F. catus* than in *F. s. silvestris*. Despite these results, the liver of domestic cat revealed a rate of steatosis higher than in wild cat. Indeed, this pathology proved to be almost absent in wild cats and can be explained by the different nature of the two species diet and microbiome composition.

This study revealed that transition from a strictly-carnivorous diet (typical of the wild cat) to an omnivorous type, has modified the nutritional intake considerably and influenced the evolution of the digestive apparatus in domestic cat.

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AMORPHOUS GLOBOSUS IN A COW: CASE REPORT

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This paper describes gross morphology and histological observations on a case of bovine *amorphous globosus* encountered. The *amorphous globosus* is a fetal anomaly occurring in twins, where the anomalous second fetus is an asymmetrical spherical mass covered by skin and without a functional heart. Occurrence of *amorphous globosus* is relatively rare, which makes it difficult to study its etiology (1, 2).

A 5-year-old Holstein-Friesian cow was presented with a history of dystocia labour. A careful exploration of the uterus was performed and a roughly spherical mass covered with a thick membrane was found deep in the uterine horn. The mass was removed through the birth canal and was diagnosed as an anomalous fetus. An oval firm mass, associated with a short, 55 cm long, pedicle was delivered to Histopathology Laboratory of Istituto Zooprofilattico della Sicilia.

The anomalous fetus was covered with pigmented skin. It was roughly spherical, measured 24x13x11 cm and weighed 1356 g.

The cranial and caudal ends could not be identified and no oral or anal openings were visible.

A medial linear incision was made to identify the development of various anatomical structures. On cross section, adipose tissue comprised most of the specimen. Undifferentiated muscle and prominent blood vessels were observed, but there was no recognizable organs.

Tissues from the anomalous fetus were fixed in neutral-buffered formalin and processed for histology. Sections of 4–5 μm thickness were cut and stained with hematoxylin and eosin.

Microscopically, the subcutaneous tissue consisted of connective tissue, muscle and well-formed nerve tissue, with mononuclear infiltration and lymphoid structures. There

were several lymphoid aggregations and blood-filled capillaries. Some areas showed scattered mononuclear cell infiltration, whereas other areas revealed dense fibroblasts arranged in various directions.

In humans, the pathogenesis of this condition is still ambiguous. It has been proposed that the malformed embryo has either a weak heart or no heart at all. This leads to a reversion of the blood flow from the normal embryo to the anomalous twin through anastomosing vessels between the two circulations. According to this theory the deprivation of of nutrition and oxygen blood results in hypoxia and malnutrition of the anomalous embryo (3). Histopathology is useful in distinguishing between *amorphous globosu*s and *acardius amorphus*.

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71P

PROSTATIC ATROPHY WITH MACROSCOPIC PROSTATIC HYPERPLASIA IN A NEUTERED AND IN AN INTACT DOG

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The most common causes of diffuse prostatic enlargement in dogs are prostatic hyperplasia and prostatic adenocarcinoma. Prostatic hyperplasia can occur in young dogs but it develops mostly in old and only in intact dogs. Two pattern are recognized: the benign glandular hyperplasia and the benign complex hyperplasia depending on the presence of cystic spaces and fibrous and muscular stroma. The WHO classification of the tumor of domestic animals divides the prostatic carcinomas in primary carcinoma of the glandular prostatic epithelium and in urothelial carcinoma.

Case 1: a 10 year-old, since 2 years-neutered male Miniature Schnauzer with history of urolithiasis and colelithiasis was presented for prostatic enlargement. The dog was asymptomatic and the prostatic alteration was detected via echographic abdominal examination as an incidental finding during the follow up for the previous lithiasis history. Fine needle aspiration was not diagnostic and prostatic massage was suggestive of carcinoma.

Case 2: a 9 year-old, mixed breed male dog was presented for prostatic enlargement.

Prostatic biopsy samples has been taken from the two dogs, the tissue fixed with 10% neutral buffered formalin and the sections stained with hematoxylin and eosin and cut at 4 microns. The sections are characterized by the presence of abundant fibro-muscular stroma with few irregular tubular structures lined by one or more layers of small flatten to cuboidal basal epithelial cells with moderate pale eosinophilic cytoplasm, round to oval central nucleus, and finely granular chromatin. Anisocytosis and anisokaryosis are mild and mitosis absent. A diagnosis of glandular prostatic atrophy was made. The dogs

did not develop any other related symptomatology during a follow up respectively of 18 and 5 months.

Prostate atrophy could develop in neutered males, but the enlargement of the organ is an unusual finding in this condition. One of our cases was on the contrary an intact male. At histological examination there is a risk of misdiagnose this pathological condition, because of the irregular glandular structures and the apparent multilayered disposition of the cells, characteristics shared with prostatic primary adenocarcinoma. Prostatic atrophy has been indeed reported in human beings for these reasons as a 'benign mimicker of prostatic adenocarcinoma'. Knowledge of this kind of pattern, as it is rare, is then important to discriminate the two conditions especially in case of enlarged rather than diminished prostate, as expected in prostatic atrophy.

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73P

DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE METHOD TO DETERMINE PESTICIDES IN CEREAL BASED ANIMAL FEED USING GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Pesticides are used in various combinations at different stages of cultivation or postharvest storage, to protect crops against a wide range of pests and fungi or to provide quality preservation. Contaminants in feeding-stuff can cause harmful health effects in animals and may be dangerous to humans through secondary exposure. Even if plant protection products may be ingested or absorbed by livestock following direct application of the product to the animals or as a result of treatment of their accommodation, the usual source of residues remains the use of pesticides in the production of crops used in the preparation of feeding-stuff (1). Published data about pesticides residues on feed are very scattered and not easy to find: results are not necessarily published and a compilation of feed monitoring data is still in the early stage (2).

The analysis of pesticides in dried matrices such as animal feed is considered to be difficult, due to the high complexity of matrices and the low concentrations in which these compounds are usually present. Animal feeds can be a complex mixture of grains, milling by-product, added vitamins, minerals, fats and other nutritional sources which make the detection and quantification of pesticide residues much more complicated if compared to matrices of higher water content(3).

A multiresidue quantitative method for the analysis of 103 gas chromatography amenable pesticides in dry cereal based animal feed was developed. The method entails a simple extraction of rehydrated sample with ethyl acetate, acidified with acetic acid, in ultrasonic bath at 30°C. The organic phase is then separated by centrifugation, after the addition of anhydrous sodium sulphate, and filtered through a syringe filter with 0.2 µm pore size prior to the final determination by GC-MS/MS. Two mass transitions were monitored for each pesticide, providing unequivocal identification of contaminants. Detected residues are quantified using tryphenylphosphate as internal standard.

Method validation was carried out, according to the European guidance document SANTE 11945/2015, on a feed for laying hens containing wheat, barley, rye, maize, soybean cake, lime, soybean oil, mineral and vitamin premix. All the parameters studied (specificity, linearity, matrix effect, LOQ, recovery, precision and ruggedness) meet the criteria set in the guideline at the three different spiking levels investigated (0.010, 0.050 and 0.10 mg/kg).

The applicability of the method has been demonstrated by the analysis of previous proficiency tests organized by the European Reference Laboratory for pesticides in Cereals and Feed showing good analytical performances both at low and high contamination levels.

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74P

THE USE OF RUMINATION COLLARS IN BEEF CATTLES

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Automatic rumination and activity monitoring are used to assess dairy cow behavior (Shirmann et al.,2009; Calamari et al., 2014), but to date no study have been performed on beef cattle.

The main objective of this study was to evaluate the rumination and activity time (RT and AT) of beef cattle, on farm and on the way to the slaughter house. In addition, the relationship between rumination time and the onset diseases symptoms was recorded.The study was performed over six month period (from October 2015 to March 2016) and involved 32 Italian crossbreed beef cattle and 37 Aberdeen Angus cattle. The animals were housed in the same farm, according two different feeding programs, based on different levels of forage inclusion. The individual RT and AT were continuously recorded using an automatic neck collar system (Hr-Tag, SCR Engineers Ltd, Israel). Data were collected at 2h intervals.

The average daily RT was 443 ± 102 minutes, while the average daily AT was 527 ± 132 bits/d. No significant differences were observed among the two distinct breeds.

A significant reduction ($p<0.05$) of RT was observed in four animals treated and later dead for respiratory desease (250±81minutes/d), compared with the healthy ones (401±111minutes/d).

Moreover, the RT and AT patterns were analyzed on a daily base using the values observed within each 2h interval. As already demonstrated in dairy cows (Calamari et al., 2014), RT was significantly greater ($P<0.05$) during night-time (54±21 night-time and 26±18 day-time) and significantly decreased ($P<0.05$) after feed distribution, while the AT showed an opposite trend (35±15 bits at night-time and 49±19 bits during day-time).

In addition, the RT and AT data of 9 healthy animals, slaughtered at 18-22 months of age, were analyzed concerning the week before slaughtering and the 2h period of transport. A significant reduction ($P<0.05$) of RT (8±4 minutes) and a significant increase ($P<0.05$) of AT (135±30 bits/d) were observed during the 2h transport (from 6.00a.m. to

8.00a.m), compared with the same 2h interval of the week before slaughtering (33±16 rumination minutes and 44±18 bits)

Our results demonstrated the efficiency of the Hr-Tag system to monitor RT and AT in order to assess physiological rumination and activity in beef cattle breeds, and highlighted a significant stress-related change in these behaviours during transport. Moreover, this electronic system allowed to record any RT and AT variations, and thus could be used to quickly identify those animals in risk of developing disease. The implementation of this system in beef cattle livestock could be useful to study the rumination behavior in various environmental and feeding conditions.

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76P

INCIDENCE OF ENDOSULFAN POISONING OF DOGS AND CATS IN CALABRIA

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The high toxic insecticide endosulfan was globally banned because of its threats to human health and the environment[1]. Despite the ban, it is still intentionally used causing poisoning events of domestic animals and wildlife, as reported by Caloni et al.[2]. Toxicological analysis of poisoned animals by the IZSM laboratories revealed that, between 2013 and 2015, this pesticide is not disappeared and an alarming presence of α and β endosulfan was registered in Calabria region.

The aim of this research was to reveal the endosulfan presence in more than 600 baits and suspected poisoned remains (liver, stomach and gastric contents) sent to the IZSM, as official control laboratory of Campania and Calabria regions, by veterinary service during institutional control activity or by private requesters, for necropsy and toxicological analysis. Homogenated sample was extracted with diethyl ether for 24 hours. The organic phase was filtered on anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in n-hexane, passed through an EXtrelut® NT3 column and washed with acetonitrile. Organic phase was evaporated to dryness, the residue was dissolved in n-hexane, filtered through a florisil prepacked column and eluted with a n-hexane, benzene, ethyl acetate (180/19/1) mixture. The extract was completely dried and the residue was dissolved in isooctane for gas chromatography-electron capture (GC-ECD) analysis. Detection was performed by comparing the retention time of peaks obtained in samples with those found in calibration standards.

Even if most of samples were negative for the organochlorine pesticide analysis, we could register the presence of 45 samples positive to the endosulfan pesticide on 440 samples (10.2%) collected only from Calabria region. In particular, 13.3% of total samples (32/241) come from Catanzaro, 11.1% (4/36) from Reggio Calabria, 7.1% (7/99) and 2.9% (2/68) from Cosenza provinces.

According to the data recorded from 1996 to 2003 in Italy by Albo et al.,[3] the dog was the most commonly poisoned species (75.5% of calls) followed by cat (6.6%). Only 15.7% of

samples were poisoned baits. From the health point of view, the danger concerns not only animals in which the pieces are addressed, but also the environment, the release of toxic substances in soils and surface waters, and people, especially children, who may accidentally come into contact with poisons. Collected data indicate how dangerous the misuse of certain agrochemicals and of many cleaning products can be. Since most cases arise from the ingestion of intentionally poisoned baits, a more severe control of marketing systems of this substance through the black market or the online sale is fundamental.

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77P

WNV VACCINES COMMERCIALIZED IN ITALY: A STUDY OF ANTIBODY DYNAMICS IN VACCINATED HORSES

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WNV is an RNA virus belonging to *Flaviviridae* family, transmitted by mosquitoes, causing zoonosis. Humans and horses are dead-end hosts. To date, there is no cure for the disease. The prevention can be achieved minimizing the exposure to the vector or through vaccination in equine species. In Italy, two vaccines are authorized: the "Equip WNV - Pfizer" (inactivated vaccine, VM-2 strain) and the "Proteq West Nile-Merial" (recombinant canarypox virus, vCP2017 strain that expresses the WNV prM/prE genes). Both vaccines protect against WNV lineages 1 and 2 strains. No vaccination is available for humans. Aim of this research was the study of the dynamic of antibodies in sera of vaccinated horses. Two groups, each consisting of 20 healthy horses, serum negative for WND, were selected by anamnestic examination and by evaluation of hematological and biochemical parameters (blood count, transaminases, creatinine), welfare indicators. The selected horses were submitted to vaccination (booster after 28 days) using authorized vaccines. After vaccination, horses were examined to evaluate the immune response from 0 to 365 days after vaccination (DAV). Specific IgG antibodies were detected through the kit ELISA: ID Screen West Nile Competition Multi-species – ID.vet. Specific IgM antibodies were detected using the kit ELISA: West Nile Virus IgM Antibody Test -

IDEXX. All sera were tested by serum neutralization (SN) test according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2013).

The results of the hematological and clinical chemistry analysis performed in both groups of horses, before and after the vaccination, showed no particular changes. All values were into the physiological range during all time of study. Data relating to IgG response showed that Pfizer vaccine induced an earlier immune response compared to the Merial one (100% of positive animals at 18 vs 38 DAV). Both vaccines produced appropriate levels of IgG for one year. SN results showed that Merial vaccine stimulated long-lasting and more intense response compared to Pfizer one (65% vs 21%). Horses treated with Merial vaccine had high neutralizing antibody titers for one year unlike of horses vaccinated with Pfizer. All horses vaccinated produced IgM.

Both vaccines gave adequate antibody titers. Data suggest to use Pfizer product during outbreaks, thanks to its capacity to produce antibodies early, instead, Merial vaccine might be used during prophylaxis plans. Both vaccines induced IgM production, therefore, DIVA (Differentiating Infected from Vaccinated Animals) strategy is not applicable. This study can be useful as model to develop the indirect prophylaxis in humans.

This study was supported by the Ministry of Health research current grant IZS SI 01/12.

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78P

DETECTION OF *LEISHMANIA INFANTUM* IN HUMAN PATIENTS FROM WESTERN SICILY WITH CUTANEOUS LEISHMANIASIS SUSPECT

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The Leishmaniases are infectious disease spread all over the world but little is known about the risk associated with occupational exposure in high-risk groups compared with general population. The objective of this study was to assess the prevalence of *Leishmania* positivity in human skin tissues collected from subjects who are residents of Western Sicily. The general characteristics of the subjects were investigated, specifically evaluating their relation to cutaneous leishmaniasis. The output of this cross sectional study is to highlight the prevalence of *Leishmania* infection positivity in skin scrapings, bone marrow and blood of Western Sicily men and women samples, of various ages and performing various work tasks, observed in the department of Dermatology of the Universitary Hospital of Palermo on suspicion of cutaneous leishmaniasis.

The spread of *Leishmania* infection in Western Sicily is strongly underestimated probably due to missed unreported diagnosis especially compared with dog's reservoirs infection. The study included 318 subjects (M/F ratio=1.0, mean age = 40±25.4 years), between 2013 and 2015, attended the Department of Dermatology of the University of Palermo; none of them was aware of any previous contact with *Leishmania infantum*. We tested their status against *Leishmania* through parasite isolation, from several human samples: skin biopsies, bone marrow, blood, and through PCR test performed on skin scrapings. All data were statistically analyzed with chi square test, comparing all positive results from the different provinces.

Positivity against *Leishmania infantum* was found in 81 (50.9%) of 159 females and 79 (49.7%) of 159 males. A higher risk for *Leishmania* positivity was found in subjects who lived in Agrigento province ($p<0.001$) and in those who lived in rural zones ($p=0.0038$). A marginally statistically significant high risk ($p=0.053$) was observed among subjects who had a pet, especially dogs, whereas no statistically significant association was found between *Leishmania* positivity and work task performed. The animals analysis of data suggests that the presence of leishmaniasis in Sicily is still really high. Although a relatively small sample size, nevertheless our results suggest that cutaneous leishmaniasis is widespread in Western Sicily and, considering that, we have observed a voluntary self-selected group of subjects, a higher prevalence can be hypothesized for the investigated geographical areas. A closer collaboration between medical doctors and veterinarians seems to be the best public health strategy for fighting against the continuous spread of *Leishmania* infection.

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79P

MONITORING OF *IXODIDAE* TICKS IN THE NATURAL RESERVE OF MONTE PELLEGRINO IN SICILY, ITALY

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Ticks (*Acari*: *Ixodidae*) are involved in the transmission of tick borne diseases, including anaplasmosis, babesiosis and rickettsiosis, posing a serious threat to humans, pets, wild animals and livestock. Tick questing activity, reproduction and survival depend on several factors, including vegetation coverage, host availability, moisture and temperature (Dantas-Torres, 2015; Randolph, 2009).

This study was aimed to the analysis of a spatial and temporal distribution of free-living ticks in the Natural Reserve of Monte Pellegrino, in Palermo (Italy), a peri-urban area of the city attended by families, walkers, companion animals.

Ticks were collected with dragging methods for two years from June 2012 to May 2014. Monthly abundance was evaluated in six different sites (1. Sede Landolina, 2. Boschetto Airoldi, 3. Pineta Ex Scuderie Reali, 4. Sito Valdesi, 5. Castello Utveggio and 6. Gorgo S. Rosalia), with different environmental characteristics. Collected arthropods were identified according to morphological keys (Manilla et al., 1998).

Tick population was analysed over the time (during the months of the year) and the space (in the different collection sites) in relation to environmental and climatic factors.

A total of 3,092 ticks (1,728 in the first year and 1,364 in the second one) was collected comprehending seven different species: *Ixodes ventalloi* (46.09%), *Hyalomma lusitanicum* (19.99%), *Rhipicephalus sanguineus* (17.34%), *R. pusillus* (16.11%) and, in less amount, *Haemaphisalis sulcata* (0.36%), *Dermacentor marginatus* (0.10%) and *R. turanicus* (0.03%).

The highest numbers of ticks were collected in June 2012 (no. 324), April 2013 (no. 256) and January 2013 (no. 225), while August 2012 (no. 15), February 2013 (no. 34) and

May 2014 (no. 38) were the months with the lower numbers of ticks. Percentage values for each collection site are reported, with the highest numbers of collected ticks in the sites no. 2 and no. 5, the lowest in the site n. 4. Data analysis showed that in many cases the same tick species was collected in sites showing similar environmental features. Tick density was related to environmental and climatic factors (altitude, land cover, vegetation, temperature and precipitation). Monthly maps with circles proportional to the tick number were created using the geographical information systems. In addition to the values for the two-year period, also the maps referred to each year of the study were created.

This monitoring provides useful information on the presence and distribution of tick species in the studied area and can be a powerful surveillance tool. Moreover, the study constitutes a premise for additional researches including hosts distribution analysis, correlation with pathogens in ticks, map risk processing and correlation with microclimate.

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80P

A MOLECULAR CHARACTERIZATION IN *E. COLI* ISOLATES FROM PET ANIMALS IN SICILY

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The aim of the study was the analysis of virulent genes and extended spectrum beta lactamases (ESBL) genes in E. coli strains isolated from pet animals. *Escherichia coli* (*E. coli*) is a bacterium that commonly lives in the intestines of people and animals, without causing health problems. However some pathogenic strains are circulating worldwide causing sometime quite severe enteric symptoms. These pathogenic *E. coli* are classified in the following groups: enterotoxigenic *E. coli* (ETEC) enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli (*EPEC) and enterohemorrhagic *E. coli* (EHEC). These categories of *E. coli* differ in their epidemiology and pathogenesis and their O:H serotypes and for the presence or absence of several genes responsible for their virulence. Another relevant public health issue related to E.coli is the presence and diffusion of antibiotic resistant genes such as the ESBL genes. Many genera of gram-negative bacteria possess a naturally occurring ß–lactamase probably due to the selective pressure exerted by ß-lactam-producing soil organisms found in the environment. The first plasmid-mediated ß-lactamase in gram-negatives, TEM-1, was described in the early 1960s. TheTEM-1 enzyme was originally found in a single strain of *E. coli* isolated from a blood culture from a patient named Temonierain Greece (TEM). TEM-1 is now found in many different species of members of the family *Enterobacteriaceae* and in other animal species. Genetic analysis for the virulence genes and the O serotypes were performed by multiplex PCRs (2). The first two PCRs target the following genes aggR, aap, aatA, astA, pet, shf , irp2, set1A and eae. Five isolates from dogs out of a total of 43 were positive for eae gene encoding an “intimin”, that in enteropathogenic *Escherichia coli* (EPEC) is necessary for the formation of attaching and effacing lesions to epithelial cells, in both piglets and humans.

The prevalence of circulation of ESBL positive *E. coli* strains isolated in pet animals in Sicily was studied by two multiplex PCRs to assay for the presence of genes encoding the following ß-lactamases: TEM, OXA, SHV, CTX-M, CMY, and DHA type β-lactamases (3). On a total of 43 *E. coli* isolates from cats and dogs 17 resulted positive for resistance genes. Over the total of 17 ESBL positive 13 were only TEM positive, 2 were SHV and 1 was CTX.

Two dog isolates carried simultaneously TEM-1 and CTX MII. The same isolates resulted resistant by the anti-biogram screening according to Kirby-Bauer's method. The more common antibiotics used in veterinary medicine were used for the resistance analysis.

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81P

EFFICACY OF DIETARY SUPPLEMENTATION IN CATS WITH ADVANCED CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is a very common disorder in elderly cats (1). A proper renal diet represents the most efficient therapeutic intervention to improve survival and life quality in feline patients with 3 and 4 IRIS stages (2). However, when diet alone is not sufficient, dietary supplementation with other substances (i.e., phosphorus chelates and alkalizing agents) is needed (3). The present study aims to evaluate the efficacy and palatability of a dietary supplementation containing calcium carbonate, calcium-lactate gluconate, chitosan and sodium bicarbonate in cats with 3 and 4 IRIS stages of CKD.
20 cats (mean age: 11.1±2.4) were considered. All animals belonged to IRIS stages 3 (80%) and 4 (20%) of CKD since at least one month and had hyperphosphatemia despite assuming a renal diet. 10 cats (T group) were administered the dietary supplementation at 0.2g/kg/die for 6 months along with the renal diet (composition: 23% CP; 17% C Fat; 4.7% C Fiber; 0.6% Ca; 0.3% P). 10 animals in IRIS stage 3 or 4 (same percentage of T group), whose owners did not give consent for any supplemental therapies apart from the renal diet, were selected from the clinical database and served as control (C) group. Haematochemical, biochemical and urine analyses were performed on 0, 15, 30, 60, 90, 120, 150 and 180 days. GraphPad Prism® software was used to perform statistical analysis. Data were analyzed by one-way ANOVA, Kruskal-Wallis, Student t and Mann-Whitney U tests ($P<0.05$).
Decrease (41% at day 180) of serum phosphorus and increase of serum ionized calcium (10% at day 180) and serum bicarbonate (7% at day 180) were observed in T group. Serum phosphorus at days 30, 60, 90, 120, 150 and 180 was lower ($P<0.01$) in T group than C.

Serum ionized calcium at days 60, 90, 120, 150 and 180 was higher ($P<0.01$) in T group compared with C one. The increase of ionized calcium fell within the physiological ranges (1.1-1.4 mmol/l).

Serum bicarbonate at days 60, 90, 120, 150 and 180 was also greater ($P<0.05$) in T group than C one. The cats remained in the same IRIS stage for the duration of the study, but they clinically improved: vomit and diarrhea disappeared and body weight remained stable.

In conclusion, the dietary supplement tested in the present study reduced serum phosphorus and increased serum bicarbonate in cats with CKD, thus it can be useful to support therapy in cats with advanced CKD improving the clinical conditions of the animals without any adverse reactions.

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82P

CHRONIC KIDNEY DISEASE IN DOGS: TOLERABILITY AND EFFICACY OF A NUTRITIONAL SUPPLEMENT

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Chronic kidney disease (CKD) is a very common disorder in elderly dogs (1). Administration of a renal diet is considered the therapeutic target to improve survival and life quality of canine patients with IRIS stages 3 and 4 (2). However, when diet alone is not sufficient for slowing down CKD, dietary supplementation with other substances (ie, phosphorus chelates and alkalizing agents) is required (2). The present study aims to evaluate the efficacy and palatability of a dietary supplementation containing calcium carbonate, calcium-lactate gluconate, chitosan and sodium bicarbonate in dogs with IRIS stage 3 of CKD.

20 dogs (mean age 10.4±2.3) were considered. All animals belonged to IRIS stage 3 of CKD since at least one month and had hyperphosphatemia despite assuming a balanced renal diet. 10 dogs (T group) were administered the dietary supplementation at 0.2g/kg/die for 6 months along with the renal diet (composition: 23% CP; 17% C Fat; 4.7% C Fiber; 0.6% Ca; 0.3% P). 10 animals, whose owners did not give consent for any supplemental therapies apart from the renal diet (the same as T group), were recovered from the clinical database and served as control (C) group. Haematochemical, biochemical and urine analyses were performed on 0, 15, 30, 60, 90, 120, 150 and 180 days. GraphPad Prism® software was used to perform statistical analysis. Data were analyzed by one-way ANOVA, Kruskal-Wallis, Student t and Mann-Whitney U tests ($p<0.05$).

Serum P at days 30, 60, 90, 120, 150 and 180 was lower ($p<0.01$) in T group than C. Alterations of Ca-P homeostasis and hyperphosphatemia negatively affect renal

functioning and survival rates in dogs with CKD (4). Serum iCa at days 120, 150 and 180 was higher ($p<0.01$) in T group compared with C one.

Serum bicarbonate at day 180 was also greater ($p<0.05$) in T group than C.

UP/UC ratio at days 150 and 180 was also lower ($p<0.05$) in T group than C.

The dogs remained in the same IRIS stage for the duration of the study, but they clinically improved: vomit and diarrhea disappeared and body weight remained stable.

Serum creatinine and proteinuria are key elements for CKD staging (2). Proteinuria is also a negative prognostic factor in dogs with CKD (5) and its reduction has been reported to slow down the declining of the renal functioning (6).

In conclusion, the dietary supplementation tested in the present study reduced serum P and increased serum bicarbonate in dogs with CKD.

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Mistery Cases

MELA

(arrhythmogenic right ventricular cardiomyopathy associated with severe left ventricular involvement in cat)

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An 8-year-old,female, domestic shorthaired cat was referred to the Veterinary Teaching Hospital of Naples University "Federico II" for dyspnea and lethargy. Physical examination showed moderately dehydrated, pale mucous membranes, normal femoral pulses (210beats/min).

A 24-h Holter recording revealed ventricular premature complexes with left bundle branch block (LBBB) morphology (25,920complexes) and right bundle branch block (RBBB) morphology (6480 complexes). Ventricular couplets (4,320) and R-on-T phenomenon (220complexes) were also noted. Average heart rate was 213 _ 12 beats/min.

A right lateral thoracic radiograph revealed pleural effusion. Thoracocentesis yielded 120 ml of serous fluid was modified transudate. B-mode echocardiography showed severe right atrial and right ventricular dilation. The right ventricular wall appeared very thin and hypokinetic. Aneurysms were detected in apical and subtricuspid regions. Flattening of interventricular septum at end-diastole and mild dilation of the pulmonary trunk were observed. The left atrium and ventricle appeared to be unremarkable. M-mode echocardiography revealed paradoxic interventricular septal motion which prevented accurate left ventricle measurement. The right ventricular chamber was severely dilated (end-diastole, 15.0 mm). Doppler echocardiography revealed mild tricuspid regurgitation and decreased maximal pulmonary artery velocity. Based upon physical examination,

24-h Holter recordings and echocardiographic findings, a diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) was made. Initial therapy included furosemide,10mg/sc/every12 h, enalapril 1 mg/os/every12 h, and sotalol 8mg/os/twice daily. The cat died suddenly 10 days after initiation of therapy. At post-mortem, gross examination of the heart revealed severe right ventricular chamber dilation with infundibular, apical and inferior aneurysms. At cross section, the right ventricle (RV) free wall was extremely and diffusely thinned (1 mm) and the wall had a "parchmentlike" appearance. Histological examination showed massive fibrous tissue replacement associated with mild fatty tissue infiltration in the right ventricular free wall.

Finally a diagnosis of ARVC associated with severe left ventricular involvement was made.

This clinical report represents the first warning of feline ARVC in Italy and was published in Journal of Veterinary Cardiology (2009)11, 41-45.

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Multiple pigmented cutaneous nodules in a dog

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A 11 year-old, male, Wiszla was presented for several cutaneous pigmented nodules. Seven of them have been excised and the histological diagnosis was of melanocytoma. After 1 year the dog presented new cutaneous nodules, and the histological diagnosis was of mildly pigmented melanoma. After 1 month from the last surgery the dog was cachectic with vomit and diarrhoea. Ultrasound examination revealed thickening and loss of the stratigraphy of the small intestinal wall. Fine needle aspiration of the mesenteric lymph nodes was diagnostic for reactive lymphadenopathy. At the same time multiple cutaneous nodules arose on the left forelimb, cytological evaluation revealed a single population of non-pigmented spindle to round cells with moderate signs of atypia. The cytological diagnosis was of spindle cell neoplasm, and, based on previous lesions, the main differential was a melanocytic tumour. The dog conditions were worsening and the dog was euthanized. The clinical presentation has been interpreted as part of the multiple dysplastic melanocytoma syndrome[1], a condition rarely seen in dogs, similar to the syndrome described in humans, a hereditary disorder of the melanocytes that can be caused also from UV radiation exposition[2]. In this syndrome, melanocytomas can be numerous, and be heavily pigmented as early lesions. They can grow progressively loosing their melanin content and the evolution to melanoma is possible.

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Multiresistent MRSA-associated pyodermitis in a dog with hypothyroidism

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A 10-year-old female Fila Brasileiro dog was referred to the Department of Veterinary Medicine and Animal Production, University of Naples, presenting a one-year history of generalized cutaneous lesions. The owner reported that the dog had dysorexia, weight loss, and depression. Physical examination revealed weight loss and depression; the mucous membranes were pale and the peripheral lymph nodes were moderately enlarged. Dermatologic disorders consisted in: multifocal alopecia, erythema, erosions and ulcers with the presence of purulent essudate on tips and interdigital level, hyperpigmentations. The dog had an offensive rancid odor but did not show pruritus. A complete haematological, biochemical and urinary profile was performed, together with skin imprints and multiple superficial and deep skin scraping procedures. Main clinicopathological alterations were hyporegenerative anaemia, a free 4- thyroxine reduction associated to a TSH increase. According to hormone concentration result and to clinical signs a diagnosis of hypothyroidism was made[1]. Skin cytological examination revealed the presence of numerous degenerated neutrophils and some macrophages with abundant foamy cytoplasm. Many coccus-shaped bacteria were seen in the cytoplasm of neutrophils. Cutaneous swab and hair sample were used for bacteriological analysis. Results were in accordance with the identification of methicillin-resistant *Staphylococcus aureus*. The isolate showed resistance to different antibiotics and was susceptible only to vancomycin and linezolid. Based on clinical signs, skin and hair, a diagnosis of severe pyoderma disease associate with multiresistant MRSA was done.

Dog was treated with Vancomycin associated to a topical therapy based on medicated antymycotic shampoo, and with synthetic sodic Levothyroxine. After one month the dog showed a good improvement of cutaneous lesions, lymph node volume reduction and mental alertness and activity increase.

The clinical sigs and clinicopathologic abnormalities associated with hypothyroidism generally should resolve within the first week of treatment through the appropriate thyroid hormone therapy. Endocrine alopecia may take several months to complete regrowth and a marked reduction in hyperpigmentation of the skin. Bacterial skin infection associated to hypothyroidism are frequent and needs a specific antibiotic treatment to avoid a spread of multi-resistant bacteria. The phenomenon of microbial resistance, which is based on genetic plasticity of bacteria, has emerged as a consequence of the selective pressure exerted by the antimicrobial usage in human medicine, veterinary medicine, animal production, agriculture and food technology[2,3,4].

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Occlusive tracheo-bronchitis in a free–living striped dolphin

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The carcass of an adult female striped dolphin (*Stenella coeruleoalba*) was found stranded in March 2016 on the Ligurian Sea coast, northwestern Italy. The most striking gross necrospy finding consisted of a severe tracheal occlusion and partial bronchial stenosis with luminal accumulation of abundant yellow, green mucous; no mucosal ulceration or cartilage destruction was apparent.

The thoracic lymph nodes showed hyperplasia. The heart presented ventricular chamber enlarged.

Aspergillus spp[1], *Mycobacterium* spp [2], *Halocercus* spp, *Holotricus* spp, and *Nocardia* spp were considered as potential etiologic agents of the respiratory disease.

Culture of the lung tissue and tracheo-bronchial exudate yielded colonies of *A. fumigatus*.

A pan-herpesvirus nested PCR assay on frozen tissue from multiple organs was positive, and the sequence amplified was classified within the cetacean alphaherpesvirus group.

Histologically, bronchial mucosal ulceration, severe necrosis and pygranulomatous inflammation with intralesional fungal hyphae, positive to Grocott and PAS stain, were observed.
The immediate cause of death was diagnosed as acute systemic aspergillosis.

Fungal identification was achieved through correlation of gross pathology, histopathology, culture and molecular characterization

In this case *Herpesvirus* infection could have played an important immunosuppressive role, predisposing the pathogenic role of *Aspergillus fumigatus* as secondary pathogen.

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What you would not expect to find in an abdominal mass of a cat

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An owned 6-year-old female cat was presented with an history of anorexia, vomiting, weight loss and abdominal distention. The cat lived outdoor in a rural setting and was routinely submitted to a large-spectre deworming. Physical examination revealed depression, weakness, lack state of nutrition, tachypnoea and fever (39.8°C). Moreover, the abdomen was distended and extremely painful at palpation. The patient was submitted to a lateral-lateral abdominal radiography that evidenced a feature compatible with a peritoneal effusion. CBC and serum biochemical analysis were carried out and the main feline infectious diseases (e.g. FIP, FeLV, FIV) were screened by serological test. A coprological examination by traditional flotation method using sugar solution was also performed. Haematological exams evidenced moderate neutrophilia, ipoalbuminemia and high hepatic enzymatic activity; serological and coprological examinations tested negative. Abdominal ultrasonography was performed and revealed the presence of a large polycystic mass (about 7 cm) just caudally to the liver and multiple, small, anaechoic cyst-like structures fluctuating within a large amount of peritoneal effusion; moreover a markedly enlargement of the mesenteric lymph nodes was observed. An abdominal cavity puncture was performed; the peritoneal fluid collected was yellow and opaque at the physical examination and, basing on cytology, biochemical and bacteriological analysis, was classified as a non-septic exudate. The cat was submitted to laparotomy and several hundred parasitic bodies resembled to solid larval cestodal stages were observed within the irremovable mass as well as free in the peritoneal fluid. Some specimens were collected and basing on their microscopic and histopathological features (e.g. pear-shaped, presence of four suckers in the enlarged part, external surface covered with minute microtriches projecting from the outer limiting membrane of the tegument, two layers of musculature, numerous well developed calcareous corpuscles within the parenchyma) the parasitic bodies were confirmed to be

cephalic forms at different stage of development of tetrathyridia, the larval stages of *Mesocestoides* sp.. A diagnosis of peritoneal larval cestodiasis (PLC) associated to massive proliferative peri-hepatitis was formulated and a treatment with fenbendazole at a dosage of 100 mg/kg q 12 h for 3 weeks was administered. A full-recovery of the symptoms was obtained but the polycistic mass was still visualised at the ultrasonographic control.

Mesocestoides sp. is a worldwide tapeworm that infects carnivores (1, 2, 3). Although the life cycle is not yet fully understood, the wild and domestic carnivores (definitive hosts) occasionally may act also as intermediate hosts harbouring metacestode stages in several body cavities (i.e. pleural and peritoneal cavities). The peritoneal colonization with larvae or larval fragments occurring in dogs is well known for causing severe peritonitis; however less common reports are described in cats. The present case highlights the importance for clinicians to include the PLC in the differential list of peritoneal effusion of uncertain origin in cats.

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Unusual cause of meningoencephalitis occurring in an Italian horse

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In August 2013, a 10-year-old Italian Warmblood gelding was referred to the Veterinary Teaching Hospital of the University of Perugia for a lateral deviation of the neck of acute onset.

On arrival the vital parameters of the horse were within normal reference ranges. Neurological examination showed: intermittent sensory blunting, a right lateral deviation of the neck and head tilt, mydriasis, ventral strabismus, absence of pupillary light reflex, photophobia and reduction of the menace reflex. The horse kept the hind limbs placed well forward, with a wide-based stance of the forelimbs.

Standard radiographic views of the head and proximal neck were unremarkable, as well as haematology, serum biochemistry and serology for West Nile and EHV1/4. Owing to the poor prognosis and the lack of response to symptomatic therapy, the animal was euthanased;. CSF was collected and analysed before death.

At necropsy, several large (> 10 cm) white-yellowish fleshy masses were found in the medial portion of the left thigh and laterally to the penis. Single similar but smaller lesions (1–4 cm) were observed in the renal cortices and below the aortic ostium. No gross lesions were observed in the CNS. At the histological examination lesions having similar appearance, consisted on aggregates of multinucleated giant cells and epithelioid macrophages occasionally associated with sections of nematodes, were observed. Similar parasitic specimens were detected in the CNS, adjacent to parenchymal vessels within small necrotic foci. Bacteriological examination of the CSF was negative. The CSF was normal at physical–chemical examination but a markedly increased of the white blood cell counts was found. Few rhabditiform nematodes were observed.

To identify the nematodes, small pieces of fresh tissue were placed in PBS pH 7.4, to allow the migration of the parasites. The specimens collected were examined by light microscopy (400×) after Lugol staining, and identified as *Halicephalobus gingivalis* on the basis of morphometric features[1].

Genomic DNA was extracted from specimens isolated from tissues and CSF, using a commercial kit. The DNA samples were submitted to PCR protocol for the amplification of a fragment of the LSU rDNA[2]. The amplicons obtained were sequenced, and the sequences were aligned with those of representative free-living and parasitic isolates. The sequence analysis by the neighbor-joining method, revealed that the isolates formed a clade with *H. gingivalis* isolates.

On the basis of the parasitological and pathological findings, a diagnosis of meningoencephalitis caused by *H. gingivalis*, associated with multi-systemic dissemination of the parasite, was formulated.

Halicephalobus gingivalis (Order Rhabditata, Family *Panagrolaimidae*) is a free-living worldwide saprophagous nematode, sporadically associated with opportunistic infections of horses and humans, frequently with a fatal outcome caused by the CNS localization[3]. The present case highlights the importance of including this verminous meningoencephalitis in the differential list of equine neurological diseases, and the importance of CSF analysis as a potential tool in achieving an ante-mortem diagnosis.

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Effusion and thoracic mass in a cat

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A 2 years-old domestic short hair was presented for weight loss and increased respiratory effort. On physical examination the cat was hyperthermic. Thoracic radiographs showed a severe thoracic effusion. Thoracocentesis was performed and approximately 290 mL of serofibrinous fluid were drained and submitted for fluid analysis and cytological examination. A complete blood cell count (CBC) and a serum biochemical analysis with serum protein electrophoresis were also performed. CBC revealed a moderate normocytic normochromic non regenerative anemia, moderate leukocytosis with neutrophilia. Biochemical profile revealed a severe hyperproteinemia with hypoalbuminemia and hyperglobulinemia. The cytological examination of effusion showed a moderate cellularity with neutrophils and macrophages. Bacteriological analysis and RT-PCR for coronavirus performed on the effusion were both negative. Despite the administration of antibiotics and NSAID therapy, after 15 days the cat showed a progressive weight loss and a recurrence of thoracic effusion. Ultrasound examination and computed tomography (CT) were performed and a large proliferation arising from the pleural membrane with pericardial involvement was noted. Moreover, multiple nodules on the liver were also identified. Based on imaging findings, the clinical most likely differential was of neoplasia of uncertain origin. Due to the worsening clinical condition, the cat was euthanized and a complete necropsy was performed. Gross findings reveled a multilacunar irregular compact whitish mass arising from the diaphragmatic pleura and involving both pericardium and pulmonary pleura. Lungs were diffusely atelectatic with widely and severely thickened surface. The liver evidenced multifocal to coalescent irregular slightly erased nodules extending from the surface to the deep tissue. At histology the thoracic mass was composed of a dense inflammatory lymphoplasmacytic infiltrate associate with scattered macrophages and fibroblasts. This infiltrate was extending and often effacing the pleura and was circumscribing wide and irregular lacunae containing fibrinous

exudate and necrotic debris. The same infiltrate was diffusely and severely effacing the lung serosa occasionally extending into the deep tissue. Severe atelectasia and hyperemia were seen into the lung. The same inflammatory infiltrate was detected into the liver nodules extending from the surface, diffusely infiltrated and effaced, to the deep tissue. In all sections, the infiltrate was occasionally perivascular and vessels (veins, presumptive) showed minimal signs of hyaline necrosis and neutrophilic exocitosis. Occasional evidence of vasculitis was seen (presumptive), thrombosis was rarely observed. The morphological diagnosis was diffuse severe lymphoplasmacytic pleuropneumonia, multifocal to diffuse polisierositis (pleura), severe mutifocal perihepatitis and hepatitis, with vasculitis and thrombosis. The etiology was most likely consistent with feline coronavirus. Immunohistochemistry on lung, pleural and liver lesions was positive for feline coronavirus (C. Giudice, University of Milano).

In this case, the results of some diagnostic test both indirect and direct, such as ultrasound/CT imaging and RT-PCR, were more capable of confusion on the diagnostic process. Once again, for the FIP diagnosis, the likelihood that the cat has FIP had to be based foremost on signalment, clinical and clinical-pathological findings[1].

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