

Comunicazioni Orali Sisvet

AN ELECTRON MICROSCOPIC STUDY ON MEMBRANE VESICLES DERIVED FROM MESENCHYMAL STROMAL CELLS: DIFFERENT MORPHOLOGIES FOR DIFFERENT FUNCTIONS?

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The biological in vitro and in vivo properties of mesenchymal stromal cells (MSCs) are eliciting great expectations in the field of regenerative medicine, immunotherapy and tumor treatment. Although their effects in vivo were initially considered as the result of integration-proliferation-differentiation, this paradigm has been recently revisited due to the observation that MSCs operate through different mechanisms that probably include, but are not limited to, differentiation in tissue specific cells of the host environment. An increasing number of experimental observations, in fact, suggests that they mainly act through a paracrine mechanism, that is mediated by the release of signaling molecules in a soluble form or embedded inside membrane vesicles (MVs), comprising exosomes (Ex) and shedding vesicles (SVs). Initially considered cellular debris, it is now ascertained that these nanometric structures are carriers of signaling molecules among cells.

The aim of this study was to characterize, by transmission (TEM) and scanning electron microscopy (SEM), MVs produced by MSCs of different mammalian species (murine, feline, canine, equine and human).

MVs isolated by ultracentrifugation of MSC supernatants were suspended in cacodylate buffer and allowed to adhere to formvar-coated copper grids. These preparations were then fixed in 2.5% glutaraldehyde and observed at TEM (Philips EM 208 – CUME, Perugia) and SEM (ZEISS - LEO 1525 Laboratorio Universitario di NANomateriali - Perugia). A morphological study was additionally carried out on monolayers of the same cells, fixed in glutaraldehyde and processed for TEM and SEM analyses.

Sample observation revealed that MVs were constitutively produced by all the tested mammalian cells and that were characterized by similar features. Within individual preparations, MVs were quite heterogeneous in size and appeared from slightly to moderately electron-dense. Some of them were characterized by cytoplasm-like texture and contained lipid droplets, as well as structural components of the cell. The different pathways of SVs and Ex formation were clearly distinguished, demonstrating that both fractions were produced.

Additionally, the detection of membrane bounded macro-vesicles containing microvesicles located in the extracellular environment was one of the most remarkable observation. It could be speculated that intracellular vesicles enclosing an undefined content are polarized underneath a distinct region of the plasma membrane and are then exocytosed through a sort of micro-apocrine secretion.

The data presented in this work, besides emphasizing the potential relationship between different morphology versus different function of MVs, have an interesting translational value, as their implications can be significant for a number of mammalian species.

György B., et al. (2011). Cell Mol. Life Sci. 68, 2667-2688. Review. - Liang X., et al. (2013). Cell Transplant. Epub ahead of print.

morfologia, cellule staminali

MSC, Membrane vesicles, electron microscopy

MORPHO-STRUCTURAL ANALYSIS OF THE MALE PIG URETHRAL MUSCLE.**Gazza F.*^[1], Ragionieri L.^[1], Botti M.^[1], Cacchioli A.^[1], Ravanetti F.^[1], Panu R.^[1]**^[1]*Dipartimento di Scienze Medico-Veterinarie ~ Parma*

The morphology and function of the urethral muscle (UM) have been extensively studied by urologists because it contributes significantly to urethral closure pressure and to the control of the lower urinary tract. Despite this, the UM remains one of the least understood and most difficult to study striated muscles of the body, because of its inaccessibility, great infiltration by connective tissue, particular innervation, and small size of its myofibers. We studied the morphological and structural characteristics of the UM of the pig to assess whether it could be a suitable model for the development of human urethral sphincter insufficiency treatments.

Six muscles were collected from male slaughtered pigs, macroscopically observed and histologically and histochemically processed.

The UM extends from the bladder neck to the central tendon of perineum and surrounds the pelvic urethra with horseshoe-like configuration, being thickest on the ventral side and thin or deficient on the dorsal aspect, where a longitudinal raphe is present. Ventrally the UM begins with a few longitudinal fibers on the neck of the bladder, continuing in caudal direction, it enriches with both circular oriented and interwoven fibers, increasing in thickness up to half of its length and then decreases slowly. By histological analysis we observed that each striated muscle fibers has very small diameter (~75µm) and is embedded in a conspicuous net of elastic fibers. Using histochemistry techniques, we proved the mixed slow-twitch and fast-twitch myofiber structure of the UM, and, using Ruffini's gold chloride method, we observed the presence of nerve trunks of various thickness, but the lack of muscle spindles and neurotendinous organs. As already demonstrated in man, the fibers of the UM seems to contribute to urinary continence through both slow and fast contraction (1, 2, 3) and have good fatigue resistance also due to their small size, that shorten the diffusion distance for metabolic substrates (3), and the help of the surrounding elastic fibers (4). The small size of the myofibers and the lack of receptors known to trigger afferent impulses in striated muscle (2), may also be in accordance with the hypothesis that these fibers could have origin by transdifferentiation of the smooth muscle-like periurethral mesenchyma into striated myotubule (5).

The structural similarity of the UM in pigs and humans suggests the suitability of the pig model for the study of human urethral sphincter insufficiency treatments.

1) Oelrich TM (1980), Am J Anat;158(2):229-46. 2) Elbadawi A. (1996) Urol Clin North Am;23(2):177-210. 3) Sumino Y et al. (2006) J Urol;175(4):1417-21. 4) Hinata N (2013) J Urol;190(4):1313-9. 5) Borirakchanyavat S et al. (1997) J Urol;158(3 Pt 2):1119-22.

anatomia normale veterinaria

pig urethral muscle, structure, morphology

IMMUNOHISTOCHEMICAL STUDY OF LEPTIN AND ITS RECEPTOR IN THE MINOR SALIVARY GLANDS OF THE DONKEY

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The minor salivary glands consist of tubulo-acinar adenomeres, characterized by the presence of mucous and serous cells and localized in the depth of the wall of the mouth or in the depth of the organs contained within the mouth. They were identified as labial, lingual, buccal glands, etc. The aim of the present work is to continue the immunohistochemical study, previously begun on the major salivary glands of various animal species, to identify the presence and localization of leptin and its receptor in the minor salivary glands. The donkey was chosen as the reference species, considering the functional characteristics of its masticatory apparatus, typical of a herbivorous animal and the very thorough mastication always accompanied by copious salivation.

Four clinically healthy adult donkeys were used. Specimens of lingual, labial and buccal salivary glands were obtained, immediately after slaughter, fixed in 4% formaldehyde solution and subsequently processed for embedding in paraffin. The immunohistochemical reaction was visualized, utilising the avidin-biotin-complex and the DAB as the chromogen. The following primary antibodies were used: anti-Ob and anti-Ob-R rabbit polyclonal antibodies.

In all glands examined, the immunohistochemical study showed a strong positivity for leptin in the cytoplasm of the serous cells of adenomeres and in the ductal cells. Mucous cells were always negative, while the ductal positivity affected all duct segments, starting from the intralobular ones through to the lobar ones. ObR-immunopositivity was localized only in the cytoplasm of the ductal cells and was most evident near the apical cytoplasmic portion, while the glandular tubulo-acinar component was always negative. Immuno-positivity for leptin and its receptor was not observed in the connective tissue or in the negative controls always included in the investigations.

Recent studies conducted on some major salivary glands have allowed researchers to highlight how the same are able to secrete leptin and may represent a target of the action of this protein [1, 2, 3, 4 and 5]. The results obtained in the present study confirm the results already obtained in humans and in some animal species about the presence of leptin and its receptor in major salivary glands, extending the knowledge to the minor salivary glands, especially those of the donkey, and allow us to hypothesize that these glands are able to produce leptin and are themselves a target of this molecule that may act in an autocrine/paracrine or an endocrine manner.

1) Leone et al., *Folia Histochem. Cytobiol.*, 50, 504-512, 2012; 2) Dall'Aglio et al., *Res. Vet. Sci.*, 93, 1116-1118, 2012; 3) Bohlender et al., *J. Endocrinol.*, 178, 217-223, 2003; 4) De Matteis et al., *J. Anat.*, 201, 363-370, 2002; 5) Dall'Aglio et al., *Acta Histochem.*, 115, 757-760, 2013.

Anatomia (Biologia)

Ob, ObR, immunohistochemistry

IMMUNOLOCALIZATION OF LEPTIN AND ITS RECEPTOR IN THE SWEAT GLAND AND SEBACEOUS GLAND OF THE HORSE.

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Leptin (Ob) is a hormone primarily secreted by adipocytes of subcutaneous and visceral fat which binds to a specific receptor (Ob-R). It is considered an important factor involved in the skin biology. Ob plays a critical role in the regulation of wound healing processes (1) and it seems to be involved in the control of hair follicle cycle (2) as well as in the biology of sebaceous gland (3).

The study of Ob in the skin of domestic animals can be of great importance to improve our knowledge in the biology of the integumentary system. We recently identified the Ob-R in the skin of some domestic animals such as dog, sheep and cow. The aim of this work is to continue this investigation by evaluating the presence of Ob and its receptor in the skin of the horse.

Normal skin samples, collected from five animals, were fixed in 10% neutral-buffered formalin. Skin sections were microwaved in 10 mM citric acid (pH 6.0) for antigen retrieval. The endogenous peroxidase activity was blocked with 3% peroxidase-blocking solution and non-specific binding was blocked with normal goat serum. The sections were incubated with mouse monoclonal anti Ob (Fitzgerald) or goat polyclonal anti Ob-R (Santa Cruz Biotechnology) antibody for 24 hours and, successively, they were incubated with the goat anti-mouse or goat anti-rabbit biotin conjugate antibody, respectively. The reaction was detected with the Vectastain ABC kit and visualized with diaminobenzidine.

By observing immunohistochemical treated sections, we evidenced an intense positivity to both Ob and its receptor in the cells of the apocrine sweat glands in all subjects considered. Moreover, leptin receptor positivity was observed in the basal cells of sebaceous glands. The staining for Ob was localized in the cytoplasm of the cells while the staining for Ob-R was both in the cell cytoplasm and membrane. No staining was observed in other structures of skin.

The result obtained suggests a role of Ob in the regulation of the sweat and sebaceous gland activity. Apocrine sweat gland cells are both source and target of leptin then, an autocrine or paracrine control by the hormone can be supposed. Since the cells of sweat glands express only the leptin receptor, it is likely an endocrine control by the leptin on sweat glands according to Chen et al., (2002).

1) Murad A, et al., (2003) FASEB J 17(13):1895-1897.

2) Poeggeler B, et al., (2010) Exp Dermatol 19(1):12-8.

3) Chen HC, et al., 2002. J Clin Invest 109(2):175-181.

Anatomia

Ob, skin, Immunohistochemistry

THE OSTEOGENIC RESPONSE AND THE OSTEOPROTECTIVE EFFECT IN VIVO OF A NANOSTRUCTURED TITANIUM SURFACE WITH ANTIBACTERIAL PROPERTIES

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The present study has the dual purpose of assessing in vivo the osteointegrative capacity and the osteoprotective potential of endosseous implants of titanium with nanostructured surface complexed with gallium (Ga + ASD) comparing it with two other surfaces, one of which treated as the previous but without complexation with Gallium (ASD) and the other treated with an acid etching (CTR).

The experimental plan is based on 12 male White New Zealand outbred rabbits, randomly divided into two groups respectively of normal models and of experimentally induced peri-implantitis models, that are implanted in the femur distal epiphysis with the tested materials and analyzed after 7 and 14 days.

For the experimental evaluation X-ray, blood tests, bacteriology culture, histological analysis, static and dynamic histomorphometric analysis (1) were performed.

In normal models both the dynamic and the quality of osteointegration resulted significantly improved, also from a statistical standpoint, for the samples ASD and ASD + Ga as compared to the CTR after 7 days, while at 14 days significant differences between the surfaces were not detected.

In the experimentally induced peri-implantitis models, after 7 days a lower level of the residual viable bacterial in the peri-implant site is documented for the ASD + Ga compared to CTR and ASD, as well as the presence of a more osteoprotective tissue less affected by the infection as borne out by the results of the histological and both static and dynamic histomorphometric parameters.

After 14 days, despite the clinical course becomes strongly affected by the infection and therefore it is vitiated also the histology, the histomorphometric investigations showed however an albeit slight better tissue response to the infection for the implant with ASD + Ga compared to the other surfaces.

From the experimental evidence we conclude that, in the normal models, the titanium with anodic nanostructured deposition surface treatment and complexed with gallium presents a dynamic and a quality of the osteointegration comparable to the surface ASD and better as compared to the CTR, moreover in the experimentally induced peri-implantitis models, the implantation of ASD + Ga exerts an antibacterial activity in vivo, in particular within the first time point considered.

(1) Dempster, D. W. et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res. 2013 (28):2-17.

Anatomia Normale Veterinaria - Multidisciplinare
Osteogenic activity, antibacterial, in vivo study

LA TECNICA TERMOGRAFICA NELLA RIABILITAZIONE EQUESTRE DEL SOGGETTO AUTISTICO: RISULTATI PRELIMINARI

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The aim of this experimental work was to verify if the thermographic technique (IRT) could be used during the sessions of hippotherapy to highlight a possible emotion and/or stress in autistic children and animals by means of any change in surface temperature. It was verified whether the different phases of the hippotherapeutic session were characterized by different temperatures that could be related to the physiological or emotional status of subjects.

The trial was carried out at "Centro Ippico Meisino", near Turin, over the period March-November 2013 on three autistic children nine to twelve years old during their equestrian rehabilitation.

The data were analyzed by the SAS[®] software. The analysis considered the periocular temperatures recorded at the various stages of the session and the differences between them, separately for children and horses. The significance of differences was tested by ANOVA model for repeated measures. The different phases were: before working; when the child goes up to the horse; during the working session; after work during the horse's care; during the half an hour after children and horses were separated.

The IRT images were able to detect significant differences of periocular temperature between the various phases, both for children than horses, when the environmental temperature was low (up to 10°C P<0,001). No significant differences were found for the classes of average and high temperatures (> 10 °C). It is extremely interesting that the eye's area trend was the same for horse and children at low environmental temperatures. The periocular temperatures started at 33.0 °C (horses) and 33.3 °C (children) in the first phase, then decreased in the two working phases (32.0 °C horses and 30.9 °C children), before recovering in the last two phases after work (32.6 °C horses and 32.2°C children) .

No hard physic work for humans or children was did during working sessions. The decreasing of the periocular temperature during the working phase may be due to vasoconstriction related to different emotional state.

This pilot study has highlighted the potential of the IRT as non invasive technique to detect small differences in periocular temperature as an objective parameter both in humans than in horses, during the hippotherapeutic sessions. Other studies are needed to better understand relation between periocular temperature and ambiental conditions and to relate periocular temperature changes to the subjects emotional status.

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Rizzolatti G., Sinigaglia C., So quel che fai, il cervello che agisce e i neuroni specchio, Raffaello Cortina Editore, 2006.

Sparrow E. M., Cess R. D., Radiant heat transfer, Brook/ Cole Pub. Co, Belmont, California, 1966.

Thermography, Hippotherapy, Autistic children

INSERTING ANIMALS IN THERAPEUTIC PROTOCOLS DRIVES POSITIVE EMOTIONS IN YOUTHS

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The skeptical debate around animal assisted therapy (AAT) as an actual supportive tool to traditional therapy can be overcome, provided that recovery is considered an improvement from a preexisting disadvantageous condition and that well-being is defined by the presence of positive emotions rather than the absence of illness. In this study we aimed at analyzing the role exerted by different animals as conveyors of positive emotions in youths affected by physical and psychological impairments, and compare it to music therapy (MT).

Nine horses, four donkeys, and five dogs assisted 26 children/adolescents (age 10,7±2,6) with different physical deficits and psychological disorders, going from disadvantage to more or less serious cognitive impairment. The study was carried out by recording clients' emotions (in terms of anxiety, indifference, happiness) at time 0, during, and at the end of each session; this consisted of 45 min. interaction with animals or instruments, doing different tasks, for overall 421 evaluations (355 AAT). The achievement of different goals, established for the clients by their medical equips, was unknown to the operator that recorded youths-animals/instruments interactions until the end of the observations.

The analyses of data carried out by ANOVA demonstrated that AAT was able to significantly change the clients' emotional status to positive, irrespective of their medical conditions ($p < 0,01$). Despite the fact that interactions with donkeys and horses started from higher baseline value than dogs, nevertheless these latter were always able to reach the same positive value of the equids. Sometimes horses were able to get better results respect to donkeys but only when sections included horseback riding. No difference was found when comparing the effect of AAT to that of MT ($p = 0,43$). Lastly, the results of clients' emotions were positively related to the degree of goal achievement as evaluated by the medical teams.

A new concept of welfare call into question that repeated and intrusive measurement of altered physiological markers could fulfill the assessment of recovery. Rehabilitation can be indeed considered as a process oriented to customized goals, where also subjective dimensions (autonomy, personal growth, mastery, self-esteem, confidence, and acceptance[^]) have to be pursued to overcome disabilities. However, while MT is included in different medical practices and it is also taught as a supporting tool to traditional therapy, the role of AAT is still largely debated.

In this report we provided evidence that, similarly to MT, animals positively drive youths' ability to perform the tasks required thus achieving different therapeutic goals. However, since AAT concerns the control of sentient beings, it needs to be attentively regulated to avoid animals' mistreatment for lucrative purpose only.

[^]Bello et al. 2008 *Psichiatria e psicoterapia*, 27-4: 283-291

anthrozoology, animal assisted therapy

animal assisted, therapy, positive emotion

SOCIAL NETWORK (SN) OF FREE-RANGING DOMESTIC CATS

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Social learning, information transfer, and alloparental care represent distinct strategies promoting successful coping in social animals. Cats living in colonies can show all those features, although very scant, often anecdotic evidence has been reported in such a field. Moreover, social behaviors in cats are too often mixed up due to neutering of adult individuals and other human interferences, which in turns lead to a distortion of naturally occurring behaviors.

In this study, the behavior of free-ranging intact cats was evaluated, analyzing the social structure of a colony and the relationship of familiar and unfamiliar subject by means of an SN-based approach.

A colony of 21 free-ranging cats was observed for five months (in spring and summer), three times a day for 30 min. each. The interactions among the subjects of the native group and foreigner individuals were classified in three different categories according to their social meaning: affiliative (Af), neutral (N), and agonistic behavior (Ag). The first and the latter were moreover defined as Af+ when implicating mutual grooming, sleeping together, rubbing, nursing, and playing; on the other hand, extremely agonistic behaviors, such as vocalizations, blowing, scratching, fighting, and scaring were classified as Ag+. The analysis of the network was then carried out by using the open source software Cytoscape 2.8.

During the experimental period, a wide range of social interactions was recorded among all the actors of the original group; sporadic interactions of external subjects were also observed, mainly ascribable to foreigner tomcats during the reproductive periods of the females. Mating behavior, delivery of offspring, and females' interaction towards kittens were observed, showing a strong relationship among some of the cats belonging to the original group. In such a context, the female matriarch showed a pro-social attitude towards every kitten, up to shared breastfeeding. Her central role was further confirmed by the SN analysis in terms of Closeness and Betweenness Centrality (CC 0.083; BC 0.090).

Specifically, the main feature of this colony could be defined as a scale-free, small world network (diameter 4; shortest paths 325; characteristic path length 1.48; average neighbors 7.332). Although at least four different individuals played a key leading role within the group, the highest centrality value was ascribable to a young cat (a one-year old male, CC 0.91; BC 0.19) that displayed a great aptitude at playing with other members of the colony as well as having a protective role against foreigners.

Such evidence was extremely interesting: individuals with high BC, in fact, tend to be information brokers in human societies(1), but that role has never been verified in cats. Further studies are currently underway aiming at clarifying that exciting and unexpected finding.

(1)Bodin et al 2006. www.ecologyandsociety.org/vol11/iss2/resp2/

Animal Behavior (Ethology VET/02)

Cat behavior, Social Network (SN), SN analysis

PLASMA IGF-I CONCENTRATIONS IN HOLSTEIN-FRIESIAN HEIFERS FROM BIRTH TO PUBERTY

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The role of the IGF system is of particular importance in prenatal as well in postnatal growth and development. IGF-I is associated with increased somatic growth. The onset of puberty involves a complex interaction of pituitary, gonadal hormones and growth factors, such as IGF-I. The aim of the study was to study IGF-I plasma concentrations in Holstein-Friesian heifers from birth to puberty.

The study was conducted on 6 female Holstein-Friesian (HF) heifers, born mature and viable. Blood samples were collected from the jugular vein into heparinized tubes, at 10 and 30 minutes (min), 3, 6, 12, 24 and 36 hours (h) after birth, at 7, 14, 21 and 28 days (d), every two weeks from 45 d to 105 d, and then monthly until after the occurrence of puberty, defined as the detection of a corpus luteum by rectal palpation. Separated plasma was stored until analysis by RIA for IGF-I. Possible IGF-I changes among each sampling time of study were statistically analysed by one way ANOVA, followed by the post Hoc Tukey test.

The 6 heifer showed a normal post natal growth and development, and did not show diseases along the time of study. Because puberty occurred at different age among the 6 heifers (8-13 months of age), IGF-I plasma concentrations (means $\pm$ SD) profile from birth to puberty was subdivided in two separate figures, one reporting the profile of IGF-I levels from birth to 28 days of age (Figure 1), and another reporting IGF-I plasma levels from puberty back to 45 days of age (Figure 2).

Results obtained in this study evidenced a significant ($p < 0.05$) increase of IGF-I concentrations from birth to the time- frame between 10 min and the first 6 hours of age, probably due to the colostrums intake. Even if the colostrum IGF-I are not absorbed by the intestinal mucosa, the elevated glucose status in colostrum-fed calves enhance the IGF-I hepatic synthesis resulting thus in elevated plasma IGF-I concentrations after colostrums intake [1]. From 7 to 28 days of age IGF-I return to values even lower to that recorded at birth, followed by a trend of increase from 45 days of age and with a significant ($p < 0.05$) increases in the last 5 months before puberty, with high levels persisting until after puberty occurrence. In humans, IGF-I are reported to markedly increase during puberty with actions amplified mutually with sex steroids in the control of growth, muscle mass increases and skeletal mineralization [2].

1) Hammon et al, 2012 Domest Anim Endocrinol 43, 171-85. 2) Mauras et al, 1996 Horm Res 45, 74-80.

Endocrinologia

PLASMA IGF-I, BIRTH, PUBERTY

Figure 1. Means $\pm$ SD IGF-I plasma levels in the 6 HF heifers from birth to 28 days

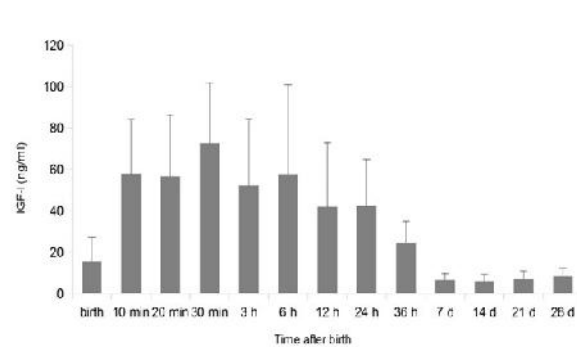
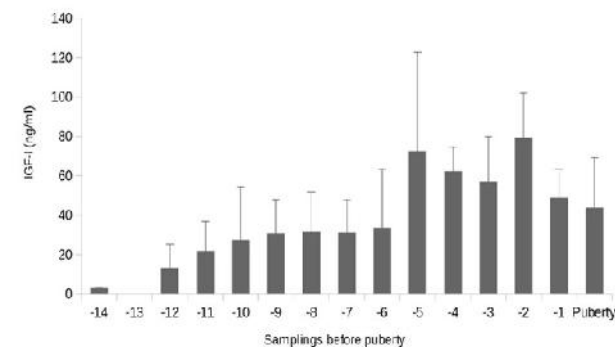


Figure 2. Means $\pm$ SD IGF-I plasma levels in the 6 HF heifers from puberty back to 45 d of age.



ROLE OF THE ENDOCANNABINOID SYSTEM IN SPERMATOGENESIS: SYSTEMS BIOLOGY APPROACH**Ordinelli A.^[1], Barboni B.^[1], Mattioli M.^[1], Di Agostino R.^[1], Bernabò N.^[1]**^[1]*Facoltà di Medicina Veterinaria ~ Teramo*

The spermatogenesis is a complex process that requires the differentiation of male gametes within the male genital tract. It involves a series of cellular events including mitosis, meiosis, cell migration, apoptosis, and differentiation of diploid germ cells (spermatogonia) to form haploid germ cells (spermatozoa). Recently, the endocannabinoid system (ECS) has been proposed to be involved in this process. It is composed by endogenous bioactive lipids, the N-arachidonoyl ethanolamine (AEA, anandamide) and the 2 arachidonoyl glycerol (2-AG), that recognize intracellular and extracellular receptors and by the enzymes responsible for their synthesis, degradation and transport (1). An innovative, computational modeling-based, approach was adopted to investigate the role of the ECS in control of spermatogenesis. In particular, we realized a network (the endocannabinoid system in spermatogenesis, ECSS) in which the nodes represented the molecules of ECS involved in spermatogenesis and the links were the interaction among them (2).

The information on ECS molecules involved in spermatogenesis, from peer-reviewed papers from PubMed, published in last 10 years, were used to realize a database (Microsoft Office Excel 2003). The data were used to build the ECSS network and the statistical analysis of main topological parameters was carried out (Cytoscape 3.0.1 software) (3, 4). In particular the node degree (the number of links per node) distribution, the clustering coefficient (the presence of nodes clusters), the network diameter (the longest shortest path among all pairs of nodes), the mean number of neighbors (the mean number of connections per node), and the characteristic path length (the expected distance between two connected nodes) were assessed.

It was found that ECSS network follows a scale free topology, characterized by a low clustering and easy navigability. In addition, the most connected ECS molecules (the hubs) are: AEA (25 links) CB1 and CB2 receptors (19 and 17 links), FAAH (11 links), NO (8 links). Thus, showing that AEA and the molecules directly involved in its signaling (its hydrolyzing enzyme, the FAAH, its receptors CB1 and CB2, and the related second messengers cAMP, Ca²⁺ and NO) play the key role in controlling spermatogenesis.

From these data it is possible to infer some relevant information, not otherwise obtainable, and to achieve an important goal, the understanding of the role of ECS in spermatogenesis, potentially opening new prospective in drug discovery, diagnosis and clinical application. The ECS appears to be a new potential target for improving reproductive health in humans.

1. Grimaldi et al. *Frontiers in Endocrinology*, 2013,4,192
2. Barabási et al. *Nat Rev Genet.* 2004, 5, 101-113
3. Bernabò et al. *BMC Systems Biology*, 2011, 30,47
4. Bernabò et al., *BMC Systems Biology*. 2010 4,87

Fisiologia Veterinaria

Biological network, Spermatogenesis, Endocannabinoid

VARIATION OF THE ENDOCANNABINOID SYSTEM EXPRESSION IN A RAT MODEL OF VARICOCELE

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Varicocele is an abnormal dilatation of the pampiniform plexus of veins within the scrotum. This pathology is highly prevalent and can impair male reproduction even if the etiology remains uncertain.

Recent evidences demonstrated that male reproduction is controlled by endocannabinoid system (ECS) with a high degree of conservation (from invertebrates to mammals). The expression of the molecular components of ECS has been demonstrated in the testis, in the reproductive fluids, in female tracts thus suggesting a continuous endocannabinoids modulation in reproduction affecting from spermiogenesis to fertilization and implantation.

Starting from these premises, the purpose of this study was to investigate whether endocannabinoid system is involved during the varicocele experimentally induced in rats.

In adult male rats varicocele was induced in left testis as described by Turner . Subsequently rats were sacrificed and left and right testis were removed while spermatozoa were collected from each epididymis.

RNA from each sample was isolated and subsequently reverse transcribed into c-DNA. Relative abundance of each mRNA species was assessed by real-time qRT-PCR. Specific primers were used to amplify sequences of the receptors CB1, CB2, TRPV1 and the enzymes NAPE-PLD, FAAH, DAGL- α and MAGL. All data were normalized to the endogenous reference gene GAPDH. Differences in threshold cycle (Ct) number were used to quantify the relative amount of PCR target contained in each tube. Relative expression of different gene transcripts was calculated by the Delta-Delta Ct (DDCt) method and converted to relative expression ratio (2- $\Delta\Delta C_t$) for statistical analysis.

The present study showed that ECS elements were differentially expressed in testis with experimentally induced varicocele (left). In particular, it has been found that CB2, CB3, FAAH and NAPE were always significantly ($p < 0.05$) over expressed, while CB1, TRPV1 and MAGL remained approximately unaltered ($p > 0.05$). The degree of response resulted quite different amongst rats thus reproducing what usually happens in humans where each patient has a different outcome of varicocele.

For the first time, it was demonstrated that varicocele is accompanied by an alteration of the endocannabinoid genes expression. However, the correlation existing amongst the ECS profile, the histopathological level of varicocele and the impact on the male reproductive function remain to be clarified thus opening new perspectives on the understanding and treatment of male fertility problems.

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fisiologia veterinaria

varicocele, endocannabinoids, rat

FATTY ACIDS, DESATURASE AND ATHEROGENIC INDICES IN THE MILK FAT IN CABANNINA, VARZESE AND FRIESIAN REARED IN NORTHERN ITALY

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The aim of the study is to evaluate the fatty acid composition of Varzese and Cabannina milk samples in several lactation stages, and to compare their characteristics with milk from Friesian cows. Moreover, the desaturase and atherogenic indices of the milk from the three breeds will be compared.

One hundred and five post partum cows of three different breeds (Friesian, Cabannina, and Varzese) were enrolled. Milk was collected from 40 days until 160 days of lactation. Milk fat was processed for fatty acid analysis by chromatography; the fatty acid profile was determined, and on C14, C16 and C18 acids and their cis-monounsaturated homologues, desaturase indices ($\Delta 9$) were calculated as reported by Schennink et al. (2008), and total desaturase index ($\Sigma \Delta 9$) was calculated according to Mele et al. (2007). The fatty acids were expressed as unsaturated (UFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, and as UFA/SFA ratio. Moreover, the atherogenic index (AI) on fat profile was determined. Data were analyzed by analysis of covariance, considering the breed as fixed factor and day of lactation as covariate.

The three groups of cows had a milk mean fat content of 3.53 ± 1.01 %, 4.05 ± 1.14 %, and 4.27 ± 0.87 % for Friesian, Cabannina and Varzese, respectively. Total MUFA content is higher in Cabannina and Varzese breeds than in Friesian, and the same conclusions can be drawn for PUFA (table 1). Total PUFAs increase their overall levels during the follow-up period (table 1). The $\Delta 9$ indices for the three breeds show a significant effect of breed for all indices, with the exception of $\Delta 918$. Cabannina and Varzese evidence higher values ($p < 0.05$) in $\Delta 914$ and $\Delta 916$; $\Sigma \Delta 9$ is significantly higher ($p < 0.05$) in Varzese than in other breeds. All desaturase indices, except for $\Delta 918$ changed significantly ($p < 0.05$ to $p < 0.001$). Atherogenic index did not show a significant temporal trend: by adjusting for time, the breed with the higher AI was the Friesian breed, while the mean lower values were reached by the Varzese cow; Cabannina cow is in an intermediate position (table 1).

Autochthonous bovine breeds yield milk with higher unsaturated fatty acids, either as MUFAs or PUFAs; these features can help the design of new functional dairy products. The FAO report on dietary fatty acids (FAO, 2010) stresses out a "convincing evidence" that replacing SFAs (C12 to C16) with MUFAs could lead to a reduction in blood cholesterol concentrations (Jakobsen et al., 2009). The higher levels in UFA reflects the higher desaturase indices in Cabannina and Varzese cows: these indirect indices of SCD activities must be confirmed through a direct determination on this enzyme.

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Lactation Physiology

Bovine, Autochthonous breeds, milk fatty acids

Fatty acid (%) index	Cabannina	Friesian	Varzese	P-value Breed	P-value Time Slope
UFA	27.61±4.25 ^a	23.22±4.47 ^b	29.55±5.28 ^a	***	n.s.
MUFA	24.07±3.99 ^a	20.93±4.30 ^b	26.64±5.07 ^c	***	n.s.
PUFA	3.54±1.62 ^a	2.30±0.93 ^b	2.91±1.31 ^b	***	7.5·10 ⁻³ *
UFA/SFA	0.38±0.08 ^a	0.31±0.08 ^b	0.42±0.09 ^c	***	n.s.
Δ ⁹ 14	6.86±1.37 ^a	5.81±2.00 ^b	6.51±2.41 ^a	*	28.7·10 ⁻³ ***
Δ ⁹ 16	5.07±1.79 ^a	4.03±1.73 ^b	5.41±2.08 ^a	**	11.0·10 ⁻³ *
Δ ⁹ 18	68.0±4.30	64.91±5.15	67.55±12.96	n.s.	59.3·10 ⁻³ **
ΣΔ ⁹	27.03±4.00 ^a	20.07±4.62 ^b	22.45±5.68 ^c	***	n.s.
AI	3.86±0.74 ^a	4.76±1.12 ^b	3.28±0.64 ^c	***	n.s.

Table 1 – Percentage content of unsaturated (UFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids, UFA/SFA ratio, Δ⁹ 14, Δ⁹ 16, Δ⁹ 18 desaturase indices and atherogenic index (AI) in the milk of the considered species. Superscripts indicate a p<0.05 difference between breeds. *-p<0.05; **-p<0.01; ***-p<0.001 significance. n.s.-not significant.

BIOACTIVABLE SCAFFOLD FOR NEOANGIOGENESIS IN TISSUE REGENERATION

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Angiogenesis is the process through which new blood vessels are formed from pre-existing vessels. This process occurs physiologically during organ and tissue development and in tissues repair. Regeneration of injured tissues is one of the main goals of tissue engineering. Angiogenesis is the process through which new blood vessels are formed from pre-existing vessels. This process occurs physiologically during organ and tissue development and in tissues repair. Regeneration of injured tissues is one of the main goals of tissue engineering implying the recovery of lost biological functions. Angiogenesis represents a key event triggering the formation of a vascular network able to provide oxygen and nutrients to the neoformed tissue. Biocompatible scaffolds represent a valuable structural support, but also as a potential guide of regenerative processes because of mechanical, topographical and/or biochemical signals. The present study aims to

To test our hypothesis, both in vitro and in vivo experiments were carried out. Scaffolds morphology and structures were characterized by scanning electron microscopy and micro-computed tomography (micro-CT), while cell-material interaction was investigated seeding Human Umbilical Vein Endothelial Cells in 2D and 3D culture systems. Cell viability, adhesion and distribution within the scaffold were evaluated at different time-points by confocal microscopy.

An in vivo trial was then performed to test and compare biocompatibility, safety and effectiveness of the two constructs. Scaffolds were implanted subcutaneously in rats and retrieved 7 and 21 days after the implant.

Alginate scaffolds induced a strong inflammatory reaction revealed by a thick fibrotic capsule; PCL scaffolds were perfectly integrated in the surrounding tissue without any sign of inflammation. No animals of either group received immunosuppressive drugs. PCL scaffold neovascularization after retrieval was evaluated by micro-CT analysis.

In conclusion, in our models both alginate and PCL constructs showed a strong in vitro biocompatibility, whereas PCL revealed a better performance in the in vivo study. On the basis of this results we plan to perform further experiments integrating angiogenic factors into the scaffold to improve the temporal control of neoangiogenesis and the functionality of the neoformed vascular network.

Functional porous hydrogels to study angiogenesis under the effect of controlled release of vascular endothelial growth factor

O. Oliviero, M. Ventre, P.A. Netti

Acta Biomaterialia 8 (2012) 3294–3301

tissue engineering

angiogenesis, PCL, alginate

THE CYTOTOXIC EFFECTS OF XYLAZINE ON EQUINE CARTILAGE CELLS: A PRELIMINARY STUDY

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joints and during arthroscopic procedures when intra and post-surgical pain can be considerable, in human and in equine medicine. IA injection of xylazine (xyl) to horses following arthroscopic surgery has been recently reported [1]. Local anesthetics administered IA should provide analgesia avoiding the potential side effects of a systemic administration, such as gastric and kidney ulcers [2]. Although studies have been carried out, the long term effects of local anesthetics on articular cartilage are still not well known. The potential toxic effects of lidocaine, bupivacaine, and mepivacaine on equine chondrocytes have been reported [3].

The aim of this study was to evaluate the effects of xyl on equine chondrocytes in vitro in order to assess the possible tissue outcome induced by its IA injection.

Primary cultures of equine chondrocytes were obtained from metacarpophalangeal cartilage by collagenase digestion (2 mg/ml for 6-8 h at 37°C) and cultured in DMEM+10% FBS at 5% CO₂ and 37°C in humidified atmosphere. Cells at the second passage of subculture were exposed to 0.05%, 0.1%, 0.2%, 0.4%, 0.8%, 1.66%, 2.5%, 5% xyl for 15', 30' and 60'. After treatments, cell viability/proliferation was evaluated by WST-8 assay. After 15' of treatments, apoptosis and necrosis of chondrocytes were analyzed with the double staining of Hoechst 33258 and propidium iodide (PI) using fluorescence microscopy, while lysosomal functionality was assayed by neutral red test (NRT).

The results indicated that xyl is able to induce a dose dependent reduction of vitality and cellular functionality of equine chondrocytes. In particular WST-8 assay evidenced that the 0.05% and 0.1 % xyl treatments had no effects whereas the decrease of mitochondrial activity was 60% with 0.4%-2.5% xyl and 20% with 0.2% xyl. No significant changes were observed between 15', 30', 60'. The NRT indicated a 10% and 30% decrease of lysosomal activity with 0.1% and 0.2% xyl respectively. Fluorescence microscope analysis showed a dose-dependent increase of PI-positive cells that was 8±1.7, 12±4.5 and 49±3.2 with 0.2%, 0.4%, 0.4%, 2.5% xyl respectively.

Although preliminary, this study indicates that xyl at common sedative dose, induces a significant chondrotoxicity. Consequently, its local use in the equine joint could potentially elicit tissue damage. Further in vivo studies with lower drug concentrations are advocated to evaluate a local analgesic effect while preserving joint viability.

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BIO 10 - VET 09

Xylazine, Chondrotoxicity, Intra-arti injection

BIOCHEMICAL CHARACTERIZATION OF 26S PROTEASOMES IN FARM ANIMALS TO IMPROVE KNOWLEDGE ABOUT ANTIGEN PROCESSING**Raule M.**^[1], Cerruti F.^[1], Cascio P.^[1]^[1]*Dip. Scienze Veterinarie ~ Grugliasco*

Aim of the present work was to perform a biochemical characterization of 26S proteasomes in farm animals through a comparative study in eight different animal species. 26S proteasome is a 2,5 MDa ATP-dependent macromolecular protease that degrades ubiquitinated and some non ubiquitinated proteins in eukaryotic cells (1). Furthermore proteasomes are involved in antigen presentation since they produce the peptides (epitopes) that are presented on cellular surface in association with MHC class-I molecules (2).

Firstly, we performed a partial purification, through differential centrifugation, of 26S constitutive proteasome (from muscle) and 26S immunoproteasome (from spleen) in eight different animal species (dog, goat, swine, cat, rabbit, cattle, horse and sheep). Then, we assessed the three proteasomal peptidase activities using specific fluorogenic peptides: 100 μ M Suc-LLVY-amc (for the chymotrypsin-like activity), 100 μ M Z-YVAD-amc (for the caspase-like activity), 100 μ M Bz-VGR-amc (for the trypsin-like activity). Finally, we also performed western blot analysis using primary antibodies against the non-catalytic subunits α 3, α 4, α 5 and α 6.

The results of the spectrofluorimetric measurements show that overall proteasomal enzymatic activity is conserved in the animal species examined despite some species-specific differences. Most importantly the ratios between different proteasomal peptidase activities are maintained, both for constitutive and immuno 26S proteasomes. Furthermore, a substantial conservation of the proteasomal active sites in animals of veterinary interest is demonstrated by a similar sensitivity of the β 5 subunit (responsible of the chymotrypsin-like activity) to three of the most specific inhibitors currently available. Finally, western blot analysis of four different non-catalytic α subunits confirm that also the non-catalytic proteasomal structure is conserved in the species examined.

Our results are consistent with a generally conserved overall structure and enzymatic activities. Nevertheless, to clarify more in detail how the proteasome system works in farm animals, we plan to test the sensitivity of the other proteasomal active sites to specific inhibitors and to study in vitro degradation of model protein substrates.

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Biochemistry

26S proteasome, antigen processing, protein degradation

EXPRESSION AND FUNCTIONALITY OF TRPV1 RECEPTOR IN HUMAN AND CANINE MAMMARY CANCER CELLS.

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Breast cancer is a common leading cause of cancer associated death in women (1). Canine mammary tumors are similar to human ones and they have been proposed as animal models for human breast cancer research. The Transient Receptor Potential Vanilloid receptor 1 (TRPV1) has been identified in both species (2, 3) and some authors have speculated on the key role of the TRPV1 in the regulation of cell proliferation.

The presence of TRPV1 receptor on MCF-7 cells was verified by performing binding assay with labeled resiniferatoxin (3[H]RTX) (4) and Western Blot using a purified goat polyclonal antibody. Displacement (in presence of 3[H]RTX 1.2M) and functionality assays (in presence of 1μCi[45Ca²⁺]/ml) were performed using decreasing concentrations of agonists (Capsaicin, RTX, Anandamide) and antagonists (Capsazepine, Sb-366791, 5-I-RTX) to measure the affinity and the efficacy of the different compounds on the TRPV1, respectively. Then a comparison between MCF-7 cells and CF.41 cells (derived from canine mammary adenocarcinoma) was performed through proliferation assays (5). Experiments were performed plating 5.000 cell/well in 96-well plates and administering the same agonists and antagonists used in the experiments explained previously. At different experimental time points (24, 48 and 72 hours), 20 μl of MTT solution (4mg/ml in PBS) per each well were added and after 4 hours of incubation the plates were read at λ=570nm.

The results of binding assay showed a B_{max} value of 1492±192 fmol/mg, K_d value of 0.03±0.004 mM and r<0.9. The images obtained from Western Blot allowed to identify a signal corresponding to TRPV1 receptor (100kDa). The results of proliferation assays were analyzed using Kruskal-Wallis test and Dunn's multiple comparison test (P<0.05) (Graph Pad Prism 4 Software). The statistical analyses allowed to identify dose-response and time-response effects of the tested drugs on MCF-7 and CF.41 cells.

Data obtained by binding assays and by Western Blot confirm the presence of TRPV1 receptor on MCF-7 cells. The results obtained by competition and functionality assays permitted to rank all drugs, as follows:

- Agonists' affinity: Anandamide > Capsaicin > RTX
- Antagonists' affinity: 5-I- RTX > Capsazepine > SB – 366791
- Agonists' efficacy: RTX > Anandamide > Capsaicin
- Antagonists' efficacy: 5-I- RTX > Capsazepine

The proliferation assays permitted to establish that both partial (Capsaicin and Capsazepine respectively) and high affinity (RTX and 5-I-RTX) agonists and antagonists are able to inhibit MCF-7 cell proliferation (from 50 to 70% at each time point and for all concentrations). The results concerning the CF.41 cells suggest that Capsaicin and Capsazepine seem to stimulate cell proliferation (80% more than control) while RTX and 5-I-RTX seem to inhibit cell growth (50% less than control).

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Farmacologia veterinaria

MCF-7, CF.41, TRPV1

THE PHARMACOKINETICS AND EX VIVO CYCLOOXYGENASE SELECTIVITY OF CIMICOXIB IN HORSES

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To evaluate pharmacokinetic (PK) and pharmacodynamic (PD) properties of cimicoxib, a novel selective cyclooxygenase-2 (COX-2) inhibitor, in fasted and fed horses

Six clinically healthy mares were used in the present study that was approved by the ethical committee of the University of Pisa (n° 001 4896/2013). A pilot study was conducted through administration of cimicoxib intra-gastrically at 2 mg/kg (canine clinical dose) to 3 fasted and 3 fed horses. The full-scale study was an open, single-dose, two treatments, two-period, crossover design (n=6) with a 2 week interval before the cross over. An increased dose of 5 mg/kg was administered due to the low drug plasma concentrations found in the pilot study. Blood (10 mL) was collected at assigned times after cimicoxib administration. The blood sample for PK analysis was placed in a lithium heparin tube and after centrifugation and the harvested plasma was stored at - 20 °C until analysis (Giorgi et al. 2013). For PD evaluation, an additional 10 mL of blood was collected at 0, 1, 4, 10, and 24 hours, split equally to a sodium heparin tube (PGE2) and a non-heparinised glass tube (TXB2). Synovial fluid was collected from the radiocarpal joint of the forelimb using aseptic arthrocentesis at 2 and 10 hours or 4 and 24 hours, after cimicoxib administration. Sample preparations for PD assays was performed according to a previously described method (Giorgi et al., 2011). TXB2 and PGE2 analysis were performed using a commercial ELISA kit (validated for horses) according to the manufacturer's protocol.

Following cimicoxib administration (5 mg/kg), the mean maximum plasma concentration was 0.16 (± 0.01) $\mu\text{g/mL}$ and 0.14 (± 0.03) $\mu\text{g/mL}$ in fasted and fed groups, respectively. The higher dose led to higher peak plasma concentrations and AUC than in the pilot study, though the increment of increase was not proportional to the dose. The mean time to maximum plasma concentration was delayed in the fed group (5.91 ± 3.23 h) compared with the fasted group (3.25 ± 1.17 h) without significant difference ($p=0.12$). In both the pilot and full-scale study, the concentration of cimicoxib in synovial fluid showed similar trends to plasma concentration. However, synovial fluid concentration of cimicoxib varied from that in the plasma at the same collection time by 1.6% to 20%. In the ex vivo pharmacodynamic assay, the mean maximal inhibition rate of thromboxane B2 and prostaglandin E2 was about 60% and 70% respectively, in both fasted and fed groups. In the present study, although the COX-2 selectivity of cimicoxib was not shown, relatively low blood drug concentration of cimicoxib exhibited good COX-1 and -2 inhibitions in horses.

Cimicoxib was found to be not COX-2 selective in the horse. Although further investigations are required at the light of these preliminary findings, the use of cimicoxib in equine practice is not recommended.

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Pharmacology
Cimicoxib, Horses, PK/PD study

PHARMACOKINETICS AND DISPOSITION OF FLUPIRTINE AFTER ADMINISTRATION OF FOUR DIFFERENT FORMULATIONS IN DOGS

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To assess the pharmacokinetics and disposition of flupirtine (FLU) after four different formulations (intravenous [IV], oral immediate release [POIR], oral sustained release [POSR], rectal [RC]) in dogs.

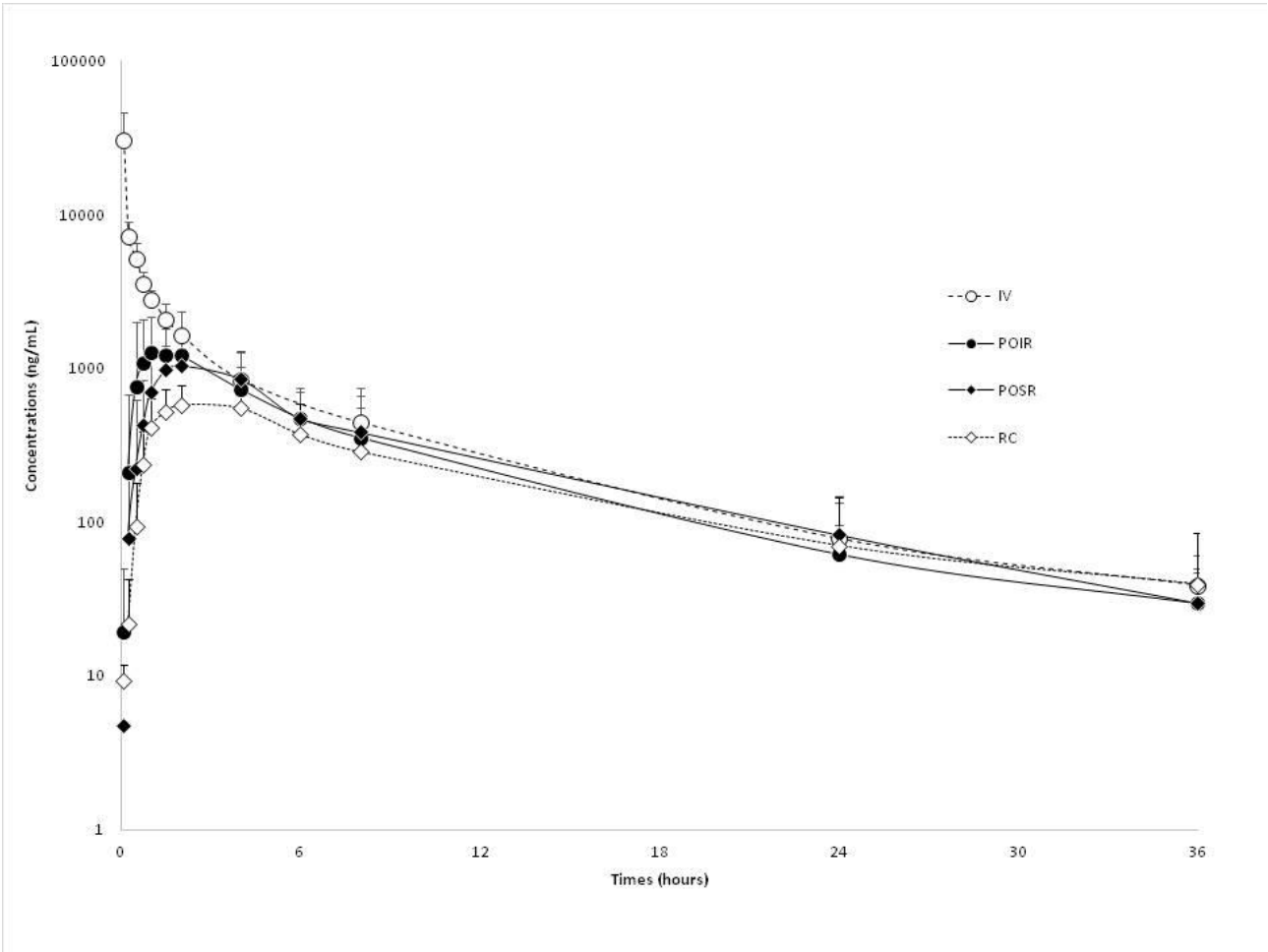
The Animal Welfare Committee of the University of Lublin approved the study protocol. One male and five female Labrador breed dogs were enrolled in the study. Dogs were randomly assigned to four-treatment groups, using an open, single-dose, four-period crossover design (4x4 Latin square). All dogs were fasted for 12 h overnight before each experiment. Dogs in group 1, 2 and 3 received a single dose of FLU 5mg/kg by IV, POIR and RC route, respectively. Group 4 received FLU 200 mg/dog by POSR. A 1-week wash out period was observed among the phases. Blood samples were collected at assigned times and analysed according to a previous validated HPLC method (De Vito et al. 2014). The pharmacokinetic calculations were carried out using WinNonLin v. 5.3 (Pharsight) according to a non-compartmental model.

After IV administration, some adverse effects including salivation, agitation and vomiting were observed in all dogs. However, they resolved rapidly and spontaneously in about 10 min. In the other treatment groups no visible side effects were shown. The average plasma concentration vs time curves are reported in Figure. After POIR, POSR and RC administrations, FLU plasma concentrations were lower than those after IV route, but detectable over the same range of time. POIR and POSR groups showed similar C_{max} and T_{max} values 1549.6±916.3 and 1256.1±353.1 ng/mL, and 1.42±0.58 and 2.16±0.93 h, respectively. Their bioavailabilities (F%) were similar (41.9±8.5 and 36.8±8.4 %, respectively). RC route showed a lower value of C_{max}, (635.3±266.4 ng/mL) obtained at a T_{max} (2.16±0.93 h) similar to the other extravascular administrations. The RC F% was 29.4±8.8 %. The terminal part of all the mean pharmacokinetic curves showed a similar trend of elimination. CL/F values of the extravascular administrations were all not statistically different from the CL after IV injection if normalized for their own F% value. The HL of elimination time after IV route (6.20±0.88 h) showed to be shorter than those from POIR (7.4±1.9 h), POSR (7.1±0.8 h) and RC (7.7±1.9 h). Although no minimal effective plasma concentrations of FLU are reported in humans and animals so far, the 5 mg/kg FLU by POIR and POSR in dogs gave plasma concentrations similar to those obtained in humans undergoing clinical treatment. Further studies are now requested to assess if this drug may be effective in canine medicine.

De Vito et al., 2014 Biomed Chromatogr, accepted

Pharmacology

Flupirtine, Dogs, Biopharmaceutics



PK/PD ASSESSMENTS OF TAPENTADOL IN YELLOW-BELLIED SLIDER TURTLES (TRACHEMYS SCRIPTA SCRIPTA).**Varoni M.V.^[4], Lee H.K.^[1], De Vito V.^[2], Rota S.^[3], Demontis M.P.^[4], Giorgi M.^[2]**^[1]College of Veterinary Medicine, Chungnam National University ~ Daejeon, ^[2]Department of Veterinary Sciences, University of Pisa ~ Pisa, ^[3]Veterinary Clinic Roma Sud ~ Roma, ^[4]Department of Veterinary Medicine, University of Sassari ~ Sassari

In reptiles, administration of opioid drugs has yielded unexpected results with respect to analgesia. Tapentadol (TAP) is a novel atypical opioid drug labelled for human use. The aim of this study was to evaluate the pharmacokinetics (PK) and the pharmacodynamics (PD) of this drug in yellow-bellied slider turtles, after a single IM injection of 5 mg/kg of TAP.

The Animal Welfare Committee of the University of Pisa approved the study protocol (n° 37070/2013). Turtles (n = 9) were randomly assigned to two treatment groups, according to an open, single-dose, single-treatment, unpaired, two-period crossover design. Group A (n = 5) received a single IM dose of TAP (5 mg/mL) at 5 mg/kg and Group B (n = 4) a single IM injection of saline. The wash out period was 1 month. Blood samples (1 mL) were collected from the dorsal cervical at assigned times. TAP plasma concentrations were evaluated according to a HPLC method (Giorgi et al., 2012) while an infrared thermal stimuli was applied to the plantar surface of the turtles' hind limbs to evaluate the thermal withdrawal latency (TWL). The PK calculations were carried out with WinNonLin v 5.3 (Pharsight) using standard non-compartmental equations. The antinociceptive effect was expressed as percentage of Maximum Possible Response (% MPR) and calculated as:

$$\% \text{ MPR} = (T_{\text{test}} - T_{\text{con}}) / (T_{\text{cut}} - T_{\text{con}}) \times 100$$

where T_{test} is TWL value after injection of TAP, T_{con} is TWL value after injection of saline (control) and T_{cut} is the cut-off time.

PK: The plasma concentrations of TAP were in the range (37-1619 ng/mL) and detectable up to 24 h, except in three subjects. C_{max} of 1641 ± 749 ng/mL was observed at 1.22 ± 0.44 h after injection. TAP was eliminated slowly with a long terminal half-life of 4.04 ± 2.10 h and a large volume of distribution of 4.30 ± 1.79 L/kg.

PD: Differences in TWL in controls (n = 9) were not statistically significant at any point tested. The T_0 was 5.66 ± 0.92 s. Animals given TAP showed a drastic increase in TWL 1 h after drug administration (13.32 ± 6.40 s). Subsequently, TWL decreased in proportion to time with significant differences from the controls still apparent up to 10 h and it was still greater than baseline, but not statistically significant, at 24 h. Mean MPR started at 1.69 ± 1.80 % (T_0), increased to a maximum of 46.68 ± 12.30 % at 1 h and decreased to a minimum of 1.62 ± 2.77 % at 24 h. The MPR difference between TAP and controls was still significant at 10 h.

PK/PD evaluation: The mean TAP plasma concentration and % MPR vs time curves were very similar. Average plasma concentration associated with maximum % MPR of 46.68 ± 12.30 % was 1619 ± 242 ng/mL. A linear relationship ($r^2 = 0.99$) between TAP plasma concentration and % MPR was found.

TAP appears to be an attractive option for antinociception in turtles, due to its rapid onset and acceptable duration of effect. However, systemic pharmacokinetics and biophase distribution should be considered along with a sound assessment of drug safety before its use in reptile clinical practice.

Giorgi et al., 2012 J Pharm Biomed Anal 2012 67-68, 148-53

Pharmacology

Tapentadol, Pharmacokinetics, Turtles

PLASMA ELIMINATION KINETIC OF PHENYLBUTAZONE IN THE HORSE**Meucci V.^[1], Minunni M.^[2], Vanni M.^[1], Sgorbini M.^[1], Intorre L.^[1]**^[1]*Dipartimento di Scienze Veterinarie ~ Pisa,* ^[2]*Dipartimento di Chimica ~ Firenze*

Phenylbutazone (PBZ) is a non-steroidal anti-inflammatory drug (NSAID) widely used in equine veterinary medicine (1). The use of PBZ and in general of NSAIDs in racehorses can configure the practice of doping when their pharmacological activity is intentionally used to conceal pathological conditions and to allow to the race animals that should be excluded. Thresholds for PBZ in equine plasma of 15 and 2 µg/ml have been proposed by United States Equestrian Federation (USEF) and Racing Medication Testing Consortium & Association of Racing Commissioners International (RMTC/ARCI). The Federation Equestre Internationale (FEI), through the European Horserace Scientific Liaison Committee (EHSLC), has proposed a detection time (DT) of 168 h for PBZ in blood based on a limit of detection of the analytical method (LOD) of 0.050 µg/ml. The aim of this study was to evaluate plasma clearance of PBZ in healthy horses following intravenous and oral administration of two commercial products marketed in Italy and to compare pharmacokinetic data obtained with both threshold limits and detection times proposed by equestrian sport authorities.

PBZ was administered to 6 horses intravenously or orally at a dose rate of 4.4 mg/kg once daily for five consecutive days. Approval to conduct this study was obtained from the Ethics Committee on Animal Experimentation of the University of Pisa (Rettoriale n. 14540 del 21/11/2011) and transmitted to the Italian Ministry of Health. Blood (10 ml) samples were collected before the last dose (t₀) and then after 1, 3, 6, 9, 12, 24 hours and every 12 hours thereafter; for 10 days. PBZ and OPBZ equine plasma concentrations were determined by using an HPLC UV method coupled to a molecularly imprinted polymers-solid-phase extraction (MISPE) procedure performed according to Meucci et al (2). Pharmacokinetic analyses were performed by WinNonlin Version 5.1 (Pharsight, Mountain View, CA).

The mean pharmacokinetic parameters values of PBZ after its intravenous and oral administration are shown in Table 1. The thresholds indicated by USEF were reached within 4-6 h after intravenous administration and were not reached after oral administration. The thresholds set by RCI were reached within 20 h both after intravenous and oral administration. At 168 h (corresponding to FEI DT for PBZ) both PBZ and OPBZ were not detectable in any horse. The withdrawal times obtained with the regression analysis were 120 and 144 hours for oral and intravenous administration of PBZ, respectively.

The detection time suggested by the FEI for PBZ is then largely protective. Taking into account that the activity of the drug has been finished at 24-36 hours post administration (3) the use of withdrawal times may be adopted for the corrected use of this drug in performance horses.

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Farmacologia

phenylbutazone, elimination kinetic, horse

Table 1 Pharmacokinetic parameters for PBZ in plasma after intravenous and oral administration of 4.4 mg/kg once daily for five consecutive days.

Parameter	Intravenous	Oral
	Mean $\pm$ SD	
C _{max} (μ g/ml)	101.40 $\pm$ 28.10	8.02 $\pm$ 2.10
AUC (h* μ g/ml)	511.80 $\pm$ 106.50	85.07 $\pm$ 17.41
Cl (ml/h)	2.23 $\pm$ 0.52	13.43 $\pm$ 3.14
V _d (l)	30.48 $\pm$ 22.06	671.40 $\pm$ 242.10
MRT (h)	6.71 $\pm$ 0.65	11.72 $\pm$ 0.35

DESCRIPTION OF GASTROENTERIC PARASITES IN THE ALPINE GALLIFORMES IN NORTH-WESTERN ITALY.

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Galliformes are species important to be preserved. Considering the few data available for Italian Alps (Florio & Gamba, 1992; Marco M.A. et al. 1999; Viganò et al., 2012;), it is indispensable to acquire more information about their sanitary status. We have analyzed 79 samples of *Alectoris graeca saxatilis*, *Lyrurus tetrix* and *Lagopus mutus helveticus*, collected in five hunting seasons (2008-2012). The samples come from four different areas of the northwest Italian Alps: Comprensorio Alpino Biella 1 "Biella valleys" (CABI1), Aosta Valley (AV), Comprensorio Alpino CN2 "Varaita Valley" (CACN2) and Comprensorio Alpino CN3 "Maira and Grana Valleys" (CACN3). The aim of the study was to evaluate i) the parasites distribution and ii) their interactions with the host species.

The gastroenteric packets were unravelled, opened and intestinal contents scraped and washed in acqua fontis. After that, it was possible to search and identify parasites. We divided the study area into: "North" including CABI1 and AV, "South" including CACN2 and CACN3.

Twenty-four samples of 79 were positives and in particular: 11 *L. tetrix* of 35 and 13 *A. graeca* of 37. Two nematodes (*Ascaridia* and *Capillaria*) and one cestode were found in *A. graeca* and *L. tetrix*. No parasite was found in *L. mutus* (N=7). The higher prevalence of infestation was found in small intestine first and in caecum after; no parasites were found in stomach. We calculated prevalence(P), abundance(A) and intensity(I) for each parasite species: *Ascaridia* was isolated with higher prevalence and with more infesting charge. *Ascaridia* showed the highest prevalence in *A. Graeca* (P= 24,32%; A= 0,018; I=0,053) while *Capillaria* was found with highest prevalence in *L. Tetrix* (P=17,14%; A=0,015; I=0,049). Cestodes were reported at low prevalence in both hosts (P=8,11% A=0,006; I=0,017 in *A.Graeca*; P=8,57% A=0,007; I=0,024, in *L. Tetrix*).

A geographic influence on parasites distribution was found with *Ascaridia* significantly prevalence in the "North" area (P=66,67% in *A.Graeca*; P=12,50% in *L. Tetrix*).

Florio F.e Gamba M. (1992), Parassitofauna dei Galliformi di montagna sull'Arco alpino italiano, Tesi di laurea in Medicina Veterinaria, Facoltà di Medicina Veterinaria, Università degli studi di Torino, 1992/1993; Marco, M.A.; Barchetti, A.; Guberti, V. (1999), Gastro-intestinal Helminths of three Galliformes species in the Italian Alps, Italian Society of Avian Pathology. 37, p. 699-704;

Viganò R., Formenti N., Ferrari N., Cerutti M. C., Lanfranchi P. (2012), Monitoraggio dello stato di salute delle popolazioni attraverso indagini sanitarie. Congresso Alcotra Italia-Francia n°88 "Galliformi Alpini". Torino, 4-5 luglio 2012.

Parasitology

Galliformes, Nematodes, Western Alps

NEOSPORA CANINUM IN SYMPATRIC WILDLIFE AND LIVESTOCK: EPIDEMIOLOGY IN NORTHWESTERN ITALY

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Neospora caninum was detected by PCR in 38% of aborted calves in Piedmont (Northwestern Italy) (Gennero et al., 2007). A domestic cycle with rural dogs acting as definitive host was demonstrated in the study area (Ferroglio et al., 2006) and possibly linked to a wild cycle maintained by rodents (Ferroglio et al., 2007). To further characterize prevalence and distribution of *N. caninum* in Northwestern Italy, we tested by means of PCR sympatric wildlife and livestock and compared sequenced isolates to assess the degree of homology between domestic and wild isolates of *N. caninum*.

we tested skeletal muscle and central nervous system (CNS) of 299 wild animals (n=81 red foxes; n=106 wild boars; n=112 roe deers) and 147 domestic ruminants (n=79 cattle and n=68 pigs). A species-specific region of *N. caninum* DNA was amplified by PCR using primers Np6plus and Np21plus (Romano et al., 2009). Maximum likelihood phylogenetic trees were used to characterize the sequenced isolates.

N. caninum prevalence differed greatly among species. It ranged from 2.83% (CI95% 0.97%-7.99%) in wild boar, 6.25% (CI95% 3.06%-12.34%) in roe deer, to 17.28% (CI95% 10.58%-26.95%) in fox. None of the swine tested positive, while *N. caninum* was detected in cattle with a prevalence of 4.08% (CI95% 1.88%-8.62%).

DNA sequencing of isolates from wild and domestic animals showed high degree of homology with isolates from sympatric wild rodents. A possible overlap between the domestic and wild cycle of *N. caninum* is confirmed, although the role of dogs and wild carnivores in *N. caninum* epidemiology requires further study.

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parassitologia fauna selvatica

Neospora caninum, wildlife, livestock

ANAPLASMA PHAGOCYTOPHILUM, BORRELIA BUGDORFERI, EHRLICHIA CANIS, DIROFILARIA IMMITIS AND LEISHMANIA INFANTUM IN DOGS FROM ITALY: A NATIONWIDE SEROSURVEY

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Vector-borne diseases (VBD) are among the most frequently emerging diseases in humans and animals (Jones et al., 2008). Human globalization and climate change are enhancing and fastening the ability of vectors to spread to new areas, adapt and establish successful transmission cycles for a wide variety of pathogens (Petric et al., 2012). Under this context we aimed to assess the distribution and sero-prevalence of 5 vector-borne parasites (*Anaplasma phagocytophilum*, *Borrelia bugdorferi*, *Ehrlichia canis*, *Dirofilaria immitis* and *Leishmania infantum*) in dogs in Italy.

A total of 2447 dogs (mean age 6.16 yrs; n=126 breeds) were sampled from 67 provinces across Italy. Approximately half (n= 1291) of the dogs referring to the 241 sampled veterinary clinics, showed clinical symptoms that could possibly be referred to the studied parasites, while n=1137 dogs showed no symptoms of VBD. All dogs were tested using SNAP 4DX (Idexx, Milan, Italy) to detect specific antibodies against *A. phagocytophilum*, *B. bugdorferi*, *E. canis*, and *D. immitis*, while SNAP Leish (Idexx, Milan, Italy) was used to detect anti-*L. infantum* antibodies.

A total of 24.64% [603/2447] dogs tested positive to at least one of the target parasites. Co-infection with two or more parasites occurred in 9.62% of the infected subjects. *A. phagocytophilum* specific antibodies were detected in 85 dogs (prevalence (P)=3.47%; confidence interval (CI) 95% 2.82-4.28%), *B. bugdorferi* in 26/2447 (P=1.06%; CI95% 0.73%-1.55%), *E. canis* in 123/2447 (P= 5.03%; CI95% 4.23%-5.96%), *D. immitis* in 156/2447 (P= 6.38%; CI95% 5.47%-7.41%), *L. infantum* in 272/2447 (P= 11.12%; CI95% 9.93%-12.42%). Traditional and newly endemic areas are delineated across the country with prevalence values in the different provinces as high as 62%.

Canine VBD are widely present in Italy. Distribution and prevalence for each of the five target parasites vary greatly across the nation and reflect the changes in vectors' presence area. The in-clinic diagnosis of VBD in symptomatic and asymptomatic dogs shall become part of routine health care in dogs.

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vector-borne diseases

Vector-borne disease, epidemiology, dogs

STRONGYLOIDES STERCORALIS INFECTION IN TWO DOGS FROM THE SAME KENNEL

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Strongyloides stercoralis is a worldwide zoonotic parasite, infecting men, dogs and cats (1). It is the unique among nematodes of veterinary importance that has both a free-living cycle and a parasitic one (2). The parasitic phase is supported entirely by females, localized in the small intestine. In the dog *S. stercoralis* infection occurs mostly via penetration of skin and mucosal surfaces or by transmammary transmission. Autoinfection also occurs and it can induce persistent strongyloidosis in absence of reinfestation from outside (3).

In dogs clinical disease varies from inapparent form to severe enteritis and pneumonia till sudden death (2). The in vivo diagnosis is difficult due to the low sensitivity of traditional parasitological methods and it is based on larvae detection in faecal samples collected from rectal ampulla. *S. stercoralis* is the only *Strongyloides* species described in naturally infected dogs. In the dog, strongyloidosis has often been reported in tropical and subtropical areas; in Italy clinical cases have never been described and a very low prevalence (0.8%) has been only recently reported in an epidemiological study on intestinal and lung parasites in owned dogs (4). Two clinical cases are described.

Two male adult dogs from the same kennel were presented with an history of chronic severe diarrhea, weight loss, depression (dog1) and of reduced appetite, diarrhea and weight loss (dog2).

Diagnosis of *S. stercoralis* infection was performed in these dogs by larvae detection (by direct and/or Baermann test) and identification in faecal samples collected from ampulla and subsequently by the finding of adult females after scraping of the intestinal mucosa at post-mortem examination. Multiple fecal pool samples from dogs living in same kennel have been examined using Baermann technique.

At clinical examination dog1 was very thin (BCS2) and depressed; in dog 2 (BCS4) a mass was clinically revealed in medium abdomen and its gastrointestinal origin at ileocolic junction was identified by ultrasound. A final diagnosis of linfoma was reached by cito/hystology. Dog1 naturally died after 24 hours from presentation, dog2 was treated for parasitic infection before surgery and monitored. The laboratory alterations common to both dogs at time of presentation were: leukocytosis, mild anemia, hypoproteinemia and increase of alpha2 fraction at serum proteins electrophoresis. Eosinophilia was not detected. The histological features showed in dog1: hemorrhagic parasitic enteritis of moderate-to-severe degree, with the presence of inflammatory linfoplasmocitic exudates in the lamina propria; several adult parasites localized in proximity of the duodenal papilla; a moderate pulmonary atelectasis associated with a moderate interstitial congestion.

Dog2 was treated with febendazole 50 mg/kg/die and metronidazole (25 mg/kg/bid) for 5 consecutive days and monitored for the following ten days.

Fecal pool from other dogs living in same kennel resulted negative to *S. stercoralis* larvae detection at Baermann test.

The clinical cases are discussed focusing on pathogenetic hypothesis (hyperinfection vs concomitant disease). *S. stercoralis* is a zoonotic parasite. Despite natural transmission between dog and man has been rarely reported (2) the potential role of the dog as source of human infection need to be taken in mind, particularly in kennel where overcrowding and reduced hygienic conditions are predisposing factors to the perpetuation of the infection.

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clinica medica

dog, clinical cases, *Strongyloides sterco*

PREVALENCE OF BLOOD PARASITES IN FERAL PIGEONS (COLUMBA LIVIA) IN NORTHWEST ITALY**Scaglione F.E.*^[1], Pregel P.^[1], Cannizzo F.T.^[1], Bollo E.^[1]**^[1] *Dipartimento di Scienze Veterinarie ~ Torino*

Blood parasites have been subject of extensive research since the beginning of the 20th century. The presence of hemoparasites in birds is very common, and it is estimated that 68% of all bird species are susceptible to haemosporidians.

Feral pigeons (*Columba livia*) are widely distributed in the world. Their number is increasing, especially in urban areas. To authors' best knowledge no data on pigeon hemoparasites distribution in Italy are available.

During the years 2010-2011, following the adoption of a regional animal containment programme, 51 Feral pigeons, from Piedmont Region (Northwest Italy) were captured and euthanized according to Italian National Bioethics Committee guidelines and subjected to a standard necropsy. Spleen was collected and frozen at -20°C for DNA amplification, as described by Hellgren et al. (2004) with some modifications. All positive samples were sequenced.

Out of the 51 Feral pigeons tested by nested PCR, 29.4% resulted positive for *Haemoproteus/Plasmodium* spp., and 15.7% for *Leucocytozoon* spp. A highly significant difference between the hemoparasites prevalence ($p < 0.001$) was detected. The coinfection with both hemoparasites was very significant ($p < 0.01$), and a greater relative risk (RR 7.2) to be infected by a second hemoparasite was recorded in already infected birds.

Regarding sex and age distribution of multiple infestation, associations were significant in males ($p < 0.05$; RR 11.0) and in adult birds ($p < 0.05$; RR 10.0).

DNA sequencing of the eight positive samples in nested PCR for *Leucocytozoon* spp. allowed to identify six different lineages: five of them were the same identified in Hooded crows investigated by the authors in the same area, the other was a new lineage in feral pigeons (L-AEMO02). In two animals, two different lineages of *Leucocytozoon* were present at the same time. DNA sequencing of the 15 positive samples for *Haemoproteus/Plasmodium* spp. showed the presence of the already described haplotypes P-SGS1 and H-HAECOL1.

The significant differences in nested PCR detection of *Haemoproteus/Plasmodium* spp. and *Leucocytozoon* spp. infection may be due to variation in vector diversity and population size, and avian community composition, or to a greater susceptibility of pigeons to *Leucocytozoon* spp.

The finding of avian hematozoa in pigeons implies the presence of ornithophilic vectors in Piedmont Region and the susceptibility of this species to infection.

Our results suggest that cross infection of feral pigeons with hemoparasites typical of other migratory or non migratory birds is possible, and should be further investigated and monitored.

Acknowledgements.

We thank the Centro di Referenza di Patologia Comparata 'Bruno Maria Zaini,' Italy.

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parassitologia

BLOOD PARASITES, Pigeons, *Columba livia*

MOLECULAR FINDINGS ABOUT CESTODES PRESENCE IN SICILIAN FOXES

The red fox is the most widespread specie of wild carnivores in Italy and have often been referred to be a resevoir of bacterial, viral and parasitic diseases some zoonotic. For it concern cestodes are largely found in intestine and stool of these animals.

Traditionally the diagnosis of this parasitic disease is made through traditional methods that are copromicroscopic for the eggs and morphological for the adults. The classification of tapeworms normally is conducted on the basis of morphological criteria, the optimization of molecular techniques permits a rapid and accurate identification. In this study a PCR protocol was applied on samples represented of intestines and stool of red foxes to find cestodes presence. Then samples of adult forms were also characterized.

Stool and intestine sample of 65 foxes (*Vulpes vulpes*) were examined after copromicroscopic test and all intestines were explored to find adult forms of parasites. 45 intestine and stool were used to extract DNA with DNeasy Blood & Tissue kit (Qiagen) and QIAamp DNA Stool Mini Kit (Qiagen). The cytochrome c oxidase (cox1) was amplified by Polymerase Chain Reaction (PCR). 1 µl or 3 µl of DNA, intestine and stool respectively, were added to a PCR mixture containing: 1X of 2X QuantiTect Probe PCR Master Mix (Qiagen) and 1 mM of each primer (CO1F 5'-TTTTTGGCCATCCTGAGGTTTAT-3' and CO1R 5'-TAACGACATAACATAATGAAAATG-3'). The volume mix was adjusted to 25 µl with sterile water. The thermal reaction was performed for 40 cycles of denaturation (94°C for 1'), annealing (58°C for 3''), and extension (72°C for 1'). PCR products were verified on 1.5% agarose. 18 PCR products were purified and used for sequencing reaction carried out by Big Dye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies). The sequencing reaction product was purified by Illustra Autoseq G-50 Dye terminator Removal kit (GE Healthcare) and was analyzed by ABI3130 Genetic Analyzer (Life Technologies).

22 intestines and 10 stool sample resulted positive to cox1 PCR. Only 5 stool and intestines sample both resulted positive.

The sequencing resultes were summarized in Table 1.

Primers used in this study could be used in PCR on routinely to confirm the *Echinococcus* presence followed by sequencing to identify many cestodes. This is part of a larger study on parasites hosted by Sicilian foxes This study moreover may give the baseline information to study the cestodes distribution in Sicilian ecosystems.

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MAPPING AND MODELING DIROFILARIA INFECTIONS IN EUROPE

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Climate change and increasing temperatures are a global phenomenon that can influence the dynamics of a number of hematophagous arthropods, vectors of pathogens with importance in human and veterinary medicine (IPCC, 2007). In fact, climatic changes, together with an increase in the movement of dogs across Europe, have caused an increase in the geographical range of *Dirofilaria immitis* and *D. repens* infections. The aim of this study was to verify if the climate of the last 30-years (1980-1989, 1990-1999 and 2000-2012) was such as to facilitate the spread of *Dirofilaria* and allow an increase in the transmission period during the season at risk.

Geographic Information System based on thermal regimen was constructed to identify areas potentially suitable for *Dirofilaria* transmission in Europe (Genchi et al., 2009). These models are based on evidence that: i) there is a threshold of 14 °C below which *Dirofilaria* development will not proceed in mosquitoes; ii) there is a requirement of 130 growing degree-days for larvae to reach infectivity, and; iii) there is a maximum life expectancy of 30 days for a mosquito vector (Fortin and Slocombe, 1981; Lok and Knight, 1998). The output of these models predicted that the summer temperatures (with peaks in August) are sufficient to facilitate extrinsic incubation of *Dirofilaria* even at high latitudes. Recently, an additional model was constructed to verify the influence of temperature in the course of three decades (1980-1989, 1990-1999 and 2000-2012) on the risk of infection by *Dirofilaria* in Italy.

The results showed an expected increasing trend of temperatures, an increase of the *Dirofilaria* generation numbers into the mosquitoes and a significant extension of the infection risk from 5-6 months (1980-1989) to 6.5 months (1990-1999), up to more than 7 months (2000-2012).

These findings show that geospatial tools are very useful for mapping, monitoring, forecasting and surveillance of both heartworm and subcutaneous dirofilariosis.

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Epidemiology

Parasitology, *Dirofilaria*, GIS

PHYLOGENETIC ANALYSIS OF RICKETTSIA PATHOGENS PRESENT IN ITALY

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Rickettsia (family of Rickettsiaceae, order Rickettsiales) is an important cause of emerging infectious disease in people and animals and rickettsiosis is one of the oldest known vector-borne diseases. This study was aimed to the phylogenetic analysis of Rickettsia strains present in Italy to depict the geographical distribution of Rickettsia species and identify the possible introduction of new pathogens, with attention to those pathogenic for humans.

A total of 98 samples was selected from a panel of samples previously identified as positive to Rickettsia spp., of which 6 samples consisted of DNA from 5 Sicilian patients with symptoms of rickettsiosis and 92 from arthropods from humans, animals or free-living. Out of the arthropods, 38 ticks were from north Italy, 14 ticks from central Italy and 40 (38 ticks and 2 fleas) from south Italy. Ticks belonged to the species Ixodes ricinus (51), I. ventalloi (10), Rhipicephalus sanguineus (9), R. turanicus (6), R. bursa (1), Hyalomma lusitanicum (12) and H. marginatum (1). Fleas belonged to the Ctenocephalides felis specie.

DNA samples were analysed by PCRs targeting the ompA (a), ompB (b) and gltA (c) genes. Amplification products were purified, quantified by the Nanodrop and sequenced. Nucleotide sequences were aligned using Bioedit software and compared with those present in GenBank. ClustalW software was used for phylogenetic trees.

Phylogenetic analysis showed that sequences obtained from 4 humans clusterized with R. conorii, while the sequences obtained from blood and tache noir of the fifth patient clusterized, respectively, with R. monacensis and R. massiliae, suggesting the possibility of a coinfection. Sequences obtained from fleas formed a cluster with R. felis.

As concerning ticks, an association between the sequences obtained from R. turanicus ticks and R. massiliae was observed, while H. lusitanicum obtained sequences were found closed to R. slovaca. The analysis showed a relationship between R. aeschlimannii and the sequence obtained from H. marginatum. The almost totality of Rickettsia species detected in Ixodes ticks clusterized together with R. helvetica or R. monacensis, confirming the spread of these pathogens in this vector genus. In particular, sequences obtained from I. ricinus ticks collected in the Northern Italy showed the highest prevalence of these two pathogens, while in south Italy and especially in Sicily, where the tick population is more varied, a greater variety of Rickettsia species was observed. R. helvetica in Sicily was found in I. ventalloi.

The study allows obtaining an overview of Rickettsia distribution in Italy supported by phylogentic analysis.

Acknowledgements: Thanks to Rosa Filippi and Pippo Bono for their technical support.

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Vector borne diseases

Rickettsia, Epidemiology, Ticks

FOCOLAIO DI INFEZIONE DA LEPTOSPIROSI NEL CANILE MUNICIPALE DI PALERMO, TEMPISTICA, OBBLIGHI LEGALI E PROTOCOLLO OPERATIVO OUTBREAK OF LEPTOSPIROSIS INFECTION IN THE MUNICIPAL DOG POUND OF PALERMO, TIMING, LEGAL OBLIGATIONS AND OPERATIONAL PROTOCOL

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Leptospirosis is a disease of humans and animals caused by infection with the motile spirochetal bacterium of the genus *Leptospira*. Leptospirosis as a zoonotic disease worldwide cannot be overstated, because it causes human disease and deaths in much of the world. Acute canine leptospirosis is well known to vet surgeons, as a complex disease. In the present work, Authors refer about an outbreak of Leptospirosis infection in the municipal dog pound of Palermo (Italy) during 2013. This event was the first case of large-scale infection over the past few years and it simultaneously occurred in both the two municipal sites of dog pounds. The purpose of this paper is also to describe some epidemiological features of the canine leptospirosis to provide scientific arguments to confirm this zoonosis as a re-emerging disease.

From the moment of the first clinical suspect of Leptospirosis infection concerning a dog found dead in its cage, the local Department for Veterinary Prevention (ASP 6) implemented the safety procedures indicated by the national laws on animal infectious diseases. Immediately after the diagnostic confirmation of the disease on the first deceased dog, the outbreak of Leptospirosis was officially formalized with an ordinance from the city Major. The Department for Veterinary Prevention ASP 6 has put in place all the needed measures to define, locate and prevent the outbreak expansion. All dogs hosted in the two affected municipal pounds were clinically controlled and their serum samples collected; these were analyzed by Istituto Zooprofilattico Sperimentale of Sicily using the microscopic agglutination test (MAT). Also the necropsy on the deceased dog was carried out at Istituto Zooprofilattico. Laboratory results confirmed the Leptospirosis infection.

Leptospirosis is increasingly diagnosed as a re-emerging canine disease in Palermo. Intervention measures were adopted for the prevention the risk of leptospirosis transmission. After establishing disease surveillance and laboratory support service, no other new infection was recordered. Reducing access to infected water environmental immediately had decreased the spread of infection as well as the reducing of kennel overcrowding and the remediation of contaminated areas.

The strict application of the related national Regulation led to resolving the outbreak.

Richard et al., (2010) Vet Clin Small Anim 40, 1091-1101

Animal health

Animal health, Leptosirosi, Canine disease

FOCOLAIO DI INFEZIONE DA LEPTOSPIROSI NEL CANILE MUNICIPALE DI PALERMO, SICILIA OUTBREAK OF LEPTOSPIROSIS INFECTION IN THE MUNICIPAL DOG POUND OF PALERMO, SICILY

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The Leptospirosis is an economically important zoonotic disease caused by a spirochaete bacterium of the genus *Leptospira*. Acute canine leptospirosis is well known to vet surgeons, as a complex disease and although vaccines are widely available, many people feel that the current products do not provide sufficient protection. Approximately 250 different serovars have been identified in the *Leptospira* complex. Many of the isolated are of unknown clinical importance in any species. Six to eight serovars are pathogenic in the dog. *L. interrogans* serovars icterohaemorrhagiae and canicola were believed responsible for most clinical cases of canine leptospirosis. Leptospirosis can be transmitted directly between hosts in close contact by urine, venereal route, placental transfer, bites, or ingestion of infected tissues as the organism through mucosa or broken skin. The reservoir hosts include dog (generally for *L. interrogans* serovar canicola), common rodents, skunks, raccoons, farm animals and deer. Indirect transmission occurs through exposure of susceptible animals or humans to a contaminated environmental, where the organism persist after exposure from urine of an infected host. In this aim we report the serological data regarding the occurrence of outbreak of Leptospirosis infection in the municipal dog pound of Palermo (Italy) during 2013.

Dog sera samples totally analyzed were 413 and the diagnosis of Leptospirosis infection was based on the detection of antibodies by serological microagglutination test (MAT). The analysis was conducted to eight common serogroups circulating in Sicily (*Leptospira australis*, *Leptospira ballum*, *Leptospira canicola*, *Leptospira grippityphosa*, *Leptospira icterohaemorrhagiae*, *Leptospira pomona*, *Leptospira serjroe*, *Leptospira tarassovi*). A real-time PCR method for the detection of the gene *lipL32* of pathogenic *Leptospira* was also set up for the diagnosis of leptospirosis and used to confirm the seropositive results obtained from serological testing.

The infection hit at the same time two sites of the municipal dog pound, geographically distant. Twenty-four of the 413 dog sera samples analyzed resulted positive to *Leptospira* spp. To confirm serological diagnosis bacterial DNA was amplified from kidney of dogs deceased during the outbreak by a PCR Real-Time. The assay successfully detected leptospiral DNA from samples and is a methodology that can provide a diagnosis in a timelier manner compared to culture for rapid diagnosis of leptospirosis.

These results demonstrate that the leptospirosis is an underestimate infection; therefore is important to think that is an actual zoonosis.

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serological surveillance

Leptospira, Canine disease, Zoonosis

HERPESVIRUS FELINO: INCIDENZA NEL TERRITORIO SICILIANO E VALUTAZIONE DI STATI DI CO-INFEZIONE CON IL CALICIVIRUS FELINO

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Feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1) are the two primary causes of upper respiratory tract disease in cats. The aim of this study was to demonstrate the distribution of FCV and FHV-1 among the feline population in Sicily.

The study was conducted on 60 cats with or without clinical signs of respiratory disease from different locations, including households, breeding catteries, veterinary clinics and animal hospitals sites on area of Palermo and Messina. The samples consisted of conjunctival swabs and sections of various organs (spleen, liver, lung) collected at necroscopy.

Thirty-seven cats were young, twenty-three were adults; thirty-seven were female and twenty three were male. Were collected 129 samples (80 organs and 49 conjunctival swabs). The virus isolation was performed in CRFK cells and, subsequently, the identification was confirmed by PCR. FHV-1 alone was isolated from 18.4% of cats, FCV alone from 11,7%, both viruses were isolated from only 8,4% of samples tested. The PCR and RT-PCR confirmed respectively FHV-1 in 10% of samples and FCV in 8,4% of samples; all resulted already positive with virus isolation. They was not significant differences on gender or age. Both viruses were isolated from cats that showed no signs of disease.

The results of our study was in according with that reported in other countries. The data suggest that a carrier state is common for both viruses in the evaluated population. Both viruses were present in Sicily, but appear a more prevalence of FHV-1. The virus isolation appear more sensible than PCR in evaluation of presence of virus; however the PCR are considered the gold standard in diagnosis of these diseases.

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Clinica medica

Feline Herpesvirus-1, FCV, cats, , Virus Isolation, PCR

CANINE LEISHMANIOSIS (CANL): AN EPIDEMIOLOGICAL SURVEY IN THE PROVINCE OF TRENTO

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In the last few decades *Leishmania infantum* infection has spread northward reaching the foothills of the Alps in northern Italy (1-2). Some areas of the Province of Trento are considered a "risk zones" due to the presence of phlebotomine sandflies, CanL clinical cases reports and serological positive dogs (2-3). In order to review and assess CanL spreading a serological and entomological survey was carried out during a two years period (2012-2013) in the Province of Trento (4).

Three study areas were identified : area A ("high risk" area) with certain previous autochthonous CanL reports, area B (potential risk area): with climate conditions potentially favorable for the sandfly life cycle and no confirmed autochthonous CanL cases, area C ("no risk" zones) with no reports of sandflies and cases.

Serum samples from 644 dogs were collected and the indirect fluorescent antibody test (IFAT) was performed. Antibody titre of 1:40 was considered the threshold to distinguish positive/negative samples.

A standard form with detailed data about each dog sampled (origin, age, sex, breed, living place, co-habitation with other dogs, vaccination, lesions) was completed.

Presence of sandfly vectors was mainly assessed using oil coated sticky traps placed in suitable sites.

Statistical significance of effect variables was tested using the likelihood ratio statistic.

Out of 644 dogs (455-area A, 166-area B and 23-area C) 10% were positive: 52 in area A and 12 in area B. Thirtysix animals resulted positive with titers between 1:40-1:80, 28 with titers $\geq$ 1:160 (maximum value 1:2560).

Overall 1281 sticky traps were set in 28 sites of 19 municipalities (in areas A and B) (average of 9-7 traps/site) during the vector activity (June-October). Sandflies were found in 2 of 28 sites of two municipalities near the province of Verona (Arco-Avio) in September 2012. Only one phlebotomine in each trap have been captured; both the sandflies were identified as *Phlebotomus perniciosus*.

Seropositive dogs, found only in area A and B defined the actual limit of the infection. Seropositive rate in valley/hill was significantly higher than in mountain sites. Living outside and living with other dogs represented relevant risk factors for *Leishmania* seropositivity according to other studies (5). Dogs housed outside at night were more likely to be positive than dogs living inside (5-1).

No differences between seroprevalences in young and old dogs have been demonstrated; on the other hand considering dogs stratified according to sampling area, in area A older animals ($\geq$ 5 years) were more likely to be seropositive than young ones (<5 years) (14% vs 9.4%) as expected in endemic areas, while in area B seroprevalence was higher in young dogs: 11.6% vs 3.9% suggesting a recently establish focus of infection.

Regarding entomological survey the present study included new monitoring areas (area B) never investigated before: phlebotomines were found only in area A, as shown in previous studies (3) suggesting a low population density. However, 4/ 12 positive dogs (area B) could represent autochthonous cases due to their local origin suggesting that an intensive trapping should be organized in the future.

A Leishmaniasis survey (serological/entomological) has been carried out in a wide area of the province of Trento for the first time. Phlebotomines were found only within sites in endemic area of the province as

previously described but seropositive dogs were found in new monitoring area. Further investigations are necessary in these latter areas to define CanL risk map.

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This work was funded by the Province of Trento (DGP n. 1395 del 28/06/2012)

parassitologia e zoonosi

leishmaniasis, epidemiology, Trento

PARENTERAL AND ORAL ADMINISTRATION OF AN ATTENUATED SALMONELLA TYPHIMURIUM VACCINE IN PIGLETS: HISTOPATOLOGICAL FINDINGS

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Salmonellosis is the first cause of gastroenteric diseases in humans, mostly due to contamination of pork products (Boyen et al., 2008). The use of vaccines in pigs could represent a valid method to minimize the spread of *Salmonella* spp. in the environment (Rostagno, 2011). Aim of this work was to compare the effects on the intestinal mucosa of *S. Typhimurium* Δ znuABC when administered orally or parenterally in piglets. Histopathological characterization was then performed, in order to evaluate the impact of vaccination and infection on intestinal tracts.

Twenty-five *Salmonella*-free weaned piglets were divided into 4 groups: Group A (5 piglets) was intramuscularly vaccinated with 10^4 CFU of *S. Typhimurium* strain Δ znuABC (an isogenic mutant strain); groups B (5 piglets) and C (4 piglets) were intragastrically vaccinated with 5×10^7 and 5×10^5 CFU of *S. Typhimurium* strain Δ znuABC, respectively; group D (11 piglets) was used as control. Six weeks after vaccination, five naïve piglets of group D were challenged with 4×10^8 CFU of wild type *S. Typhimurium* ATCC 14028, and all the groups were allocated in the same barn for two weeks to allow the contact of vaccinated and naïve animals with the shedder ones. Piglets were euthanized four weeks. Samples of ileocecal lymph nodes, ileum, caecum and colon were collected and submitted to histological analyses. A numerical value based on the degree of lesions was assigned to each examined intestinal section, taking into account epithelium, submucosa and Peyer's patch conditions, congestion and lesion patterns.

Histologically, naïve piglets showed the most severe lesions in all the intestinal tracts. Epithelial conglutination and necrosis were associated to vascular congestion and lymph nodes depletion. Group C, being the most affected of the vaccinated groups, showed microscopical lesions similar to those observed in naïve piglets. Conglutination was present in 94.4% of examined tracts in group D and in 50.0% in group C, whereas only 26.7% and 20.0% of intestinal tracts of group A and B were involved ($p < 0.0001$). Group A and B showed a milder degree of lymphocytic and eosinophilic inflammation.

The infection, acquired by direct contact with piglets shedding wild type *S. Typhimurium*, showed marked differences with the pattern observed in animals infected by oral challenge, revealing a clear involvement of the ileum, with evident signs of reactivity. When wild type *S. Typhimurium* is intragastrically administered, a high number of salmonellae reaches the gut and invades the mucosa of the colon and the cecum in a very limited period of time. At the opposite, when piglets are infected by a continuous contact with *S. Typhimurium* released by shedding piglets, they are exposed to a reduced number of salmonellae, but for a protracted period of time. In such condition, the ileum represents a preferential route of *Salmonella* entry.

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S. Typhimurium, Swine, Intestine

MONITORING ANTIBIOTIC CONSUMPTION AGAINST MASTITIS IN UMBRIAN DAIRY COW HERDS: A PRELIMINARY STUDY.

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Veterinary pharmacovigilance consists of an integrated system dealing with evaluation and improvement of safety and efficacy of veterinary medicines. Antibiotics are one of the most used medicines in dairy cows to treat and prevent mastitis and they are still used massively with possible presence of drug residues in milk, which can cause the slowdown and the destruction of fermentation bacteria, possible allergic reactions in hypersensitive humans and the selection of resistant germs (1). In Italy a systematic research of drug residues is done by the implementation of the PNR but we must realize that only certain classes of antibiotics are considered and little is known about their real usage. The aim of this work is to evaluate the use of antibiotics in Umbrian dairy herds and the presence of residues in bulk milk and in single cow milk using Delvotest®, which is one of the most sensitive techniques in discovering β -lactams and sulphonamides (< MRL)(2).

Classes of most used antibiotics were obtained by a control of registers of treatments after farmers' interviews. A total of 540 samples (July-December 2013) of bulk milk samples were analyzed using Delvotest®. Farmers were also asked to sample milk from treated single cow; for each cow 2 samples were collected: one during the last milking inside withdrawal periods and one during the first milking after withdrawal periods. 12 samples from 6 cows were obtained and analyzed using Delvotest®.

All the analysis regarding bulk milk were negative. This may be due to both the observance of withdrawal periods and the dilution factor of bulk milk. Two samples, from a cow treated with an association of penicillin G (PG) + dihydrostreptomycin (DS) were positive at microbiological screening. Therefore they were analyzed with a confirmatory method using liquid-chromatography high-resolution mass spectrometry technique. No residues of PG were found. Whereas the results showed the presence of DS at 1600 $\mu\text{g/kg}$ and 1100 $\mu\text{g/kg}$ respectively.

MRL for DS is 200 $\mu\text{g/kg}$ so the level measured immediately after the withdrawal period was about five-fold higher than the MRL. Our results may be explained considering individual peculiarities in antibiotic metabolism, pharmaceutical factors and events associated with drug administration (3). However, the high DS concentrations detected may be the results of a different way of administration in contrast with line guides in the leaflet. Nevertheless, after the analysis, the farmer was advised to practice a more responsible use of antibiotics in daily routine. Furthermore all the farmers were encouraged to carry out individual residues monitoring before cow's reintroduction in the milking group, which is a good control strategy.

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Farmacologia, Farmacovigilanza, Ricerca di residui
Antibiotic residues, Milk safety, Pharmacovigilance

MULTIDRUG RESISTANCE ASSOCIATED TO CEPHALOSPORINS-RESISTANT ESCHERICHIA COLI FROM BOVINE MASTITIS

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The aim was to compare the rate of multidrug resistance in *Escherichia coli* selected randomly and based on resistance to ceftiofur. The study targeted strains isolated from bovine mastitis.

E. coli (n = 107) isolated from bovine subclinical and clinical mastitis in Northern Italy dairy farms were included. *E. coli* isolation and identification were carried out following the National Mastitis Council guidelines (1). The isolates were selected following two criteria: A) 53 *E. coli* were randomly chosen to represent each farm; B) 54 *E. coli* were selected on MacConkey agar with 8 mg/L of ceftiofur (2). Ceftiofur was used as a representative of cephalosporins class (3). Antimicrobial susceptibility was tested in vitro by minimal inhibitory concentrations (MICs) of 16 antibiotics using Sensititre susceptibility plates according to supplier instructions (Trek Diagnostics System, East Grinstead, UK). CLSI recommendations and resistance breakpoints were used (4). A χ^2 test was used to compare the two group A and B. Results were considered significant if $P < 0.05$.

All the isolates, regardless to the group, were susceptible to Amikacin, Imipenem and Cefoxitin ($P=0.061$). The resistance frequencies to most antimicrobials were significantly different between group A and B. The *E. coli* of group B were more frequently resistant to other antibiotic classes than group A. P was 0.001 for ampicillin, ticarcillin, cefpodoxime, Ticarcillin/clavulanic acid, Trimethoprim/sulfamethoxazole, Cefazolin, Doxycycline, Ceftiofur, Marbofloxacin and Chloramphenicol, 0.030 for Amoxicillin/clavulanic acid and 0.070 for Gentamicin.

The results confirmed the usefulness of ceftiofur to detect resistance to cephalosporins (3) and the link with multidrug resistance. A growing number of cephalosporin-resistant *E. coli* are extended-spectrum beta-lactamases (ESBL) producers and multidrug-resistant (5). A monitoring based on a selective medium could reveal a higher than expected prevalence of resistant strains.

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Malattie infettive degli animali domestici

Mastitis, *Escherichia coli*, Multidrug resistance

ATRIO-VENTRICULAR VALVULAR LESIONS IN A WILD LONG-FINNED PILOT WHALE (GLOBICEPHALA MELAS)

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Aim of this work is to describe a case report of atrio-ventricular valvular lesions in a wild Long-finned Pilot Whale (*Globicephala melas*) stranded in Italy.

A wild female 3 years-old Long-finned Pilot Whale, measuring 360 cm in length, was found death stranded in October 2013 along the ligurian sea cost of the Savona province (Italy). Gross examination was performed. Tissue specimens were fixed in 10% buffered neutral formalin, paraffin embedded sections were stained with hematoxylin and eosin, Alcian PAS, Weigert - Van Gieson and Toluidine blue, and examined microscopically.

At gross examinations the heart revealed a moderate increase in volume due to dilation of the right ventricle, and an intimal roundish, 0.5 cm in diameter nodule in the first part of the aortic arch. The epicardic fat was multifocally brown and the pericardium appeared thick and opaque.

Mitral and tricuspid valves were thick with an irregular atrial surface. Valve edges were translucent, rounded, with some foci of whitish proliferation, and leaflets did not fit together. Moreover a mitral leaflet appeared thick and dome-shaped, causing a valvular prolapse.

The aortic valve appeared also thick, with proliferation on the ventricular surface, and a dilation of the artery was present over the semilunar valve.

Microscopic examination showed the presence of a severe pericardial lymphocytic steatitis. The aortic nodule was composed by fibro-elastic fibers probably due to jet impact lesions caused by abnormal blood flow resulting from the valvular insufficiency.

The valves showed a fibrous proliferation with an increasing in myofibroblasts consistent with a diagnosis of valvular fibrosis and an enhancement of the fundamental substance. Deposition of eosinophilic interstitial matrix, and degeneration of the fibrosa and of the spongiosa were observed, allowing a diagnosis of endocardiosis. In this animal, proliferation and endocardiosis coexist as occurs in cattle and horses.

Gross and microscopic investigations allowed to detect a mitral, tricuspid and aortic valvular disease combined with dilation of the aortic arch and presence of impact jet lesions associated to chronic valvular insufficiency, causing dilation of the right ventriculum and of the pulmonary artery.

Acknowledgements. We thank the Centro di Referenza di Patologia Comparata 'Bruno Maria Zaini,' Italy.

Anatomia patologica

VULAR LESIONS, PILOT WHALE, GLOBICEPHALA MELAS

SUBLEUKEMIC ACUTE MYELOID LEUKEMIA WITH CONCURRENT MYELODYSPLASIA IN A HORSE

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Acute myeloid leukemia (AML) is rarely reported in horses and myelodysplastic syndrome (MDS) has been described in one case. AML is defined based on the presence of at least 20% blasts in the bone marrow or blood, while MDS is characterized by morphologic abnormalities in one or more cell lineages with a hypercellular bone marrow, < 20% blasts and concurrent cytopenias due to ineffective hematopoiesis. The use of flow cytometry (FC) and cytochemistry (CC) best supports the diagnosis and classification of AML in humans and dogs, but only a few cases have used these techniques in horses (1). We report a case of acute myelomonocytic leukemia (AML-M4) with myelodysplastic features in a horse.

A 6-year-old female WB horse with a 4-month history of poor performance and pancytopenia was referred. At admission, the horse was in good body condition with pale mucous membranes. Blood and bone marrow samples were obtained for hematological, cytochemical and immunophenotypical analysis and for cytologic and histologic examination, respectively.

CBC showed severe, non-regenerative, macrocytic anaemia, thrombocytopenia and neutropenia. On blood smears a left shift in neutrophils to myelocytes, promonocytes, 7% blasts and numerous small RBC fragments, all with the same shapes, were seen. Bone marrow cytology showed a dominance of myeloid cells comprised of 30% blasts, with evidence of neutrophilic (37% of cells) and monocytic (32% of cells) differentiation. Dysplasia was evident in neutrophils (giant forms) and erythroblasts (asynchronous maturation). Histology confirmed a dominance of myeloid progenitors with high mitotic rate, rare erythroid precursors and no megakaryocytes. CC revealed that blasts were positive for the neutrophil marker, chloroacetate esterase and negative for alkaline phosphatase (ALP) and α -naphthyl butyrate esterase. FC showed that the majority of cells were neutrophils and monocytes, based on size and granularity, positive expression of CD172a, and mixed expression of CD14, MHCII, CD2, CD4, CD8 and CD21. The diagnosis of AML-M4 with concurrent myelodysplasia was made.

AML-M4 is characterized by at least 20% blasts (myeloblasts, monoblasts, promonocytes), plus 20% or more neutrophils and 20% or more monocytes on marrow (1). CC supported a granulocytic origin for blasts. The lack of ALP in myeloid cells was aberrant. The variations of cytochemical behavior of leukemic cells is not documented in horses. FC excluded a diagnosis of lymphoid neoplasia and supported neutrophil and monocytic differentiation of the leukemia. The expression of lymphoid markers on the myeloid cells was also aberrant. The presence of MDS with AML is associated with a poorer prognosis and the long history of illness suggests that a prior MDS had progressed to the AML (2).

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Hematology, Clinical Pathology

Myeloid Leukemia, Myelodysplasia, Horse

FLOW CYTOMETRY IS USEFUL TO DETECT CYTOLOGICAL MISDIAGNOSIS OF CANINE LARGE CELL LYMPHOMA

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Canine large cell lymphoma is routinely detected by means of cytology. Flow cytometry is useful to define immunophenotype and to stage the disease [1]. Acute leukemias can infiltrate lymph nodes and cytological differential diagnosis is challenging [2; 3]. Aim of the study is to describe immunophenotypic and haematologic features of acute leukemias infiltrating lymph nodes

Thirteen cases of acute leukemias with lymph node involvement were included. All cases were previously diagnosed as large cell lymphoma by cytology. Flow cytometric analysis of lymph node (LN) and bone marrow (BM) aspirates and peripheral blood (PB) was carried out using a panel of antibodies including lymphoid (CD3, CD5, CD4, CD8, CD79, CD21), myeloid (CD11b, CD14, NSA), pan-leukocytic (CD45) and precursor (CD34, CD117) markers. CBC and cytologic examination of BM smears were performed. Diagnosis was based on immunophenotype and bone marrow infiltration >20% [2].

According to the expression of lineage-specific antigens leukemias were classified as myeloid (AML, n=8), lymphoid (ALL, n= 2, B lineage) and undifferentiated (AUL, n=3). All dogs were purebred except two; 6 were golden retrievers. Age ranged 1 to 11 years (median 6 years). Forward scatter properties showed a prevalence of medium-sized cells in AML and AUL, and of large-sized cells in B-ALL (resembling those of large cell lymphomas). All neoplastic cells expressed CD45 at low intensity and CD34 at least partially. CD117 was detected in 7 cases. Four AML showed partial expression of CD14, suggesting a myelomonocytic differentiation. Differences in antigen expression by neoplastic cells in LN, PB and BM were noted in some cases. Leukocyte count in PB was within reference interval in 1 case; 7 cases showed leukocytosis (16000 – 572000 WBC/ul) and 5 cases were leukopenic (420 – 5100 WBC/uL). Non-regenerative anemia and thrombocytopenia were detected in all cases except one (B-ALL) and two (1 AML and 1 AUL), respectively. While BM infiltration by neoplastic cells was always obvious both at microscopic examination and flow cytometric analysis, PB involvement was very variable (blasts count: 0-98%). Follow up data were available for 7 cases; overall survival ranged from 3 to 111 days (median 13 days).

Cytologic examination of lymph nodes can lead to misdiagnosis of lymphoma. Flow cytometric evaluation of lymph node aspirates allows an easy and quick identification of myeloid neoplastic cells leading to a diagnosis of AML. CBC should always be run beside cytologic examination in case of suspected lymphoma, because it can reveal an overt acute leukemic picture. However, a very low or undetectable infiltration of PB is possible. BM evaluation is always diagnostic and it is mandatory to differentiate between large cell lymphoma and ALL

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PATOLOGIA CLINICA, ONCOLOGIA, DIAGNOSI DI LABORATORI
canine lymphoma, flow cytometry, cytology

EPIDEMIOLOGICAL DATA OF CANINE MULTIPLE MYELOMA IN ITALY: A RETROSPECTIVE CASE-CONTROL STUDY.

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Multiple Myeloma (MM) in dogs has been rarely reported. In dogs and humans it is an incurable malignant plasma cell disorder. A rate of <1% of all canine malignant tumours and 8% of canine hematopoietic tumours has been previously reported (1). A large study including 60 cases of canine MM established prognostic criteria (2).

The aim of this study was to present epidemiological data of canine MM in Italy.

The electronic medical data-base P.O.A System Plus 9.0® of the San Marco Veterinary Clinic, Padua, Italy was searched between October 2002 and February 2014 for dogs with diagnosis of MM (Group 1, n=65). All dogs included in Group 1 met the three following criteria: bone marrow plasmacytosis (≥20% of plasma cells), osteolytic lesions and serum clonal gammopathy. All the other dogs presented within the same period of time to the clinic were included in the study as control (Group 2, n=47,949). Sex (including neuter status) and age were compared between dogs with MM and control dogs. Normality of data was assessed with the Shapiro-Wilk test. Difference in age between groups was analysed by Mann-Whitney test and difference in sex (including neuter status) by Pearson's X2 test. Risk to develop MM within the different breeds was evaluated by Odds ratio using the frequency of MM in mixed breed as baseline. For all statistical analyses, the significance level was set at p<0.05.

A total of 47,949 canine medical records were included in the study. Seventy-six dogs with MM were identified (prevalence 0.16%; 95% CI 0.12-0.20) and 65 were incident cases (dogs who has just developed MM for the first time). Dogs affected with MM were more likely to be female neutered (p=0.005) or intact male (p=0.024) compared to controls. There was a significant difference in age between Group 1 (median 124 months; range: 68-145) and Group 2 (median 78 months; range: 0-203; p<0.0001).

Twenty-two different breeds were affected with MM. The Giant Schnauzer was at increased risk to develop MM (p< 0.0001; OR = 16.52; 95% CI 5.72-47.7).

To our knowledge this is the first epidemiological study on canine MM conducted in Italy. Giant Schnauzer, female neutered, intact male and older dogs are at increased risk to be diagnosed with MM.

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medicina interna oncologia

multiple myeloma, dog, epidemiological

CANINE MAMMARY CYTOLOGY: CAN WE IMPROVE THE DIAGNOSTIC POWER?

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To evaluate the cytologic features that may help in achieving a diagnosis of malignant mammary tumours Cytological specimens and their histopathological samples from subcentimetric mammary nodules in bitches of variable age and breed reached at the Veterinary Teaching Hospital of the University of Pisa from January 2012 to May 2013 were collected. All cytologic samples were evaluated for nineteen specific cytological features as well as for mean nuclear area by NIS-Elements D 3.1 (Nikon). Data were analyzed with Mann-Whitney U test by SPSS 21.0.0 program

40 nodules were included; 25 malignant carcinoma and 15 benign lesions. Reticular or granular chromatin pattern ($p=0,026$), abundant macrophage infiltration ($p=0,01$), severe anisokaryosis ($p=0,04$) and greater mean nuclear area ($p=0,019$) were frequently observed in samples from malignant tumours and their frequencies statistically higher than in benign tumours

Mammary gland tumors are common in dogs and represent the most common neoplasms in sexually intact female dogs(1). To date, unlike in human medicine, cytological diagnosis of canine mammary nodules remains a challenge (2,3). Maybe the systematic observation of chromatin pattern, macrophage infiltration, grade of anisokaryosis and mean nuclear area can help to discriminate between benign and malignant lesion, improving the diagnostic power of canine mammary cytology.

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Clinical Pathology, Cytopathology, Canine cytology

Canine cytology, Nuclear morphometry, Mammary gland tumors

PERIOULAR CUTANEOUS MAST CELL TUMORS IN THREE CATS: SURGICAL APPROACH AND LONG TERM FOLLOW-UP.

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To describe surgical approach and long term follow up in three cases of feline periocular cutaneous mast cells tumor (CMCT).

Signalment and history, clinical features, surgical approaches, histological evaluations and long term follow up in three cats with periocular cutaneous mast cell tumor were recorded. En bloc resection of CMCT by removing > 50% of the upper lid in two cases and > 40% of the lower lid with medial canthus were performed without other adjunctive treatment for tumor. The lids defect was reconstructed with a transposition skin flaps derived from the frontal region. During reconstruction upper palpebral or third eyelid conjunctiva were dissected and sutured with continuous suture over the cutaneous edge of the surgical wound in order to avoid trichiasis. Post-surgical evaluations at 2, 4 and 6 months were recorded in all cases.

All mast cell tumors were restricted to the eyelids. Complete excision were performed in all cases but one. Satisfactory cosmetic and functional results were achieved in all cats. All CMCTs were classified as low-grade based on histopathology. Local recurrence and metastatic disease were not detected during minimum follow-up of 6 months (media follow-up time of 670 days).

In according with previous reported series, also in our cases surgical approach was an effective treatment option in cats, even following incomplete surgical excision.

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Ophthalmology

mast cell tumor, cat, periocular

CONJUNCTIVAL BACTERIAL AND FUNGAL FLORA IN HEALTHY DONKEYS IN ITALY

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To evaluate the normal ocular bacterial and fungal flora of healthy eyes of mixed breed donkeys (*Equus asinus*) living in Marche region (central Italy).

Fifty-five healthy mixed breed donkeys (44 females and 11 males, age ranging between 1-16 years) housed in Marche Region (central Italy) with no history of clinical signs related to eye disorders were included in the study. For each eye, two ocular specimens were obtained running a sterile swab on the ventral conjunctival fornix. Samples were spread onto Columbia agar plate containing 5% sheep blood, with and without Streptococcus supplement, Mannitol Salt agar, Mac Conkey agar, Pseudomonas cetrimide agar and Burkholderia cepacia selective agar (Oxoid, Milan Italy). Plates were incubated at 37°C for 24-72h in aerobic conditions. Samples for micological investigations were maintained in 1mL of sterile saline added to 50µL/mL gentamicin and stored at 4°C for 24h. They were seeded onto Sabouraud dextrose agar (SDA), incubated at 25°C and examined daily over a 21-day period. The effect of age (young if age<3 years; adult if age≥3 years) and sex was determined using an independent-samples t-test, χ² test or Fischer's exact test, where appropriate. Significance was set at P < 0.05.

Both eyes of all donkeys were positive for at least one microorganism. A total of 10 Gram positive bacteria and 13 fungi and yeast were recovered (Table 1-2).

There was no significant effect of sex and age on frequency of fungal isolation (P>0.05), while female (P=0.04) and adult animals (P=0.004) had a significant average amount of CFU per eye.

The study revealed an exclusive Gram positive flora in contrast with what observed in an other study carried out on donkeys (1). Staphylococcus spp. strains are the most prevalent microorganism isolated and Aspergillus spp., Mucor spp. and yeast were the fungal flora more represented, as reported in horse (2). With the exception for Streptococcus spp, none of the isolated microorganism belonged to the most frequently isolated pathogens in equids.

The association between gender or age and bacterial flora needs further investigation. Risk factors for conjunctival fungal flora in equids could be linked to the environment as suggested by other authors (3-4).

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The first two authors contribute equally to the work.

internal medicine

Donkey, Ocular disease, Conjunctival swab

Table 1. Prevalence (%) and CFU/ml (mean and SD) of bacterial isolates.

Bacteria	Frequency	Percentage (%)	CFU/ml mean (SD)
<i>Staphylococcus aureus</i>	83	22.7	61.3 (157.6)
<i>Staphylococcus</i> spp. coagulase negative	38	10.4	31.4 (54.1)
<i>Staphylococcus intermedius</i> group	3	0.8	5 (0.1)
<i>Arcanobacterium pyogenes</i>	53	14.5	29.1 (40.2)
<i>Corynebacterium jeikeium</i>	30	8.2	40 (45.2)
<i>Streptococcus</i> spp.	3	0.8	5 (0.1)
<i>Bacillus cereus</i>	66	18.1	9.5 (6.9)
<i>Bacillus subtilis</i>	58	15.9	8.4 (4.4)
<i>Bacillus licheniformis</i>	29	8.0	11.7 (10.5)
<i>Bacillus thuringiensis</i>	2	0.6	10 (0.1)

Table 2. Prevalence (%) and CFU/ml (mean and SD) of fungal isolates.

Fungi and Yeast	Frequency	Percentage (%)	CFU/ml mean (SD)
<i>Absidia</i> spp.	4	2.2	10 (5.8)
<i>Acremonium</i> spp.	6	3.3	10 (0.1)
<i>Alternaria</i> spp.	8	4.5	5 (0.1)
<i>Aspergillus flavus</i>	9	5.0	6.1 (2.2)
<i>Aspergillus nidulans</i>	39	21.8	10 (5.5)
<i>Aspergillus niger</i>	2	1.1	5 (0.1)
<i>Aspergillus ochraceus</i>	12	6.7	8.3 (4.4)
<i>Aspergillus penicilloides</i>	13	7.3	9.6 (5.2)
<i>Aspergillus terreus</i>	4	2.2	5 (0.1)
<i>Mucor</i> spp.	28	15.6	6.1 (2.1)
<i>Penicillium</i> spp.	12	6.7	6.7 (2.5)
<i>Pichia anomala</i>	22	12.3	23.4 (32.3)
<i>Rhodotorula</i> spp.	20	11.2	12.5 (6.2)

CONJUNCTIVAL IMPRESSION CYTOLOGY IN HORSES

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Conjunctival impression cytology (CIC) is a technique described both in human and veterinary medicine (1;2). Information about CIC in the horse are scant (3).

Aim of the study was to evaluate feasibility, protocol standardization and normal cytologic pattern of CIC in healthy horses.

Samples were taken from the bulbar conjunctiva of 25 healthy horses.

The first 10 horses were used to standardize the method. CIC smears were collected following Bolzan's protocol (4), after the instillation of oxybuprocaine hydrochloride. Impressions were taken applying a 5x7 mm strip of Millipore filter with a pore size of 0.45µm (Merck Millipore, Milan) on the temporal bulbar conjunctiva and fixed in 95% alcohol. Specimens were stained with Periodic Acid–Schiff (PAS) and with Hematoxylin–Eosin (H&E) stains. The Bolzan's protocol was modified, reducing the time of immersion in Schiff's reagent (3 min) and elongating the immersions in sodium metabisulfite (10 min). Stained samples were immersed in xylene and then mounted on slides cover-slipped.

Conjunctival and inflammatory cells were differentiated with a light microscope, counting 200 cells for each eye, by scanning each sample in a sinuous and continuous pattern.

Cellularity, cell distribution and damage were scored following the criteria of Bauer et al. (5).

Cells' count and quality scores are reported in table 1.

A nose twitch was used in 5 cases. The technique allowed collecting a sufficient quantity of well-preserved cells. Goblet cells and erythrocytes were not observed. Epithelial and inflammatory cells, debris, naked nuclei and keratinized cells were found for both stains.

CIC can be considered a quite good technique for the study of the conjunctiva in the horse. Even if difficulties in the placement on the bulbar conjunctiva and the maintenance of the open eye were observed, instillation of local anesthetic facilitated samples collection. CIC is non-invasive and well tolerated by the animal, as demonstrated by the absence of erythrocytes in the smears. It is important to assess the size of the strip, because smaller strips leaked from the biocassette.

Samples had a good cellularity and cells were well distributed and preserved.

The staining methods influenced the evaluation. PAS stain gave a strongly pink background, although the time of staining was reduced and the time of discoloration was elongated. The H&E stain gave less background and a better definition of morphologic cellular features and allowed the differentiation of inflammatory cells. Thus, H&E may be considered advisable when an inflammatory disease is suspected.

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equine ophthalmology

conjunctiva, impression cytology, horse

	Periodic Acid – Schiff (PAS)		Hematoxilin – Eosin (H&E)	
	Mean cells ($\pm$ SEM)	Percentage	Mean cells ($\pm$ SEM)	Percentage
Basals Cells	20 ($\pm$ 6)	10,1	33 ($\pm$ 8)	16,5
Intermediates Cells	95 ($\pm$ 13)	47,3	111 ($\pm$ 11)	55,6
Superficials Cells	85 ($\pm$ 13)	42,4	55 ($\pm$ 11)	27,7
Neutrophils	1 ($\pm$ 0)	0,2	1 ($\pm$ 0)	0,2
Eosinophils	0 ($\pm$ 0)	0	0 ($\pm$ 0)	0
Lymphocytes	0 ($\pm$ 0)	0	0 ($\pm$ 0)	0
Cellularity score	1.5 (0-3)		1 (0-3)	
Distribution score	2 (1-3)		2 (1-3)	
Damage score	2 (0-3)		1 (0-3)	

EFFECT OF EXERCISE ON INTRAOCULAR PRESSURE (IOP) AND BLOOD PRESSURE (BP) IN DOGS.**Ragusa M.*^[1], Zhang K.^[1], Quarta M.^[1], De Majo M.^[1], Pugliese M.^[1]**^[1]*Dipartimento di Scienze Veterinarie – Università degli Studi di Messina ~ Messina*

The intraocular pressure (IOP) is a dynamic value influenced by many factors as age, sex, diurnality, season, type and time of exercise (1). The physiological changes in the eye during exercise are studied in human, but not fully understood (2-3).

The purpose of the present study was to gain more knowledge about how transient changes in systemic blood pressure, induced by exercise, affect IOP.

Fifteen healthy dogs (8 male, 7 female) of different breeds participated in the present study. The median age was 2.9 y.o., the median body weight 14.14 Kg. The dogs were not familiar with the equipment. They underwent an exercise test on a motorized treadmill (professional canine treadmill, ® Grillo, Modena, Italy) for 45 min at the following speeds: 2.5 Km/h for 15 minutes, 5.0 Km/h for 20 and 2.5 Km/h for 10. IOP and BP was measured respectively with a Tonopen Vet (Medtronic, Solan–USA) and oscillometry equipment (VET HDO; S and B MedVet, Babenhausen, Germany) in three times: at rest (T0), before starting the treadmill session, immediately after the exercise (T1) and after 1 hour to it (T2). Also, at the recovery venous blood samples obtained from the cephalic vein were used for lactate dosage. Applicable data of IOP and BP are presented as the mean $\pm$ SEM. Student's t-test was used to calculate significance between means value before the exercise, during the recovery and after 1 hour to conclusion of exercise. $P < 0.05$ was significant.

The results are summarized in table 1. During the exercise, there was a gradual decrease on IOP and it not is directly related to systemic blood pressure. These values were not significant immediately at the end of exercise, but they were significant until at 1 hour after the exercise.

Aerobic exercise is known to reduce intraocular pressure (IOP) in human (4-5). The mechanism lowering IOP it is not clear. It has also been suggested that exercise increases the facility of outflow leading to small increases in ocular blood flow (6-9). This study demonstrates in a population of young adult dogs that a period of dynamic exercise leads to significant changes in IOP and it seems not related to the changes of blood pressure.

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veterinary ophtalmology and clinical medicine
intraocular pressure, blood pressure, exercise

	T0	T1	T2
IOP Eye dx	12.86	12.64 (p=0.17)	11.71(p=0.023)*
IOP Eye sn	13.57	11.71(p=0.44)	11.43(p=0.0088)**
SYS	154.33	158.14(p=0.31)	164.21(p=0.010)**
MAP	103.57	106.36(p=0.23)	112.75(p=0.0007)**
DIA	76.62	78.39 (p=0.38)	85.18(p=0.0026)**

A RESTROSPECTIVE STUDY ON ATRIAL "MARGINAL" ANEURYSMS IN CATTLE

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After a retrospective study in which 6348 bovine atrial aneurysms were submitted to pathological examination- 2493 in 6-7 months old calves and 3855 in 18-20 months old beef cattle - (Guarda et al., 1993; Guarda, 1994), the authors describe the results of a systematic study on 77 atrial aneurysms providing some hypothesis about the aetiopathogenetic mechanism.

From January 2013 to March 2014 a systematic macroscopic and histological study of 77 bovine aneurysms was performed. Affected animals belonged to several breeds (Charolais, Blonde d'Aquitaine, Piemontese), but mainly cross- breed, regularly slaughtered at the Turin slaughterhouse.

Affected animals were beef cattle 10-30 months old (35%; 0.042 prevalence) and veal calves 6-9 months old (65%; 0.034 prevalence). The observed aneurysms involved the right atrium and particularly the edge, the upper and lower surface. They were rounded, sometimes filled with blood, sometimes empty, with a diameter of 2-6 mm. They were single, isolated or organized in small clusters. Histologically it was possible to differentiate two types of aneurysms: parietal (characterized by the presence of a neck that divides the atrial cavity from the aneurysmal cavity) or originated from the dilatation of pectinated muscle arterioles. One *Cysticercus bovis* was present into an aneurysm.

Although histological investigations seems to have clarified the morphology of the aneurysms, it remains to define the aetiopathogenetic mechanism. The authors speculate about different mechanisms underlying the two types of aneurysms. In the first place the existence of a genetic predisposition or a congenital connective tissue defect, that may predispose the aneurysms formation in presence of increased (or perhaps normal), venous blood pressure in "loci minoris resistentiae" as a result of cardiac-lung disease and an increased arterial blood pressure for a cardiac "overwork" due to a non-physiological breeding system in the fattening phase. A systematic study of the heart and lung lesions and an ultrastructural investigations of atrial aneurysms and normal atrial arterioles will clarify the pathophysiology of the two different types of aneurysms and if they are related to the increased venous or arterial pressure, respectively.

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Cardiac Pathology

Heart, Aneurysm, Cattle

ECG MONITORING IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE

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Electrocardiography (ECG) is currently the most sensitive and effective diagnostic technique for the identification and evaluation of electric diseases of the cardiac muscle, and though nowadays echocardiography is the most effective diagnostic technique to identify structural and kinetic diseases of the heart, ECG is still amply used in private practices. This study aims to quantify the sensitivity and the specificity of ECG in the diagnosis of atrial enlargement in subjects affected by myxomatous mitral valve disease (MMVD). A closer attention was given to the correlation between P wave deflection and some echocardiographic measures.

As MMVD is the most common heart disease in dogs, many veterinarians use the ECG exam as first choice in their diagnostic procedure. For this reason we studied the correlation between ECG sensibility and echocardiographic measures.

This study was carried out at the Department of Veterinary Science of Pisa University between Jan/2010 and Nov/2013. 155 dogs (64 females and 91 males) were enrolled in this study. The MMVD group included different breeds: mixed breeds (n=62), CKCS (n=15), Poodle (n=13), Yorkshire Terrier (n=9), English setter (n=8), Spitz (n=7), Espaniel Breton (n=7), Miniature pinscher (n=5), Chihuahua (n=4), Pekingese (n=4), and other breeds (n=21). These patients were divided into 4 age-related groups: <6 years, between 7 and 10 years, between 11 and 14 years, and > 14 years old. Their body weight range was between 2 and 40 kgs. Each dog was examined and a cardiological evaluation, including complete ECG and echocardiographic exam, was performed. For each patient, values of the P wave duration were obtained by means of the ECG exam. While the considered echocardiographic measures were: EF, ES, La/Ao, EDVI, ESVI, LV diastolic, LV systolic. Dogs affected by MMVD were staged according to ACVIM classification and divided into 3 groups: B, C and D. Data were statistically analyzed through MedCalc® version 12.5.0.0.

The correlation between the P wave duration and La/Ao, EDVI and ESVI measures is statistically significant in group C and D (p<0.01). No statistically significant correlation between ECG and echocardiographic values was found in group B.

The study has highlighted that a good correlation between the P wave duration and echocardiographic values exists: EDVI, ESVI and particularly atrial enlargement. Our data reveal that echocardiography is more sensitive in the diagnosis of cardiac chambers enlargement caused by MMVD. ECG exam is also necessary to keep under control the disease. ECG, however, is an adequate instrument to identify atrial enlargements in patients with MMVD and belonging to ACVIM C and D classes.

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electrocardiogram, mitral valve disease, dog

SODIUM AND POTASSIUM IN DOGS AND CATS AT DIFFERENT STAGES OF CKD: RETROSPECTIVE STUDY

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The aim of the present study was to evaluate serum sodium (Na⁺) and potassium (K⁺) levels in dogs and cats at different stage of severity of chronic kidney disease (CKD).

436 dogs and 214 cats referred to the Veterinary Teaching Hospital of Pisa University for CKD were enrolled and classified according to the IRIS guidelines.(7) Patients were evaluated for serum Na⁺ and K⁺. Data were statistically analysed by GraphPad Prism®.

dogs were classified as IRIS stage 1 (1%), IRIS stage 2 (36%), IRIS stage 3 (39%), IRIS stage 4 (24%); cats were classified as IRIS stage 1 (4%), IRIS stage 2 (57%), IRIS stage 3 (18%), IRIS stage 4 (21%). No significant difference in the average concentration of Na⁺ was found among IRIS stages in both dogs and cats, although in cats the number of hypernatremic patients increased with the progression of CKD. In both dogs and cats average K⁺ levels and the percentage of hyperkalemic patients increased significantly with IRIS stage (p=0.0061 and p=0.0001 respectively). A significant increase (p=0.0001) of hypokalemic subjects was found in cats of IRIS stage 1 and 2.

at late stages of CKD the higher prevalence of K⁺ disorders in dogs and cats seemed to be similar to what found in human CKD patients.(4) The increased percentage of patients with hyperkalemia in IRIS stage 3 and 4 may be due to a progressive loss of renal function and reduced excretion of K⁺, but also to a higher prevalence of metabolic acidosis, which is responsible for the extracellular shift of K⁺. The higher prevalence of cats with hypokalemia in the early stages of the disease seemed to be in agreement with what previously found in veterinary medicine (1,5) and should be taken into consideration, as several studies in cats hypothesized a relationship between K⁺ depletion and progressive loss of renal function.(5,3) No previous report of significant increase in K⁺ levels according to the progression of CKD was found in dogs.

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clinica medica

CKD, potassium, sodium

LUNG ULTRASOUND IN DOGS WITH CHONIC MITRAL VALVE DISEASE

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The aim of this study is to compare sonographic and radiological signs of the lung in dogs with chronic mitral valve disease, and to verify if there is a correspondence between these signs and the severity of the disease.

Our study considers 15 dogs affected by chronic mitral endocardiosis received at "M. Modenato" Teaching Hospital from 1st December 2012 to 1st December 2013. These dogs were classified as B1, B2 and C, according to AHA/ACC classification. Echocardiography, ultrasonographic and x-ray examination of the chest were performed in all dogs.

We used X-ray to evaluate cardiac enlargements, presence and distribution of alveolar pattern.

We used ultrasound to evaluate the pleural line, presence of pleural effusion and pulmonary edema observing the presence of lines B, their distribution and number.

Patients included in the study are 12 males and 3 females, with a higher prevalence of small breeds, aged between 7 and 19 years old. Two dogs belong to the class B1, 5 to the class B2 and 8 to the class C. The X-ray of the thorax showed in 7 cases left cardiac enlargement, in 6 generalized cardiomegaly and in 2 normal silhouette. Slight edema appeared in 3 cases, moderate edema in 4 cases, severe edema in 5. In 3 cases pulmonary edema was not detected. At ultrasound the pleural line resulted thickened and regular in 7 cases, thickened and smooth in 1 case, normal in 7 cases. B lines are viewed in 7 cases: rare in 1, dense in 5, confluent in 4. In 5 cases they have not been displayed. A slight layer of anechoic pleural effusion was seen in 2 cases.

The thoracic ultrasound is an easy, fast (<5'), non invasive examination and non stressfull exam for critical patients.

It always detected moderate and severe edema, which showed an intermediate and peripheral distribution at X-ray; it did not detect slight perilare edema because the air in the peripheral parts of the lung did not allow the passage of ultrasounds.

There was always correlation between radiographic, ultrasonographic signs and severity of the disease.

Chest ultrasonography could be a primary aid to differentiate, in the large class of patients B2, dogs coming to present pulmonary edema from those which will never present it. This is important to evaluate a possible drug therapy.

Finally the lung ultrasound is a fundamental tool in monitoring the efficacy of the therapy.

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medicina interna, diagnostica per immagini

lung, ultrasound, endocardiosis

CLINICAL AND HEMATOLOGICAL RESPONSES OF CATS UNDERGOING BLOOD DONATION USING AN ANESTHETIC COMBINATION OF TILETAMINE AND ZOLAZEPAM

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The aim of this prospective study was to investigate the effect of blood donation on selected clinical and hematological variables in healthy cats anesthetized with a tiletamine-zolazepam combination.

Thirty one cats were enrolled in the study (18 males and 13 females) as blood donors at the Veterinary Transfusion Unit (REV) of the University of Milan, Italy. Mean body weight of cats in the study was 5.2 kg (range 4.7-8.4 Kg), mean age was 5 years (range 1-8 years), and all cats were European domestic cats. Each cat was anesthetized with a combination of 2.5 mg/kg of tiletamine and 2.5 mg/kg of zolazepam administered intramuscularly. Each cat received 90 ml of saline solution subcutaneously before the donation, and 60 ml by intravenous infusion beginning half-way through the donation. Blood (10 ml/kg body weight) was collected from the jugular vein. The clinical parameters evaluated at the pre- and post-donation points included rectal temperature (RT) measured using a digital thermometer, systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), and heart rate (HR) measured using a high definition oscillometric instrument. Blood was collected, both pre- and post-donation, for complete blood count using an automated hematology analyzer to evaluate white blood cells, red blood cells, hemoglobin, hematocrit, lymphocytes, neutrophils and platelets count.

All the variables studied were normally distributed at the pre- and post-blood-collection time points. Significant decreases in RT ($P < 0.001$) occurred after blood donation. The mean $\pm$ standard deviation (SD) values for pre- and post-blood collection RT were 38.3 ± 0.5 (range 37.5-39.4°C) and 37.4 ± 0.6 (range 36.5-38.6°C), respectively. The RT change ranged from 0 to -2.1°C, and the mean change was -0.7 ± 0.5 °C. Significant increases in SAP ($P = 0.030$), MAP ($P = 0.003$) and DAP ($P = 0.001$) occurred after blood donation. The mean $\pm$ SD values for pre- and post-blood collection for SAP were 141 ± 17.5 (range 105-180 mmHg) and 153 ± 23 (range 106-199 mmHg), respectively; for MAP were 98 ± 13 (range 72-124 mmHg) and 109 ± 15 (range 86-136 mmHg), respectively; and for DAP were 75 ± 12 (range 53-95 mmHg) and 86 ± 12 (range 64-108 mmHg), respectively. The SAP change ranged from -27 to +66 mmHg and the mean increase was 13 mmHg. The MAP change ranged from -23 to +66 mmHg and the mean increase was 12 mmHg. The DAP change ranged from -22 to +49 mmHg and the mean increase was 11 mmHg. There was no statistically significant change in any of the hematological variables or HR after blood donation. Blood collection lasted less than 40 minutes, with a mean duration of blood collection of 39 ± 11 minutes (range 24-76 minutes). None of the cats showed evidence of pallor or collapse after recovery from anesthesia.

Collection of 10 ml blood/kg of body weight in healthy donors using tiletamine and zolazepam combination anesthesia appears to be safe and well tolerated by feline donors.

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medicina trasfusionale, medicina interna
feline, transfusion medicine, blood donation

CLINICAL EVALUATION OF CBC PARAMETERS OBTAINED BY LASER CELL COUNTER IN THE DOG

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The complete blood count (CBC) is an important part of the common diagnostic procedure in the laboratory. Microscopic evaluation of blood smears by experienced and qualified Clinical Pathologist (ClinPath) is an important tool to confirm the data generated even by the most modern and sophisticated automated cell counter in veterinary field like the Idexx ProCyte®.

In a period of 6 months 1,649 canine CBCs were examined and 350 CBCs with moderate/severe hematological abnormalities were selected to evaluate the ability of the ProCyte® to perform leukocyte differential count. The following parameters were studied: flag reporting for band neutrophils (Band), nucleated RBC (nRBC) and aggregates of platelets (agg PLT); reticulocyte (Rets) count; neutrophilia, eosinophilia, lymphocytosis, and monocytosis. The comparison was carried-out using the manual differential count performed by experienced clinical pathologist.

An excellent specificity for all flags (Band 0.86; nRBC 0.99; Agg PLT 1.00) was established, meanwhile the sensitivity to detect nRBC (0.06) and Agg PLT (0.16) was poor and was moderate for Band (0.60). The agreement for sensitivity for Band flag increased if a 500/mcL cut-off was adopted (K 0.83). The correlation (R) and the agreement (K) for neutrophilia were excellent (R 0.89, K 0.93) as well as the (R) for Rets count (0.84) and moderate for monocytosis (R 0.67, K 0.60), while were poor for eosinophilia (R 0.36, K 0.47) and lymphocytosis (R 0.40, K 0.16). A correlation between the appearance of Band and the decrease of Rets count has been documented. The threshold value beyond which the Procyte® would increase the reliability of flags for nRBC and Rets appeared to be set at 5% for nRBC and 500/μL for Rets, but the sensitivity was still low for nRBC

This work highlighted the instrumental limitations for flags, and confirm once a time, the review of the blood film by an experienced ClinPaths is still very important. On the other hand, many of the indication by flags provided by the instrument are important because they can immediately provide clues to guide timely ClinPath to confirm or improve the overall interpretation of the CBC and the clinician to arrange quick adjustments of diagnostic or therapeutic procedures.

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Hematology
CBC, Procyte®, dog

RED BLOOD CELL DISTRIBUTION WIDTH IN DOGS WITH PRE-CAPILLARY AND POST-CAPILLARY PULMONARY HYPERTENSION

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Red blood cell distribution width (RDW) is a component of the CBC and is a quantitative measurement of anisocytosis.¹ In humans, increased RDW has been recently associated with different cardiovascular and cardiopulmonary disorders, including pulmonary hypertension (PH).² The aim of the present study was to evaluate RDW values in dogs with pre-capillary PH (Pre-PH) and post-capillary PH (Post-PH).

Dogs were prospectively enrolled and were submitted to physical examination, CBC and serum biochemical profile, and cardiovascular examination including ECG, thoracic radiography and complete echocardiographic and echo-Doppler examination. Based on results of these exams, dogs were divided in healthy dogs (control, n = 39 dogs), dogs with chronic degenerative valvular disease without PH (CDVD-noPH, n = 52 dogs), dogs with CDVD with PH (Post-PH, n = 42 dogs) and dogs with pre-PH (n = 24 dogs). The diagnosis of PH was based on the presence of a tricuspid regurgitation jet maximal velocity (TRVmax) ≥ 2.8 m/s in absence of increased right ventricular outflow tract obstruction. Selected echocardiographic, CBC and serum biochemical parameters were compared among groups using ANOVA and Kruskal-Wallis tests for normally and non-normally distributed variables, respectively. The Spearman's correlation test was used to analyze the correlation between RDW and selected echocardiographic, CBC and serum biochemical parameters. The minimum level of significance was chosen for $P < 0.05$.

The median RDW value in dogs with Pre-PH (14.0% [range 11.3-22.4%] $P < 0.001$) was significantly higher compared to that of healthy dogs (13.10% [range 11.8-14.4]). The median serum urea concentrations of dogs with Pre-PH, Post-PH and CDVD-noPH were significantly higher than that of control dogs ($P < 0.001$, $P = 0.001$, and $P < 0.001$, respectively). The median white blood cells value of dogs with Pre-PH ($P < 0.001$), Post-PH ($P < 0.01$) and CDVD-no PH ($P < 0.001$) were significantly higher compared to that of the control group. The RDW has a significant, positive but weak correlation with white blood cells (correlation coefficient 0.290), serum urea concentration (correlation coefficient 0.300) and TRVmax (correlation coefficient 0.240). In dogs with Pre-PH, the RDW had a significant, positive correlation with serum urea (correlation coefficient 0.720) and negative correlation with serum total protein concentration (correlation coefficient - 0.50).

Results suggest that the RDW can play a role in dogs with Pre-PH, but not in dogs with CDVD with or without PH, likely as a consequence of thromboembolic and/or inflammatory mechanisms involved in the pathophysiology of pre-PH as well as deterioration of renal function.

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Medicina interna

Canine, Cardiology, Echocardiography

CHARACTERIZATION OF LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD OF DOGS

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^[1]~ *Parma*

The aim of the study is to evaluate changes of lymphocyte subsets (B, T, NK, TCR $\gamma\delta$) in dogs related to sex, age and body size. Blood of 49 dogs (pure and cross breed) was investigated.

The 49 dogs were clinically healthy, regularly vaccinated and treated against parasites. Dogs were divided into age groups in function of body size (table 1). The investigation was performed using flow cytometry. Table 2: antibody panel.

Tables 3, 4 and 5 represent the statistical results in function of gender, age and size.

Table 6 represents the most significant comparison between parameters.

Significant changes were observed, sometimes in contrast, sometimes in agreement with previously published data in literature (1-5). The most interesting results have regarded changes affecting NK and TCR $\gamma\delta$ lymphocyte. Data show that the younger, the higher values of NK percentage. This trend might suggest that these innate cells could have some important role when the acquired immunity is not yet well developed. Regarding TCR $\gamma\delta$ cells in circulating blood we observed statistically significant changes in function of both variables (size-age).

It could be argued that the decrease of the percentage of TCR $\gamma\delta$ from young subjects to the elderly is a function of their location predominantly in the intestine. This district is, for extension and physiological functions, a first line in which the subject, either newborn or young, interfaces with the external environment and takes contact with new antigens, towards which he has to build a balanced immune responsiveness.

An hypothesis that would justify the increase in the percentage of TCR $\gamma\delta$ according to dog's size may lie on the greater extension of the intestine. In this case, a greater extension could correspond to a higher concentration of TCR $\gamma\delta$ lymphocytes, in absolute number into the intestinal mucosa and, probably, this could lay to a greater concentration percentage in the circulating blood.

Obtained data open the way to clinical applications because it is possible to set available ranges of normal values and so to compare them with the results performed on blood samples of subjects in diagnostic process. Based on knowledge of the role of lymphocyte populations individually considered and of their relationships and interactions, theoretically, it is possible to apply the obtained results in every dysfunction in which the immune system can come in any way involved.

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immunology immunopathology
dog, immune-system, age

TABLES

tab 1: EXPERIMENTAL GROUPS OF DOGS

GROUP	DOGS (Nr)	WEIGHT (Kg)	SIZE	AGE (years)
Young	13	all	all	first year
Adult	23	<9	S	<9
		<23	M	
		23>40	L	<7
		>40	XL	
Elderly	13	<9	S	<9
		<23	M	
		23>40	L	<7
		>40	XL	

tab 2: ANTIBODIES USED FOR FLOW CITOMETRY

antibody	format	isotype	clone	company
mouse anti dog CD3	FITC	IgG1	CA17.2A12	ABD Serotec
anti-CD56	PE	IgG1	MOC-1	Merck Millipore
mouse anti canine CD21	PE	IgG1	CA2.1D6	ABD Serotec
anti-mouse (NC)	PE	IgG1	MOPC-21	Merck Millipore
rat anti dog CD4/CD8	FITC/PE	cocktail	YKIX302.9/ YCATE55.9	ABD Serotec
TCR δ	null	IgG2a	CA20.8H1	LAL
goat anti mouse IgG2a	PE	IgG2a		Invitrogen

tab 3: ESTIMATION BY GENDER GROUP

	F	ds	M	ds	sig
CD21	11,418	1,811	14,03	1,705	0,273
CD3	49,114	5,119	61,941	4,818	0,6
CD4	29,429	2,994	37,385	2,818	0,047
CD8	13,26	1,723	17,035	1,599	0,097
CD4:CD8	2,393	0,265	2,546	0,25	0,659
CD56	3,682	1,672	7,541	1,574	0,083
TCRδ	2,239	0,555	2,593	0,457	0,607

tab 4: ESTIMATIONS BY AGE GROUPS

	Y	ds	A	ds	E	ds
CD21	18,525	2,258	9,499	1,826	10,148	2,411
CD3	64,005	6,381	57,151	5,159	45,427	6,813
CD4	40,14	3,732	36,147	3,018	23,933	3,985
CD8	18,77	2,118	15,57	1,712	11,102	2,316
CD4:CD8	2,455	0,331	2,318	0,267	2,636	0,353
CD56	6,975	2,085	6,904	1,686	2,955	2,226
TCR$\gamma\delta$	4,144	0,596	2,24	0,565	0,864	0,732

tab 5: ESTIMATIONS BY SIZE GROUPS

	S	sd	M	sd	L	sd	XL	sd
CD21	9,384	3,435	14,514	1,967	14,41	1,81	12,588	3,065
CD3	45,236	9,709	48,057	5,56	62,227	5,117	66,591	8,662
CD4	22,847	5,678	29,544	3,252	37,123	2,993	44,114	5,066
CD8	13,835	3,223	13,436	1,849	17,719	1,72	15,599	2,88
CD4:CD8	1,998	0,503	2,311	0,288	2,374	0,265	3,196	0,449
CD56	1,901	3,172	7,413	1,817	10,891	1,672	2,242	2,83
TCR$\gamma\delta$	2,113	1,071	1,985	0,547	2,289	0,51	3,276	0,822

tab 6: SUMMARY TABLE OF MOST SIGNIFICANT COMPARISONS BETWEEN PARAMETERS

dependent variables	fixed factors and interactions			linear covariates			quadratic covariates	
	parameters	P		parameters	P		parameters	P
CD3	E-Y	0,05	$\leq 0,05$	W	0,018	$< 0,05$		
				A	0,048	$< 0,05$		
CD21	A-Y	0,005	$< 0,01$				A	0,000 $< 0,001$
	E-Y	0,014	$< 0,05$					
CD4	F-M	0,047	$< 0,05$	W	0,000	$< 0,001$	A	0,019 $< 0,05$
	L-S	0,039	$< 0,05$	A	0,001	$\leq 0,001$		
	M-XL	0,019	$< 0,05$					
	S-XL	0,009	$< 0,01$					
	A-E	0,011	$\leq 0,01$					
	E-Y	0,004	$< 0,01$					
CD8	E-Y	0,018	$< 0,05$	A	0,021	$< 0,05$		
CD4/CD8							A	0,037 $< 0,05$
CD56	L-S	0,021	$< 0,05$					
	L-XL	0,01	$\leq 0,01$					
TCR$\gamma\delta$	A-Y	0,035	$< 0,05$	A	0,001	$\leq 0,001$	W	0,018 $< 0,05$

E-Y	0,001	$\leq 0,001$	A	0,019	$< 0,05$

EQUINE MULTISYSTEMIC EOSINOPHILIC EPITHELIO TROPIC DISEASE (EMEED): TWO CASE REPORTS

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Equine Multisystemic Eosinophilic Epitheliotropic Disease (EMEED) is an uncommon syndrome that affects horses characterized by eosinophilic infiltration of multiple organs of ectodermic origin such as skin, gastrointestinal tract, liver, pancreas, lungs, lymphnodes, etc (1). Clinical signs include weight loss, diarrhea, exfoliative dermatitis and, less frequently, respiratory distress (2). The aetiology of EMEED is unknown, although parasitic, allergic, toxic and viral causes have been suggested.

Case 1 was a 2 y.o. Standardbred colt with anorexia, multiple oral ulcerations, ventral and limb edema and weight loss. Laboratory findings showed eosinophilia, increased ALP, GGT and LDH, hypoalbuminaemia, hypergammaglobulinaemia and hyperfibrinogenaemia. Oral glucose tolerance test revealed total malabsorption. Bone marrow aspirate demonstrated increased maturative stages of the eosinophilic series. Abdominal ultrasonography showed enlargement of the liver with hyperechoic parenchyma, dilated bile ducts, increased peritoneal fluid and fibrin deposition. Liver biopsy indicated an eosinophilic hepatitis with fibrosis and cholestasis. Post mortem examination revealed marked enlargement of mesenteric lymphnodes, liver and pancreas, with a severe infiltration of eosinophils.

Case 2 was a 13 y.o. Pony gelding referred for labored breathing. Clinical examination showed the presence of nasal discharge, cough, prolonged expiration and inspiratory crackles on auscultation. Laboratory findings showed leukocytosis with marked eosinophilia and increased PLT, PT and aPTT. Blood gas analysis revealed hypoxaemia and hypercapnia. Thoracic ultrasonography showed disseminated comet tail artifacts, and abdominal ultrasonography showed several renal and hepatic small hyperechoic nodules. Thoracic radiography revealed a markedly increased interstitial pattern. Endoscopy of the upper airways demonstrated the presence of small white nodules on the mucosal surface; cytological examination of bronchoalveolar lavage showed eosinophilic inflammation. Biopsy of the liver and the of the airway nodules showed eosinophilic infiltration. Post mortem examination showed the presence of small calcific nodules on the liver, lymphnodes, lung and kidney, that were largely infiltrated with eosinophils.

According to these findings, the diagnosis of EMEED was confirmed for both cases.

This paper contributes to increase the low number of EMEED cases reported worldwide. Interestingly, in case 2 the pulmonary involvement is described, which represents a quite uncommon feature. Amongst possible different aetiologies, neoplasia should be considered, because of several common features with human clonal hypereosinophilic syndromes (3).

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Equine Internal Medicine
Equine, Eosinophil, EMEED

COMPARISON OF THREE IN-CLINIC AUTOMATED BLOOD CELL COUNTERS (SEAC HECOVET, CELL-DYN 3500 AND ADVIA) FOR ERYTHROCYTE, LEUKOCYTE AND PLATELET COUNT IN BLOOD DONOR DOGS

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The aim of this retrospective study was to compare CBC results generated by four University Blood Bank using 3 different blood cell counters on homogeneous healthy dog groups selected as blood donors.

EDTA blood samples were collected for routine blood testing from 204 healthy blood donor adult dogs (1) selected at 4 University Blood Banks (UBB): Veterinary Transfusion Units of University of Milan (REV), Perugia (EMOVET-UNIPG), Pisa and Bologna. All dogs from the different sites were from both sex, ranged from 2 to 8 years old, weighting more than 25 kg and belonging to various breeds (Mongrel, Bernese Mountain Dogs, Golden Retriever and various mastiffs).

In all UBB the samples (0.5-1 ml EDTA-anticoagulated blood) were analyzed within 8 h and before analysis tubes were placed on a roller plate for at least 1 minute to ensure proper mixing. CBC parameters were assessed using a validated veterinary impedance cell counter (HeCoVet, SEAC, Calenzano, Florence, Italy) (1) for UBB of Perugia and Pisa, a dual optical and peroxidase-based hematology analyzer (ADVIA 120, Siemens, Deerfield IL USA) for UBB of Bologna and an impedance and laser-based hematology analyzer (CELL_DYN 3500, Abbott Laboratories, IL, USA) for UBB of Milan (2), and included: RBC count, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), PLT count and WBC count. Data obtained from each site were analyzed for normal distribution and groups of results were compared using Mann-Whitney test or t-test ($p < 0.05$).

The mean or median $\pm$ SD values obtained on samples collected with HeCoVet (107 samples), ADVIA 120 analyzer (37 samples) and CELL_DYN 3500 (60 samples) were respectively: RBC 6.8 ± 0.6 , 7.22 ± 0.7 , 6.8 ± 0.7 ($\times 10^{12}/L$); Hgb 15.2 ± 1.6 , 16.5 ± 1.4 , 16.5 ± 1.7 (g/dL); Hct 46.9 ± 4.4 , 50.6 ± 4.2 , 41.8 ± 4.7 (%); MCV 70 ± 2.9 , 70.4 ± 3.2 , 60.4 ± 4.02 (fL); MCH 22.5 ± 1.8 , 23.3 ± 1.8 , 24.6 ± 1.8 (g/dL); MCHC 32.6 ± 2.4 , 32.8 ± 1.9 , 41 ± 4.2 (g/dL); PLT 305 ± 69.95 , 248 ± 55.5 , 284 ± 90.9 ($\times 10^9/L$) and WBC 9.13 ± 2.1 , 9.52 ± 2.29 , 10.9 ± 2.21 ($\times 10^9/L$). All the results from these samples were within their respective reference ranges set for each cell counter. The main significant differences were found for Hct between all three analyzers, for the erythrocyte indices between CELL_DYN 3500 and HeCoVet and ADVIA 120; for Hgb and WBC between CELL_DYN 3500 and HeCoVet and for PLT between HeCoVet and ADVIA 120 ($P < 0.0001$). RBC count showed significant difference between ADVIA 120 and CELL_DYN 3500 ($P = 0.006$) and ADVIA 120 and HeCoVet ($P = 0.001$).

The CBC parameters obtained with different hematology analyzers on different but homogeneous group of samples collected from healthy blood donor dogs, show statistically significant differences.

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Medicina interna, medicina emotrasfusionale
dog blood donor, hematology, blood cell counters

EFFECTS OF THE ADMINISTRATION OF XYLAZINE OR DEXMEDETOMIDINE ON PLASMA CONCENTRATION OF CORTISOL AND SUBSTANCE P IN CALVES UNDERGOING DISBUDDING AND SIMULATED DISBUDDING

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The aim of this study is to compare the behavior of cortisol and substance P plasma concentrations in dairy calves undergoing disbudding or simulated disbudding, after administration of xylazine, dexmedetomidine or placebo. Moreover this work evaluates the efficacy of dexmedetomidine versus xylazine during disbudding, in order to increase the number of analgesic molecules available for the bovine specie.

The protocol for this study was approved by the Institutional Ethical Committee for Animal Care at University of Milan (protocol No. 28/2011). This study was a blinded, randomized, prospective and placebo-controlled trial, performed in healthy calves. Cortisol and substance P were analyzed in plasma in 60 calves divided in 6 groups of 10 animals and submitted to disbudding (BUD) or simulated disbudding (SIM) after the administration of a placebo (Groups 1 PLA-BUD and Group 2 PLA-SIM), 0.2 mg/kg b.w. xylazine (Groups 3 XYL-BUD and Group 4 XYL-SIM) or 5 µg/kg b.w. dexmedetomidine (Groups 5 DEX-BUD and Group 6 DEX-SIM). Blood samples were collected for each group 30 minutes before disbudding or simulated disbudding (basal), within 5 minutes from the procedure (T0), 20 minutes (T1) and 1, 2, 3 and 4 hours after the procedure (T2, T3, T4 and T5 respectively).

Cortisol plasma concentration increased at T0 and T1 especially in calves of groups 1 PLA-BUD, 2 PLA-SIM, 3 XYL-BUD; a little increase was detected also in group 4 XYL-SIM at T1. Substance P was higher only at T1 in group 1 PLA-BUD.

Substance P has been suggested as biomarker of pain in assessment of analgesic efficacy instead of cortisol, that can be influenced by many stressors as transport, manipulation, or alpha-2 agonist administration (1, 2). Also in our study, substance P seems to be a better indicator of pain than cortisol because cortisol was influenced in groups 1 PLA-BUD, 2 PLA-SIM, 3 XYL-BUD and 4 XYL-SIM by stress caused by handling or hemodynamic and respiratory distress induced by xylazine administration.

Even if society concern regarding animal welfare and suffering has grown up exponentially, there are really few sedatives or anesthetics registered to alleviate pain during zootechnical mutilation in food animals (3), the present study proposes to consider the employment of dexmedetomidine, a new effective alpha-2agonist in calves submitted to disbudding.

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vet 08- clinica medica veterinaria
disbudding, substance P, calves

PERIENDOSCOPIC BRONCHOALVEOLAR LAVAGE TECHNIQUE: RESULTS OF CITOLOGICAL AND BACTERIOLOGICAL TESTS

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Bronchoalveolar diseases of the dog are an important part of the clinical work, and the therapy is based on the precise identification of the cause. It is fundamental, therefore, to establish a diagnostic protocol as accurate as possible, that leads to a definitive diagnosis. Bronchoalveolar lavage (BAL) is based on the infusion of saline in the distal lung of the patient and a collection of the liquid: the sample obtained is used for bacteriological and cytological tests.

Dogs presented with a history of coughing underwent endoscopic examination, followed by a bronchoalveolar lavage. The BAL was performed using a periendoscopic techniques, through a urinary catheter for male dogs. Bacteriological and cytological results of BAL from 39 dogs were collected, and confronted with the endoscopic findings.

From an endoscopic point of view, acute tracheobronchitis is the main diagnosis (44%), followed by the chronic-reacutized (28%) and chronic (20%) ones. The majority of the bacteriological results are negative (51%), with no bacteria isolated. Among the germs found, the most frequent is *Pseudomonas* spp, and then *Streptococcus* spp. The primary finding in cytology results is a chronic tracheobronchitis (51%).

In the literature, the main cause of coughing in dogs is chronic bronchitis, while in our endoscopic findings this is not true: this difference could be related to the time of year we conducted this study (March-January), when referring vets send patients for endoscopic examination at the beginning of the symptoms, due to concerns related to the presence of cereal ear foreign bodies in the lungs. Endoscopic evaluation differs from cytology and this dichotomy could be due to the fact that the first technique is not sensitive in detecting acute cases, while it is useful for the chronic ones. Bacteriology results line up with what can be found in international papers regarding the germs isolated. It is worth discussing, though, the high number of negative results obtained: the majority can be found in the acute cases, when, as said before, dogs are send for endoscopic evaluation during the first few days of the symptoms, and this could make it difficult to isolate the germs from the airways. Moreover negative results indicate very low risks of contamination allowing the clinician to select the correct therapy based on sensitivity tests, reducing antibiotic resistance. Based on these results, the technique described is reliable and useful for a correct diagnosis in a coughing dog, it is cheap and can be used to monitor the disease, as it progresses and to check response to therapy, even without endoscopic examination.

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Internal Medicine

Dog, BAL, endoscopy

CLINICOPATHOLOGICAL FEATURES OF 48 CATS WITH CHRONIC GASTROINTESTINAL SIGNS (CGIS)

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To describe the clinical presentation and to evaluate the clinicopathological data in cats with chronic gastrointestinal signs (CGIS).

Medical records of cats referred between June 2008 and December 2013 to the Veterinary Teaching Hospitals of Bologna, Teramo and Turin Universities, with CGIS (> 3 weeks) were retrospectively reviewed. Clinical examination, laboratory analysis, imaging findings, histopathology of bowel samples and follow up were analysed. Cats with CGIS caused by non-gastrointestinal diseases were excluded. Based on the response to subsequent empirical therapeutic trials and/or histopathological results, all cats were classified as having alimentary lymphoma (AL), food-responsive enteropathy (FRE), or idiopathic inflammatory bowel disease (iIBD). A statistical analysis (by applying parametric and non-parametric tests) was performed in order to define possible predictive parameters useful for the characterization of CGIS. The variables analysed between groups were: age, presence of vomiting, diarrhoea, weight loss, muscle wasting, body condition score, lethargy, jaundice, packed cell volume, white blood cells and platelets count, serum total protein, albumin, globulin, cholesterol, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, bilirubin, feline pancreatic lipase immunoreactivity, cobalamin, folate concentrations, urinary specific gravity and abdominal ultrasound.

Eight cats with AL, 19 with iIBD and 21 with FRE were included. Twenty-three cats were male (20 castrated) and 25 female (19 spayed). Median age at diagnosis was 97 months (range 5-190 months). European short-haired cats were overrepresented (n=39). The median BCS was 2.7 (range 1-5). Eleven cats died within 48 weeks of the initial diagnosis, while 37 cats are still alive. Cats with alimentary lymphoma were older (ANOVA, $p < 0.05$) and showed greater weight loss (Kruskall Wallis, $p < 0.05$) than cats with FRE. Moreover, cats with alimentary lymphoma showed greater muscle wasting (Kruskall Wallis, $p < 0.05$), lower BCS (Kruskall Wallis, $p < 0.05$), differences in WBC count (Kruskall Wallis, $p < 0.05$) and abdominal ultrasound abnormalities (Kruskall Wallis, $p < 0.05$) than cats with FRE or iIBD.

Cats of this study were mostly affected by FRE and iIBD and showed classic gastrointestinal signs (1). Hypoalbuminemia was observed most frequently in cats with AL compared to FRE and iIBD, however the difference was not significant. The absence of significance may be related to the small number of cats with AL included in the study. Clinical outcome was variable, but many cats experienced remission of clinical signs and prolonged survival time. In addition, this study found that cats with poor body conditions, WBC count and abdominal ultrasound abnormalities are potentially affected by alimentary lymphoma (2).

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Internal medicine

Cat, Alimentary lymphoma, IBD

CLINICOPATHOLOGICAL FEATURES OF 44 DOGS WITH PROTEIN-LOSING ENTEROPATHY (PLE) OF NON-NEOPLASTIC ORIGIN

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To describe clinical presentation and to evaluate clinical activity scores (CIBDAI/CCECAI) and clinicopathological data in dogs with PLE of non-neoplastic origin

Medical records of dogs referred between January 2009 and November 2013 to the Veterinary Teaching Hospital of Turin and Veterinary Clinic of Valdinievole, with a diagnosis of non neoplastic PLE were retrospectively reviewed. Dogs with history of chronic gastrointestinal signs (> 3 weeks), hypoalbuminemia of gastrointestinal origin and histopathological evidence of non-neoplastic intestinal inflammation were included. All dogs were scored using the canine IBD activity index (CIBDAI) and the canine chronic enteropathy activity index (CCECAI). Based on the response to subsequent empirical therapeutic trials, dogs were classified as steroid-, food- or antibiotic responsive. In addition, they were divided into 4 groups based on hypoalbuminemia severity. Finally, a score (Score Evaluation of Response to Therapy; SERT) of 2, 1 or 0 was given to each dog based on satisfactory, poor or no clinical response to treatment, respectively. Steroid-, food- and antibiotic-responsive enteropathies were diagnosed in 40, 3 and 1 dogs, respectively. Twenty-four dogs were male and 20 female. Median age at diagnosis was 6.4 years. German Sheperds were overrepresented (n=9). Presenting complains included vomiting (n=26), appetite variations (n=26), diarrhea (small bowel n=30; large bowel n= 6; mixed n= 8), peripheral edema, abdominal or pleural effusion (n= 21), muscular twitching or convulsions (n=4) and pruritus (n=3). Pre-treatment CIBCAI and CCECAI ranged from 4 to 17 and 5 to 19, respectively. Post-treatment CIBCAI and CCECAI ranged from 1 to 14 and 1 to 15, respectively. SERT was 2 in 19, 1 in 14 and 0 in 11 dogs, respectively. Total protein, globulins, total calcium, cholesterol, magnesium and cobalamin were decreased in 40/44, 37/44, 35/43, 23/43, 13/35 and 9/33 dogs, respectively. Twenty-nine dogs survived more than 6 months, while 11 dogs died within 6 months of the initial diagnosis; 4 dogs were lost at follow up. No correlation (Spearman Rho, $p>0.05$) between albumin concentration and pre-/post-treatment clinical scores was found. Significant differences (Student's t test $p<0.01$) in albumin concentration and clinical scores before and after treatment were found. Pre-treatment albumin concentration was not associated with survival (Student's t test $p>0.05$), nor to SERT (ANOVA $p>0.05$). No significant differences were found between hypoalbuminemia severity groups and age (ANOVA F $p>0.05$), weight (ANOVA F $p>0.05$), pre- and post-treatment clinical scores (Kruskal test $p>0.05$; Chi Squared $p>0.05$), survival and SERT (Chi Squared $p>0.05$)

In addition to classic gastrointestinal signs, dogs of this study often showed clinical signs associated with hypoalbuminemia and low oncotic pressure. Clinical outcome was variable, but many dogs experienced remission of clinical signs and prolonged survival. This study found that not all PLE dogs with high clinical scores and severe hypoalbuminemia at presentation have a guarded prognosis (1). Furthermore, the severity of hypoalbuminemia could not be a reliable indicator of survival or positive response to treatment (2).

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2. SM Simmerson et al Clinical features, intestinal histopathology, and outcome in protein-losing enteropathy in Yorkshire Terrier dogs. JVIM 2014, Jan 27

Gastroenterologia cane
chronic enteropathy, canine, hypoalbuminemia

RECURRENT DIARRHEA IN A FLAT COATED RETRIEVER: A CASE REPORT.Fusi E.^[1], Valdez Lumbreras A.E.^[1], Bontempo V.^[1], Vatta E.^[2], **Rossi L.*^[1]**^[1]Università degli Studi di Milano, Dept. of Health, Animal Science and Food Safety ~ Milano, ^[2]self employer-VETERINARI ASSOCIATI ~ Monza

The goal of this clinical study was to solve the recurrent diarrhea in a 2 years old male Flat Coated Retriever by the dietary approach.

A 2-year-old male Flat Coated Retriever was examined for a 18 months recurrent diarrhea. The physical examination revealed a normal appearance. The dog weighed 42 kg, and had a BCS of 5/9 and a normal MCS. The dietary history was complex because more of 12 different commercial diets were offered to the dog. The owner, in fact, changed petfood frequently and suddenly, most of the time after the recurrence of episodic diarrhea, which severity varied over the months (fluid or mucous feces). The vaccinations and deworm treatments were current.

Diagnostic evaluation included complete blood count (normal), serum biochemistry profile (ALP 524 IU/L; cobalamin 150 picog/ml) and abdominal ultrasounds (normal).

The following conditions antibiotic-responsive enteropathy, diet-responsive enteropathy inflammatory bowel disease (IBD) (mild form), and chronic idiopathic large bowel diarrhea and other intestinal disorders were considered.

According to clinical data, malabsorption associated to intestinal inflammation was present in the patient. Limiting the intestinal mucosa exposure to potential antigens was the dietary strategy adopted. In particular, an exclusion homemade diet, consisting in a novel protein (pork meat) source and carbohydrate (potatoes) source, was designed in relation to the dog's body condition.

The cobalamin supplementation (25 µg/kg once/week SC) was associated to the elimination trial. The owner reported that diarrhea had resolved completely after a week, so a lipid source (linseed oil) was introduced after 4 weeks. Later, psyllium powder (1 table spoon) and non-flavored vitamin and mineral supplements were added.

Due to the owner attitude to changing petfood, the dog's oral tolerance could be breached. An inflammatory process, that increased the intestinal permeability was the main cause of the possible absorption of allergens and the following sensitization of the patient.

The basis for the dietary recommendation mentioned above is that the more rapidly intestinal inflammation can be controlled, the more rapidly the intestinal permeability barrier will be restored and the less exposure the dog will have to intestinal antigens.

The dietary elimination trial could have sustained the rapid remission of the clinical signs. The evolution of the dog's clinical conditions in the following weeks were good, determining the complete resolution of the food-responsive chronic enteropathy (CE).

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animal nutrition

recurrent diarrhea, canine nutrition, homemade diet

SENSITIVITY AND SPECIFICITY OF ULTRASONOGRAPHY FOR THE DIAGNOSIS OF MALABSORPTION SYNDROME IN THE HORSE

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Malabsorption syndrome in horses is frequently due to infiltrative bowel disease (IBD): the infiltration of the intestinal mucosa impairs the absorptive function with the onset of chronic wasting syndrome. Histology represents the gold standard diagnostic procedure and it can be performed by surgical biopsy of the small intestine, rectal biopsy or endoscopic duodenal biopsy. Biopsy techniques present many practical disadvantages in equine practice, therefore finding more practical complementary diagnostic tests to be used for screening purposes is advisable. Abdominal ultrasonography is both easy to perform and capable of detecting intestinal wall thickening that is a pathological feature of infiltrative disorders(1). The aim of the present study was to assess the sensitivity and specificity of ultrasonographic measurement of small intestinal wall thickness (UMSIT) to evaluate IBD, using the histology of rectal biopsies as the reference standard(2).

55 horses with a history of chronic wasting were retrospectively selected. In all patients, repeatability of UMSIT was statistically evaluated. All the UMSIT were analyzed and an "individual scoring system" defining the distribution of thickened intestinal loops (>3 mm) in each horse was elaborated to subdivide the population into two groups: general group (GG) including all horses and subgroup A (SA) including only those horses which had a "diffuse" thickening of both duodenum and jejunum. Eventually, sensitivity (Se), specificity (Sp) and ROC curve were statistically calculated both for GG and SA.

Since no statistical differences were observed among measurements, UMSIT resulted repeatable ($p < 0.05$). For GG, the best UMSIT cut off was 5.7 mm with a Sp of 87.5% and a Se of 36.8%. For SA, the best UMSIT cut off was 5 mm with a Sp of 100% and a Se of 50%.

The repeatability of UMSIT, previously demonstrated under experimental conditions(3), was confirmed in the present study in a clinical population. Specificity of UMSIT was high but linked to inclusion criteria. Sensitivity of UMSIT was low due to variable ultrasonographic explorability of the small intestine and to the different extension of the thickening: sensitivity was higher for SA because only horses with "diffuse" lesions were included. Study limitations include the use of rectal biopsy as the reference standard (whose selection was due to clinical practical reasons) and the absence of a standardized protocol for intestinal ultrasonographic evaluation (due to the retrospective nature of the study). However, this study demonstrates the UMSIT promising diagnostic potential, highlighting the necessity for more detailed perspective studies in the future.

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Internal Medicine

horse, malabsorption, ultrasonography

USEFULNESS OF ULTRASONOGRAPHY TO DETECT SUBCLINICAL MASTITIS IN 216 SHEEPS

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Subclinical mastitis represents a pathological condition of the mammary gland of economic and sanitary interest. Diagnosis of subclinical mastitis in the sheep is made when the macroscopically normal udder secretion, in the absence of abnormalities on clinical examination of the mammary gland, presents mastitis pathogen bacteria and a somatic cell count (SCC) greater than a conventional value.

The objective of this study is to compare the diagnostic tools for subclinical mastitis with ultrasonography, in order to evaluate the usefulness of the latter in the diagnostic of disorders of the mammary gland of the sheep.

216 pluriparous Lacaune and Sarda breed ewes without abnormalities on physical examination of the udder or on macroscopic appearance of the mammary gland secretion were included in the study.

Ultrasonographic examination of each half of the udders was performed using 3 views, to evaluate echogenicity and echotexture of the parenchyma, as well as the presence of structural changes and the corpuscular echogenicity of the fluid content at the level of the cistern.

The ultrasonographic findings were compared with the results of the cyto-bacteriologic exam. On the basis of the SCC and culture results, udder halves were classified as follows:

- control: SCC<500.000 cells/ml, no mastitis pathogen bacteria isolated
- positive: SCC>500.000 cells/ml and mastitis pathogen bacteria isolated
- questionable: SCC>500.000 cells/ml or mastitis pathogen bacteria isolated

The statistical analysis shows an association between udder secretion with SCC>500.000/ml and hyperechogenicity of the parenchyma (Pvalue = 0.003).

Correlation between udder secretion with SCC>500.000 cells/ml and morphological modifications of parenchyma was statistically significant (Pvalue < 0.001).

The udder secretion in which the number of somatic cells is > 500.000/ml has a probability almost triple compared to milk of control group to belong to half-udder whose ultrasound examination shows a hyperechoic echogenicity and almost three times more likely to show morphological changes on ultrasound as compared to controls.

Ultrasonographic screening of the udder can be considered a useful diagnostic tool, easy to perform in the field, and its application may allow early identifications of ewes potentially affected by subclinical udder pathology, on which to perform cyto-bacteriological analysis of the udder secretion.

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Internal medicine, Ultrasonography
mammary gland, ultrasonography, sheep

ULTRASONOGRAPHY EVALUATION OF UMBILICAL STRUCTURES IN CLINICALLY HEALTHY ITALIAN HOLSTEIN FRIESIAN NEWBORN CALVES DURING THE FIRST WEEK OF LIFE

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The aim of the present study was to determine ultrasonographically the size of the umbilical structures in clinically healthy newborn calves and to evaluate their involution during the first week of life.

Fourty five Italian Holstein Friesian newborn calves were included in this study. All the calves were born in the University dairy farm (CIRAA, Pisa). Inclusion criteria: 1) physiological gestational length (>260 days) and vaginal delivery (1); 2) APGAR score ≥ 7 within 10' from birth (2); 3) no clinical symptoms revealed by clinical examination. The ultrasonographic examination of umbilical remnants was performed at 24 hours (T1), 3 (T3) and 7 (T7) days of life with a 5-7.5 Mhz convex transducer (Esaote, Italy). Calves were in standing position, not sedated but only restricted manually. Umbilical vein was imaged just cranial to the umbilicus in trans-sectional scan, vertical and horizontal diameters were measured and a mean (M1) of them was calculated. Arteries were visualised at the apex of the bladder, the diameter of right and left vessels was measured in trans-sectional scan, then a mean (M2) of the two measures was calculated. Data were expressed as mean and standard deviation (SD). Distribution was performed using KS test, thus T-student test was applied to verify differences for M1 and M2 related to time. Statistical differences were set at $p < 0.05$.

The mean diameter of umbilical vein was 0.9 ± 0.2 cm at T1, 0.7 ± 0.2 cm at T3, 0.6 ± 0.2 cm at T7, while the mean diameter of arteries were 1.1 ± 0.2 cm at T1, 0.8 ± 0.20 at T2 cm and 0.6 ± 0.2 cm at T3. T student test showed statistical differences, both for M1 and M2, between T1 vs T3, T1 vs T7 and T3 vs T7.

A good quality imaging was reached with calves in standing position in no clipped areas. Arteries diameter at T0 was slightly wider if compared with some authors (3), but similar for others (4). The mean vein diameter is similar to those reported by others at 24h (4) and in 7 days old calves (3,4). Vessels diameter at T3 are not comparable to literature, because to the best of our knowledge no data were reported for this time. If compared to other species, the diameter of both vessels is similar with a previous study performed on equine foals (5,6) and wider than in donkey ones (7). The narrow SDs highlight the homogeneous size of umbilical vessels in our calves and stress the great reliability of ultrasonography examination. Our statistical results showed an involution of the umbilical vessels during the first week of life, as already reported in other species (5-7).

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vet08, medicina interna

umbilicus, calf, ultrasonography

ULTRASOUND AS A SUPPORT TOOL FOR CAUDAL EPIDURAL INJECTION IN THE HORSE

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The aim of the study was to describe the sonoanatomy of the epidural space at first, second and third coccygeal space in horses.

Phase 1: two tails, obtained from a slaughterhouse, were used to perform the ultrasound anatomical study of the epidural space at the coccygeal level. One tail was first used to obtain CT images and then was used to make anatomical sections. The second tail was dissected to individualize the ligaments and the muscular planes, then the tail was completely stripped to bone in order to obtain the coccygeal bones to make a water immersion ultrasound study.

Phase 2: Fifteen standardbred female horses, undergoing perineal surgical procedures, were enrolled in the study. After individuation of anatomical landmarks done in phase 1, images of the sacro-coccygeal zone were first collected from the horses always by the same operator in order to acquire the technique. Then each horse was sedated with xylazine 0.5 mg/kg IV and the registration of data started; one expert operator and different inexperienced operators individuated the needle insertion point for the epidural space with the classical method described by Skarda et al., 2009. The insertion point was marked with a specific dot for inexperienced and expert operators. Then ultrasound images of the coccygeal (Co) spaces were taken and the most appropriate insertion point was detected. The distance between the expert and the inexperienced points, the expert and the ultrasound technique points and between the inexperienced and the ultrasound technique points were measured with a caliber. After the measurements were acquired, a surgical scrub of the area was done and an epidural needle insertion was performed at the ultrasound individuated point. The depth of the epidural space was individuated by ultrasound. The Thouy needle was then inserted with a 90° angle respect to the skin and the right positioning of the tip needle was confirmed by the hanging drop technique.

Stage 1: CT and ultrasound images of the tails confirmed that the spinous processes of coccygeal vertebrae are incomplete. Specific ultrasound images can be individuated and the depth of the epidural space can be measured.

Stage 2: mean depths of epidural space recorded at the spaces between Co1 and Co2 and between Co2 and Co3 were 3.069 ± 0.36 and 2.63 ± 0.29 respectively. Mean distance between the expert and inexperienced points was 1.18 ± 0.94 cm, between the inexperienced and ultrasound points was 2.16 ± 1.77 cm and between the expert and ultrasound points was 1.26 ± 1.46 cm. The hanging drop technique confirmed the right positioning of the needle in all horses.

Ultrasound evaluation of the epidural space at coccygeal level is feasible and quite easy. This study puts in evidence that the ultrasound technique can be more accurate than both inexperienced and expert operators in individuating the epidural injection point. This technique can also be used to know the depth of the epidural space. The technique may be a support tool and not a guide because the 90° angle of the needle does not allow the direct visualisation under the ultrasound probe during the procedure. Further studies are to be performed to evaluate the possibility to make the procedure under the ultrasound direct visualization.

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Anaesthesiology and pain therapy
epidural anesthesia, horse, ultrasound

MINI-INVASIVE ENDOSCOPIC-ASSISTED GASTROPEXY FOR DOGS AT RISK OF GASTRIC DILATATION-VOLVULUS

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Gastric dilatation-volvulus (GDV) is a frequent acute syndrome found in many large or giant breeds of dogs, that can be prevented demonstrably only by gastropexy¹. This study will analyze an endoscopic-assisted gastropexy procedure. Surgical times, complications, post-operative recovery and suture strength will be evaluated. Furthermore, Gdv is known to be multifactorial and is thought to be associated to chronic enteropathy²; because of this correlation, a CECCAI clinical scoring index was handed to the owners.

13 healthy dogs, weighing >25kg, over 18 months old, underwent an endoscopic-assisted gastropexy procedure. 1 out of 13 dogs previously manifested a GDV episode, solved with an orogastric tube two weeks before surgery. A blood sample was taken for full haemochromocytometric analysis, serum protein electrophoresis, C-reactive protein, fibrinogen and creatinine at the time of surgery (T0) and three months later (T1). The results of the CECCAI were registered and analyzed. The dogs were able to go home the same day of the surgery.

At T1 an abdominal ultrasonography was performed to evaluate the gastropexy adhesion.

Mean $\pm$ SD surgical time was 19 $\pm$ 6 minutes and mean $\pm$ SD gastropexy length was 3,2 $\pm$ 0.4 cm.

At T1, ultrasound examination of the gastropexy site confirm the complete adhesion between the portion of the stomach evaluated and the abdominal wall (no sliding motion detected)³. Inflammatory pattern not found in the laboratory analysis.

According to this study, the endoscopic-assisted gastropexy truly results mini-invasive in many different ways⁴. Performing surgery in such a little incision minimizes the surgical field exposure and substantially decreases the anesthesiological duration, with all the benefits intra and post operator associated. At the same time, the endoscope becomes really important for early diagnoses of chronic enteropathy, even in absence of clinical evidence. Furthermore, this procedure keeps the costs relatively low, even cheaper than other mini-invasive procedures.

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3 Rawlings CA, Mahaffey MB, Bement S., et al: Prospective evaluation of laparoscopic-assisted gastropexy in dogs susceptible to gastric dilation. J Am Vet Med Assoc 221:1576– 1581, 2002

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Mini invasive surgery

gastropexy, canine, endoscopy

A NEW FEMORAL BLOCK: LATERAL LOWER-ILIAC APPROACH IN THE PSOAS COMPARTMENT WITH ULTRASOUND TECHNIQUE IN DOGS; PRELIMINARY STUDY.

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The aim of the study was to describe a new ultrasound-guided approach to the femoral nerve in the psoas compartment and to evaluate the clinical efficacy when combined with a parasacral sciatic nerve block in dogs undergoing surgery of the pelvic limb.

Stage 1- Four cadaver dogs were used to investigate the ultrasound anatomy details of the femoral nerve within the psoas compartment and to describe the approach to the nerve. To obtain a good image of the femoral nerve the ultrasound probe was positioned in the middle of the angle created by a tangent line drawn to the cranial margin of the iliac wing and a tangent line drawn to the ventral angle of the iliac wing. After localization of the femoral nerve, 0.1 mL/kg of a methylene blue solution were injected. Then dissections were performed to determine the dyeing of the nerve.

Stage 2- Seven client-owned dogs anaesthetized for elective pelvic limb surgery (TPLO and TTA), were enrolled in the study. Dogs were premedicated with acepromazine (0.01 mg/kg IM) and after 20 minutes fentanyl (5 mcg/kg IV) was given as a bolus. Anaesthesia was induced with propofol IV titrated to effect and maintained with isoflurane. Nerve blocks were performed with 0.5%, ropivacaine, 0.2 mL/kg each nerve (femoral and sciatic). The new femoral block approach was achieved by the use of the ultrasound and the nerve stimulator. The sciatic nerve block was done using only the nerve stimulator as described by Portela et al. During surgery heart rate (HR), respiratory rate (RR), mean blood pressure (MAP) with invasive technique, end-tidal carbon dioxide (EtCO₂), end-tidal isoflurane percentage (Fe%Iso) were recorded every five minutes and during specific time points until the end of anaesthesia. Increase of more than twenty per cent of the HR, RR and MAP values was considered nociception and rescue analgesia was provided with fentanyl 2 mcg/kg IV. If more than 2 boluses of fentanyl were required to re-establish normal cardiorespiratory parameters a variable rate fentanyl infusion (5-20 mcg/kg/h) was started. After extubation, carprofen 2 mg/kg IV was administered. Post-operative pain assessment was measured with the Glasgow pain scale, until the first injection of rescue analgesia, using methadone 0.2 mg/kg IV.

Stage 1: The femoral nerve was visible as a hypoechoic round structure in the middle of the ileopsoas compartment. Anatomical dissection showed the right positioning of the methylene blue (dyeing of the entire circumference of the nerve) in all the 4 cases.

Stage 2: the femoral nerve was localised in all dogs. During surgery four dogs received one or two boluses of fentanyl, but any of them required an infusion. Nociceptive stimulation was recorded during capsular, muscular and skin suture. Mean values of HR, RR and MAP were 109 ± 18 bpm, 11 ± 2 bpm and 78 ± 16 mmHg respectively. Mean FeIso was 1.06 ± 0.09 %. In the postoperative period mean time to first rescue analgesic treatment was 10 ± 2 hours after the execution of the block.

The ultrasound-guided lateral lower-iliac femoral nerve block is a feasible technique to perform with the dog in lateral recumbency. The use of 0.2 mL/kg of 0.5% ropivacaine provided adequate intra- and post-operative pain relief for about 10 hours in dogs undergoing surgery of the pelvic limb.

Portela DA, Otero PE, Tarragona L, Briganti A, Breggi G, Melanie P. Combined paravertebral plexus block and parasacral sciatic block in healthy dogs. *Vet Anaesth Analg*. 2010 ;37(6):531-41.

Anaesthesiology and pain therapy

femoral nerve block, ultrasound guided, dog

SOLAR FOOT PENETRATING INJURY IN A HORSE: THE USE OF COMPUTED TOMOGRAPHY TO GET THE DIAGNOSIS AND TO AID THE SURGICAL APPROACH

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Describe the use of CT scan in a case of septic osteitis of the distal phalanx (P3) and septic arthritis of distal interphalangeal joint (DIP) secondary to street nail.

A 19 yo tb cross gelding was presented to the VTH for a severe chronic lameness after a penetrating nail on the sole. At admission there were severe lameness (AAEP 4-5/5), swelling of distal limb and a puncture wound of the lateral paracuneal sulcus of the left forelimb was detected. On lateromedial (L-M) radiographic view of the foot, a smoothly outlined depression of the flexor surface of the navicular bone (NB) and a sclerotic appearance of the spongy bone dorsally to the flexor surface of P3 were identified. On dorsoproximal-palmarodistal oblique view an ill-defined localized irregular radiolucent area on the lateral solar margin of P3 was detected. LM view with a probe showed a penetrating wound extended to the flexor surface of the NB. Ultrasound (US) of the foot revealed moderate effusion of the navicular bursa and severe distention of the palmar recess of the DIP joint with echogenic synovial fluid, hyperechogenic spots and thickening of the synovial membrane. A ill-defined irregularity of the hyperechogenic line with some echoes penetrating deep into the solar surface of P3 were identified. Synoviocentesis confirmed the septic arthritis of the DIP joint. CT scan of the foot was performed before surgery and a well-defined circular, hypodense area surrounded by a sclerotic rim in the lateral part of the glenoid cavity of P3 was detected; increased number and dimension of the synovial invaginations of the NB were identified. On CT findings diagnosis of severe septic osteitis of P3 was made. The patient underwent a "street nail" procedure to debride the necrotic tissue and a through-to-through lavage of the DIP joint was performed. During hospitalization the horse was treated with systemic and local antimicrobials and antiinflammatory therapy for 15 days. Radiographic follow-up demonstrated a significant thinning of the articular surface of the DIP joint, consequence of the advance stage of the septic arthritis at admission. At discharge the patient was improved but still lame (AAEP 3/5).

Penetrating injuries to the sole may involve deep structures of the foot resulting in damage that may be life-threatening. Radiographic assessment of soft tissue is limited and involvement of bone is not always easily recognized in early stage. US exam may yield additional information but an unsatisfactory image quality is a common problem in acute emergencies. CT exam allows the acquisition of more details and provides a detailed examination of bone to get relevant information for surgical planning and prognosis.

Dabareiner R.M. et al. Trauma to the Sole and Wall, in Diagnosis and Management of Lameness In The Horse, (2003) Eds: M.W. Ross, S.J. Dyson, W.B. Saunders Co, Philadelphia. pp 275-282

Diagnostica per immagini

Equine CT, foot, injury

UTEROPEXY IN 3 MARES USING AN ABSORBABLE SUTURE WITH UNIDIRECTIONAL SHALLOW BARBS

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Pendulous ventrally angled positioned uterus is an important cause of delayed uterine clearance leading to reduced fertility in old multiparous mares(1). Uteropexy is the laparoscopic technique used to elevate uterus to a horizontal and physiological position suturing uterine body and horns to the dorsal part of mesometrium (imbrication). This procedure is effective in improving uterine position and in increasing reproductive capability (2). The aim of this study is to describe the surgical technique, complication and outcome of uteropexy in 3 mares using an absorbable suture with unidirectional shallow barbs. The suture device allows the apposition of the uterine wall to the dorsal part of the mesometrium in a continuous pattern without making knots neither at the begin nor at the end of the procedure making the surgical technique easier and decreasing surgery time.

3 mares were included in the study: they ranged from 15 to 20 years, barren for 2-3 years and multiparous. In all cases were reported regular follicular activity, fluid accumulation unresponsive to common treatments and ventrally angled uterus diagnosed by palpation and ultrasound. Mares were fasted for 36-48 h, treated with Benzylpenicillin 22.000 ui/kg IM, Gentamicin 6.6 mg/kg IV and Flunixin 1.1 mg/kg IV, were sedated with Romifidine 30 µg/kg IV or Detomidine 10 µg/kg IV and Butorphanol 20 µg/kg IV in bolus, maintained with the same alfa-2 agonist in CRI and restrained in stock. In all mares was performed epidural anesthesia with Morphine 50 mg or Methadone 60 mg. Standing laparoscopic surgery included trocars placement (3 to 4 each side), insufflation of CO2 and visualization of uterine structures. The suture strand used was an absorbable 2-0 (copolymer of glycolic acid and trimethylene carbonate) end-looped device with unidirectional shallow barbs (V-Loc™ 180)(3) swaged to a 26 mm, round bodied, taper point, half-circle needle. The device allows the apposition of tissues in a simple continuous pattern without making knots because of its intrinsic tissue-holding capacity. The uterine body was grasped and hold in place by 2 Babcocks forceps and the needle is inserted through seromuscular layers of the dorsolateral aspect of the body of the uterus, adjacent to the reflection of the mesometrium onto the uterus, and then through the mesometrium about 3 cm dorsal to its attachment on the uterus. After the first stitch through the uterus and mesometrium, the suture was anchored, rather than tied, by passing the needle through the small loop of the device. The dorsal aspect of the mesometrium and the seromuscular layers of the uterus were then sutured together from the cranial aspect of the uterine body to the tip of the uterine horn using a continuous suture pattern avoiding the opening of the oviduct. At the end of the procedure the suture was tied firmly without knot and was cut with scissors. The abdominal wall, sub cutis and cutis were closed with a standard technique. The post-operative period is described elsewhere (4). Follow-up information was obtained thorough telephone communication with the referring vet.

The mean duration of the entire surgical procedure was 170 mins (210-125 mins). There were neither intraoperative nor postoperative complications. The device maintained the juxtaposition of tissues at the end of the procedure in 3/3 cases. Transrectal palpation and ultrasound exam at 4-6 months confirmed the dorsally elevated position of the uterine body and horns in all cases. 2/3 mares remained pregnant without other therapies at the beginning of the breeding seasons and delivered two live foals.

Uteropexy has been proved to be efficacious in repositioning pendulous uterus into normal position affecting positively the fertility of old multiparous mares. Horses eligible for surgery should be fasted for a minimum of 48h as colon mass could impair correct visualization of abdominal structure. This is the first study, to our knowledge, on the use of an absorbable end-looped suture with unidirectional shallow barbs

in laparoscopic surgery of the reproductive tract and it has demonstrated to be practical, safe and efficacious in imbrication of the uterine wall to the dorsal part of the mesometrium. Operating times drastically decrease as the surgeon skills improve (210-174-125 mins) as reported in bibliography. The main limitation of this study is the small number of cases but the preliminary results are encouraging. Future perspectives include preoperative biopsy to investigate the relationship between endometrial histology and postsurgical reproductive outcome and laparoscopic control of the uterine position post-pregnancy.

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SURGERY

LAPAROSCOPY, UTEROPEXY, EQUINE

SPYING AND NEUTERING EXPERIENCE IN A GROUP OF RACCOONS

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The raccoon (*Procyon lotor*) was originally a North and Central American species. Raccoon are also kept as zoo animals and their popularity as pet increases. A reproductive management is recommended to prevent fertile animals to escape in to the wild, stabilise the population structure and reduce the risk of genital pathologies as described for in non breeding female individuals.

This work describes the surgical techniques of sterilization in a population of raccoons caught for translocation in a larger outside enclosure and castrated for birth control before release.

Eleven adult raccoons (4males and 7females) weighting 7.6 ± 1.5 kg were included in the study. The animals were injected IM with a mixture of ketamine(7mg/kg) and dexmedetomidine(7ug/kg). After induction, the trachea was intubated and connected to a circle anesthetic system for maintenance with sevoflurane in 100%O₂ together with sufentanyl CRI.

In females the ventral abdomen, from the xiphoid to pubis, was clipped and aseptically prepared. The surgical incision of 4-6 cm was performed caudal to the umbilicus, including skin, subcutaneous tissue and fat to expose the linea alba. Hacking the fascia and peritoneum, it was possible to access the abdominal cavity and exteriorize both ovaries. By tearing the broad ligament caudal to the ovarian pedicle 2 braided suture resorbable USP2-0 were performed; following the uterine horn caudally until the body of the uterus and exerting traction on the uterus in a cranial direction, a double bind with Miller node was performed with the same suture on the body cranial to the cervix of the uterus.

Once hemostasis was verified, the uterine stump was repositioned into the abdomen and the abdominal belt was sutured as in domestic carnivores.

In males a proper clipping and scrubbing of the surgical area preceded a pre-scrotal opening. After a skin incision of 2cm along the median raphe above the testis dislocated the testicles were manually compressed to get external exposition. The incision was then deepened as far as to affect the spermatic fascia and tunica vaginalis, then the tail of the epididymis ligament tunic was separated by blunt dissection, leading to a further external exposition of the testis by pulling. The spermatic cord was closed with a double ligature, placing the hemostatic forceps and cutting the cord. Finally the tunica vaginalis was sutured, followed by subcutaneous and intradermal layers.

Surgery was easily performed in all the raccoons including 3 pregnant females and the anesthetic protocol was excellent with a smooth induction and recovery and a stable surgical depth.

Although the surgical techniques used in this study are very similar to that for dog, we found some interesting anatomical differences, which were never been described before. In females, the ovarian ligament appeared looser than in the bitch, determining an easier exteriorization of the ovary, without the need to tear it. The raccoons are pet with subcutaneous fat of considerable varying thickness between 3 and 5cm, which moreover varies according to seasonal requirement, up to obesity during winter. The amount of fat can interfere with anesthetics distribution and elimination and possibly with laparotomy healing.

Fossum(2013) Mosby ed.; Vogler et al(2010) Proc Int Conf Dis Zoo Wild Anim, 6-9

CHIRURGIA VET 09

raccoons , *Procyon lotor*, sterilization

URETER'S DAMAGE AFTER SURGICAL REMOVAL OF A LARGE GRANULOSA THECA CELL TUMOUR (GCT) IN A MARE

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This report describes ureter's damage after removal of a large GCT in a 5yo mare.

The mare was referred for recurrent colics and a previous diagnosis of uterus' broad ligament hematoma. Behavioural abnormalities, physical and transrectal examination, abdominal and rectal ultrasonographic (US) findings, which revealed an enlarged mass in proximity of the left ovary, and peritoneal fluid cytology, led to two differential diagnosis of ovarian neoplasia or abdominal abscess. A standing laparoscopy was planned to visualize the abdominal mass and its relationship with viscera, reducing the incidence of anesthetic and surgical complications of a midline celiotomy. The mare was sedated with detomidine CRI1 and analgesia was provided with epidural lidocaine and IV flunixin meglumine. After surgical exploration the left ovary (30 cm diameter) adhered to the ipsilateral uterine horn so that, after administration of lidocaine 2% into the ovarian pedicle, it was decided first to partially dissect the mesovarium (LigaSure®)3, and then to perform a midline celiotomy under general anesthesia. Laparotomy was deemed necessary both to fully release the enlarged ovary from its pedicle and to make a partial hysterectomy of the left uterine horn, which was adhering to the mass. Definitive diagnosis of GCT was based on histopathological examination of ovarian tissue.

The next days the mare was depressed with fever, high RR, HR, serum creatinine and urea (3.5 and 103 mg/dl) and electrolytes imbalance (Na⁺168; K⁺5.97; Cl⁻131.5 mEq/l). An abdominal US revealed an increased peritoneal fluid, due to uoperitoneum. A cystoscopy showed that only the right ureter was draining the urine into the bladder while the left ureteroscopy revealed a reduction of ureter's lumen that blocked the endoscope passage. Another standing laparoscopy confirmed that the left ureter was broken leading to a continuous leakage of the urine into the abdomen. The gap between the ureter stumps was so large that nephrectomy was the only possible surgical approach, but the owner elected for euthanasia.

Although adhesions to abdominal structures are uncommon complications of GCTs3, our report indicates that they can occur. Generally a standing laparoscopy ensures a good visualization of mesovarium during and after its transection with a direct assessment of an ovarian stump suitable haemostasis, but this approach sometimes enables a complete evaluation of significant adhesions with other organs if a large mass is involved. Moreover in some cases a midline laparotomy is necessary to remove the extremely enlarged ovaries. In conclusion a primary mini invasive standing approach is the gold standard for ovariectomy but it doesn't always give a complete picture of all the possible structures involved in the adhesions with large GTCs.

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chirurgia

granulosa theca cell, ureter's damage, GTC's adhesions

INFLUENCE OF ARYTENOIDEAL ABDUCTION ANGLE IN THE LONG TERM RESULTS IN DOGS UNDERWENT "TIE BACK" LARYNGOPLASTY.

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The clinical criteria to determine both the prognosis and the long term results in dogs underwent laryngoplasty for laryngeal paralysis are far to be standardized and effective. Even in case of high number enrolled patients studies, as in the case of Crico-Arytenoid Laryngoplasty, no standardized data are evident concerning the follow-up (1,2,4,5,6).

In the horse a convenient method based on post surgical laryngoplasty has been proposed by Davidson in 2010 and resulted to be sensitive and effective (3).

The aim of this study is to evaluate the relationship between the degree of arythenoid abduction and the clinical results in long terms follow up in dogs underwent cricoarythenoid laryngoplasty (tie back).

This retrospective study evaluate 12 consecutively enrolled patients with definitive diagnosis of laryngeal paralysis underwent to tie back laryngoplasty at the Department of Clinical Science and Public Health – University of Milan from January 2012 to December 2013.

All patients were presurgically classified according to the severity of the disease by means of a scoring zero-one system based on the detection of standards signs in each patient. All dogs were clinically evaluated for the presence of severe dyspnea, indirect detectable stridor, direct detectable stridor, after exercise stridor, hyperventilation, voice changes, exercise intolerance, salivary coughing, post drinking coughing and ataxia at the time of admission and at 10 and 30 days after surgery.

Each dog underwent a standard tie back laryngoplasty by the same surgeon and a post operative laryngoscopy was performed to evaluate the arytenoid abduction angle. The preoperative and post operative arytenoid angles have been evaluated electronically by a dedicated software (Osirix . Mac)

The clinical results scheduled by the scoring system and the degree of laryngeal abduction have been matched to determine the distribution of the patient according to their long term clinical conditions

All the patients with the high clinical response belonged to the class of high arythenoid abduction.

The proposed technique could be considered an objective method to determine the prognosis for dogs which underwent tie back laryngoplasty.

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Chirurgia dei piccoli animali
dog, laryngeal paralysis, surgery

PARTIAL ILEAL BYPASS AS TREATMENT OF ILEAL OBSTRUCTION: SHORT AND LONG TERM FOLLOW-UP IN 8 CASES

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The aim of the study is to describe short and long term follow-up and possible complications in a group of horses that underwent exploratory laparotomy and jejunocaecostomy with no resection of the ileum (partial bypass) after a diagnosis of ileal obstruction secondary to several diseases.

Medical records of patients admitted for signs of colic that underwent an exploratory celiotomy were reviewed retrospectively. Eight (8) horses, in which an ileal obstruction was found after exploration of the abdomen and it was treated by a partial bypass, were identified. Signalment, history, physical examination findings at admission, surgical findings and post operative hospitalization data were obtained from the records. 6/8 horses were affected by ileocecal intussusception, 1 had ileal hypertrophy and 1 had a severe/long standing ileal impaction. The short term survival, defined as horses that were discharged from the hospital, and long term outcome, defined as survival at least 6 months after surgery and possible complications occurred were examined. Follow-up informations were obtained by a telephone interview with owner\trainer\referring vet.

All 8 patients were discharged from the hospital; all of them survived at least 6 months after surgery and no complications were recorded with the telephone interview.

Ileal obstructions can be considered the most frequent obstructive lesions of the small intestine diagnosed during exploratory celiotomy. The causes are mostly ingesta impaction, jejuno-ileum ileo-ileum or ileo-caecal intussusception or ileal hypertrophy. Different surgical treatments are considered in literature as extraluminal massage of the ingesta into the caecum, ileal resection with jejunoleo-caecostomy (end-to-side or side-to-side; complete bypass) or jejunoleo-caecostomy alone (partial bypass). Complications of these procedures are well represented in literature and they can be post operative adhesions after excessive manipulation due to serosal damage, post-operative ileus, leakage or dehiscence of the anastomosis site or the ileal stump, and backward movements of ingesta from the caecum to the jejunum through a too large or ventral anastomosis. Resection of the ileum especially if the stump is remote from the abdominal incision or manual resolution of long-term intussusceptions can also be a huge challenge for the surgeon. In author's knowledge there are no reports in literature regarding the long term follow up of horses that underwent a partial bypass of the ileum to treat an ileal impaction diagnosed during an exploratory celiotomy. In our case series none of the patients had postoperative complications or long term problems related to the surgical procedure (recurrent colic; poor growth or body condition). According to our results partial bypass of the ileum carries a good prognosis and can be considered the procedure of choice in the surgical treatment of ileal impaction of various origin for the reduced surgery duration compared to other techniques and the absence of short and long term complications.

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chirurgia grandi animali

equine, colic, bypass

COMPARATIVE EVALUATION OF THE SEDATIVE EFFECTS OF BUTORPHANOL AND METHADONE IN COMBINATION WITH DEXMEDETOMIDINE AND ACEPROMAZINE IN DOGS

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To compare the effects of methadone (MET) and butorphanol (BUT) in combination with dexmedetomidine (DEX) and acepromazine (ACP) on degree and onset of sedation, side effects and quality of recovery Prior informed owner consent was obtained in each case. Fifteen client-owned dogs undergoing diagnostic procedures requiring sedation were used. The dogs received intramuscular (IM) administration of a mixture of BUT 0.2 mg/kg, DEX 5 µg/kg and ACP 5 µg/kg (BUT group). After 30 days the same dogs were sedated with a mixture of MET 0.3 mg/kg, DEX 5 µg/kg and ACP 5 µg/kg IM (MET group) for the same diagnostic procedures and with the same method of BUT group. The degree of sedation was assessed using a numeric descriptive scale (NDS). The NDS consisted of a scale ranging from 0 to 3: 0, no sedation; 1, mild sedation (less alert but still active); 2, moderate sedation (drowsy, recumbent but attempts to get if stimulated); 3, intense sedation (very drowsy, still, unable to walk). The NDS scores were recorded at 5, 10, 20 and 30 minutes after IM injection. Side effects were recorded. At the end of procedures atipamezole 0.05 mg/kg was administered IM.

NDS score was analyzed by a Mann-Whitney U test.

In BUT group eleven dogs had intense sedation (NDS=3), three after 10 minutes and eight after 20 minutes; four dogs had a moderate sedation and three of these received propofol 1 mg/kg IV for completing the diagnostic procedure. Median NDS score was: 5 minutes, 1 (range 0-2); 10 minutes 2 (1-3); 20 minutes 3 (2-3); 30 minutes 3 (2-3). Significant differences were recorded in NDS score between 5 and 10 minutes and between 10 and 20 minutes. In one dog, muscle spasms and tremors were recorded.

In MET group eleven dogs had intense sedation (NDS=3), four after 10 minutes and seven after 20 minutes; four dogs had a moderate sedation and two of these received propofol 1 mg/kg IV. Median NDS score was: 5 minutes, 1 (range 0-2); 10 minutes 2 (1-3); 20 minutes 2 (2-3); 30 minutes 3 (2-3). Significant differences were recorded in NDS score between 5 and 10 minutes and between 10 and 20 minutes. In two dogs, muscle spasms and tremors were recorded; in one case mild reaction with wagging tail; in one dog, diagnostic procedures stimulated aggressive reaction.

No significant differences were recorded between NDS scores of the groups.

In both groups, recovery was sweet and devoid of complications.

When used with DEX and ACP, butorphanol and methadone provide the same degree of sedation, the maximum effect was obtained after twenty minutes and no considerable side effect was produced

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anaesthesia

anaesthesia, dog, methadone

POSTOPERATIVE ANALGESIC EFFECTS AND PHARMACOKINETIC PROFILE OF A CONSTANT RATE INFUSION OF TRAMADOL IN DOGS UNDERGOING ORTHOPEDIC SURGERY

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The aim of this study was to evaluate the postoperative analgesic effects of a constant rate infusion of tramadol in dogs undergoing orthopedic surgery.

After written owner consent, twenty dogs were anesthetized with acepromazine and morphine, propofol and isoflurane in oxygen. At the end of surgery, 2 hours after extubation (T0), dogs were randomly assigned to tramadol (TRM) or control (CTR) groups. The TRM group received a loading dose (1.5 mg/kg) followed by a constant rate infusion (1.3 mg/kg/h) of tramadol for 12 hours. The CTR group received a loading dose and an infusion of saline solution up to 12 hours. In the TRM group serum samples were obtained before (time 0) and from 0.08 to 24 after tramadol administration to quantify drug concentrations. In all dogs at 0.5 (T0.5), 1 (T1), 2 (T2), 4 (T4), 8 (T8), 12 (T12), 16 (T16), 20 (T20) and 24 (T24) hours after the beginning of the infusion heart rate (HR, beats/min), mean arterial pressure (MAP, mmHg), respiratory rate (RR, breaths/min), rectal temperature (T °C), sedation and the score of the Short Form of the Glasgow Pain Scale (SFGPS) were recorded. In case the SFGPS score was ≥ 5 , 0.1 mg/kg of morphine were administered. Sedation was assessed with a visual analogue scale with a score between 0 (awake) and 10 (deep sedation). Moreover in all dogs of the TRM group the pharmacokinetic profile of the drug and its metabolites was determined. Pharmacokinetic parameters were obtained with a two-compartment model and also with a non-compartmental analysis. Parametric data were analyzed with the one way ANOVA for repeated measurements, non parametric data were analyzed with the Kruskal-Wallis test.

Mean values of HR, RR, T and MAP were similar between the two groups at each study time. Sedation score was higher in the TRM group compared to the CTR group at T1, T2, T4, T8 and T12. The average number of doses of morphine per dog was higher in the CTR group (4.5 ± 2.0 doses/dog) compared to the TRM group (0.8 ± 1.1 doses/dog). All dogs in the CTR group required the administration of at least 1 dose of morphine in the 24 hours of observation while in the TRM group only 4 dogs required morphine administration. In the last 12 hours of observation (T12 - T24) dogs in the TRM group did not require any morphine while in the CTR group morphine was administered in 8 dogs. At the first sampling time (0.08 h) tramadol mean concentration were 0.57 ± 0.3 µg/ml, the concentrations attained in a range of 0.3-0.9 till 14 h, then decreased till 0.18 ± 0.2 at 24 h. At the first sampling time (0.08 h) O-desmethyl mean concentration were 0.05 ± 0.04 µg/ml, the concentrations increased gradually till a maximum value of 0.3 ± 0.15 µg/ml at 14 h and then decreased till 0.12 ± 0.11 at 24 h.

The results of this study demonstrated that a constant rate infusion of tramadol in dogs could be a valid and safe analgesic treatment after orthopedic surgery. It produces some degree of sedation and drastically reduces the use of morphine up to 24 hours after surgery.

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Anestesiologia

Tramadolo, Analgesia, Morfina

INTRANASAL ADMINISTRATION OF FENTANYL IN DOGS FOR THE TREATMENT OF SEVERE POST-OPERATIVE PAIN

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Aim of this study was to assess in dogs the analgesic effects of intranasal (IN) administration of fentanyl citrate for the treatment of severe post-operative pain.

N. 8 privately owned dogs of different breeds and sex, 2,69±2,8 years and 19,45±14,08 kg, undergone routine orthopedic procedures or laminectomy under isoflurane anesthesia, were randomly enrolled in the study under owner consent. After recovery, the dogs were hospitalized for the treatment of intense post-operative pain. Pain score (PS) was measured every 5 minutes starting from extubation by a modified multiparametric algometric scale (University of Melbourne Pain Scale) (1). When PS exceeded 6/23 (severe pain) 4 µg/kg of fentanyl citrate (Fentanest® Pfizer Italia S.r.l., 0.1mg/2ml) were administered by intranasal route by a nasal Mucosal Atomization Device (MAD® Wolfe Tory Medical Inc., Salt Lake City, UT, USA). Analgesic effect onset and duration were noted and vital parameters (T°C, RR, HR, NIBP and SPO2) were continuously recorded by a multiparametric monitor (Mindray PM-9000 Express®) before (T0) and up to 60 minutes after IN fentanyl administration. Analgesic treatment was continued by IM buprenorphine (20 µg/kg) according to patient needs. Physiological parameters and PS were reported at 5 minutes intervals (T0-T60) on an Excel sheet and processed by Analysis of Variance for repeated measures (ANOVA). When a significant difference was identified post hoc Tukey's (P≤ 0,05) was applied.

Intranasal administration of fentanyl citrate effectively reduced severe pain in our patients showing an onset time of 9,38±4,17 minutes, reaching a peak analgesic effect in about 20 minutes (PS median=3,5) and an overall duration of 35,63±8,21 minutes. PS showed significant differences during the observation interval (P<0,0001) identified between time points: T50>T15; T55>T20; T60>T20; T60>T15. No significant differences in T°C, RR, HR, NIBP and SPO2 were recorded. No adverse effects were observed in any subject. This study substantiates the IN administration of fentanyl for the treatment of severe postoperative pain in dogs as a suitable alternative to standard IV route and other analgesic treatments commonly used in the intensive care unit, due to the short latency and the full analgesic effect observed. Intranasal administration of opioids is commonly used in humans, especially in case of severe and sudden pain due to its rapid absorption and clinical effectiveness (2). A new fentanyl citrate formulation is now marketed for the treatment of breakthrough pain in human cancer patients for its rapid onset and ability to bypass first pass metabolism (2; 3).

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Veterinary Anesthesia

Fentanyl, Intranasal, Dog

SEVOFLURANE-SUFENTANIL OR PROPOFOL-SUFENTANIL ANAESTHESIA FOR BILATERAL PLEURODESIS UNDER THORACOSCOPY IN PIGS: PRELIMINARY STUDY.

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The aim of this study is to compare 2 different anaesthetic protocols in pigs undergoing bilateral pleurodesis.

After approval by Ministerial Decree n°298/2013-B, sixteen pigs were randomly divided in 2 groups differing in the maintenance regimen; propofol-sufentanil group (PG = 8 pigs) and sevoflurane-sufentanil group (SG = 8 pigs).

Premedication was provided by ketamine 8 mg/kg, methadone 0,2 mg/kg and dexmedetomidine 20 ug/kg, administered together IM behind the ear. Anesthesia was induced with propofol 4-6 mg/kg and trachea was intubated with a 7-7.5 mm cuffed tube. General anaesthesia was maintained with sevoflurane in 100% O₂ in the SG, and by propofol CRI (0,1-0,5 mg/kg/min) in PG and 100% O₂ administered by the same circuit. Animals were mechanically ventilated. Respiratory rate was adjusted to achieve normocapnia (EtCO₂ 35-45 mmHg) according to surgeon requests. A sufentanil CRI was started just after induction (0,1-0,5 ug/kg/h); a bolus of 0,1 ug/kg was given if signs of pain occurred. At surgeon request (once per side), a bolus of vecuronium bromide (0,1 mg/kg) was administered and animals were kept in apnoea for 90-180" by stopping ventilation in order to perform surgical treatment. Heart rate (HR), pulse rate (PR), periferic oxygen saturation (SpO₂), invasive blood pressure (IBP), airways pressure (Paw), gas inspiration and expiration % in SG, propofol infusion (0,1-0,5 mg/kg/min) in PG, tidal volume and body T° were continuously monitored and recorded every 5'. Arterial blood gas samples were collected as well. As anesthesia was turned off, times for palpebral reflex, extubation, head movement and standing were recorded as minutes from the end of anesthesia. Carprofen (3 mg/kg IM) was administered at the end of procedure.

Physiological values were analyzed at baseline (15' before start of surgery), incision, 5,10,15,20,25,30' after first and second apnoea by Mann-Whitney test.

Groups resulted homogeneous for weight, gender and age, and no significant differences between cardiovascular and respiratory values were detected. A statistical significant difference was found in standing between SG and PG, resulting longer in PG.

Both anesthetic protocols resulted adequate to maintain vital parameters in physiological ranges and were considered equivalent in terms of anesthetic depth and cardiorespiratory responses in pigs under thoracoscopic treatment.

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anesthesia

propofol, sevoflurane, pigs

XYLAZINE FOLLOWED BY XYLAZINE OR DEXMEDETOMIDINE VARIABLE RATE INFUSION FOR STANDING SEDATION IN THE HORSE

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The aim of the study was to evaluate the effectiveness of two different sedation protocols for a brief standing procedure in horses.

Fourteen standardbred horses undergoing gastroscopy for diagnostic purposes were involved in the study. Approval for ethical committee of University of Pisa was obtained. Animals were divided in two groups: group XX, that received a sedation with a bolus of xylazine 0.75 mg/kg IV followed by a xylazine variable rate infusion and group XD, that received a sedation with a bolus of xylazine 0.75 mg/kg IV followed by a dexmedetomidine variable rate infusion. The infusion rate was modified by an operator based on the evaluation of the sedation state.

Sedation was assessed with a multiparametric index of sedation by considering posture, response to a vocal stimulus, position of head and neck, position of the lower lip and tongue and position of the eyelid. During the procedure heart rate (HR), respiratory rate (RR), mean arterial pressure (MAP) and tidal CO₂ (EtCO₂) were recorded before the administration of sedation (Tbase) and every five minutes for 25 minutes.

No differences were detected between Tbase and the subsequent time points for HR, RR and EtCO₂ in both groups. Concerning MAP in the XX group a significant difference of Tbase values was found in comparison to T5, T10, T15 and T20 time points. No differences were found for XD group for MAP. Sedation score in XD was significantly higher at T10, T15 and T20 compare with Tbase, while in XX group Tbase values were significantly lower than T10, T15, T20 and T25. Median infusion dosage of dexmedetomidine was 5 mcg/kg/h (range 2-9) while median xylazine infusion dosage was 0.4 mg/kg/h (range 0.26-0.69). Mean time from infusion stop and coordinate movement of the horses was 12.5 ± 5 minutes for XX group and 6.8 ± 2 minutes for XD group.

Both sedation protocols resulted appropriate for the procedure. The infusion rate was modified in order to adequate the sedation plane to the stimuli in both groups. In the XD group a lower hypertensive effect was registered in comparison to XX group. The sedation plane in XX group was more stable but horses required a longer time to move out from the stock.

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Anaesthesiology and pain therapy

standing anesthesia, horse, alfa2-agonist

CONSTANT INFUSION OF XYLAZINE IN HORSE IN GENERAL ANESTHESIA WITH ISOFLURANE

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The purpose of the study was to evaluate the influence of a constant infusion of xylazine in healthy horses undergoing elective surgery under general anesthesia with isoflurane.

In the study were included 22 horses of various breeds anesthetized to perform elective surgery on the limbs. All horses were premeditated with acepromazine intravenously (IV) (0.02 mg / kg), flunixin meglumine (1.1 mg / kg IV) and procainic benzylpenicillin intramuscularly (IM) (9000 IU / kg IM) one hour before induction of general anesthesia. At the time of induction, all horses received xylazine 0.4 mg / kg IV and after 5 minutes diazepam 0.1 mg / kg IV ketamine and 2.2 mg / kg IV. All horses were intubated, connected to the anesthesia machine via a rebreathing circuit for large animals and mechanically ventilated. General anesthesia was maintained by inhalation of a mixture of isoflurane in oxygen. Also in 12 horses (group XIL) was simultaneously infused of xylazine at a dose of 1 mg / kg / h (Ringer et al., 2013) while the remaining horses (SF group, n = 10) received an infusion of saline. During anesthesia were monitored the following parameters: heart rate (HR, beats / min), mean arterial pressure invasive (MAP, mmHg), respiratory rate (RR, breaths / min), partial pressure of end-tidal carbon dioxide (EtCO₂, mmHg), percentage of isoflurane at end expiration (EtISO, %) and esophageal temperature (T, °C). The plan of anesthesia was assessed by monitoring the standard clinical parameters (palpebral reflex, position of the eyeball, tearing, and sympathetic response to surgical stimuli). In the case of values of PAM below 70 has started an infusion of dobutamine at the starting dose of 0.5 mcg / kg / min which was then increased if necessary to obtain a value of PAM lies between 70 and 80 mmHg. At the end of the surgery was interrupted administration of isoflurane and the infusion of xylazine or saline. During the recovery phase were given additional doses of xylazine (100 mg, IV) in the case of attempts to move earlier than 15 minutes after arrival in the recovery room and / or in the presence of nystagmus. The animals did not receive any type of assistance during the awakening and the quality was assessed using a scale previously validated in horses (Schauvliege et al., 2011), which provides values between 1 (excellent awakening) and 6 (awakening disastrous). In all patients was recorded the time between the arrival in the recovery room and the assumption of the station quadrupedal (time of awakening). The data were tested for normal distribution using the Kolmogorov-Smirnov test and analyzed with repeated measures ANOVA (parametric data) or the Kruskal-Wallis test (nonparametric data). Were considered significant for P values < 0.05.

The two groups were homogeneous with regard to the weight (XIL = 446.7 ± 98.5 kg, SF = 473.6 ± 78.4 kg), age (XIL = 7.4 ± 5.6 years, SF = 8.3 ± 4.3 years) and the duration of anesthesia (XYL 75.4 ± 13.3 min; SF = 83.6 ± 21.6 min). The average values of EtISO were significantly lower in the group XIL (1.30 ± 0.11 %, range 1.6 to 0.9) compared with the SF group (1.72 ± 0.13 %, range 2, 1 to 1.4) at all times of the study. All other physiological parameters monitored during anesthesia showed no significant differences between the two groups. The average consumption of dobutamine was lower in the XIL (0.14 ± 0.15 g / kg / min) compared with the SF group (2.64 ± 0.38 mcg / kg / min). XIL 6 horses in the group of 12 (50%) required the infusion of dobutamine, whereas in the group SF all subjects (100%) required an infusion of dobutamine. In the wake of the group XIL two horses and all horses in the SF group required additional boluses of xylazine for an average of horse bolus of 2.4 ± 0.5 in the SF group and 0.1 ± 0.3 in group XIL. The duration of the awakening was shorter in the group XIL (34.00 ± 6.04 minutes) compared with the SF group (55.80 ± 7.84 minutes). The median quality of awakening was better in the XIL (1.00, range 1-2) compared with the SF group (3, range 3-5).

The data of this study demonstrate that a constant infusion of xylazine at a dose of 1 mg/kg/h during general anesthesia in the horse reduces the dose of isoflurane required to maintain general anesthesia, reduces the consumption of dobutamine and improves quality of awakening.

Ringer, S.K., Portier, K., Torgerson, P.R., et al. 2013. The effects of a loading dose followed by constant rate infusion of xylazine compared with romifidine on sedation, ataxia and response to stimuli in horses. *Vet Anaesth Analg* 40, 157-165.

Schäivliege S., Marcilla M.G., Verryken K., et al. 2011. Effects of a constant rate infusion of detomidine on cardiovascular function, isoflurane requirements and recovery quality in horses. *Vet Anaesth Analg* 38, 544 – 554.

anesthesia

anesthesia , horses, xylazine

RABBIT DEEP SEDATION AND PLASMA CONCENTRATIONS AFTER TRANSNASAL ADMINISTRATION OF A DEXMEDETOMIDINE BUTORPHANOL AND MIDAZOLAM COMBINATION

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Aim of this study was to correlate the clinical and sedative effects of a combination of Dexmedetomidine, Midazolam and Butorphanol (DBM) with their respective plasma concentrations after transnasal (TN) administration in healthy rabbits.

This study was approved by the Ethics Committee of the University of Naples (CESA). A combination of 0.1 mg/kg of Dexmedetomidine (D- Dexdomitor® Pfizer Italia srl, 0.05%), 0.4 mg/kg of Butorphanol (B- Dolorex® Intervet, 1%) and 2 mg/kg of Midazolam (M- Midazolam IBI®, 0.5%) (DBM), was delivered by trans-nasal (TN) catheterization (Levin probe CH 6) onto the nasopharyngeal mucosa of 5 NZW Rabbits. Vital parameters were recorded (Mindary PM-9000 Exspress®) for 60 minutes along with sedation score (SS) and pain response by a numeric (0-12) rating scale (1). SS was classified (1) as insufficient (0-3), moderate (4-7) or deep (8-12). Blood samples (2 mL) were collected before (T-0) and at T-: 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 (minutes) after TN DBM administration. Plasma samples were then frozen and stored at -80°C. Single drug pharmacokinetics were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using AB Sciex 3200 triple quadrupole with positive ion electrospray ionization. Chromatographic separation (LC Agilent 1200) was performed on column ZORBAX DB-C18 (4.6 x 50 mm, particle size 1,8 mm). Identification and quantification was based on selected reaction monitoring (SRM). Calibration curves were prepared using fortified plasma samples.

All numerical data were processed by ANOVA for repeated measures. Significant differences were analyzed by a post hoc Tukey's HSD test ($P \leq 0.05$).

Deep sedation ensued in all rabbits 1.5' after TN DBM administration along with moderate respiratory and cardiovascular depression. Peak sedation and analgesia (T-5) coincided with mean plasma concentrations of about 23 ng/ml for D, of 34. ng/ml for B and of 560 ng/ml for M. Rabbits were still fairly sedated (T-60) at plasma levels of about 4 ng/ml for D, 12 ng/ml for B and 118 ng/ml for M. Insufficient sedation (T-90) was scored at plasma levels of about 2 ng/ml for D, 12 ng/ml for B and 85.40 ng/ml for M. No anesthetic complications were observed in any of the subjects.

TN administration of our DBM combination in healthy rabbits produced a deep, long-lasting sedation and analgesia. Plasma concentrations of single molecules correlated well with sedation scores.

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Veterinary Anesthesia

rabbits, trans nasal sedation, plasma concentration

COMPARISON OF THE ANALGESIC AND PHYSIOLOGICAL EFFECTS OF CAUDAL EPIDURAL ADMINISTRATION OF XYLAZINE OR CLONIDINE IN CATTLE

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The aim of this study was to compare the cardiovascular, respiratory, analgesic and sedative effects of caudal epidural administration of xylazine or clonidine in cattle.

After the approval from the ethical committee of the University of Bari and the written owner consent, in the study were included six cows, which required two caudal epidural anesthetics (within 1 week) for gynecological procedures in the perineal region. Each patient was randomly treated with xylazine (0.05 mg/kg) or clonidine (0.002 mg/kg) diluted with saline solution in a total volume of 5 mL injected in the caudal epidural space. Observers were blinded to the drugs administered. Heart rate (HR), respiratory rate (RR), mean arterial pressure (MAP), rectal temperature (T) were monitored and recorded before drugs administration and at 5, 10, 15, 30 minutes following drugs administration and at each 15 minutes thereafter. Thirty minutes after drugs administration an arterial blood sample was withdrawn for the determination of pH, arterial oxygen tension (PaO₂), arterial carbon dioxide tension (PaCO₂), arterial hemoglobin oxygen saturation (SaO₂), hemoglobin (Hb) and HCO₃⁻ concentrations. The onset, duration, intensity and level of sensory nociceptive block were assessed by pinpricks using a 18-Gauge x 3.8 cm needle. Bilateral stimulation was performed at defined regions using a scoring system of 0-3, as described by Skarda and Muir (1996). The regions where analgesia was tested are: tail, perianal area, perineal area, lateral surface of the hip and lumbar dermatomes. Positive nociceptive responses were defined as gross purposeful movements of the head, neck, trunk or limbs. The degree of sedation was assessed immediately prior to pinprick, based on the degree of struggling and the response to hand clap near the head. The degree of ataxia was assessed by the evaluation of the resistance to a lateral push on the pelvis and the frequency of fetlock flexions and swaying of the animal. Analgesia, sedation and ataxia were assessed before drugs administration and at 5, 10, 15, 30 minutes following drugs administration and at each 15 minutes thereafter. Parametric data were analyzed with the one way ANOVA for repeated measurements, non parametric data were analyzed with the Kruskal-Wallis test.

The onset, duration, intensity and level of sensory nociceptive block were similar between the two treatments. The degree of sedation and ataxia was greater in patients treated with xylazine as compared to clonidine between 15 and 90 minutes after drugs injection. In patients treated with xylazine PaO₂ was lower and intrapulmonary shunt was higher as compared to clonidine.

The results of this study demonstrated that xylazine and clonidine produce a similar intensity and spread of anesthesia when injected in the caudal epidural space in cattle. Xylazine causes more sedation and ataxia and a greater derangement of gas exchange compared to clonidine.

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Anestesiologia
epidural, clonidine, xylazine

PRELIMINARY CLINICAL EXPERIENCE ON HYALURONIC ACID TO ENHANCE THE HEALING OF CANINE SPONTANEOUS OPEN WOUNDS: AN OBSERVATIONAL STUDY ON 12 CASES

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Many in-vitro and in-vivo studies confirmed that hyaluronic acid (HA) is closely involved in fibroblast proliferation, enhanced formation of granulation tissue and even in keratinocyte proliferation and migration during wound healing process.¹

The aim was to explore the application of available in commerce HA-containing wound dressing on naturally occurring canine skin open wounds healed by second intention. In addition to wound area the progress of healing was also monitored using two well-recognized wound assessment scales of human medicine that quantitatively described the status of salient physiologic wound characteristics.

Twelve canine spontaneous cutaneous open wounds healed completely by only second intention were prospectively enrolled. All wounds were treated using a range of wound dressing containing HA from admission to complete re-epithelialization of the wound assessed by clinician. Because wound healing is a dynamic process, the choice of which HA-containing dressing had to be used was modified during healing phases according to salient physiologic wound characteristics and to manufacturer's instruction.

At all clinical control and/or bandage change, for each wound, the wound area and the scale scoring assessments were performed and recorded in double blinded modality by two clinicians with same experience in wound healing assessment. An agreement between two clinicians' evaluations in the application of both scoring systems was also investigated.

Based on wound etiology, 7 were traumatic, 2 were primary closure complication and 3 were excised wounds. At admission, the mean and median values of wound area were 72.8 and 32 cm², respectively. After debridement an increased wound size was obtained while an improvement was determined by both scale score systems. The median number of return to clinic was 5 times, respectively. The mean and median time to complete wound healing were 42.3 and 34.5 days, respectively. The mean wound area at day 7 was 90.4%, at day 14 was 47.7%, at day 21 was 22.4% and at day 28 was 14.8% of the original size. All products were well tolerated. The agreement between the two operators was considered high using both wound healing assessment tools.

Even if no control group was available, considering the short median time of complete epithelialization HA-containing dressing could be consider a possible wound treatment to enhance healing of open wounds in dogs. The assessment of wound parameters in addition to wound area could be useful especially in the earlier healing period and also to direct clinical decision-making. Further studies are needed to confirm these results and to compare HA-containing dressing with other wound therapies.

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chirurgia
hyaluronan, second intention, dog

ENHANCING BONE HEALING AND REGENERATION: CORTICAL AND CANCELLOUS MORSELIZED ALLOGRAFT SAMPLING AND BANKING.

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Critical size defects represent a huge challenge in both human and veterinary orthopedic surgery. Tissue engineering is a new approach to enhance bone regeneration. Combining a 3-dimensional scaffold with cells and growth factors, will allow to develop bone tissue equivalents able to induce a total regeneration of the affected area. In order to provide a proper 3D structure, several requirements for scaffolds are advocated as biocompatibility, a highly porous microstructure, strength and controlled degradation. In orthopedic reconstructive surgery a fresh graft of autologous cancellous bone with hematopoietic bone marrow is considered the most effective for major bone defects. However, the clinical application of such grafts is sometimes limited for amount, patient pain, donor site morbidity. On the other hand, the collection of allogenic bone samples in tissue banks would lead to the availability of an unlimited amount of material to be used for bone allograft or as a scaffold for cells led to the wide use of the freeze-dried bone allograft. We describe a new technique applied in the horse, which would aim to establish a Bank for bone allograft.

The donor's physical examination includes serological tests for EHV 1-4, EVA, EIAV before its euthanasia. The gluteal area is clipped and prepared for aseptic technique. The operator wears 3 pairs of sterile gloves and removes one of them at each step. An incision of about 20 cm from the tuber coxae is performed ventro-caudally. The scalpel runs over the surface underlying the bone, cutting the superficial gluteal muscle, the caudal margin of the tensor fascia lata and the gluteus medius. Then 2 perpendicular incisions will expose the bone surface. Four samples 2X4 cm of cortical-cancellous bone are obtained with an oscillating saw from the central portion of the tuber coxae toward the neck of the ileum. Swabs from both cortical and cancellous surface are taken for bacteriology, as well as a biopsy specimen from the cancellous bone. Bone samples are washed in sterile distilled water, dried out with sterile gauze and finally packed in 3 sterile plastic bags and frozen at -80° C. Microbiology negativity is ascertained in 7 days. Allografts can be processed as a powder, granules, chips, wedges or blocks. The morselized allografts are prepared with a bone mill as chips of 3-4.5 mm to preserve the bony consistency. The morselized is washed 3 times with PBS.

This technique is rapid with a reduced risk of contamination. The collected material is adequate for engraftment of msc, if the samples maintain a good ratio cortical-medullary bone with a reduced amount of bone marrow toward the tuber coxae leading to a lower risk of antigenicity.

Freeze-drying is an advantageous method that further decreases antigenicity and produces no biochemical changes focusing primarily on a promising bioengineering approach.

chirurgia ortopedica

bone healing, morselized, scaffold

BIODEGRADABLE POLYMERIC THREEDIMENSIONAL SUPPORTS FOR BONE REGENERATION: RESULTS OF A LONG-TERM STUDY IN VIVO.

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Bone defects with substance loss represent a common clinical problem with a difficult therapeutic approach. The aim of this study was to evaluate the effects of a long-term application of three-dimensional, biocompatible and biodegradable anatomically-shaped star poly-ε-caprolactone (*PCL) scaffolds in a critical-sized bone defect (CSBD) in a rabbit model.

Anatomically-shaped *PCL three-dimensional scaffolds, made with wet-spinning technique, were implanted in 6 New Zealand White rabbits, after the creation of a 20mm CSBD in the radial diaphysis. After surgery and at 4, 8, 12, 16, 20 and 24 weeks post-implantation, X-rays orthogonal images were obtained to evaluate new-bone formation with a scoring system point-based. At 24 weeks each scaffold was both explanted and morphologically and histo-pathologically evaluated. Findings concerning radiological scoring were evaluated with the one-way ANOVA test. The significance was defined for $p < 0.05$.

All subjects showed good tolerance to implants, the functional recovery was fairly rapid, and only one subject presented pain at limb manipulation without macroscopic signs of fistulas or skin lesions. Mean regeneration score obtained from radiological evaluation was 12.75 ± 4.57 . Radiological study of all images collected showed that bone regeneration on scaffolds had been evident as early as 4 weeks after implantation in 5 animals (83.3%); the regeneration was particularly evident on the lateral side of the scaffold near the ulna; the scaffolds were covered mostly by newly formed tissue with bone-like opacity. Macroscopical and histological data confirmed radiological findings. A new-regenerated bone was evident at the lateral side of the scaffold, near the ulna, with a new formed medullary cavity containing a tissue similar to bone marrow. The new-formed bone was hystologically characterized by the presence of mature and immature bone tissue. Neoangiogenesis was particularly abundant.

The *PCL scaffolds employed in this study showed a good capacity of osteointegration, osteoconduction and osteoinduction. Further investigations regarding functionalization with osteogenetics substances and/or the application on bone segments diverse from radius are necessary in order to validate this scaffolds as an effective aid for the orthopaedic surgery in the treatment of CSBD bone lesions.

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Dini F, Barsotti G, et coll. Supporti tridimensionali in materiale polimerico biodegradabile per la rigenerazione ossea: risultati dello studio in vivo Atti LXVI Convegno Nazionale SISVet, Roma – Auditorium, Ministero della Salute, 12-14 Settembre 2012; 325-328.

Chirurgia

Polycaprolactone, Critical Size Defect, Bone Regeneration

AMNIOTIC EPITHELIAL CELLS TRANSPLANTATION INDUCED ALTERNATIVE M2 MACROPHAGE ACTIVATION SUPPORTING TENDON REGENERATION

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^[1]~ TERAMO

Amniotic epithelial cells (AECs) have a therapeutic potential in tissue repair because their multipotent characteristics and ability to modulate the immune response (1,2). AECs, directly and secreting paracrine factors, modulate tendon healing inducing a prompt regeneration in experimentally injured tendons (3). In the present research, the role exerted of AECs on macrophages (M ϕ) polarization from pro-inflammatory M1M ϕ to anti-inflammatory M2M ϕ phenotype were studied and related to the early phases of tendon healing.

Ovine PKH-26 labeled AECs were transplanted into experimentally induced calcaneal tendons defects (3). Control (CTR) and AEC-tendon explants were explanted at 7, 14, and 28 days for morphological/molecular analyses. Tendon healing was described using Hematoxylin and Eosin stain and immunohistochemical (IHC) analyses to detect the expression of collagen type I an extracellular matrix protein (COL1). IHC and RT-PCR analyses were carried out to identify M ϕ (PanM ϕ CD68) and the incidence of pro-inflammatory M1M ϕ (CD86, IL12) and anti-inflammatory M2M ϕ (CD206, YM1, IL10) sub-populations.

AECs were always retrieved within the host tissue enhancing the early phase of tendon healing. In parallel, tendons displayed a different evolution in inflammatory events. PanM ϕ CD68 expression indicated that inflammation progressively decreased in both tendons. However, while polarized M ϕ and cytokines were similarly expressed in AECs and CTR tendons until day 7, the M2M ϕ became prevalent in the presence of AECs at day 14 when the tissue started to recover its microarchitecture displaying COL1 parallel oriented fibers. Analogously, the mRNA content of M2M ϕ anti-inflammatory cytokines YM1, IL10, CD206 increased, while M1M ϕ pro-inflammatory markers IL12 and CD86 decreased. M ϕ and cytokine expression were considerably reduced in regenerated AEC-tendons after 28 days, while they were still expressed in CTR tendons that maintained a disorganized tissue structure.

In summary, these findings suggest that AECs modulate macrophage recruitment enhancing the M2M ϕ regenerative phenotype and down regulating M1M ϕ , pro-inflammatory phenotype contributing to tendon healing. The present results suggests novel insights into potential mechanisms underlying tendon regeneration, associated with extracellular matrix remodeling amelioration, induced by exogenous ovine amnios epithelial layer stem cells delivery.

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REGENERATIVE MEDICINE

AMNIOTIC CELLS, TENDON REGENERATION, MACROPHAGE M1 AND M2

PHARMACOKINETICS AND SAFETY OF INTRA-ARTICULAR ADMINISTRATION OF LIDOCAINE PLUS ADRENALINE IN DOGS.

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Evaluation of the pharmacokinetics and safety of intra-articular (IA) lidocaine plus adrenaline in dogs undergoing arthroscopy.

The study was approved by Bioethical Commission of the University of Perugia.

Twelve dogs scheduled for arthroscopic surgery of the elbow were enrolled in this study and randomly divided into two groups. Once anesthetized, dogs were injected IA saline (S) or lidocaine (L) 1.98% plus adrenaline 1:100.000. The administered doses of L ranged between 6.40 and 9.43 mg/kg. The occurrence of cardiotoxicity was evaluated during surgery by monitoring the heart rate, the respiratory rate and the arterial blood pressure. As the nervous system toxicity could have been masked by the general anesthesia, L neurotoxicity was evaluated by assessing if neurotoxic levels of L could have been reached after the IA treatment. Thus, blood was sampled at scheduled time points up to 6 h from administration. The analytical determination of L in serum was performed by LC-MS/MS.

No occurrence of bradyarrhythmia, hypotension or impaired respiratory function was recorded after L administration. Lidocaine appeared in the systemic circulation 5 minutes after the administration, and the C_{max}, ranging from 0.19 to 2.19 µg/mL, was achieved between 30 and 240 minutes post-administration. At 6 hours, L was still detectable in all dogs at a mean concentration of 0.28 µg/mL.

The IA administration of L in dogs at the employed dose rates was not associated with the occurrence of cardiotoxicity. Achieved L serum concentrations were lower than those able to produce toxicity at the nervous system level, estimated for dog equal to 2.7 µg/mL (Lemo et al., 2007).

Lemo et al., (2007) Veterinary Record 160, 374-375.

Farmacologia veterinaria

Lidocaine, Intra-articular, dog

TIBIAL TUBEROSITY ADVANCEMENT MODIFIED BY A CIRCULAR CUT: CLINICAL EXPERIENCE IN SIX CASES.**Paragnani K.L.*^[1], Dini F.^[1], Gabellieri P.^[2], Carlucci F.^[1], Raschi A.^[1]**^[1]*Dipartimento di Scienze Veterinarie ~ Pisa*, ^[2]*Unità di Ortopedia e Traumatologia- Ospedale di Cecina ~ Livorno*

Cranial cruciate ligament insufficiency is a common cause of lameness in dogs as a result of instability of the stifle. TPLO and TTA are the most popular techniques to treat it. Both procedures are able to neutralize the cranial tibial thrust during the weight bearing even if by different rationales.

According to the TTA inventors, the femorotibial compressive forces are almost parallel to the patellar tendon. The finality of the TTA is to move the patellar tendon cranially to make it perpendicular to the tibial plateau in order to neutralize the cranial tibial shear force through a linear frontal plane tibial osteotomy and inserting a spacer between the tibial tuberosity and the corpus of the tibia. The tibial tuberosity is fixed in the new advanced position by use a dedicated implant. A modified TTA procedure was described in 2010. In this procedure, admitting the same theoretical principles of the TTA, the advancement of the tibial tuberosity is performed by a circular cut by a radial blade. The osteotomized fragments were fixed in the new advanced position using a T shaped locked plate. The aim of this study was to evaluate this modified TTA using different type of implants by a novel preoperative planning.

In this study is described the preoperative planning to perform the modified TTA by a circular cut and the modalities to replicate the landmarks in the surgical theatre and shift the osteotomized tibial tuberosity proximally along the surface of the radial cut, in order to create a post operative angle between patellar tendon and tibial plateau (PTA) nearly 90°.

Six clinical cases are described. One dog had previously a tradition TTA on the contralateral stifle whereas another dog was treated first with a TPLO. One dog had a bilateral CrCL rupture and both stifles were treated by modified TTA. Different implants were used to fix the tuberosity after his shifting, as linear and T shaped locked plates and tension bands. For all cases degree of lameness was recorded before surgery and during the follow up and radiographs were taken during the post operative period with follow up of two years and ten months.

The outcomes of the procedures are considered satisfactory in all cases but one without post operative F.U.. In a case Fracture of the tuberosity was recorded after a month. In this dog, the tuberosity was fixed previously by a locked plate and after surgical revision by a tension band.

Total recovery of the weight bearing was recorded after 2 months.

This modified TTA can be considered a valid alternative to traditional TTA with the advantage of avoiding dedicate instrumentation to perform it. In this clinical report, a variety of implants were used to fix the tibial tuberosity as locked plates and tension bands. Therefore, differently from traditional TTA, no cancellous bone grafting is required to fill the osteotomy gap because of the maintained contact between the tibial tuberosity and the rest of the tibia during the shifting of the fragment

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Chirurgia

modified TTA, Tension Band, Patellar Tendonangle

RADIOGRAPHIC EVALUATIONS OF DISTAL NAVICULAR BORDER FRAGMENTS AND FOREFEET CONFORMATION IN THE HORSE

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Radiographic examination has good sensitivity in revealing fragments located at the distal border of the navicular bone (DSBF) in the dorsoproximal-palmarodistal oblique (DPr-PaDiO) view with a proximo-distal angle between 55° and 65° (1). DSBF is a common finding in lame horses as well as in radiographic screenings (2).

Aim of the study was to evaluate the correlation between presence of DSBF and navicular and forefeet conformation.

The radiographs of 120 horses (Italian Saddlebred and Angloarabian, aged 3-15 years), obtained during pre-purchase or for diagnostic purposes, were used. Feet were classified in 3 groups: 1. lame feet with DSBF, 2. lameness-free feet with DSBF, 3. feet from sound horses without DSBF. Location of the lameness at the foot was confirmed by diagnostic anesthesia in lame horses.

The averages of 3 consecutive measurements of 3 ratios and 1 angle were statistically compared among the 3 groups: 1. distal navicular border length / navicular maximal width ratio (Y/Z), 2. height of sole at the toe / height of the heels ratio (E/D), 3. length of the toe / length of the foot ratio (C/B), 4. angle of the dorsal hoof wall to the ground (F).

In feet with DSBF, Y/Z, E/D and C/B ratios were higher if compared with feet without fragment ($P < 0.05$). In feet with fragment, feet without lameness showed higher E/D ratio than feet with lameness ($P < 0.05$). Although the difference was not statistically significant, Y/Z and C/B ratios were also higher in lame feet if compared with non lame feet.

Even if not statistically significant, F angle was smaller in feet with both fragment and lameness and in feet without fragment if compared with feet with fragment without lameness.

DSBF seem to be more present in navicular bones with more "rectangular" rather "trapezoidal" shape in the DPr-PaDiO 60° oblique view (high Y/Z ratio) and in feet with long toe-low heel conformation (high E/D and C/B ratios). This correlation between conformation of the foot, navicular shape and presence of DSBF suggests an important role of mechanical forces in the occurrence of DSBF in horses's foot and highlight the importance of early foot trimming and management.

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Surgery

horse, foot conformation, navicular fragment

CT STUDY OF PELVIC LIMB ALIGNMENT IN ENGLISH BULLDOG

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To define the pelvic limb alignment in English Bulldog (EB) breed dogs (with and without medial patellar luxation MPL) through the execution of CT studies and the measurement of femoral and tibial angles in order to identify any specific alignment abnormalities associated with MPL.

The study included 39 pelvic limbs of 21 EB older than one year of age divided into five groups: normal (15), grade I MPL (15), grade II MPL (5), grade III MPL (2) and grade IV MPL (2). The limbs that had orthopedic surgeries performed before the CT studies (1) and the limbs with lateral patellar luxation (2) were excluded from the work. A CT scan (Siemens®, Somatom Emotion) was performed for each patient and for each pelvic limb the following angles were calculated:

Femur: inclination angle (AI), anteversion angle (AT), anatomic lateral proximal femoral angle (aLPFA), anatomic lateral distal femoral angle (aLDFA)¹.

Tibia: mechanical lateral proximal tibial angle (mLPFTA), mechanical lateral distal tibial angle (mLDFTA), mechanical cranial proximal tibial angle (mCrPTA), mechanical cranial distal tibial angle (mCrDTA), trans-condylar tibial axis (TC), cranial-distal tibial axis (CnT), CnT – TC = Tibial torsion (TTA)².

The Mann-Whitney U test was used to compare each variable for normal and affected subjects, the values with $p < 0.05$ were considered to be statistically significant.

Significance (p) for each variable: AI = 0.08, AT = 0.078, aLPFA = 0.95, aLDFA = 0.0001, mLPFTA = 0.31, mLDFTA = 0.09, mCrPTA = 0.64, mCrDTA = 0.47, TTA = 0.066.

The statistical analysis of the data showed that "p" was less than 0.05 (<0.05) for the variable aLDFA, while it was close to significance for the AT and TTA angles.

From the results analysis, it was possible to appreciate how patients with MPL show an increased aLDFA (mean value $100.02 \pm 8.41^\circ$), a decreased AT angle (mean value $6.92 \pm 12.78^\circ$) and an increased external TTA (mean value $5.92 \pm 8.82^\circ$) compared to normal subjects (mean values are respectively $92.33 \pm 4.75^\circ$, $11.36 \pm 6.41^\circ$, and $4.004 \pm 7.37^\circ$).

These anatomical and functional aspects are described to cause, alone or in combination, MPL in other dog breeds³. This study has proved that in EB as well an increased varus angle of the distal femur can cause MPL, while, considering the small number of patients with grade III and IV MPL, future studies are needed to identify also insufficient femoral neck anteversion and increased external tibial torsion as other factors.

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2. Fitzpatrick C et al. (2012) Evaluation of Tibial Torsion in Yorkshire Terriers with and without Medial Patellar Luxation. Vet Surgery, 41, 966-972

3. Soparat C et al. (2012) Radiographic measurements for femoral varus in Pomeranian dogs with and without medial patellar luxation. Vet Comp Orthop Traumatol, 25, 197-201

Orthopedy

Pelvic limb, CT Study, English Bulldog

NONUNIONS TREATMENT WITH A POLYAXIAL LOCKING PLATE SYSTEM (PAX) AND BMP-2 IN FOUR TOY BREED DOGS

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Most pseudarthrosis are due to technical errors. However, in toy breed dogs, there's an inherent tendency to nonunion.

Three Italian greyhounds with distal radio-ulnar nonunion and a Pomeranian with femoral nonunion were brought to our attention. In the first case ulna k-wiring and radius plating with a 2.7DCP were performed, in the second case fracture fixation was achieved by means of a 2.0 T-plate, while in the third case the fracture was treated with a type II external fixator. In the fourth case, the fracture was initially treated with a 2.0DCP plate, after implant failure, a revision surgery was performed with a tie-in configuration external fixator. In the first case refracture occurred during implant removal. In the other cases there has been implant failure accompanied, in two cases, by severe bone resorption.

In the first three cases nonunions were treated with 2.4PAX plates applied on the dorsal surface of the radius (2proximal screws and 2distal screws), associated with BMP2 (Truscient) implants. A plate and rod construct was used for revision of the femoral nonunion (a 2.4 PAX plate and a 1.5mm rod).

Serial radiographs were performed every 15days and the implants were first dynamized by removing one screw respectively from the proximal and the distal side and then completely removed after fracture healing at different times for each case.

Angular stable implants offer the advantage of better preserving the periosteal vascularity, a fundamental aspect for fracture healing in toy breeds, it also allows for a stable fixation using a reduced number of screws. The internal osteosynthesis PAX system gives the surgeon the freedom of up to 10° multidirectional screw insertion. This characteristic was found to be essential during surgery, it allowed for an easy and proper implant placement associated with a correct alignment of the skeletal segment, which is not always uncomplicated to achieve with "traditional" locking systems.

The use of a titanium-made osteosynthesis system could be theoretically useful for a more physiological mechanical stimulation of the healing fracture site resulting in a faster maturation of bone callus. We believe that the results obtained represent a good starting point for further studies on off-label use of BMP2 as ancillary to surgical treatment of nonunions in dogs.

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Orthopedy

pseudarthrosis, BMP, polyaxial locking

USING A STRUCTURAL CORTICO-CANCELLOUS BONE GRAFT AND BONE MORPHOGENETIC PROTEINS (rhBMP-2), IN AN ATROPHIC NONUNION OF A CAT.

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Atrophic nonunion with substance loss represents a common clinical problem and difficult therapeutic approach. The aim of the work is to document the treatment of radio-ulnar atrophic nonunion of a cat, treated by internal fixation associated with structural cortical autograft and bone morphogenetic proteins rhBMP2.

A male six year old cat, has been taken to the Veterinary Teaching Hospital "Mario Modenato" for persistence of the left front limb lameness. Owners reported a diaphyseal fracture of the radius and ulna, happened 18 months before and 3 surgeries performed due to implant loosening and delayed union. At the orthopedic clinic visit the patient presented lameness of second / third grade, mild algia on palpation of the radial diaphysis, muscles atrophy of the arm.

The radiographic examination showed a severe atrophic nonunion, with thinning and loss of density of the bone stumps, the ulna was well established and with an increased diameter compared to the contralateral side. The fourth surgery was performed in compliance with the principles of treatment of atrophic nonunions, with the aim of stimulating the bone vascularization and osteogenesis by realizing a debridement and ostectomy of the apices and recanalization of the stumps and managed 2 cm deficit bone of the radius (20% of the entire length), with a transplantation of a segmental cortical bone (osteoconductive), taken from the cranial-dorsal portion of the ilium wing.

We proceeded with the stabilization of the radius and of the segmental bone graft by Synthes 1.5 LCP plate and by 1.5 locking screws with buttress technique, expanding two screws in proximal and distal radius and two screws into the bone graft.

After fixation an osteoinductive biomaterial was applied constituted by bone morphogenetic proteins rhBMP-2, (Pfizer, Truscient ®) to a total dose of 0.4 mg carried by two strips of a osteoconductive membrane bovine collagen.

The clinical control at 25 days after surgery showed no lameness, no algia on palpation of the radio, the absence of reaction of the soft tissues.

Radiographs in 25 days, performed in two standard orthogonal projections showed the total corticalization of the bone segment with the integration of the bone graft.

Key factors for the management of this atrophic nonunion, were the application of bone mechanical and biological needs:

osteogenesis was guaranteed by the management of the fracture with debridement, ostectomy of the apices and recanalization of the bone, in order to stimulate the formation of a new vascular network and cellular supply.

The osteoconduction is derived from cortico-cancellous bone graft taken from the ileum that guaranteed scaffold effect.

The osteoinduction was stimulated by the rhBMP-2, bone morphogenetic proteins, belonging to the group of growth factors, derived from recombinant DNA technology, with chemotactic function and differentiation of multipotent cell lines.

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chirurgia

bone regeneration, rhBMP-2, nonunion

DESCRIPTIVE EPIDEMIOLOGICAL STUDY OF FRACTURES IN RAPTORS ADMITTED TO THE WILDLIFE RECOVERY CENTER (C.R.A.S.) IN NAPLES.

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The aim of the study was to assess the prevalence and the distribution of bone fractures in free-living raptors as well as to evaluate the influence of hunting activity.

A retrospective study was performed on clinical records of the Wildlife Rescue and Rehabilitation Center (CRAS) of Naples from January 2010 to February 2014. Bird species, age and sex, rescue area, and date of admission were recorded. Bird, classified in three age classes: pullus, subadult and adult, belonged to three Orders: Accipitriformes, Strigiformes, Falconiformes. Fractures were characterized by using radiographs; in one case, a CT study was also performed. Recorded data were analysed by using a χ^2 -test to compare between age classes, their predisposition to single or multiple fractures, the months of the year in relation to numbers of shot subjects. Fisher's test was used to correlate sex to single or multiple fractures, the prevalence of species in our sample and of shot species. Significance was set at $P < 0.05$.

Out of 1068 raptors admitted, 402 (37.64%) were fractured with a total of 566 limbs included. The sample was composed of 57.6% males and 43% females with a significantly higher prevalence of males ($P = 0.0462$). Regarding ages, adult class was significantly higher ($P < 0.0001$). The fractures involved mainly thoracic limbs (87.46%) compared to pelvic ones (12.54%); bone localization included ulna (34.63%), radius (21.38%), humerus (19.79%), metacarpal bone (10%), tibiotarsal bone (7.77%), femur (2.83%) and others (3.53%). The most frequent fracture line patterns were oblique (47.35%), transverse (30.56%) and spiroid (20.14%). The majority of shot raptors were admitted during the hunting season ($P < 0.0001$). Among the nine species recorded for fractures, Buteo buteo and Athene noctua were the most prevalent ($P < 0.0001$). Falco tinnunculus was the most prevalent species admitted for gunshot fracture ($P < 0.0001$).

Descriptive epidemiological studies about bone fractures in birds of prey showed to be an important source of information on natural distribution and anthropogenic hazards for raptors living in urban environments.

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SURGERY

BIRD OF PREY, FRACTURES, EPIDEMIOLOGY

CARPAL ARTHRODESIS WITH EXTERNAL SKELETAL FIXATION IN DOG: 12 CASES

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In small animals, arthrodesis is a salvage procedures commonly used to treat muscular, orthopedic and neurologic diseases of carpal joints unresponsive to conventional surgical and medical management. Previously reported techniques include internal fixation using plates applied to either the dorsal, medial, or palmar aspect of the carpus, cross pinning, and linear or circular external skeletal fixation (ESF). The aim of this study was to compare the clinical outcome of carpal arthrodesis obtained by the use of linear ESF and acrylic ESF.

A retrospective study was made on the records of the years 2010-2013. Inclusion criteria for pancarpal or radio-carpal arthrodesis were pain and lameness attributable to carpal joints diseases nonresponsive to previous surgical or nonsurgical management, and severe neurological deficiency. Surgical procedure consisted in carpal joints fusion through debridement of cartilage from the articular surfaces, perforation of the subchondral bone plate, cancellous bone transplantation and external skeletal fixation (traditional or acrylic implants). Age, breed, body weight, type of implant and time for ESF removal and were recorded for each dog and groups were compared with Mann-Whitney U-test ($P < 0.05$).

The sample was composed by 12 patients. Mean duration until ESF removal, based on clinical and radiographic evidence of joint fusion, was 73 days (range 54–86 days): mean time to implant removal was 64.6 days (range 54–70 days) for acrylic ESF and 81.4 days (range 77–86 days) for traditional ESF.

Mean time for implant removal is less in patients treated with acrylic ESF, about 8 weeks, than in patients treated with traditional ESF, about 12 weeks. This difference is statistically significant with a P-value of 0.001, and animals with acrylic ESF achieved bone fusion earlier than animals treated with traditional ESF.

The best results of the acrylic ESF were probably due to two factors: less incidence of complication (i.e. pin tract infections); and then because the less weight of the implant that is associate to a faster use of the limb. The choice of implant was correlated to body weight, in fact large dogs were treated with traditional ESF. After 4-5 weeks osteolytic areas around the K-wires determinate a reduction of the system working; this condition, associated to a good use of the limb, increases the mechanical loading on the damaged joint and a high response to bone healing and bone matrix deposition.

In our experience, ESF arthrodesis demonstrated to be a good option for obtaining carpal bones fusion. This technique offer satisfactory mechanical strength with low trauma to soft tissue during surgery and consequently minor risk of postoperative complications. In small sized dogs the use of acrylic resin is a valid alternative to traditional implants.

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Chirurgia

Dog, Arthrodesis, External Fixation

LUMBOSACRAL LUXATION IN DOGS: CLINICAL OUTCOME OF TWO DIFFERENT SURGICAL PROCEDURES

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The aim of this study is to evaluate clinical outcome of 2 surgical procedures performed to stabilize the traumatic luxation of the lumbosacral joint (LSJ) in 3 dogs

three paraplegic dogs with LSJ traumatic dislocation were included in the study. After premedication with Acepromazine at the dose of 20 µg/kg and Morphine at the dose of 0.3 mg/kg IM, anesthesia was induced by Propofol (4 mg/kg IV) and maintained with Isoflurane in 100% O₂. Case 1: a 30 kg female mixbreed, was treated with external skeletal fixation (ESF) utilizing 2 transilial pins, externally locked with 2 clamps. Case 2 and 3: males of 15 and 30 kg respectively, were treating performing lumbosacral fixation utilizing 6 pins. Two pins were inserted, across articular process L7/S1, into the body of the sacrum; two pins were inserted into the L6 and L7 pedicle and body respectively. All pins were cut and bent to achieve maximum overlap. Cerclage wire and polymethylmethacrylate (PMMA) was applied to bond free ends of pins

Case 1: the ESF was removed 50 days after surgery although it has become loose 30 days before. By 7 months there was a marked improvement and the dog was able to walk and run with a slight neurological deficit. Case 2: the dog was able to stand and walk within 48 h after surgery. 90 days after surgery the dog still had mild urinary and faecal incontinence. Case 3: the dog was able to stand and walk 24 h after surgery without presenting neurological alterations

To the authors' knowledge, different techniques have been described to stabilize fracture or fracture/luxation of L7: nevertheless single traumatic LSJ dislocation and its treatment are not widely described in dogs¹. In our experience the ESF is able to stabilize L7 fractures because the bone healing via callus formation needed a shorter time, while the healing via fibrosis requested for L7 luxation lasted longer. On the other hand ESF shows several advantages, including limited dissection of soft tissues, adjustability after application and removal after repair. The stabilization technique with pins and PMMA provides a relatively rigid construct to reduction of L7 luxation and have several advantages such as excellent strength and stability, minimal need of postoperative care and short recovery time. Disadvantages can include soft tissues dissection, relatively long time of surgery with higher risk of infections that we controlled by using strict aseptic technique and antibiotic administration. In our experience the stabilization with pin and PMMA shows better clinical outcome with a short recovery time and mild complications, while ESF seems to not stabilize completely a LSJ luxation because of the early implant loosening and the high risk of infections

Weh and Kraus Vet. Surg 36:775–782, 2007

ortopedia

lumbosacral luxation, PMMA, ESF

SHEEP OVERGRAZING: TURF AND SOIL DEGRADATION

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Sheep breeding, traditionally linked to pasture, has economic advantages by reducing feed costs, but it can cause serious damage to environment. Besides leading to plant cover reduction and species replacement, sheep overgrazing, in fact, negatively affects physical soil properties, and, in consequence, microbial community functioning, and soil organic matter cycling (Prieto et al., 2011). The aim of this study was to verify sheep overgrazing damages on soil and turf at different intensities of grazing.

The trial was carried out on an flat area located in S. Piero a Grado (Pisa), characterized by spontaneous turf and never used for grazing. In a fenced plot of 150 m², 15 Massese sheep were introduced to grazing about eight hours per day. A high stocking density (about 1 head/ 10 m²) has been chosen in order to recreate a situation of extreme overgrazing. In two different experimental phases, spring and autumn, turf and soil samples were collected. On turf sampling were carried out at the starting of the trial and at autumnal sprout, to evaluate the floristic changes due to grazing, through botanical analysis. Soil was sampled after 100 hours and 250 hours of grazing for two seasons respectively. On soil samples the analysis of the major physical, chemical, biochemical and chemical structural parameters, appropriate indicators of soil fertility and quality, were carried out.

At the end of the first phase of grazing, botanical analysis showed changes in floristic composition, highlighted by Leguminosae disappearance (25% vs 0%) and by increase of Graminaceae (40% vs 47%) and Others Species (35% vs 53%). Chemical parameters linked to soil organic matter content did not change significantly with grazing impact, thus showing a balance in nutrient cycling. Moreover, a deeper investigation on mineralization and humification processes was carried out through the evaluation of chemical structural soil properties by Pyrolysis Gas Chromatography technique. Sheep grazing generally affected soil physical and biochemical characteristics, mainly in the second phase of grazing (autumn). A significant reduction of soil total cracking (17,9% of cracked area vs 9,1%) was observed while bulk density did not change significantly. Biochemical parameters of soil (dehydrogenase and B-glucosidase) dramatically decreased in the second phase of grazing (autumn), thus demonstrating the negative effect of animal pressure on soil microbial activity.

Overgrazing by sheep negatively affected turf quality while no greatly affected soil organic matter content and its dynamic. On the other hand, soil physical and biochemical characteristics seem to be totally compromised. In conclusion, mismanagement can lead to irreversible damages to plant-soil system.

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Environmental impact and livestock management

Livestock, soil quality, mismanagement

		Undisturbed soil	I phase of grazing (spring)	II phase of grazing (autumn)
Total Cracks (%)	100 h	17.93 a	18.97 a	6.24 c
	250 h		17.01 b	9.11 d
Bulk Density (g/cm ³)	100 h	1.33 ns	1.09 ns	1.17 ns
	250 h		1.14 ns	1.17 ns
Total Organic Carbon (%)	100 h	0.95 ns	1.24 ns	0.98 ns
	250 h		1.04 ns	1.07 ns
Total Nitrogen (%)	100 h	0.11 a	0.16 a	0.09 b
	250 h		0.14 a	0.07 b
Dehydrogenase (µg INTF/g dw*h)	100 h	2.55 b	6.23 a	0.89 d
	250 h		1.36 c	1.24 d
B-glucosidase (µg PNF/g dw*h)	100 h	49.4 c	110 a	54.8 cd
	250 h		81.2 b	45.4 d
B/E3	100 h	0.79 b	0.67 bc	0.61 c
	250 h		0.89 b	1.06 a
N/O	100 h	0.86 b	1.32 a	1.31 a
	250 h		1.48 a	0.84 b
O/Y	100 h	1.88 a	1.05 b	1.22 b
	250 h		1.31 b	1.08 b

Tab 1. Chemical, biochemical, chemical structural and physical characteristics of soil after sheep grazing in spring and autumn, respectively and in undisturbed soil. Different letters mean statistically different value; ns, not significant ($p < 0.05$)

	I phase of grazing (spring)			II phase of grazing (autumn)		
	0 h	100 h	250 h	0 h	100 h	250 h
Graminaceae	40 %	45 %	-	47 %	-	-
Leguminose	25 %	3 %	-	-	-	-
Other species	35 %	52 %	-	53 %	-	-

Tab 2. Percentage of turf abundance after sheep grazing in spring and autumn, respectively and in undisturbed soil.

BIRTH WEIGHT AND POSTNATAL GROWTH OF MAINE COON KITTENS

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The Maine Coon cat, the biggest recognized feline breed, is enjoying a great popularity in Europe and in the rest of the world. Although this cat is well known for the late somatic and sexual development, scientific studies on its body growth, as well as of the other cat breeds, are still limited, though this kind of knowledge may be very important from a veterinary perspective.

Aim of the present study was to study the growth of Maine Coon kittens of both sexes.

The growth of 68 pure-bred kittens (37 males and 31 females), belonging to 14 different litters, was monitored during 75 days after birth. Body weight of each kitten was recorded immediately after birth and, on a daily basis, from the 1st to the 15th day of life, then every 4 days until kittens reached 43 days of age. After this period, the body weight of 45 of the 68 initial kittens was recorded at Day 51, 59, 63, 67 and 75. All kittens were breastfed and were weaned starting from the 3rd week of life with a mainly dry diet for growing kittens. The same feed management was used also at the end of the weaning, at about 8 weeks of life. All kittens remained in good health throughout the study. Data were analyzed by one-way ANOVA with sex as the main effect. In addition, linear regression was performed to study the association between the age and body weight of kittens.

Average weight of kittens at birth was 126±16 g in females and 134±17 g in males; these values are higher than those observed by other authors in Maine Coon cats (1,2,3) and in other feline breeds such as Siamese, Norwegian Forest cat, Birman and Persian (2). As expected, regression analysis showed a highly significant relationship between age and body weight of kittens ($r^2 = 0.9994$ and 0.9992 , in males and females, respectively; $P < 0.0001$). Growth curve during the first 75 days of life showed that body weight of kittens, compared to body weight at birth, was twofold higher at Day 10, threefold higher at Day 19, fivefold higher at Day 35 and tenfold higher at Day 63. This trend seems to confirm data from a previous study (2), although, in this latter case, body weight of kittens (79 animals) was measured only at birth and again after 28 and 84 days of life. Daily weight gain of kittens was not influenced by sex. Conversely, body weight was significantly higher in male kittens from Day 1 ($P < 0.05$) throughout the study ($P < 0.001$ starting from Day 39) (Figure 1).

The present investigation showed that body growth of Maine Coon kittens followed a similar trend in both sexes, despite the fact that body weight of male kittens was already higher at the first day of life and remained higher throughout the study.

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2. Moik K & Kienzle E. Br J Nutr 2011;106:S32-34.
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Breeding of companion animals

Maine Coon, Kitten growth, Weight curve

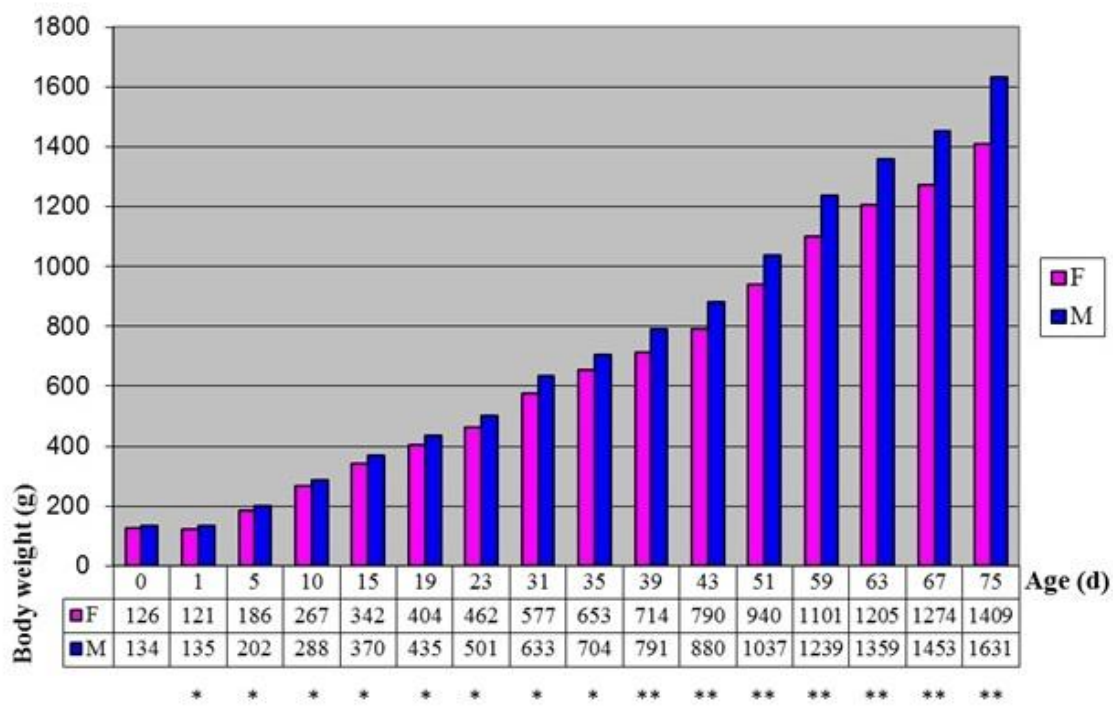


Figure 1: Average body weight gain of 68 Maine Coon kittens from birth to 75 days of life
(*: $P<0.05$; **: $P<0.001$)

PRODUCTION OF BACTERIAL ANTIGENS IN PLANT EXPRESSION SYSTEM

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The aim of this study was to evaluate tobacco plants, transformed by stable agroinfection, as expression system of bacterial antigenic proteins as a model of edible vaccine for swine. The attention was also focalized on the analysis of tobacco progeny in order to assess the stability of the bacterial genes into the plant genome.

Three independent lines of transgenic tobacco plants (Rossi et al., 2003a; Rossi et al., 2003b), respectively expressing the F18 adhesive fimbriae and VT2e B-subunit genes from Verocytotoxic Escherichia coli strains and the flgK flagellin from Salmonella typhimurium strain were previously obtained by Agrobacterium-mediated stable transformation technique (Rossi et al., 2013; Rossi et al., 2014a). The chimeric constructs pBlpGLOB-F18, pBlpGLOB-VT2eB, pBlpGLOB-flgK were used to transform Agrobacterium tumefaciens strain EHA105. The GLOB promoter is the soybean basic 7S globulin promoter (DDBJ no. AX006477) and was used for the seed-specific protein expression (Reggi et al., 2005; Rossi et al., 2014b).

The entire genes, codifying respectively for F18, VT2e-B and flgK, were amplified from genomic DNA of transgenic plants by PCR (table 1). The mRNA was evaluated by Northern blot analysis on the seeds 12 days after pollination. The total proteins were extracted from all mature transformed tobacco lines by homogenization with liquid N₂ in a mortar with the solubilization buffer (50 mM Tris, pH 8, 5 mM EDTA, 200 mM NaCl, 0.1% Tween 20) and were estimated by a Bradford assay (using bovine serum albumin as the standard). The expression of each antigen in the total protein samples was evaluated by Western blotting with specific polyclonal antibodies. The R1 and R2 generations, propagated in a greenhouse were evaluated using the same experimental conditions previously described.

The presence of genes codifying for the antigenic proteins in the genome of the three transgenic plants were confirmed by PCR. Transgenes were identified by electrophoresis on agarose gel by the presence of an amplified product of 0.5 Kb, representing the gene encoding F18 fimbriae, by the presence of an amplified product of 0.25 Kb, representing the gene encoding VT2e-B and by the presence of 1.6Kb, representing flgK. Northern blot and Western blot analyses detected specific signals in all samples, and by comparison with positive controls, the amount of antigenic proteins was estimated to be about 0.6 mg per gram of seeds corresponding to 0.33% of the total soluble protein in tobacco seeds.

For each line the obtained amount of antigens is sufficient for subsequent oral immunization trials (Verdonck et al. 2007, Lamphear et al. 2002,). Obtained data showed the inheritance of transgenes in the R0, R1, R2 generations and the stable integration of VT2e-B, F18 and flgK genes into tobacco genome.

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Lamphear B, Streatfield SJ, Jilka JM, Brooks CA, Barker DK, Turner DD, Delaney DE, Garcia M, Wiggins B, Woodard SL, Hood EE, Tizard IR, Lawhorn B, Howard JA. Delivery of subunit vaccines in maize seed. J Control Rel 2002, 85(1/3), 169–180.

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Nutrizione e alimentazione animale

expression system, edible vaccine, transgenic plants

Antigens	Pair of primers	PCR conditions
Vt2e-B (<i>Escherichia coli</i>)	5'ggATCCATgAAGAAgATgTTTATAgCgg 3'gAgCTCTTAgTTAACTTCACCTgggCAA	25 cycles of Den.: 1' at 95 °C, Ann.: 1' at 50 °C Ext.: 1' 30" at 72 °C.
F18 (<i>Escherichia coli</i>)	5'ggATCCATgAAAAgACTAgTgTTTATTTCTTTg 3'gAgCTCTTACTTgTAAgTAACCgCgTAAgC	25 cycles of Den: 1'at 94 °C, Ann: 1'20" at 56 °C Ext: 1'30" at 72 °C.
flgK (<i>Salmonella Typhimurium</i>)	5'ggATCCATgTCCAgCTTgATTAATCACgCC 3'AAACTACgCAATAACTTATAAgCgATTCTCgAg	25 cycles Den.: 1'at 94°C, Ann.: 1' 20" at 55°C, Ext.: 1' 30" at 72°C.

ALOE ARBORESCENS SUPPLEMENTATION IN DOG DIET: IN VITRO EVALUATION**Musco N.^[1], Calabrò S.^[1], Grossi M.^[1], Ferrara M.^[1], Tudisco R.^[1], Addi L.^[1], Cutrignelli M.I.^[1]**^[1]DMVPA, Università di Napoli, Federico II ~ Napoli

The genus *Aloe* comprises about 600 species. *Aloe* has been used for centuries for a multiplicity of unrelated human ills. *Aloe* plant contains anthraquinone glycosides (aloins), mucilage, a resinous material, sugars, mucopolysaccharides (acemannan, β -mannan), fatty acids, glycoproteins, enzymes and other compounds. This complex composition confer to the *Aloe* laxative properties, anti-inflammatory, immunostimulant and antiseptic activities (Capasso et al., 1998). Aim of the present study was to evaluate the hindgut effects of *Aloe* in dog using the in vitro gas production technique

Four neutered adult dogs were fed with a commercial diet (ME 3114 kcal/kg, CP 27,8 %; EE 16.5%, CF 3.0 %) for 20 d before to collect feces, which were used as inoculum for in vitro trial. The same diet supplemented with different ratios (0; 0.7; 1.6 and 3.2 %) of a commercial product (NUTRIZOO s.a.s., Italy) containing *Aloe arborescens* was incubated in vitro according to Cutrignelli et al. (2009). In particular, each diet was weighted in serum flasks with a medium and inoculum. The flasks were incubated under anaerobic condition for 48 hours at 39°C. The inoculum was composed by fecal samples pooled and diluted with sterile saline solution. Gas production of fermenting cultures was recorded every two hours using a manual pressure transducer. After 48 h the fermenting liquor was analyzed for pH, short chain fatty acids (SCFAs) by gas chromatograph, lactic acid and ammonia (NH₃) by spectrophotometer. The organic matter disappearance (OMD) was measured by filtering flasks content through glass crucibles and burning the residual for 5 h at 550°C. The influence of *Aloe* supplementation was analysed by ANOVA.

The OMD and short SCFAs were significantly ($p < 0.01$) decreased by *aloe* supplementation, while gas volume related to the incubated organic matter (OMCV) were not affected by the supplementation. On the other hand, lactic acid production increased progressively in function of the *aloe* dose. Both parameters related to the protein degradation showed a trend not-related to the supplementation dosage. The pH values were surely affected by the content of acid and basic end-products.

Aloe supplementation affected most of in vitro fermentation parameters. The complex *aloe* composition surely interfered on digestibility and fermentability of the diet. In particular, the mucilaginous and resinous compounds inhibited the faecal bacteria activity on fermentable nutrients decreasing digestibility and gas production. These last results partially explain the laxative effect of *aloe*. The low SCFAs productions appeared to deny the trophic effect on the gut mucosa. However, the direct relation between lactic acid and *aloe* supplementation suggests an increasing use of lactic fermentation and, consequently, a higher *Lactobacillus* spp. development in the gut.

Capasso F., Borrelli F., Capasso R., Di Carlo G., Izzo A.A., Pinto L., Mascolo N., Castaldo S., Longo R 1998. *Phytotherapy Res*, Vol. 12, S124-S127.

Cutrignelli M.I., Bovera F., Tudisco R., D'Urso S., Marono S., Piccolo G., Calabrò S. 2009. *J Anim Physiol Anim Nutr*, 93: 305-312.

Animal nutrition

gut fermentation, carbohydrates, gas production

Table 1. Fermentation parameters.

Treatment	OMD	OMCV	pH	SCFA	Lactic acid	BCFA	NH ₃
	%	ml/g OM		mmol/g OM			
Diet 0	72.26A	94.48	6.76ab	79.88A	0.04Bc	0.066	30.12a
Diet 0.7	56.51B	94.48	6.81ab	55.55C	0.08Bb	0.075	28.82ab
Diet 1.6	55.86B	96.46	6.86a	59.93C	0.14ABa	0.074	23.60b
Diet 3.2	56.87B	96.68	6.75b	69.41B	0.23A	0.065	25.96ab

OMD: organic matter disappearance; OMCV: cumulative volume of gas; SCFA: short chain fatty acid; BCFA: branched chain fatty acid. A,B,C and a, b, c: along column letters different for p<0.01 and p<0.05, respectively

INFLUENCE OF GENOTYPE AND PROCESSING OF CORN GRAIN ON THE EXTENT OF RUMINAL STARCH DEGRADATION

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This trial was conducted to determine the effects of genotype, grinding size and commercial processing of corn grain on the extent of ruminal starch degradation using the in vitro method.

Samples of yellow dent and flint corn grain and an extruded corn product were used in this study. In the first trial 7 hybrids samples were ground through a Wiley Mill at 1,2,4,6 mm screen. In a preliminary study to evaluate and compare the effect of heat and grinding treatment on starch degradability; a corn sample was ground at 1,2,4,6 mm through a Wiley Mill and extruded after 2 mm grinding. Samples were incubated in in vitro batch for 3,6,9,12,18,24,36,48 h with rumen fluid and rumen buffer (1:4). Starch was analysed enzymatically based on a procedure involving gelatinization and partial hydrolysis of starch at 95°C using thermostable α -amylase followed by complete hydrolysis at 50°C using amyloglucosidase and a spectrophotometrical determination (1).

Starch degradability was not significantly ($P=0.338$) affected by corn genotype.

In Table 1 are presented degradation profile of starch degradability in relation to the different grinding size. Our data showed a significant difference among the different grinding sizes (Tab. 1); lower grinding size resulted to higher starch degradation.

Heat treatment were generally found to increase the rate and the extent of starch degradation (2), which was also confirmed by our in vitro results; in fact after 3 h of incubation 71.00% of the starch was degraded for extruded corn compared with 28.79% starch for corn ground at 2 mm (Tab. 2).

Our data showed that increasing the grain size decreased starch rumen degradability. The accessibility of starch granules to ruminal microorganism increased because of the fineness of the particles.

Characteristics that make a grain or a hybrid ideal for livestock differ with processing method. For ground corn incomplete starch digestibility is of primary concern, but with processing, starch degradation was altered suggesting that could be the simplest way to increase its content of digestible energy.

1) Hall M.B., 2009. Journal of AOAC International L. VOL. 92, No.1.

2) Offner A. et al.,2003.Animal Feed Science and Technology 106,81-93.

Animal Nutrition

Starch degradation, corn grain, in vitro method

Table 1. Effect of corn grinding size on starch degradation profiles (%starch disappearance) using the *in vitro* system.

	1mm	2mm	4mm	6mm	SEM	P-value
In vitro degradation, h						
3	45.57 ^b	44.15 ^b	40.62 ^a	36.41 ^a	2.173	0.030
6	63.15 ^b	58.52 ^b	55.87 ^a	49.67	3.027	0.032
9	75.99 ^b	70.99 ^b	67.57 ^a	61.73 ^a	2.267	0.001
12	83.93 ^b	80.03	77.52 ^a	75.13 ^a	1.767	0.012
18	93.33 ^d	90.15 ^{cd}	86.70 ^{ac}	83.16 ^{ab}	1.821	0.004
24	97.22 ^c	96.12 ^{bc}	94.25 ^b	90.92 ^a	0.863	< 0.001
30	99.81 ^d	99.00 ^{cd}	96.76 ^b	93.88 ^a	0.646	< 0.001
48	101.42 ^c	101.04 ^{bc}	101.01 ^b	97.57 ^a	0.457	< 0.001

^{a,b,c,d} Within a row means without a common superscript differ ($P < 0.05$)

Table 2. Corn grinding vs corn extruded (Ext) (%starch disappearance) using the *in vitro* system.

	1mm	2mm	4mm	6mm	Ext
In vitro degradation, h					
3	35.50	28.79	28.02	21.95	71.00
6	61.24	40.83	35.72	32.50	86.22
9	88.08	64.16	46.16	41.19	94.07
12	93.44	77.04	62.91	52.82	96.96
18	99.93	92.06	78.30	69.21	98.34
24	100.00	96.89	91.61	83.00	98.81
30	100.00	99.94	97.75	93.78	100.00
48	100.00	100.00	100.00	97.27	100.00

HAY QUALITY PRODUCED IN CAMPANIA REGION

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Forages represent an extremely important source of nutrients in livestock nutrition. Additionally, they provide effective fiber in the ration, which enhances a proper digestion in forage-consuming animals. The feed costs represent the largest expense in the livestock systems; the production and correctly preservation (i.e. haymaking) of high-quality forages can help reduce the feed costs (Schroeder, 2013). For all these reasons the forage evaluation represents one of the first step to formulate adequate nutritional planes for livestock in order to improve animal welfare and milk or meat nutritional quality and to reduce the environmental impact of animal husbandry. Aim of the present study was to evaluate the mean quality of hays produced in Campania Region.

For the investigation 27 mixed hays were collected in livestock farm of Campania Region, since July 2013 to January 2014. The sensorial evaluation was carried out separately by three evaluators: a form including some quality parameters (i.e. colour, number of species, presence of powder, texture, smell, legumes/grasses ratio and leaf presence). For all parameter the score (from 1 to 10 points) was assigned and the maximum score was fixed at 70 points. For each parameter, with the exception of powder presence, the highest score corresponded to the best condition. All the hays were also analysed for the proximate analysis (i.e. dry matter, crude protein, NDF and ash) according to the standard procedures. All the sensorial parameters were related to the chemical data using the PROC CORR (SAS, 2000).

The mean values of total score obtained (43 ± 5) as well as crude protein and NDF values (10 ± 3 and 54 ± 6 % DM, respectively) (Patra, 2010) evidenced that in Campania Region the haymaking procedure needs to be improved yet. The correlation between the sensorial evaluation parameters and chemical values evidenced interesting significance. In particular, the most related parameters were the presence of leaf and the texture, while colour and smell resulted related with any chemical parameters. The presence of powder was inversely related with ash content (more powder corresponded to more ash). The total score was significantly related with crude protein content and CP/NDF ratio.

These preliminary results indicate that the sensorial evaluation could be useful to predict the main nutritional characteristics of hay. It should also be emphasized that the tested hays have proved to be of modest quality either in terms of sensorial and chemical characteristics. In particular, the most common mistake errors would be attributed to the late harvest and/or to the intercropping of species characterized by different growth rate or by not-complementary nutritional characteristics.

A lot of work is still needed to make it clear to the farmers that in order to reduce production costs, improve milk and meat characteristics, as well as to reduce the environmental impact it is necessary to improve the quality of hay produced/used.

Patra A.K. (2010) Effects of supplementing low-quality roughages with tree foliages on digestibility, nitrogen utilization and rumen characteristics in sheep: a meta-analysis JAPAN 94: 338-353

SAS (2000) SAS/STAT Software: Changes and Enhancements through Release 8.1 (Cary, NC, SAS Institute Inc.).

Schroeder J.W. (2013) Forage Nutrition for Ruminants www.ag.ndsu.edu

Animal nutrition

forages, NDF, crude protein

Table 1 Correlation among sensorial evaluation parameters and chemical values.

	Hash	CP	NDF	CP/NDF
Colour	0,0622	0,1251	0,0139	0,1111
Cultivars	0,4179*	0,1410	0,1775	0,0841
Powder	-0,5209**	-0,0675	-0,1720	-0,0114
Texture	0,2388	0,4454*	-0,5080**	0,4997**
Smell	-0,0235	0,2344	-0,2985	0,2626
Leg/gra	-0,0684	-0,0412	0,2521	-0,1032
Leaf	0,4404*	0,5505**	-0,1769	0,5052**
Total	0,2660	0,5076**	-0,3140	0,5077**

* P<0.05; **P<0.01

FEED AND PIG PRODUCTS: EFFECTS OF OCHRATOXIN A IN DIFFERENT DIETS AND BREEDS

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Ochratoxin A (OTA) is a mycotoxin produced by several species of fungi, genera *Aspergillus* and *Penicillium* (El Khoury e Atoui, 2010). It is a toxic metabolite, the presence in feed can lead to the contamination of animal products specially pork and poultry. Moreover the presence of OTA is potentially dangerous for human and animal health (Abrunhosa et al., 2010). OTA is nephrotoxic to all animal species studied and particularly can be toxic to pigs for the longest half-life for its elimination. Several studies indicates that OTA is hepatotoxic, neurotoxic, teratogenic, carcinogenic and immunotoxic (Duarte et al., 2011). The aim of the trial was to evaluate the effect of OTA levels on pig products for different diets breeds.

The experiment was carried out in Garfagnana area (Tuscany). Twenty pigs were slaughtered at 140 kg of live weight about. Ten pigs of Large White breed (LW) and Nero di Parma breed (NP) were fed with a traditional (COM) diet: corn meal, barley meal, soybean meal; others ten pigs were fed with a by-products (BYP) diet: spelt meal, spelt bran, chestnut meal. After slaughtering, samples of muscle (*Longissimus dorsi*) and backfat were carried out, immediately vacuum-packaged and stored. The different tissue of pigs (muscle, backfat) and a pork meat-derived product (salami) were analyzed for OTA contamination. Moreover samples of feeds of diets used during the trial were analyzed. All samples were subjected to a process of extraction and purification and analyzed with HPLC-FLD method.

The different breed and diets showed a high variability, however below the legal limit ($> 1 \mu\text{g/kg}$) in most of case (Table 1). LW of both diets showed a low levels of OTA contamination. Animals fed with COM diet showed a greater contamination that BYP diet. NP group showed a higher contamination than LW group, with a similar concentrations for COM and BYP diets, with a decreasing trend from salami, backfat and muscle. Salami of NP showed a contaminations levels exceeding the limits of the law ($> 1 \text{ ppb}$) in both diets. The presence of OTA in tissues and particularly in meat derived product appears to be influenced both by the diets and breed. This result combined to the difficult of experimental data regarding the OTA contamination of pig products, highlights the importance of carrying out further investigation about the presence of OTA in feed for human consumption.

El Khoury A., Atoui A., 2010. Ochratoxin A: general overview and actual molecular status. *Toxins*, 2, 461-493.

Abrunhosa L., Robert R., Paterson M., Venancio A., 2010. Biodegradation of Ochratoxin A for food and feed decontamination. *Toxins*, 2, 1078-1099.

Duarte S.C., Lino C.M., Pena A., 2011. Ochratoxin A in feed of food-producing animals: An undesirable mycotoxin with health and performance effects. *Veterinary Microbiology*, 154, 1-13.

Animal production
pig, ota, by products

Table 1. OTA contamination in muscle, backfat, salami.

		mean ($\mu\text{g/kg}$)	min - max ($\mu\text{g/kg}$)
LW-COM	Muscle	0.775	0.17 - 1.88
LW-COM	Backfat	2.110	0.07 - 7.84
LW-COM	Salami	0.860	<LOQ - 1.97
LW-BYP	Muscle	0.310	<LOQ - 1.13
LW-BYP	Backfat	0.405	<LOQ - 1.14
LW-BYP	Salami	0.162	<LOQ - 0.52
NP-COM	Muscle	0.223	<LOQ - 0.44
NP-COM	Backfat	1.543	<LOQ - 6.00
NP-COM	Salami	2.558	<LOQ - 5.36
NP-BYP	Muscle	0.730	0.04 - 1.62
NP-BYP	Backfat	1.327	<LOQ - 6.71
NP-BYP	Salami	2.412	1.30 - 5.05

LOQ: Limit of quantification

PACKAGING OF MEAT PRODUCTS

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The research aims at the evaluation of the properties of most types of packaging used in the food industry, analyzing the advantages, disadvantages and the microbial flora that develops.

This review is based on the articles found on the main food journals and is enriched by texts from the library of the University of Perugia.

Packaging is one of the technological aspects most interesting and constantly evolving. The term "packaging" refers to the technological intervention aimed at the protection of food from a variety of factors. The functions of packaging are: i) containment (in particular free flowing products) at any stage of the cycle of production, storage and transport; ii) protection from mechanical, chemical and biological attacks; iii) marketing and advertising. The packaging (food packaging) is then defined as a coordinated system which prepare the products for transport, distribution, storage, marketing and consumption. Numerous challenges are the basis of different approaches but the purposes remain those to guarantee a quality product which maintains the organoleptic and sensory characteristics as long as possible.

There are three main types of packaging for meat products: i) aerobically; ii) under vacuum; iii) in a protective atmosphere. Food are dynamical systems with a very limited shelf-life and the nutritional and organoleptic properties may change due to physical and chemical changes that occur during storage of the product, and because of the microbiota and microbial activity. The study of packaging systems that lead to a extension of the shelf life, the maintenance sensorial characteristics at low cost is essential. Learn about the optimal solution depending on the characteristics of the product is a prerequisite.

1. Borch E., K.-M. M. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology* , 103-120.
2. Colavita. (2008). *Igiene e tecnologie degli alimenti di origine animale*. Milano: Le Point Vétérinaire Italia.
3. Hueck. (1968). The biodeterioration of material- an appraisal. *Biodeterioration of materials. Microbiological and allied aspects. Proceedings of the 1st international symposium* (pp. 6-12). Southampton: Elsevier Publishing Co.
4. Philips (1996). Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce *International Journal of Food Science and Technology*, 463-479

Food microbiology and Meat hygiene
Packaging, Vacuum, MAP

ENTEROTOXIGENIC STAPHYLOCOCCUS AUREUS IN FOOD SAMPLES

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Staphylococcus aureus is a well known agent for its causative role in food poisoning outbreaks associated with several food including milk and dairy products. *S. aureus* is also the most important agent responsible for bovine mastitis and other different diseases in animals and humans. The objective of this study was to characterize *S. aureus* recovered from different food matrices through phenotypic and genotypic methods. Between 2012 and 2013, 38 coagulase positive staphylococci were isolated from different food samples (1 from sandwich; 1 from butter; 8 from cooked meat and sausages; 1 from egg; 9 from raw milk and 16 from cheese samples). All the strains were cultured with standard methods and tested for susceptibility to oxacillin by disk diffusion. Contemporarily the DNA was extracted from *S. aureus* strains using a protocol described in literature (Cremonesi et al., 2006) and the isolates were further analysed by both RS-PCR (Fournier et al., 2008) and multiplex PCR assays. The first method, based on PCR amplification of the 16S-23S rRNA intergenic spacer region, was previously used to differentiate between different subtypes associated with bovine mastitis. The multiplex PCR assays tested the presence of genes encoding for enterotoxins (sea, seb, sec, sed, see, seg, seh, sei, sej, sel) and other virulence genes such as leukocidins and leukotoxins.

All the strains analysed in this study were found to be *S. aureus* (nuc positive). Genotyping by RS-PCR of all isolates revealed the presence of 11 (28,9%) GTB, 3 (7,8%) GTAC, and other 18 minorities genotypes with low frequencies. Eight strains isolated from butter (1), milk (5) and from meat (2) were oxacillin resistant (21%), confirmed also by the analysis with *mecA* gene.

Moreover, 89.4 % of the isolates were shown to be enterotoxigenic (SEs), and the most common genes present were sea, sed, seh, sei, sej. One strain isolated from sausage was positive for SEB while SEL was recovered in only one strain isolated from a milk sample. The majority of the isolates (63.1%) carried two or more enterotoxin genes with the combination of sea, seg, sei (13.1%), or sea, sed, sej (31.5 %), prevalently in GTB. See gene was not detected while seh gene occurred in 10 non-GTB strains (26.3%) in combination with sea gene. Moreover, all the strains were negative for other virulence genes such as *tsst*, *eta*, *etb*, and positive for *lukM* (7.8%), *sak* (68.4%), *fmtb* (97.3%), *scn* (60.5%), *chp* (55.2%), *cna* (84.2%).

Mastitis-associated *S. aureus* strains, such as GTB, carrying different SE genes, were most frequently found in contaminated cheeses and a relevant number of MRSA were also found. These findings emphasize the need to prevent in the herd the presence of enterotoxigenic and MRSA *S. aureus* strains that might have implications in public health.

Cremonesi P., et al. (2006). J. Dairy Sci., 89:163–169; Fournier C., et al. (2008). Res. Vet. Sci., 85: 439-448.

ISPEZIONE ALIMENTI

S. aureus, food poisoning, enterotoxins

IN VITRO AND IN VIVO COMPARISON OF ENROFLOXACIN METABOLISM IN TURKEY.**Merlanti R.*^[1], Lucatello L.^[1], Cagnardi P.^[2], Capolongo F.^[1], Montesissa C.^[1]**^[1]*Dept. of Comparative Biomedicine and Food Science, ~ Padova,* ^[2]*Dept. of Health, Animal Sciences and Food Safety ~ Milano*

Enrofloxacin (E) is a synthetic fluoroquinolone antimicrobial agent, administered orally to turkeys for the treatment of respiratory and intestinal diseases. The aim of the study is to assess, in turkey, the extent of the in vitro hepatic biotransformation of Enrofloxacin (E) into Ciprofloxacin (C), its active metabolite, as the sum of E+C is the marker residue for edible tissues from treated turkey fixed by EU to minimize the risk for human health (1).

Liver microsomal fractions of 2 healthy turkeys were prepared and incubated at 38°C, for 15 minutes with E (500 ppb) and the target analytes were extracted with Acetonitrile(3ml). Blood, lungs, livers and intestinal content were collected from 8 healthy turkeys, 24h after a last treatment with E (20 mg/Kg in medicated water for 5 days) to evaluate C and E concentrations by a liquid chromatography, coupled to a mass spectrometry method (LC-MS/MS) (2).

The average percentage of transformation of E in C, is $2,21 \pm 2,37\%$, in agreement with in vivo plasma concentration of C, collected from 8 turkeys treated with E (20mg/kg in medicated water for 5 days) for kinetic purposes. The tissue distribution obtained from turkey sacrificed 24h after the kinetic study, confirmed a good distribution in lungs and a high hepatic and intestine accumulation of the metabolite, probably due to its biliary excretion and entero-hepatic recycle. After 24 hours from the last treatment the contribution of C, to the total sum of active compounds E+C(%) was, in decreasing order, of $28,58 \pm 7,43$; $10,54 \pm 2,42$; $3,92 \pm 0,70$, $2,30 \pm 0,73$ respectively in liver, intestinal content, lung and plasma.

Fluoroquinolones are reported to be sufficiently lipid soluble to cross the cell membrane and to accumulate intracellularly (macrophages and neutrophils) with concentrations 4-10 times more higher than those reached in plasma (3). The extent of biotransformation of Enrofloxacin to its active metabolite Ciprofloxacin evaluated in vitro in liver is low and the sum of residue in edible matrices in turkey tissues, E+C, do not reach levels that could raise concerns, for consumer safety. The present research confirm the convenience of in vitro metabolism experiments to predict fate of veterinary drugs and the usefulness of in vitro and in vivo comparison.

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Pharmacology, Drug metabolism, food safety

In vitro Metabolism, Enrofloxacin, Turkey

ISOLATION OF SALMONELLA SPP. IN LIVER OF WILD BOARS HUNTED IN EASTERN LIGURIA

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Salmonella species represent zoonotic bacteria able to infect humans, livestock, companion animals and wildlife [1,2]. In Liguria, during the last decades, the wild boar population (*Sus scrofa*) growing has induced a consequent increase of meat consume derived from these animals. The animal slaughter is frequently performed by hunters; in certain cases, the application of inappropriate hygiene procedures could determine poor sanitation of meats (i.e. contamination by pathogens, such as salmonella spp) responsible for human health risk [3,4]. Few data regarding prevalence, type of serovars and drug resistance of salmonella isolated from liver and meat of wild boars are to date available [5].

The aim of the present study is to evaluate the meat safety of wild boars hunted in our territory. The presence of pathogen was investigated in liver, a target-organ for bacteria, whose the habitual undercooked consumption is associated to a significant food safety risk.

In the last hunter season (1 October 2013-31 January 2014), 521 livers of wild boars hunted in eastern of Liguria were sampled. All samples were analyzed by Real-time PCR (IQcheck salmonella, Biorad, cat. 357-8123) and the positive cases were confirmed according to ISO 6579-2002. Suspected colonies were confirmed using biochemical tests; moreover, the isolates were checked for antibiotic resistance [6]; in particular the resistance to the follows antibiotic were verified: trimethoprim-sulphamethoxazole (SXT), tetracycline (TE), ampicillin (AMP), amoxicillin and clavulanic acid (AMC), colistin (CT), chloramphenicol (C), streptomycin (S), cephalothin (KF), ceftazidime (CAZ), Triple-Sulfa (sulfacetamide, sulfathiazole, and sulfabenzamide, 3SU).

A total of 56 liver samples was positive for Salmonella spp. (10,7%) classified as follows: *S. enterica* subsp. Enterica (80,35%, 12 serovars), *S. enterica* subsp. Salamae (3,57%, 2 serovars), *S. enterica* subsp. Houtenae (5,35%, 2 serovars) and *S. enterica* subsp. Diarizonae (10,71%, 1 serovars). Moreover, in our study 8 international outbreaks associated to Salmonella Enterica subsp. Enterica were identified: *S. Typhimurium*, *S. Napoli*, *S. Coeln*, *S. Enterica*, *S. Goldcoast*, *S. Kottbus*, *S. Stourbridge*, *S. Thompson*. The prevalence values in male and female wild boars, evaluated by Fisher's Exact Test ($P > 0.05$), were comparable. The majority of the examined cases showed antibiotic resistance (87,24%): the 85.10 % of isolated were characterized by resistance to 3SU, the 14.89% to KF, the 2.12% at CAZ, C 4.25%, CT 4.25, S 8.51%, SXT 6.38%, TE 19.14%, AMP 14.89%, AMC 12.76% (table 1).

As reported (EFSA 2014) [7], the decrease of human salmonellosis cases in EU in 2008-2012 could be induced by successful control programs in poultry populations. Our results suggest nevertheless the Salmonella spp. presence in european wild boar populations, representing a possible risk for public health. This evidence may be related to the carcass contamination that could occur when animals are eviscerated and skinned under insufficiently hygienic conditions [3,4].

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6.NCCLS M31-A3 VOL.28 N° 8-Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animal; approved standard third ed.

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Food Control and Animal Health

salmonella, wild boars, hunting

Table 1: Antimicrobial drug resistance in *Salmonella* spp. isolates from 521 livers of European wild boars in eastern Liguria

N° of Strains	% of Resistance to *										% of Resistance to Drugs **				
	3SU	SXT	TE	AMP	AMC	KF	CAZ	CT	C	S	0	1	2	3	≥4
56	85.10	6.38	19.14	14.89	12.76	14.89	2.12	4.25	4.25	8.51	12.76	53.19	17.02	4.25	12.76

Abbreviations: Triple-sulfa (3SU, 250 µg); Trimethoprim–sulphamethoxazole (SXT, 1.25-23.75 µg); Tetracycline (TE, 30 µg); Ampicillin (AMP, 10 µg); Amoxicillin and clavulanic acid (AMC, 20-10 µg); Cephalothin (KF, 30 µg); Ceftazidime (CAZ, 30 µg); Chloramphenicol (C, 30 µg); Colistin (CT, 10 µg); Streptomycin (S, 10 µg);

*Data expressed as percentage of strains resistance to single molecules.

**Multi-drugs resistance of strains indicated for molecular number.

OVERVIEW OF IMPORTS OF PRODUCTS AT LIVORNO BIP DURING LAST TEN YEARS

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We try to analyze the curve trend of imports issues from third countries through Livorno BIP
Review of the official records on imports advertised every year on website of Ministry of Health, then we analysed these records

The trend analysis spotlight an increase from 2002 to 2008, then, from 2010 there is a substantial decrease
Later years we have noticed a decrease of imports and, this trend we highlight for all Italian BIPs, in according to the records published by Ministry of Health

Linee guida operative PIF – 1 edizione novembre 2007 – Sito web del Ministero della Salute

Dott.ssa D. Cambiaghi (PIF Malpensa) - Criteri per la valutazione degli alimenti di origine animale provenienti da paesi terzi – Sito web del Ministero della Salute

Dott.ssa D. Cambiaghi (PIF Malpensa) - Criteri per la valutazione degli alimenti di origine animale provenienti da paesi terzi – Aggiornamento 2013 – Sito web del Ministero della Salute

Attività dei Posti di Ispezione Frontaliera anni 2002 – 2012 – sito web del Ministero della Salute

Inspection and control of aliments

Animal product, Imports, BIP

A PRELIMINARY STUDY ON THE PRESENCE OF FIVE NEONICOTINOIDS IN SHEEP MILK IN JORDAN

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Neonicotinoids, a relatively new class of pesticides, comprise 24% of the insecticide market in Europe, equal to organophosphorus and carbamates combined, and 80% of the insecticidal seed treatments [1]. In Jordan, pesticides including neonicotinoids are used in agriculture, mainly to protect citrus trees cultivated in the Jordan Valley [2,3]. During the treatment of the trees, these compounds can be dispersed into the environment contaminating the surrounding grasses and vegetation on which the sheep graze. Pesticide residues in animal feed can be deposited in animal tissues and can be detected in milk intended for human consumption. The aim of this study was to develop a sensitive method using LC-MS/MS and to analyse ewe milk samples collected in Jordan for the purpose of verifying the presence of five neonicotinoid insecticides: acetamiprid, imidacloprid, clothianidin, thiacloprid and thiamethoxam.

Between April and May 2011, 37 samples of sheep milk have been collected randomly from five flocks in the Jordan Valley. A method based on a single cleanup extraction step with diatomaceous earth material was developed and samples were analyzed by LC-MS/MS.

The calculated limits of quantitation (LOQ) for the compounds studied were 1 ppb, being much lower than the maximum residue limits (MRLs) established by European and Jordan legislation (10-50 ppb). Average recoveries for the five neonicotinoids ranged between 75.1% and 88.3% at spiking levels 1-20 ppb. The 37 samples analyzed were consistently negative for all neonicotinoids sought.

The results demonstrate the safety of tested milk for human consumption because the concentration of the five compounds searched are all below the limit of determination. This could be due to a proper use of these compounds aimed to prevent contamination of the meadows for grazing or to the hydrophilic characteristics of neonicotinoids that could prevent their retaining in milk which is an emulsion of fat in water. There is a need for further studies aimed at establishing the residual behaviour of these substances in milk, as in scientific literature there are no studies regarding the disappearance trends of neonicotinoids in sheep milk.

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Sicurezza alimentare

Neonicotinoids, Sheep milk, LC-MS/MS

PHENOTYPICAL CHARACTERIZATION OF STAPHYLOCOCCUS AUREUS SEA POSITIVE STRAINS ISOLATED FROM RAW MILK: ENTEROTOXIN A PRODUCTION AND ANTIBIOTIC-RESISTANCE

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^[1]~ Pisa

The aim of the study was to assess the antibiotic-resistance profile of 43 *S. aureus* sea positive strains isolated from Tuscan bovine bulk tank milk and evaluate their ability to produce enterotoxin A (ent A) under different growth conditions.

Forty-three *S. aureus* sea positive strains (1) were evaluated for ent A production in BHI. Positive strains were evaluated for the ability to produce ent A in raw milk. Two bacterial suspensions (10^3 CFU/ml and 10^4 CFU/ml) were obtained using McFarland scale. One ml of each suspension was dispensed in 9 ml of BHI or milk and incubated at 37°C, room temperature (RT) and refrigerated conditions (4°C +/- 3°C) for 24 h. Ent A detection was carried out by ELISA RIDASCREEN® SET Total (Biopharm). Microbiological analysis of milk were performed. Antibiotic-resistance profile of strains was determined according to Kirby Bauer method for the following antibiotics: linezolid (LZD), vancomycin (VA), amoxicillin-clavulanic acid (AMC), oxacillin (OX), gentamycin (CN), quinopristin-dalfopristin (QD) and mupirocin (MUP).

At both concentrations, 15/43 strains (34.88%) were able to produce ent A at RT and at 37°C in BHI. Ent A production was not detected at 4°C +/- 3°C. At both concentrations, 14/15 strains (93.33%) incubated at 37°C were able to produce ent A in milk. All strains incubated at 4°C +/- 3°C and at RT didn't produce ent A, regardless the tested concentrations. Microbiological analysis of milk revealed a good hygienic profile. As concern antibiotic-resistance, 26/43 strains (60.46%) were susceptible to all antibiotics tested; 17/43 (39.53%) showed resistance to OX and CN and intermediate susceptibility to MUP; 12/43 (27.9%) were resistant to QD, 2/43 (4.65%) showed intermediate susceptibility to QD; all the strains (100%) were susceptible to LZD, VA, AMC.

Enterotoxins are usually produced in a range temperature of 10°C–45°C (optimum 35°C–40°C) (2). Thus, ent A production at 37°C could be considered as an expected result due to the promoted growth of *S. aureus* in BHI and in milk. Only 34.88% sea positive strains was able to produce ent A in BHI at 37°C and at RT; among these strains 14/15 (93.33%) produced ent A at 37°C in milk, but not at RT. All strains incubated at 4°C +/- 3°C didn't produce ent A, neither in BHI nor in milk. As concern antibiotic-resistance, 17/43 strains (39.53%) were multi-drug resistant; among these only one strain produced ent A in BHI, but not in milk. Our findings suggest the potential correlation between the antibiotic-resistance phenotypic profile and ent A production. As previously reported (3,4), the frequent presence of sea in *S. aureus* from bovine milk is not always associated with enterotoxin production. Moreover, enterotoxin production is temperature dependent, consequently a proper maintenance of the cold chain in milk and dairy products is effective in preventing food borne staphylococcal intoxications; however the presence of resistant strains remains a possible risk.

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Applied microbiology

Staph. aureus sea +, enterotoxin A, resistance phenotype

PRELIMINARY DATA ON HYDROLYTIC ACTIVITY OF LACTIC ACID BACTERIA ON β -LACTOGLOBULIN IN MILK

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β -Lactoglobulin (β -lg) is the major whey milk protein and it represents the main allergen in cow and sheep milk (1). Microbial fermentation produces some proteolytic enzymes and leads to the degradation of milk protein allergens. In this study, the results of a screening on the ability of lactic acid bacteria (LAB) isolated from typical Sicilian cheeses to reduce the β -lg are shown. This screening was carried out through an indirect competitive ELISA. The lactic acid fermentation was found to be suitable for decreasing milk immunoreactivity.

Twenty three strains of cheese LAB, belonging to six genera (Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus), were cultured for 48h in their optimal growth medium and, after washing and re-suspension in Ringer's solution, inoculated (1% vol/vol) in UHT milk. After 24h incubation at the optimal growth temperature, all samples were diluted (1:10) in distilled water and analysed by Ridascreen β -Lactoglobulin kit (r-biopharm-Germany) according to the manufacture's instructions. Un-inoculated UHT milk was used as control.

After milk fermentation by LAB, the amount of β -lg decreased in twelve samples with an inhibition rate ranging between 21% and 96%, as compared with unfermented milk. Eleven samples did not show a significant β -lg decrease. The strains that showed a higher capacity to hydrolyze the β -lg were represented by Leuconostoc mesenteroides, followed by Lactococcus lactis subsp. lactis (Figure 1). It is important to observe that two different strains of Leuconostoc mesenteroides give different results.

Since β -lg is recognized as the major milk allergen and it is known that its hydrolysis may reduce milk allergenicity (2), this study can contribute to give some help in the development of hypoallergenic milk products. Future studies will be needed to evaluate the efficacy and suitability of the selected LAB useful in the processes of cheese-making.

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Animal production

β -Lactoglobulin, LAB, allergenicity

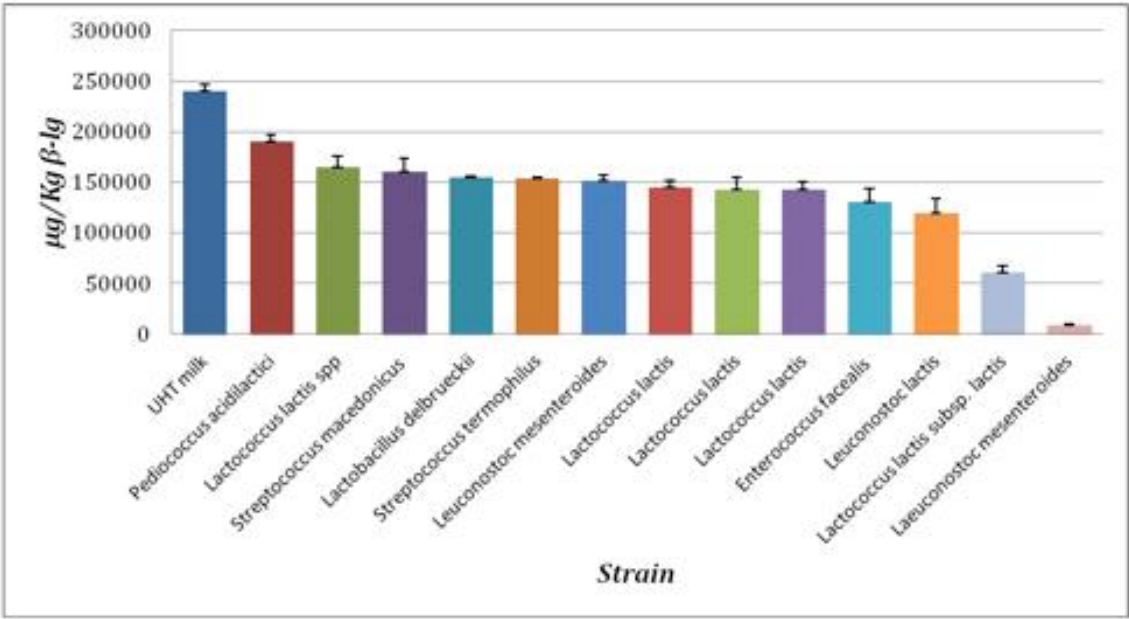


Figure 1: Changes in β -Ig content during milk fermentation by LAB strains compared with unfermented milk.

EXCRETION OF AFLATOXIN M1 IN DONKEY MILK FOLLOWING EXPOSURE TO AFLATOXIN B1**Tozzi B.*^[1], Liponi G.B.^[1], Meucci V.^[1], Casini L.^[1], Intorre L.^[1], Gatta D.^[1]**^[1] *Dipartimento di Scienze Veterinarie ~ Pisa*

The Commission of the EC has set limit for Aflatoxin B1 (AFB1) for feedstuffs (20 ppb) and for . Aflatoxin M1 (AFM1) in ruminant milk (0.05 ppb) but not for donkey milk.

Donkey milk, for its protein profile, is an excellent substitute to the breast milk for infants allergic to cows milk thus expand knowledge about this topic will help European legislators to define the limits for this kind of Aflatoxin (AF) (in feed and milk) in order to ensure infants health. Donkey ration is based on forage, thus to increase milk production, it's necessary to supplement the diet with energy sources such as corn. AF are natural contaminants of a variety of agricultural products; in particular the most contaminated is corn. The addition of this cereal in lactating donkey ration could result in the passage of AF in milk as observed in other mammals AFM1, the major metabolite of AFB1, is classified by the IARC as class 2B, possible human carcinogens. No data are present in the international literature about the amount of AFM1 in donkey milk in response to feeding AFB1. The aim of this study was to assess the transmission into donkey milk of AFM1 after the administration of AFB1 naturally contaminated corn to 5 lactating donkeys.

Each animal was fed hay, mixed flakes and 1 kg of corn for 21 days. After 7 days adaptation period until 19th day corn was replaced with a corn naturally contaminated (CNC) by 202 µg/kg of AFB1 (according with the 86/609/EEC). Individual milk samples were collected at -24,0,4,8, 24,32,48,56,82,106,154,202 and 250 hours after the first AFB1 administration. At the day 19th the animals were fed for the last time with CNC and individual milk samples were collected at 0,4,8,12,24,32,48 hours after the last administration. AFM1 determination was performed by an HPLC FLD method according to Meucci et al. [1].

Milk AFM1 concentrations are showed in Table 1. The steady-state condition was reached at 32h. In the clearance phase, AFM1 quickly decreased and it was no longer detected at 24h post the last AFB1 administration. The carry over (0.03%) was lower than that reported for ruminants: dairy cattle (from 0.3 to 6.6%) [2;3] goat (0.4%) [4] and sheep (from 0.26 to 0.33%) [5] and similar to sow (0.06%) [6].

In the present study we observed a quick decrease (24h) of AFM1 concentrations in milk after the last administration. Furthermore the administration of a naturally contaminated corn by AFB1 to lactating donkeys, showed that total AFM1 excretion in milk is lower than that observed for ruminants but close to the value found in lactating sow.

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Animal Nutrition and Toxicology
Aflatoxins, Donkey milk, Carry over

Table 1. Average excretion pattern of AFM1 in donkeys milk

Time (hour)	AFM1 (mean $\pm$ SD, ng/l)
1	8.82 $\pm$ 1.39
4	11.90 $\pm$ 1.36
8	18.32 $\pm$ 2.96
24	14.88 $\pm$ 6.31
32	31.48 $\pm$ 13.08
48	25.22 $\pm$ 8.17
56	30.10 $\pm$ 6.20
80	31.02 $\pm$ 11.42
104	33.50 $\pm$ 8.04
128	28.24 $\pm$ 5.92
152	24.50 $\pm$ 2.03
176	24.64 $\pm$ 3.33
184	21.60 $\pm$ 3.61
188	23.53 $\pm$ 2.73
192	20.30 $\pm$ 5.05
196	25.00 $\pm$ 3.46
208	19.00 $\pm$ 0.23
216	4.30 $\pm$ 3.69
232	<LOQ

„D - ALLELE" FREQUENCIES IN MILK ? - S1 CASEIN FROM BULGARIAN LOCAL SHEEP BREEDS

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The aim of this study was to determine the presence and frequency of the "Welsh gene" in α s1 - casein in the milk of different Bulgarian local sheep breeds. The study is based on polymorphism in the DNA fragments containing milk protein genes in sheep, in particular D-allele in α s1. In α s1 casein are distinguished two genotypic variants, i.e. "normal" genotypes and "Welsh" genotype that contains D - allele.

Tissue samples from eight breeds were analyzed (569 sheep reared in 18 herds): White Maritza sheep (66 sheep in four herds), Patch-faced Maritza sheep (88 sheep in four herds), Stara Zagora sheep (56 sheeps in two herds), Pleven blackhead sheep (67 sheep in 4 herds), Karakachan sheep (31 sheep in one herd), Karnobat sheep (80 sheep in one herd), Central Rhodope sheep (84 sheep in one herd), Central Balkan sheep (97 sheep in one herd).

DNA samples were taken and preserved through an innovative technology for sealing the tissue sample taken from the ear into a container with preservative substances. This technique allows marking the animal and at the same time taking a DNA sample. The conventional PCR method was used to determine the polymorphism in α -s1 (CSN1S1) casein and distinguishing the different allelic variants, particularly the frequency of the D-allele in α -s1 casein. The detection of a 237-bp fragment including the polymorphic exon 9 for the D allele was amplified using 50 pmol of the following primers D1: 59CAACATATTTTAAATAAATTGACAAT39 and D2: 59AATTAACATAAAA-AATGGCATACGTC39, for 30 cycles. The annealing temperature and time were 53 °C and 30 s, respectively; 1.5 mm MgCl₂ final concentration, 200 mm each dNTP and »100 ng of genomic DNA. Tissue samples were examined in the DNA laboratory in the University of Padua - Italy. The data were processed using the software product SYSTST 13.

Chianese et al. (1996) establishes the presence of five alleles in α s1 casein: A, B, C, D, E. The allele – featuring the highest frequency is the C allele (0.85 to 0.97). The frequency of the other alleles is from 0.03 to 0.15 (it varies between breeds). Barillet et al. (2005) indicates a lower rate of variation of the "Welsh gene" later referred as the "D-variant" with frequencies in the range from 0 to 0.03 compared to the frequencies stated by Banykó (2007) and varying from 0 to 0.17. Bolla et al. (1989) found in their research that there is a negative correlation between the "D-variant" and the fatty substances and protein in sheep milk. In the samples taken from sheep with this allele of α s1 casein observed is a reduced casein content and impaired coagulation ability (longer time for coagulation, loose-texture of the coagulum, etc.). This negative performance of this gene from technological point has been also reported in the study findings of Pirisi et al. (1999) and Pirreda et al. (1993). They found that the presence of this allele reduces the amount of casein which in turn leads to impaired coagulation ability of milk (Pirisi et al., 1997). From the results on table 1 it is seen that in three of the eight entered in the study breeds the presence of the D allele is found. The White Maritza sheep breed gives the biggest number of animals with the Welsh gene in α s1 – casein.. From 66 tissue samples taken, the Welsh gene is found in 3 animals.. The frequency of the D allele in White Maritza sheep is 0.028. This is the highest established frequency of this allele from all breeds entered in our study. Another breed in which the Welsh gene is detected is the Patch-faced Maritza sheep. From 88 samples taken, the Welsh gene is found in only one animal and respectively the allele frequency of the D allele is - 0.011. Obtained results for the Patch-faced Maritza sheep are similar to those for the Central Balkan sheep. For the Central Balkan sheep from 97 samples, the above- mentioned gene is found in only one animal. The allele frequency of the D allele in that breed is a bit lower, i.e. 0.010. Table 2 shows the allelic frequencies of the D - allele in α s1 - casein in different sheep breeds. In France, Italy and Spain there

are animals from different breeds carrying this unwanted gene. The highest frequency of this allele is established in the Italian breed Sarda, i.e. from 0.027 to 0.028. The presence has been found also in the Merino breed in Spain with the frequency of 0.009. The French breed Lakaune also carries this gene and its allelic frequency varies from 0.001 to 0.007. In Bulgaria this technology for identification and separation tissue samples from DNA has been used for the first time. We explore interrelationship between the different genotypes of casein and the quantity and quality of sheep milk. At this stage we have not found a reliable link between the quality of milk in the animals studied and carrying the Welsh gene because of their small number.

Expanding the range of the research studies related to the establishment of the polymorphism of milk proteins in sheep, in particular detecting the D – allele, is of significant importance for the technological properties of milk and the economic efficiency of breeding animals.

The presence of the D allele in α s1 – casein has been detected in three of eight breeds entered in our study: Central Balkan, White and Patch-faced Maritza sheep.

Comparing the results obtained by us and those presented in Table 2, it can be concluded that in the frequency of the D-allele in the Bulgarian local sheep breeds similarities are found with the most common local breeds in France, Spain and Italy.

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Welsh gene, milk, α -s1 casein, sheep

Welsh gene, milk, α -s1 casein

„D - allele” frequencies in milk α -s1 casein in Bulgarian local sheep breeds

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Abstract

The aim of this study was to determine the presence and frequency of the so-called "Welsh gene" in α s1 - casein in milk provided by Bulgarian local sheep breeds. Tissue samples from eight breeds were analyzed (569 sheep reared in 18 herds). The study is based on polymorphism in the DNA fragments containing milk protein genes in sheep, in particular D-allele in α s1. PCR was used as a method for determining polymorphism in α -s1 - casein and for establishing the frequency of D-allele. Three of the studied breeds revealed the presence of D-allele with the following frequencies: White Maritza sheep - 0,028; Patch-faced Maritza sheep - 0.011; Central Balkan sheep - 0.010. Our results correspond with published research findings by a number of authors for the presence of the unwanted "Welsh gene".

Key words: Welsh gene, milk, α -s1 casein, sheep.

Table 1: Frequency, number and percentage of the animals with D allele in the α -s1 - casein in Bulgarian local sheep breeds.

Breed	Number of Animals	α -s1 - casein		
		Number of Animals with the D-allele	Frequency of the D-allele	Percentage of animals with the D-allele
White Maritza sheep	66	3	0,028	4,55%
Patch-faced Maritza sheep	88	1	0,011	1,14%
Stara Zagora sheep	56	0	0,000	0%
Karnobat sheep	80	0	0,000	0%
Pleven blackhead sheep	67	0	0,000	0%
Central Balkan sheep	97	1	0,010	1,03%
Karakachan sheep	31	0	0,000	0%
Central Rhodope sheep	84	0	0,000	0%
Total for all breeds	569	5	0,006	0,9%

Table 2: Frequencies of the D - allele in α -s1 - casein in different sheep breeds reared in France, Italy and Spain. /Barillet F.et al.,2005 /

Breed	Country	Number of Animals	Allele frequencies	
			α s1 casein	
			D - allele	Normal
Lakaune	France	1054	0.007	0.993
Lakaune	France	517	0.001	0.999
Lakaune	France	992	-	-
Manech (blond faced)	France	222	0.000	1.000
Massa	Italy	54	-	-
Sarda	Italy	2700	0.027	0.973
Sarda	Italy	652	0.028	0.972
Sarda	Italy	2957	-	-
Churra	Spain	901	-	-
Merino	Spain	168	0.009	0.991
Merino	Spain	340	-	-
Manchega	Spain	238	0.000	1
Segurea	Spain	50	0.000	1

PHENOTYPIC CHARACTERISTICS OF THE INDIVIDUAL PARAMETERS OF MILK COAGULATION ABILITY IN INDIGENOUS COWS FROM ISKAR AND RHODOPE SHORT-HORNED CATTLE BREED IN BULGARIA

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The aim of this study is to establish the phenotypic characteristics of the parameters of the coagulation ability of the milk from cows of the indigenous Iskar and Rhodope Short-Horned cattle breeds. Three traits (rennet coagulation time – RCT, min, curd firmness – A30, mm, and curd firming time – K20, min/ of 67 individual milk samples from 67 cows reared in 4 herds were studied.

Data: 67 individual milk samples were studied from the Iskar and Rhodope Short-Horned cattle breeds reared in 4 herds. The milk samples were taken by hand milking of the cows. For this purpose, the calves were separated from the mother cows the previous night.

The animals from the Iskar and Rhodope Short-Horned cattle breeds are reared extensively on pasture. Calves are reared side by side with their mothers and the duration of the suckling period is 180 days. Feeding is mainly grazing with feeding up with hay and straw during the suckling period.

Studies were conducted from July, 2009 to May, 2010.

Laboratory analysis: The analysis of the individual coagulation ability of milk was performed in the laboratory of the Agricultural Institute - Stara Zagora using Computerized Renneting Metter - Polo Trade, Italy. The milk was tested by the third hour of taking the samples. Ten milliliters of milk were heated up to 35 °C and 0.2 ml (rennet chymosin) were added and placed in the apparatus for analysis. Analysis of the coagulation ability of milk lasted 30 min.

For data analysis the software program Systat 13 was used and the graphic data processing was done by Excel.

From the table it is seen that the curd-firming time is characterized by the same values of variation in the milk from the Iskar and Rhodope Short-Horned cattle breeds, i.e. 0.200 min and 3.490 min. The results related to traits are also very interesting, i.e. rennet clotting time and curd firmness. For both breeds very similar values were determined for the studied parameters. The probable reason for this can be summarized from the results of previous studies on the genetic characteristics of these breeds. From a study in 2009, it is clear that the Iskar and Rhodope Short-Horned cattle have relatively small genetic distance, substantial however, which means that they can be considered as quite separate breeds even if the results suggest possible common ancestors and somewhat admixture especially between the Rhodope Short-Horned and the Iskar cattle breeds (Dalvit et al., 2009).

The highest standard deviation is reported in curd firmness in the cows from the Iskar cattle breed, i.e. 11.423. In the Rhodope Short-Horned cattle breed also is registered a high standard deviation for this indicator. The lowest value for standard deviation was found in the coagulum-firming time in cows from the Iskar cattle breed - 0.858.

The coefficient of variation is in the range from 0.41 to 0.92, as its highest value is for the curd-firming time in cows from the Rhodope Short-Horned cattle breed - 0.92. For both breeds, object of our study, the highest values of the coefficient of variation were determined for the curd-firming time and the lowest ones for the curd firmness - 0.41. The coefficient of variation of the rennet clotting time trait is 0.48 in the Iskar cattle breed and 0.44 in the Rhodope Short-Horned cattle breed.

Many authors have studied the qualitative composition of milk and the signs describing the coagulation ability of milk from local breeds (Joudu et al., 2007, 2009; Kubarsepp et al., 2005; De Marchi et al., 2007; Cassandro, 2010).

Diagram 1 shows the results from the study on traits describing the coagulation ability of milk, i.e. time for

coagulation (RCT, min), curd firmness (A30, mm), curd - firming time (K20, min). The Iskar cattle breed stands out with a higher value of the rennet clotting time, i.e. 14.121 min, while for the Rhodope Short-Horned cattle it is 12.330 min. For the other trait describing the curd firmness, it was found that the milk from the Iskar cows has better parameters, i.e. 28.150 mm. The milk from the Rhodope Short-Horned cattle breed features a looser coagulum, i.e. 26.963 mm. Curd- firming time is longer for the milk from Iskar cows, while the curd-firming process is faster for the Rhodope Short-Horned cattle breed.

A significant phenotypic variation has been found in the studied traits. The highest values of the coefficient of variation were found in the curd-firming time in both breeds.

The milk from cows from the Rhodope Short-Horned breed is characterized by a shorter rennet clotting time while the milk from cows from the Iskar cattle breed features higher curd firmness.

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coagulation ability of milk; local breeds, indigen
coagulation ability, of milk; local breed, indigenous

Phenotypic characteristics of the individual parameters of milk coagulation ability in indigenous cows from Iskar and Rhodope Short-Horned cattle breed in Bulgaria

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Abstract

The aim of the study is to establish the phenotypic characteristics of the individual parameters of coagulation ability in indigenous cows from Iskar and Rhodope Short-Horned cattle breed.

Three traits /rennet coagulation time-RCT, min, curd firmness-A₃₀, mm, and rate of firming-K₂₀, min/ of 67 individual milk samples from 67 cows reared in 4 groups (herds) were studied. The analysis of an individual coagulation ability of milk was performed in the laboratory of Agricultural Institute - Stara Zagora through Computerized Renneting Metter - Polo Trade, Italy, instrument for evaluation the milk coagulation ability by automatic milk classification.

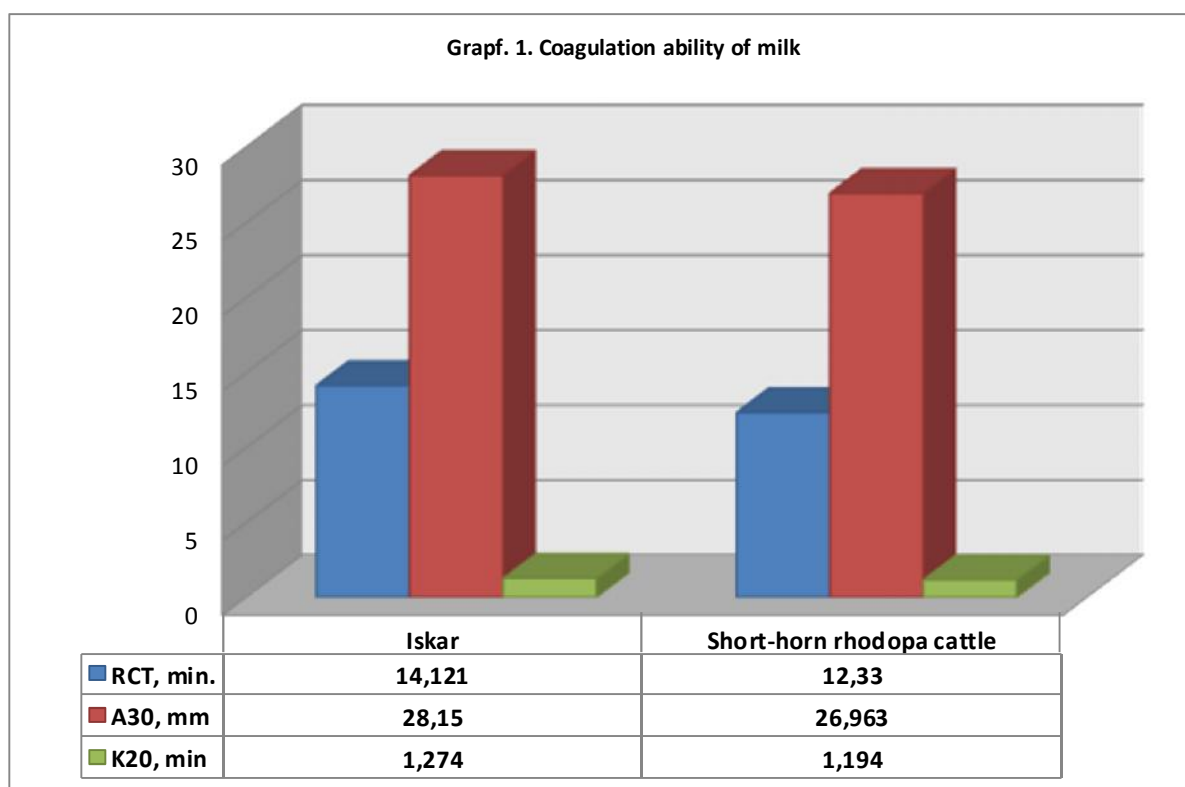
For statistical analysis of the data was used Systat 13 software product.

Significant phenotypic variability of the studied traits in both breeds was established. There were coefficients of variation for RCT, min from 0.44 to 0.48, A_{30} , mm and K_{20} , min from 0.67 to 0.92. The highest values of the coefficient of variation are found in the rate of firming- K_{20} , min in both breeds. The milk from cows of Rhodope Short-horned cattle is characterized by a shorter rennet coagulation time, while for the Iskar cattle established is greater curd firmness.

Key words: coagulation ability of milk; local breeds, indigenous, cows

Table 1. Statistical parameters describing the coagulation ability of milk from the Iskar and Rhodope Short-horn cattle breeds

Breed	Variable	Mean	Min	Max	Stand. dev. SD	Coef. var. CV
Iskar	RCT, min	14.121	6.010	30.000	6.792	0.48
	A_{30} , mm	28.150	4.000	44.000	11.423	0.41
	K_{20} , min	1.274	0.200	3.490	0.858	0.67
Short-horned Rhodope cattle	RCT, min	12.330	5.160	30.000	5.425	0.44
	A_{30} , mm	26.963	4.000	47.000	11.005	0.41
	K_{20} , min	1.194	0.200	3.490	1.095	0.92



MICROFLORA EVOLUTION DURING RIPENING OF CACIOCAVALLO PALERMITANO CHEESE.

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The Caciocavallo palermitano is a traditional Sicilian cheese manufactured within the provinces of Palermo and Trapani. It is produced in small size farms using indigenous cattle breeds - as Cinisara - and typical wood instruments (1). The ripening lasts from three to 12 months and the final product is a stretched cheese with a rectangular shape and a weight between 8 and 12 kilos. It can be consumed both fresh and seasoned. The quality characteristics of this cheese are influenced by several factors - especially by the microflora in milk and cheese - and in particular during the ripening period (2). Lactic acid bacteria (LAB) presents in raw milk have an important role in the process of cheese-making. LAB are subjected to constant qualitative and quantitative changes and so they influence the organoleptic characteristics of the final product.

This work was performed to examine the microflora evolution during the Caciocavallo palermitano ripening.

Six dairies in the area between Palermo and Trapani were selected to follow the Caciocavallo palermitano production process. From each dairy were taken: 1)milk, 2)paste before stretching (PBS), 3)Caciocavallo at six days, 4)Caciocavallo at three months. All the samples were processed according to the Istituto Zooprofilattico Sperimentale della Sicilia standard operating procedures to evaluate the LAB variability, total bacteria count at 30°C, total coliforms, enterococci, coagulase-positive staphylococci, E. coli, Salmonella spp. and Listeria monocytogenes.

The total bacteria count had a concentration from $8.0 \cdot 10^2$ to $1.6 \cdot 10^7$ cfu/ml. Total coliforms, E.coli and coagulase-positive staphylococci were found in milk or in product taken during cheese making processes but no pathogenic bacteria were found in the cheeses. The results concerning LAB evolution are shown in Figure 1; some of these strains were isolated and are currently being identified.

This work may contribute to increase knowledge about the indigenous lactic microflora of the Caciocavallo palermitano cheeses.

The absence of pathogenic bacteria, including Salmonella spp and Listeria monocytogenes, confirms that all the final products had good healthy characteristics.

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processi produttivi e sicurezza alimentare

Caciocavallo, microbiology, LAB evolution

EVALUATION OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA ISOLATED FROM TYPICAL SICILIAN CHEESES: COMPARISON BETWEEN TWO DETECTION METHODS

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Bacteriocins from lactic acid bacteria (LAB) are a group of antimicrobial proteins offering potential as biopreservatives. Bacteriocin-producing LAB have attracted great interest in terms of food safety. In this study, the authors describe the results of a screening on LAB from typical Sicilian cheeses, for the production of these antimicrobial substances, by comparing two methods.

The study was conducted on 783 LAB previously isolated from typical Sicilian cheeses ("Caciocavallo Palermitano", "Vastedda della valle del Belice DOP", "Pecorino Siciliano") and genetically characterized. Their antimicrobial activity was first detected by screening all the strains using the spot on the lawn method (1). Among the strains showing positive results, we selected 61 cultures isolated from different farms and from different cheese-making processes, on which we subsequently conducted the well diffusion assay (2). *Listeria monocytogenes* was used as indicator strain in both methods.

A total of 255/783 strains were found to inhibit the growth of *L. monocytogenes* in the spot on the lawn method (Fig 1). In this step the possible inhibitory effect of the organic acids and of hydrogen peroxide was not excluded. Only 17/61 strains were confirmed by the well diffusion assay, showing a measurable clear inhibition zone around the well (Fig.2). In this study, most of bacteriocin-producers were *Lactobacillus* spp. and *Enterococcus* spp.

Since bacteriocins have been recognized as antibacterial compounds that may be used in the food industry, the present study may contribute to detect and characterize the LAB from milk products, for their potential use as microorganisms during the manufacture and ripening process and in biopreservation, to improve the safety and quality of the final product.

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Processi produttivi e sicurezza alimentare

Bacteriocins, Detection methods, Sicilian cheeses

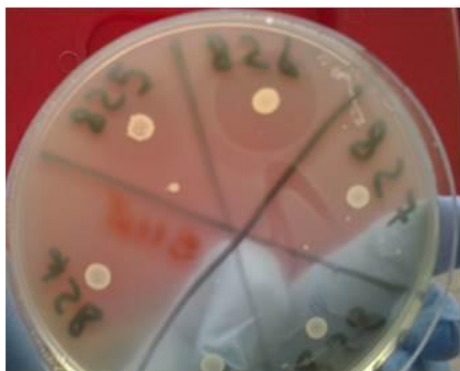


Fig.1: Inhibition zones detected by spot on the lawn

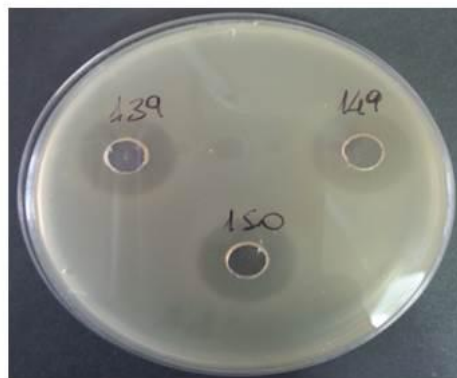


Fig.2: Inhibition zones detected by well diffusion assay

APPLICATION OF SELECTED LACTIC ACID BACTERIA FOR THE YEAR-ROUND PRODUCTION OF VASTEDDA-LIKE CHEESE

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The autochthonous lactic acid bacteria (LAB) of PDO Vastedda della valle del Belice cheese were investigated for the development of a starter culture for the year-round production of this cheese.

Winter and spring PDO cheese productions were analysed by plate counts on several media commonly used for isolation of LAB. All colonies showing different appearance were differentiated phenotypically and genotypically. All strains were subjected to a technological screening consisting of acidification capacity, diacetyl formation and production of antimicrobial compounds (1). Based on the technological performances evaluated in vitro, 12 LAB strains were selected and used in different combinations (all strains belonging to each species in triple combinations, all thermophilic strains and all mesophilic strains) to produce experimental cheeses by means of a dairy pilot plant. The different bacterial combinations (107 CFU/mL) were tested in different conditions: 1) growth in the optimal synthetic media, re-suspended in Ringer's solution and inoculated in pasteurised ewes' milk; 2) growth in whey-based medium (WBM) and inoculated in pasteurised ewes' milk; 3) growth in WBM and inoculated in raw ewes' milk (2). Final cheeses were evaluated for microbial counts, pH and total titratable acidity, sensory properties and subjected to the analysis of the volatile organic compounds (VOC) carried out by gas chromatography coupled with mass spectrometry (GC/MS) (3).

Plate counts showed the total microbial counts till levels of almost 10⁹ CFU g⁻¹ and all cheese samples were dominated by coccus LAB. A total of 72 strains were found to represent 13 LAB species belonging to five genera (*Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*). All lactococci were able to perform the rapid acidification of the curd in winter conditions (Tab.1). The sensory evaluation of the resulting cheeses indicated the cheeses processed with lactococci in single and multiple combinations as those well appreciated by the judges.

On the basis of the results shown for the winter and summer productions, at pilot scale and industrial level, respectively, and combining VOC and sensory evaluation, the multi-strain combination of lactococci was selected to act as starter preparation for the four-season production of Vastedda-like cheese.

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Processi produttivi e sicurezza alimentare

LAB, Pilot plant, ewes' milk cheese

Tab. 1 Changes of pH during experimental Vastedda-like cheese making and microbial counts curd before stretching.

Production process*	Inocula	pH curd at T ₀	Time of curd pH in the range of 5.2–5.4	pH cheese at T ₀	pH cheese at 15 days	Curd before stretching			
						PCA-SkM 30°C	M17 30°C	MRS 42°C	M17 44°C
3	Control cheese	6.48 ± 0.00	72 h (5.35 ± 0.00)	5.60 ± 0.01	5.57 ± 0.01	7.3 ± 0.4	7.6 ± 0.1	4.6 ± 0.5	5.3 ± 0.4
3	Lc. lactisubsp. cremoris PON36	6.66 ± 0.01	5 h (5.29 ± 0.01)	5.49 ± 0.00	5.29 ± 0.02	9.0 ± 0.1	9.5 ± 0.2	n.d.	n.d.
3	Lc. lactisubsp. cremoris PON153	6.67 ± 0.00	24 h (5.28 ± 0.01)	5.45 ± 0.02	5.30 ± 0.00	8.7 ± 0.3	9.4 ± 0.5	n.d.	n.d.
3	Lc. lactisubsp. cremoris PON203	6.65 ± 0.02	5 h (5.33 ± 0.01)	5.52 ± 0.00	5.32 ± 0.01	8.6 ± 0.5	9.3 ± 0.4	n.d.	n.d.
3	Multi-strain combination of three lactococci	6.11 ± 0.00	6 h (5.32 ± 0.02)	5.50 ± 0.00	5.10 ± 0.01	8.9 ± 0.1	9.5 ± 0.1	n.d.	n.d.

*3, growth in WBM and inoculated in raw ewes' milk.

ROPY SLIME FORMATION ON MEAT PRODUCTS : AN OLD PROBLEM, A NEW CONCERN

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The aim of this study was to focus on spoilage of cooked cured meat and, in particular, on the formation of ropy slime on the surface of vacuum-meat products. The research aims at identifying the etiology, the origins of contaminations and the way to avoid it, raising the attention to an issue still unsolved and existing.

This review is based on the articles found on the main food journals and is enriched by the partnership with an important Italian company that produces cooked meat products.

The shelf-life of a product is the period of time in which the food retains its qualitative characteristics, if properly stored. The shelf-life of the product is strongly linked to the deterioration: the bacteria associated with the spoilage of meat and cooked meat products produce unattractive odors and flavors (fruity, putrid, sulphurous and cheesy), discolouration and gas production. Depending on the contaminant population that predominates, in certain cases the production of filaments occurs. The shelf-life of cooked meat products could be significantly limited by the survival and proliferation of microorganisms of *Lactobacillus* spp. and *Leuconostoc* spp., which can have different forms of resistance to heat treatments and, in many cases, be introduced through cross-contamination after heat treatment. Although food safety is likely to be guaranteed, the macroscopic appearance of the product, when the package is opened, can be particularly unpleasant, and it becomes unsuitable for further processing or marketing.

In food production, the quality of products must be ensured. Consumers' needs are becoming more sophisticated and it is not acceptable to present a product that is not at the best of its characteristics. The presence of filaments is a problem, still unsolved, that causes significant economic losses associated with the deterioration of the products and the consequent elimination from the trade.

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Makela P.M., K. H. (1992). Ropy slime-producing lactic acid bacteria contamination at meat processing plants. *International Journal of Food Microbiology*, 27-35.

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Food microbiology and Meat hygiene

Leuconostoc, Ropy slime, Meat

BACTERIAL AND ARCHAEAL COMMUNITIES OF EQUINE HINDGUT CONTENT ANALYSED WITH PCR-DGGE

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Comparison of bacterial and archaeal population community structure of fecal samples with samples of cecum and different parts of colon.

The total DNA was extracted from frozen and lyophilised samples of cecum, right and left ventral colon, left and right dorsal colon and feces. Bacterial and archaeal communities structure in these samples were revealed by DGGE (Denaturing Gradient Gel Electrophoresis) using bacterial and archaeal universal primers. Q-PCR analysis of Bacteroidetes, Firmicutes, Actinobacteria and γ -Proteobacteria was performed by real-time PCR using specific primer pairs.

Bacterial DGGE analyses revealed differences among bacterial profiles of cecum, left ventral colon and dorsal colon. Interesting shift in bacterial bands was noticeable between right and left ventral colon, while both left and right dorsal colon had similar pattern. Feces bacterial profile was similar to left ventral colon. Archaeal DGGE analyses revealed distinction between cecum and colon. Only different intensity of bands indicated archaeal profile divergence between right and left ventral colon and similarity of right ventral and both part of dorsal colon. Feces pattern was again similar to left ventral colon. Quantitative PCR detected the highest number of total Eubacteria in cecum. Firmicutes (the most numerous group), Bacteroidetes and Actinobacteria achieved the highest numbers in left ventral colon, but no dramatic changes were found among the other parts of the colon. Interesting trend has been observed for γ -proteobacteria. Their number plunged along the hindgut and increased only in feces.

These observations indicate the important fermentative activities in cecum and different fermentative activities in distinct parts of horse lower digestive tract.

Mura, E. Diversity of anaerobic fungi population (phylum Neocallimastigomycota) in the horse hindgut - PhD Thesis 2013.

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PRODUZIONI ANIMALI-ALIMENTAZIONE

EQUINE HINDGUT, BACTERIAL - ARCHAEAL, PCR-DGGE

BACTERIAL AND ARCHAEAL COMMUNITIES OF EQUINE HINDGUT CONTENT ANALYSED WITH PCR-DGGE

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Introduction: Equine colic is a non-infectious hindgut disease with the hindgut forming large fermenting chamber for a high density, complex and dynamic microbial population. Gut microorganisms form a closely integrated ecological unit among themselves and the host animal, playing a vital role in the energy extraction, immune stimulation, and pathogen exclusion, but compared with other animals, little research has been conducted. The latest research indicates considerable differences among equine cecal, colonic and faecal microbial populations (L. Murai 2014, Douglas et al. 2017).

The aim of work: Comparison of bacterial and archaeal population community structure of fecal samples with samples of cecum and different parts of colon.

Material and methods: The total DNA was extracted from frozen and lyophilized samples of cecum, right and left ventral colon, left and right dorsal colon and feces. Bacterial and archaeal community structure in these samples was revealed by DGGE (Denaturing Gradient Gel Electrophoresis) using bacterial and archaeal universal primers. Q-PCR analysis of Bacteroidetes, Firmicutes, Actinobacteria and γ -Proteobacteria was performed by real-time PCR using specific primer pairs.

Results: Bacterial DGGE analyses revealed differences among bacterial profiles of cecum, left ventral colon and dorsal colon. Increasing shift in bacterial bands was noticeable between right and left ventral colon, while both left and right dorsal colon had similar pattern. Feces bacterial profile was similar to left ventral colon. Archaeal DGGE analysis revealed distinction between cecum and colon. This difference intensity of bands indicated archaeal profile divergence between right and left ventral colon and similarity of right ventral and both parts of dorsal colon. Feces pattern was again similar to left ventral colon. Quantitative PCR detected the highest number of total bacteria in cecum. Firmicutes the most numerous groups. Bacteroidetes and Actinobacteria achieved the highest numbers in left ventral colon, but no dramatic changes were found among the other parts of the colon. Increasing trend has been observed for proteobacteria. Their number plunged among the hindgut and increased only in feces. These observations indicate the important fermentative activities in cecum and different fermentative activities in distinct parts of horse lower digestive tract.

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EFFECT OF A PROTECTIVE CULTURE ON THE EVOLUTION OF MICROBIOLOGICAL AND PHYSICO-CHEMICAL PARAMETERS OF FRESH SAUSAGES STORED IN THERMAL ABUSE: PRELIMINARY DATA

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The action of protective cultures, particularly *Lactobacillus sakei*, is well known in dry sausages where the technological conditions allow a suitable growth; in fresh sausages their use is mostly focused on inhibiting spoilage and pathogen microflora, particularly in thermal abuse conditions. Data about this effect in “salsiccia” are scanty, so the aim of the study was to preliminarily investigate the evolution of spoilage microflora and some physico-chemical parameters in this product in presence of a commercial protective culture.

Two batches of fresh sausages (“salsiccia”) were produced in a Tuscan plant with (T7+) and without (T7-) a protective culture (PC) for fresh cured meat products (*Lactobacillus sakei* and *Staphylococcus xylosus*) and stored over-wrapped in PVC at 7°C for 9 days. A control without PC, stored at 2°C, was also analysed. At 0, 3, 7 (end of the shelf life) and 9 days of storage, the following microbiological and physico-chemical analyses were carried out as described in a previous study (Nuvoloni et al., 2012): total mesophilic aerobic bacteria (TMB), total psychrophilic aerobic bacteria (TPB), lactic acid bacteria (LAB), Micrococcaceae (MC), Enterobacteriaceae (EB), pseudomonads (PSEU), *Brochothrix thermosphacta* (Bt), pH and colour (surface with casing). Thiobarbituric acid reactive substances (TBARS) were determined following Ke et al. (1977) and Dal Bosco et al. (2009). Data were statistically processed by one-way ANOVA using Tukey test for post-hoc comparisons.

As shown in the Table, storage at 2°C was effective in controlling the microbial spoilage, while T7- showed counts of at least 2 log higher, except for MC. Due to PC, TMB, TPB, LAB and MC counts of T7+ were the highest. *L. sakei* grew well, as indicated by pH evolution, while *S. xylosus* did not show significant increase. PC caused a good control of EB and PSEU but not of Bt. The colour parameters evolution showed a statistically significant superficial change (a*, b*) in T7+ after 7 days, together with an important pH decrease. TBARS values (not shown) remained low in all samples up to 9 days.

Lactobacillus sakei has a technological use in the preservation and fermentation of various meat products mainly due to the production of organic acids, hydrogen peroxide and bacteriocins (Champomier-Vergès et al., 2002; Ammor and Mayo, 2007). In our study the examined PC, and particularly *L. sakei*, demonstrated to have a positive role in limiting Gram – bacteria in “salsiccia” stored at 7°C, but not in limiting Bt. The colour and pH modifications, probably related (Torrieri et al., 2011), appeared only at the end of the shelf life.

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Champomier-Vergès et al. (2002) Res Microbiol 153 115-123.

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Torrieri et al. (2011) Food Sci Technol Int 17 223-232.

Ispezione degli alimenti - Tecnologia alimentare
fresh sausages, thermal abuse, *Lactobacillus sakei*

Table: Evolution of the examined microbiological and physico-chemical parameters (means $\pm$ SD) in sausages produced with and without a protective culture and stored in abuse conditions vs. refrigerated control sausages

		t0	t3	t7	t9
TMB (log CFU/g)	T2	4.39 $\pm$ 0.17aA	4.19 $\pm$ 0.25aA	4.21 $\pm$ 0.25aA	4.43 $\pm$ 0.21aA
	T7+	7.11 $\pm$ 0.21aB	7.54 $\pm$ 0.33aB	8.69 $\pm$ 0.18bB	8.57 $\pm$ 0.14bB
	T7-	4.39 $\pm$ 0.17aA	5.07 $\pm$ 0.17aC	6.80 $\pm$ 1.31bC	7.51 $\pm$ 0.59bC
TPB (log CFU/g)	T2	3.42 $\pm$ 0.14aA	3.45 $\pm$ 0.32abA	3.99 $\pm$ 0.43abA	4.17 $\pm$ 0.44bA
	T7+	6.66 $\pm$ 0.29aB	7.16 $\pm$ 0.23bB	8.72 $\pm$ 0.11cB	8.60 $\pm$ 0.08cB
	T7-	3.42 $\pm$ 0.14aA	5.03 $\pm$ 0.36bC	6.88 $\pm$ 1.22cC	7.60 $\pm$ 0.62cC
LAB (log CFU/g)	T2	2.57 $\pm$ 0.32aA	2.39 $\pm$ 0.11aA	2.42 $\pm$ 0.33aA	3.37 $\pm$ 0.26bA
	T7+	6.84 $\pm$ 0.10aB	7.69 $\pm$ 0.63bB	8.65 $\pm$ 0.15cB	8.64 $\pm$ 0.13cB
	T7-	2.57 $\pm$ 0.32aA	4.03 $\pm$ 0.97abC	5.76 $\pm$ 2.44bcC	7.33 $\pm$ 0.80cC
MC (log CFU/g)	T2	1.59 $\pm$ 0.68aA	2.24 $\pm$ 0.07aA	3.04 $\pm$ 0.09aA	2.22 $\pm$ 1.42aA
	T7+	6.57 $\pm$ 0.24aB	6.71 $\pm$ 0.27aB	6.95 $\pm$ 0.23aB	6.64 $\pm$ 0.25aB
	T7-	1.59 $\pm$ 0.68aA	3.14 $\pm$ 0.19bC	4.00 $\pm$ 1.15bA	5.89 $\pm$ 0.46cB
EB (log CFU/g)	T2	2.01 $\pm$ 0.82aA	2.27 $\pm$ 0.68aA	1.47 $\pm$ 0.58aA	2.21 $\pm$ 0.65aA
	T7+	2.16 $\pm$ 0.54aA	2.54 $\pm$ 0.81aA	2.51 $\pm$ 0.60aA	2.72 $\pm$ 0.83aA
	T7-	2.01 $\pm$ 0.82aA	2.91 $\pm$ 0.96abA	4.07 $\pm$ 0.63bcB	4.76 $\pm$ 0.77cB
PSEU (log CFU/g)	T2	2.54 $\pm$ 0.54aA	2.72 $\pm$ 0.66aA	2.14 $\pm$ 0.96aA	2.57 $\pm$ 0.42aA
	T7+	2.66 $\pm$ 0.67aA	3.37 $\pm$ 1.40aA	2.87 $\pm$ 1.07aA	3.77 $\pm$ 0.53aB
	T7-	2.54 $\pm$ 0.54aA	3.64 $\pm$ 0.34aA	4.98 $\pm$ 0.81bB	6.29 $\pm$ 0.68cC
Bt (log CFU/g)	T2	1.79 $\pm$ 0.54aA	2.55 $\pm$ 0.50aA	3.90 $\pm$ 0.50bA	4.08 $\pm$ 0.54bA
	T7+	2.08 $\pm$ 0.29aA	4.55 $\pm$ 0.64bB	5.64 $\pm$ 0.71cB	6.26 $\pm$ 0.12cB
	T7-	1.79 $\pm$ 0.54aA	4.70 $\pm$ 0.40bB	6.16 $\pm$ 0.50cB	6.99 $\pm$ 0.09cC
pH	T2	5.55 $\pm$ 0.07aA	5.62 $\pm$ 0.04bA	5.66 $\pm$ 0.04bcA	5.69 $\pm$ 0.04cA
	T7+	5.52 $\pm$ 0.05aA	5.51 $\pm$ 0.14aB	5.13 $\pm$ 0.10bB	5.12 $\pm$ 0.10bB
	T7-	5.55 $\pm$ 0.07aA	5.62 $\pm$ 0.03aA	5.60 $\pm$ 0.08aA	5.58 $\pm$ 0.09aC
colour: L*	T2	53.10 $\pm$ 3.02aA	51.64 $\pm$ 2.35aAB	50.91 $\pm$ 2.66aA	51.48 $\pm$ 4.27aA
	T7+	52.68 $\pm$ 3.52abA	49.72 $\pm$ 2.88aA	51.13 $\pm$ 2.15abA	53.42 $\pm$ 5.11bA
	T7-	53.10 $\pm$ 3.02aA	52.57 $\pm$ 3.75abB	50.04 $\pm$ 3.16bA	50.06 $\pm$ 2.62bA
colour: a*	T2	15.15 $\pm$ 1.88aA	14.52 $\pm$ 1.72aA	14.29 $\pm$ 1.55aA	14.58 $\pm$ 1.00aA
	T7+	13.17 $\pm$ 1.51aB	14.17 $\pm$ 1.43aA	9.56 $\pm$ 1.30bB	9.17 $\pm$ 2.30bB
	T7-	15.15 $\pm$ 1.88aA	14.33 $\pm$ 2.33aA	14.14 $\pm$ 1.74aA	11.77 $\pm$ 1.68bC
colour: b*	T2	7.92 $\pm$ 1.05aA	8.56 $\pm$ 2.07aA	8.27 $\pm$ 1.50aA	9.11 $\pm$ 1.10aA
	T7+	6.55 $\pm$ 1.20aB	8.10 $\pm$ 1.21bA	6.63 $\pm$ 1.37aB	6.17 $\pm$ 1.96aB
	T7-	7.92 $\pm$ 1.05aA	8.56 $\pm$ 1.99aA	8.30 $\pm$ 1.20aA	8.19 $\pm$ 0.79aA

T2: control sausages produced without a protective culture and stored at 2°C

T7+: sausages produced with a protective culture and stored at 7°C

T7-: sausages produced without a protective culture and stored at 7°C

t0, t3, t7, t9: days of storage

TMB: total mesophilic aerobic bacteria

TPB: total psychrophilic aerobic bacteria

LB: lactobacilli

MC: *Micrococcaceae*

EB: *Enterobacteriaceae*

PSEU: pseudomonads

Bt: *Brochothrix thermosphacta*

For each parameter different letters in the same row and in the same column

indicate significant differences (P<0.05)

OCCURRENCE OF OCHRATOXIN A IN ARTISAN SALAMI PRODUCED IN VENETO

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Ochratoxins are mycotoxins produced by various *Aspergillus* and *Penicillium* spp. and ochratoxin A (OTA) is the most common and toxic. It is a very stable and persistent molecule and it is responsible of many genotoxic effects on laboratory and farm animals. OTA is classified as possibly carcinogenic to humans (IARC, 2003). The aim of this study is to develop a suitable analytical method for the evaluation of ochratoxin A in highly heterogeneous meat products like salami and carry out monitoring to assess the presence of OTA in artisan salami sampled in Veneto. In this region there is a long tradition in the production of handmade salami from small family-run farms and holiday farms. Not having these small farms self-control systems like the major meat product factories, it becomes interesting to assess if in these small productions OTA is a real risk to human health.

A total of 50 samples of artisan salami were surveyed. They were purchased in the provinces of Vicenza and Treviso. For analysis of OTA, we developed an economic method based on the extraction procedure described by Bozzo et al. (2012); our method can be optionally improved using immunoaffinity columns (IAC) in case of salami rich in fat. The analyses were performed by high performance liquid chromatography with fluorescent detection (HPLC-FD).

The sample preparation procedure without IAC has proved a suitable and effective method for OTA evaluation in 39 samples; for the other 11, it was necessary a clean-up step by IAC. This more performing procedure lets to lower the method detection limit (LOD) from 0.25 to 0.06 ppb, as well as the limit of quantitation (LOQ) from 0.5 to 0.2 ppb, but on the other hand, IAC have a relatively high cost. Ochratoxin A was detected in 5 samples but only one exceeded the guideline level (1 ppb) established by the Italian Ministry of Health.

The sample preparation procedure with IAC and analysis via HPLC-FD is a satisfactory method for the determination of OTA in heterogeneous meat products like salami. This preliminary study that considered a relatively low number of samples, would seem to suggest that salami made with the traditional, non-industrial production method, as regards contamination by ochratoxin A, can be considered healthy like the same of industrial production. We believe it would be interesting to continue the research extending it to salami producers in other areas.

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Sicurezza alimentare

Ochratoxin A, Salami, HPLC/FD

DETECTION OF SALMONELLA ON CARCASSES AND ENVIRONMENT IN PIG SLAUGHTERHOUSES IN NORTHERN ITALY

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The aim of the present study was to evaluate the presence of Salmonella on carcasses and environment in pig slaughterhouses.

Three slaughterhouses (A-B-C) with high slaughter capacity (~400 pigs/h) were selected for the survey and visited 6 times with 4-week interval. On each visit, samples were collected from n.5 carcasses by using sponges (10×10 cm), caecal contents (225g pools of the 5 pigs) and the slaughterhouse environments: a surface 10×10 cm per site was sampled using sponges in three different places: floor after the bleeding stage, gut container, runoff pit. The samples were pre-enriched in buffered peptone water and analyzed according to the Salmonella Precis method (OXOID - Milan). Presumptive colonies were submitted to phenotypic identification (API ID 32E - bioMérieux, France); serotyped by agglutination tests with specific O and H antisera and then genotyped by pulsed field gel electrophoresis (PFGE) using the Xba enzyme.

From a total 216 samples, Salmonella was recovered from 11 (5%) samples in two slaughterhouses: A-B. From a total 90 pre-chill carcasses sampled in 18 batches, Salmonella was isolated from 4 (4.44%) in only one slaughterhouse (B). Two out of the 18 batches (each batch: ~140 animals), originating from different farms, were positive for Salmonella (11.1%). From the 108 environmental samples (54 collected before and 54 during slaughter activities), Salmonella was detected only in the samples collected during the slaughter activities (7/54 - 12.9%). None of the pooled faeces were positive. Four different serotypes were detected: S.Rissen (n=5), S.Tiphymurium (n.1) and the monophasic-variant of the serotype Tiphymurium (n.3), S. Derby (n.2). The serotypes from the carcasses were S.Rissen and S.Derby whereas those from the environmental samples were S.Rissen, S.Tiphymurium and the monophasic-variant. In serotype S.Rissen isolates, two different PFGE restriction profiles could be distinguished; among S.Tiphymurium monophasic-variant isolates two PFGE patterns were identified; the S.Derby strains showed the same pulsotype.

The European Union (EU) is currently discussing Salmonella control in pigs and deliberating on setting targets for such control programs. In this study, differences in Salmonella contamination were observed in relation both to the sampling day and to the slaughterhouses. Salmonella was recovered in two of the three abattoirs tested. These findings are in accordance with the results of Botteldoorn et al.(1), indicating that sampling results depend on the slaughterhouse, the sampling day and the origin of the pigs. The detected percentage of carcass contamination (4.44%) is in accordance with other studies (2). Regarding serotype, our results are consistent with the Baseline EU survey (3). As for as the PFGE analysis is concerned, a diversity of strains has been identified. However, it was not possible to isolate the same pulsotype within the same slaughterhouse in different sampling days. S.Rissen isolates recovered from carcasses had the same PFGE profile and the same isolate was also detected from the floor after the bleeding stage during the same sampling visit in slaughterhouse B. The slaughterhouse environment may be a source of carcass contamination of the slaughtered pigs passing along the slaughter line.

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Food Safety

Salmonella, pig, slaughterhouse

OPTIMIZATION OF PRACTICAL DIETS FOR FLATFISH REARED IN EUROPE: A REVIEW AND PERSPECTIVES

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Salmonids, gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.) play a lead role in the European fish production; however, other farmed fish species are required to differentiate and widen the market supply. Flatfish have long been of interest for aquaculture in Europe. In particular, turbot (*Psetta maxima*) is the most important cultured flatfish species in Europe, widely reared also in other countries such as East Asia, whereas the sole species common sole (*Solea solea*) and Segenegal sole (*Solea senegalensis*) represent an interesting alternative for the diversification of the European and Italian aquaculture, due to the high price and high market demand. To date, the production of these species in Europe and Italy are still minimal compared to other species and further improvement are needed to achieve a full sustainability of the production cycle. Above all, pursuing the ideal feed formulation and an adequate nutrient utilization are of particular relevance for the success of the aquaculture production of any given species.

In this context, a critical review of our studies aimed at optimizing the diets for these flatfish species and conducted during the last ten years will be presented.

Results indicated that soles species require innovative ingredients and have specific nutritional needs to improve feed intake and growth. In particular different dietary protein levels demonstrated a considerable influence on growth, feed utilization and nitrogen excretion with the highest growth and feed utilization achieved with a diet containing 57 % of crude protein (1). A feeding trial with different dietary energy levels evidenced that increasing dietary lipid level lead to a substantial decline in performance and affect gut health. Dietary lipid levels higher than 12 % depress growth and lipid utilization. High lipid diets lead to moderate to severe intestinal steatosis and ultrastructural evaluations display cellular suffering due to a lipid overload. Soybean meal (SBM) seems to be a good protein source in diets for Egyptian sole. A level of 30 % of SBM can be added in the diet without any reduction in growth rate and any effect on gut histology. Soy products may be promising protein sources for inclusion in sole diets (2). The substitution of fish meal by mussels meal improved performance and feed utilization. The mussels meal could mimic the characteristics of the sole's natural prey, in terms of attractiveness and nutrient utilization. It is advisable to consider mussel meal, rather than fish meal as a reference diet to determine the growth potential of common sole. Turbot showed low tolerance to high plant protein inclusion, since vegetable ingredients negatively affected feed intake, growth and fish welfare. A substitution of a mixture of plant protein for up to 52 % of fish meal protein did not reduce feed intake, and at 39 % substitution, turbot maintained optimal growth rate and nutrient utilization. Worsened feed utilization in diets containing higher plant protein levels was not associated with a reduced digestibility of ingredients or alterations of gut histology (3). The administration of diet containing 35 % of fish meal ensured growth performance close to that containing 50 %, without significant effects on health and welfare of turbot juveniles. Diet containing 20 % of fish meal produced sub-optimal growth performance, metabolic stress and an immune response with consequences on health and welfare status. A 5 % fish meal diet caused a worsening of growth performance and fish welfare, probably due to an insufficient feeding and nutrients intake (4).

The results of the different experiments will be provided and discussed in order to identify new perspectives for optimizing practical diets for turbot and sole in aquaculture.

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Aquaculture

Sole, Turbot, Nutrition

DNA BARCODING FOR THE IDENTIFICATION OF SPARIDAE SPECIES OF COMMERCIAL INTEREST ON THE INTERNATIONAL MARKET

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The aim of this work was to use the DNA barcoding for the identification of Porgies (Sparidae) species of commercial interest on the International market.

Two hundreds and ninety-six reference samples (89 fresh specimens and 207 ethanol preserved tissues) from Museums and Research Institutions and 55 commercial samples from Italian resellers were collected. Thirty-four fresh specimens were cooked to test the heat degradation effect on DNA. After the DNA extraction (1), the first part (~650 bp) of the mtCOI gene was amplified with universal primers (2). The obtained sequences were elaborated with Clustal W in Bioedit 7.0.9.0 (3), then analyzed by the Identification System (IDs) on BOLD and by a BLAST analysis on GenBank. A top match with a sequence similarity of at least 98% was used to designate potential species identification (4). Moreover, the genetic distance was assessed using the Kimura 2-parameter (5).

Sixty-four species, out of the 133 of the family Sparidae, were collected. Of these, 58 are included among the 85 species of commercial interest according to the International lists (6-13). The DNA amplification for the reference samples was successful in 75% of samples (82.5% for the fresh specimens, 63% for the ethanol preserved samples, and 50% for the cooked samples). Overall, 199 sequences were obtained (at least one full barcode for 57 species). Kimura 2p distance values were observed to increase with taxonomic level. The IDs on BOLD, supported by the BIN discordance report, assessed the concordance between the morphological and the genetic identification for 161 (80.9%) sequences belonging to 43 (75.4%) species, while using the BLAST analysis these values were 134 (67.3%) and 34 (59.6%). The impossibility to identify some of the species can be explained considering both the close phylogenetic relationships between some species of the family and the unavailability of some reference sequences in the databases. In particular, while in GenBank 15 species (26.3%) were absent, on BOLD only 7 (12.3%) were missing. All the DNA from the commercial samples was successfully amplified and sequenced. The comparison with the databases revealed that 21 samples (38%) were mislabeled.

Our findings confirm that the DNA barcoding is a useful tool for the "molecular inspection" of most of the Porgies species and that it can be used to enforce the traceability of fishery products in the light of the European legislation (Reg. (CE) 104/2000 (14) and Reg. (UE) 1379/2013 (15)).

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Ispezione degli Alimenti di origine animale
DNA barcoding, Identification, Sparidae

POLYCYCLIC AROMATIC HYDROCARBONS IN WILD MYTILUS GALLOPROVINCIALIS COLLECTED IN CAMPANIA REGION

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Some polycyclic aromatic hydrocarbons have been shown to be genotoxic and carcinogenic for humans and high levels of these contaminants have often been found in mussels and other seafoods collected in polluted marine waters. Regulation EC/2004/1881 fixed a maximum level of 6 µg/kg for Benzo(a)pyrene and 35 µg/kg for the sum of four hydrocarbons (Benzo(a)pyrene, Chrysene, Benz(a)anthracene, Benzo(b)fluoranthene) in mussels.

Aim of this study was to evaluate the hydrocarbons contamination in wild mussels (*Mytilus galloprovincialis*-Lamarck, 1819), manually collected in 21 stations of coastal areas of the Gulf of Naples (Italy). The marine areas of sampling were not under official health surveillance, but did not lie far from authorized off-shore mussel plants.

The samples were extracted by cyclohexane, and then a HPLC analysis of low and high molecular weight 13 hydrocarbons were carried out.

The concentrations of PAH marker were higher of maximum established level in n.15 samples. The concentrations of marker Benzo(a)pyrene were higher in n.15 samples of mussels (max value 131.78 µg/kg), and the sum of the four hydrocarbons was above the maximum level in n.13 samples (max value 384.56 µg/kg).

Environmental polycyclic aromatic hydrocarbons contamination showed to have a role on the contamination of wild mussels collected in Gulf of Naples. Hydrocarbon levels are indicative of a prevailing pyrolytic hydrocarbon source related to anthropogenic activities. Results confirmed that the mussels showed to be not only useful as bioindicators of marine contamination, but also raised public health concerns, if they are

consumed. Because of the presence of high levels of PAH markers, and other possible/probable carcinogenic hydrocarbons in wild mussels of Gulf of Naples, more effective controls on the marine habitat and cultured mussels are required.

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food safety

PAH, *M. galloprovincialis*, Food safety

A SURVEY ON GOOD MANAGEMENT PRACTICES FOR THE PREPARATION OF RAW FISH PRODUCTS

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This survey was aimed to outline the main issues related to the management of the risk associated to the presence of parasites in the preparation and administration of raw fish products in food activities located in the area of the Local Sanitary Unit n°10 of Florence.

Thirty-six catering activities serving ethnic raw food were identified on the basis of the registry of Local Sanitary Unit n° 10 of Florence or after performing an internet search. All the activities were contacted by telephone to verify their catering procedures, their availability in participating to the survey and to answer to an anonymous questionnaire. The questionnaire was developed together with the staff of the LSU n° 10. Among the 36 catering activities that were contacted, 12 were not included in the survey because 2 were closed, 2 did not exist and 8 did not prepare raw fish products. Of the 24 activities that participated to the survey 68% were managed by Chinese Food Business Operators (FBOs), 27% by Italian and 4.5% by Japanese. 13% were restaurant, 61% restaurant and takeaway and 26% restaurant, takeaway and catering. The raw foods were prepared daily (52.2%), daily and on order (39%), during "theme events" (4.5%) and only on order (4.3%). Most of the FBOs supplied at wholesalers or at the large-scale distribution, while 8.6% at fishmongers. Fish were mainly purchased fresh (87%) and only a small proportion (3%) frozen. Among the most used fish species were Tuna, Salmon and Sea Bass. All the FBOs stated to have a self-control plan and a procedure for the management of the Anisakis risk: 70% is aware of the need to apply a preventive treatment before the administration of raw fish products. 70% of the activities was equipped with blast chiller but only 40% used it according to the regulatory parameters: 60% of FBOs adopted combinations of time/temperature which were non-compliant and, in some cases, ineffective to devitalize larvae of Anisakids. In case of detection of larvae, 70% of FBOs would proceed with a notification to the supplier, 4.5% to the ASL or to the person in charge of their HACCP plan, 8.7% would apply the preventive treatment, 13% would reject the fishes without any notification and the remaining 3.8% would not know how to proceed.

The result of this survey showed that the level of training of the FBOs that operate in raw fish products catering activities is insufficient for a correct management of the risk associated to the presence of parasites (1). In particular, it is evident the confusion regarding the parameters (time/temperature) to be used for the preventive freezing treatment and the procedure to adopt in case of noncompliance. It would be desirable to improve the level of FBOs training, without neglecting the language barriers, which represent one of the major hindrance to the promotion of correct operating procedures in a multiethnic context.

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ISPEZIONE DEGLI ALIMENTI DI ORIGINE ANIMALE

GOOD MANAGEMENT PRAC, RAW FISH PRODUCTS, PARASITES

MICROFLORA EVOLUTION DURING RIPENING OF CACIOCAVALLO PALERMITANO CHEESE.

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The Caciocavallo palermitano is a traditional Sicilian cheese manufactured within the provinces of Palermo and Trapani. It is produced in small size farms using indigenous cattle breeds - as Cinisara - and typical wood instruments (1). The ripening lasts from three to 12 months and the final product is a stretched cheese with a rectangular shape and a weight between 8 and 12 kilos. It can be consumed both fresh and seasoned.

The quality characteristics of this cheese are influenced by several factors - especially by the microflora in milk and cheese - and in particular during the ripening period (2). Lactic acid bacteria (LAB) presents in raw milk have an important role in the process of cheese-making. LAB are subjected to constant qualitative and quantitative changes and so they influence the organoleptic characteristics of the final product.

This work was performed to examine the microflora evolution during the Caciocavallo palermitano ripening

Six dairies in the area between Palermo and Trapani were selected to follow the Caciocavallo palermitano production process. From each dairy the following products were taken : 1) milk, 2) paste before stretching (PBS), 3) Caciocavallo at six days, 4) Caciocavallo at three months. All the samples were processed according to the Istituto Zooprofilattico Sperimentale della Sicilia standard operating procedures to evaluate the LAB variability, total bacteria count at 30°C, total coliforms, enterococci, coagulase-positive staphylococci, *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*.

The total bacteria count had a concentration from $8.0 \cdot 10^2$ to $1.6 \cdot 10^7$ cfu/ml. Total coliforms, *E.coli* and coagulase-positive staphylococci were found in milk or in product taken during cheese making processes but no pathogenic bacteria were found in the cheeses. The results concerning LAB evolution are shown in Figure 1; some of these strains were isolated and are currently being identified.

This work may contribute to increase knowledge about the indigenous lactic microflora of the Caciocavallo palermitano cheeses.

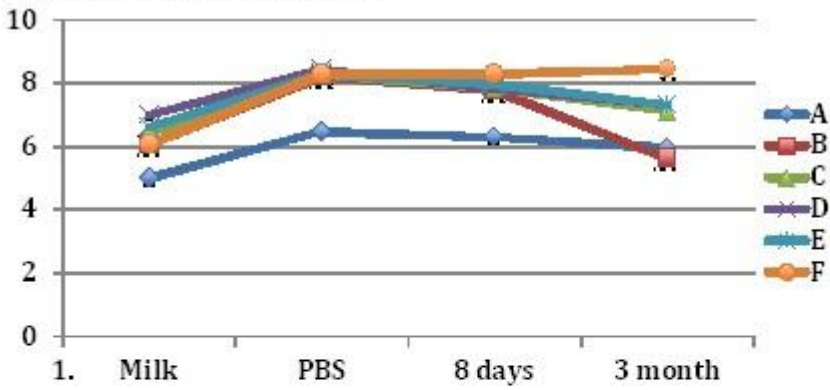
The absence of pathogenic bacteria, including *Salmonella* spp and *Listeria monocytogenes*, confirms that all the final products had good healthy characteristics.

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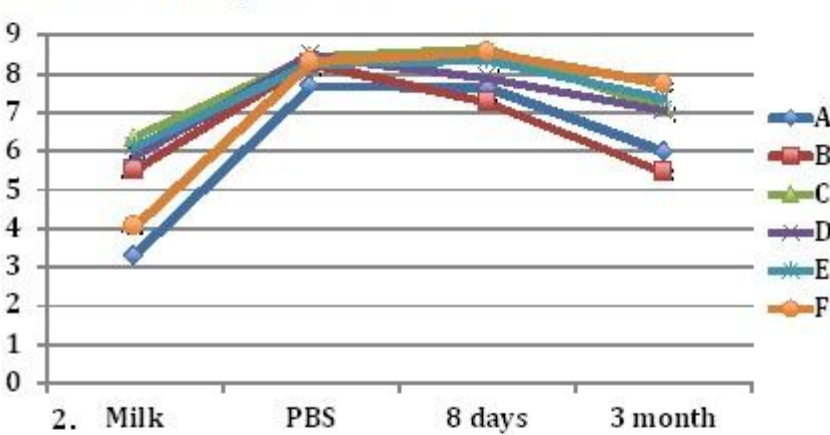
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processi produttivi e sicurezza alimentare
Caciocavallo, microbiology, LAB evolution

1. Variations of Mesophilic Lactococci



2. Variations of Thermophilic Lactococci



3. Variations of Lactobacillus

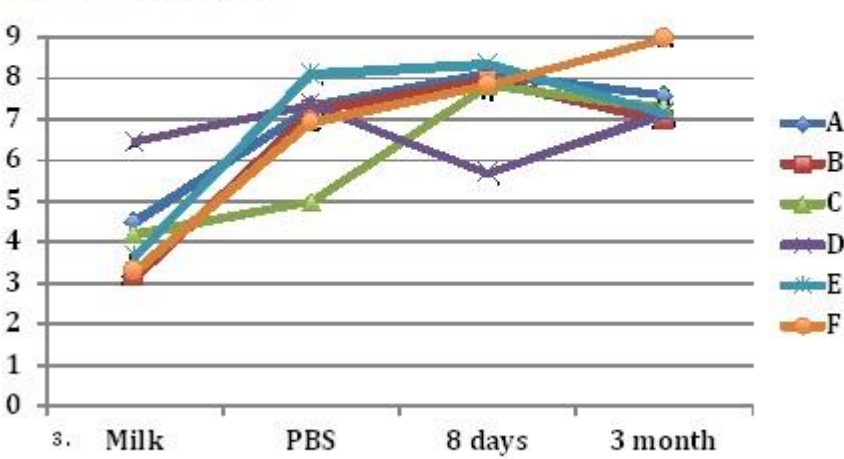


Figure 1. LAB evolution in six dairies (A-F) during cheese making processes and ripening. PBS (Paste Before Stretching)

Sisvet Poster

5-LOX EXPRESSION IN THE BRAIN TISSUE OF STRANDED STRIPED DOLPHINS (STENELLA COERULEOALBA) AND BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS), WITH OR WITHOUT INFECTIOUS ENCEPHALITIS/MENINGO-ENCEPHALITIS

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Dolphin Morbillivirus (DMV), *Toxoplasma gondii* and *Brucella ceti* are regarded as pathogens of major concern for both free-ranging striped dolphins (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiops truncatus*) (1). Although a more or less severe encephalitis/meningo-encephalitis is known to occur in striped dolphins and, to a lesser degree, in bottlenose dolphins infected by the aforementioned agents, very little information is available on the neuropathogenesis of brain lesions, including the neuronal and non-neuronal cells targeted during infection and the molecular mechanisms leading to neurodegeneration (2, 3).

With this in mind, we investigated the Western blot (WB) expression of 5-lipoxygenase (5-LOX), a key-enzyme for mammalian infections, within the brain tissue of 11 striped dolphins and 5 bottlenose dolphins stranded along the Italian coastline.

Three striped dolphins and 2 bottlenose dolphins showed no morphologic evidence of central neuropathies, while the remaining 8 striped dolphins and 2 additional bottlenose dolphins exhibited encephalitic/meningo-encephalitic lesions of various degree, associated with DMV (1 striped dolphin), *T. gondii* (5 striped dolphins and 2 bottlenose dolphins) and *B. ceti* (1 striped dolphin) infection, as well as with DMV-*T. gondii* co-infection (1 striped dolphin).

All the 8 striped dolphins affected by encephalitis/meningo-encephalitis showed an intensity of 5-LOX WB bands which was more pronounced than that observed in the 3 dolphins without any morphologic evidence of brain lesions, with the most prominent band intensity being detected in the *B. ceti*-infected animal. The same was not true for *T. gondii*-infected as compared to *T. gondii*-uninfected bottlenose dolphins, 1 of which had the most consistent 5-LOX band intensity. Nevertheless, malacic areas, associated or not with cholesterol clefts, were seen scattered throughout this animal's brain.

In summary, we believe that the finding related to a higher expression of 5-LOX enzyme in the 8 striped dolphins affected by infectious encephalitis/meningo-encephalitis is of interest. The finding that this was not paralleled by a simultaneous increase of 5-LOX expression in the brain from *T. gondii*-infected in comparison to *T. gondii*-uninfected bottlenose dolphins is also of concern, likely reflecting the mutual host-parasite adaptation of the latter ("inshore") in comparison to the former ("offshore") species (4).

Since 5-LOX is a putative neurodegeneration biomarker both in human patients (5) and in experimental animal models (6), further investigation on this challenging issue is warranted.

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Neuropatologia comparata

5-LOX, Stranded cetaceans, Brain lesions

PHARMACOKINETICS OF FLUPIRTINE IN HEALTHY CATS FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATIONS

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Flupirtine (FLU) is a non-opioid analgesic drug without antipyretic or antiphlogistic effects used in the treatment of a wide range of pain states in human beings. There is a substantial evidence on the efficacy of FLU in humans however this is inadequate to recommend its off-label use in veterinary clinical practice. The aim of this study was to evaluate the pharmacokinetic profiles of FLU after IV and PO administration in healthy cats.

The Animal Welfare Committee of the University of Lublin approved the study protocol. Cats (n=6) were randomly assigned to two treatment groups, using an open, single-dose, two-treatment, two-phase, paired, cross-over design. All cats were fasted for 12 h overnight before each experiment. During the first phase each cat in group 1 (n = 3) received a single dose of 5 mg/kg of FLU (Katadolon®, AWD Pharma) injected IV into the jugular vein. Group 2 (n = 3) received the same dose via PO route (Efiret®, Meda Pharma). A 1-week wash out period was observed. Blood samples were collected at assigned times and plasma was analyzed according to a previous HPLC method (De Vito et al., accepted). The pharmacokinetic calculations were carried out using WinNonLin v 5.3 (Pharsight). A weighting (1/[actual plasma concentration]²) was used.

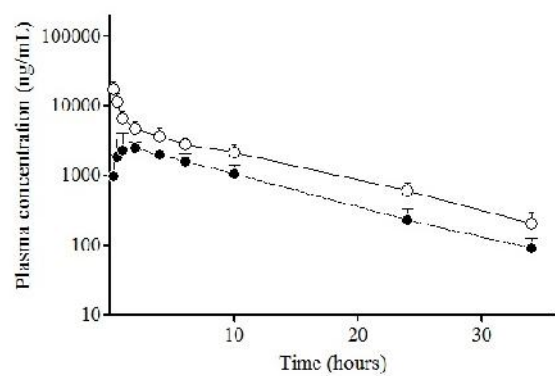
The HPLC method was re-validated in the cat plasma. Briefly, FLU was linear ($r^2 > 0.99$) in the range 10-2000 ng/mL. The intraday repeatability was lower than 6.1 %, whereas accuracy, was lower than 5.9 %.

No adverse effects at the point of injection and no behavioral or health alterations were observed in the animals during or after (up to 7 days) the study. A bi-compartmental model best fitted the plasma concentrations after IV and PO administrations in all the 6 cats. The average plasma concentration vs. time curves after both the administrations are reported in Fig. 1. After IV administration FLU plasma concentration was largely variable, especially at the first points of collections. FLU was detectable in plasma up to 36 h, then at 48 h the drug concentrations dropped down the LOQ of the method. After oral administration the FLU plasma concentrations were lower than after IV route, but detectable in the same range of time. The C_{max} (2460 ng/mL) was shown at a T_{max} of (2.78 h). The oral bioavailability (F%) was $39.3 \pm 9.7\%$. The HL of elimination (B_{HL}) values after either routes were similar. The terminal part of both mean pharmacokinetic curves showed a similar trend of elimination.

This is the first study concerning FLU in animal species of veterinary interest. The pharmacokinetic profiles of FLU in cats were somewhat different compared to the FLU disposition in humans. Its PO F% was about 39%. This study could pave the road for the use of this active ingredient in the veterinary field. Further studies need to be undertaken to assess if this drug may be adequate in feline medicine.

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De Vito et al., 2014 Biomed Chrom, accepted
Pharmacology
Flupirtine, Cats, Pharmacokinetics



PK/PD EVALUATION OF PARECOXIB AND ITS ACTIVE METABOLITE VALDECOXIB IN CATS.

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Parecoxib (PX) is a injective pro-drug of Valdecoxib (VX), known to be a selective cyclo-oxygenase-2 (COX-2) inhibitor. The aim of the present study was to evaluate pharmacokinetics and pharmacodynamics in vitro/ex vivo cyclooxygenase selectivity of PX and VX in cats.

In vitro evaluation of effects of PX and VX was performed using blood collected from 6 adult male cats (Ethic committee's protocol number 2473 - University of Pisa) in sodium citrate and anticoagulant free tubes for TXB2 (marker for COX-1 isoform) and PgE2 (marker for COX-2 isoform) measurements, respectively. For COX-1 evaluation, blood aliquots were mixed with dymethyl sulfoxide (DMSO) containing VX or PX (0.0508-1,000 μ M). Blood aliquots with only DMSO were used as controls. After incubation and centrifugation, supernatants were analyzed using a commercial TXB2 ELISA kit. Samples for COX-2 evaluation were incubated with VX or PX and with 5 μ l of lipopolysaccharide (LPS) solution to stimulate PgE2 synthesis. Supernatant was mixed with methanol (1:5) to allow protein precipitation. Supernatants were analyzed using a commercial PgE2 ELISA kit. In ex vivo study, the same method used in in vitro study was performed using samples collected by cats treated with 2.5 mg/kg of PX IM. For HPLC analysis, blood samples were collected into lithium-heparinized tubes and plasma samples were prepared according to a previous method (1) with some modifications. Pharmacokinetic analyses were described by a non-compartmental model (WinNonLin 5.3, Parsight).

The IC50 values of VX for COX-2 and COX-1 were 0.45 and 38.6 μ M, respectively. PX did not affect COX enzymes. HPLC analysis showed that PX is rapidly converted to VX with a relatively short half-life of 0.4 h. VX achieved peak plasma concentration ($2.79 \pm 1.59 \mu\text{g/mL}$) at 7 h following PX injection. The mean residence times for PX and VX were 0.43 ± 0.15 and 5.94 ± 0.88 h, respectively. The ex vivo study showed a COX-2 inhibition rate of about 70% in samples taken at 1, 2, 4 and 10 h after injection of PX. COX-1 inhibition ranged from 0.7% to 9.7% compared to the control without any significant difference for 24 h after PX administration.

Although PX has not been studied as extensively in veterinary medicine as it has been in humans, some preliminary studies reported that this drug might also be effective and safe (2, 3) in animal species. Results of the present study seem to encourage further experiments on feline species to investigate whether this drug could be successfully used in feline medicine.

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Farmacologia veterinaria
Parecoxib, Valdecoxib, Cat

ANISAKIS-INDUCED ANAPHYLAXIS: A CASE REPORT.

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This study describes a clinical anisakiasis case. On Tuesday 24th December, a 50 years old woman was admitted to Ceva (CN) hospital reporting abdominal pain, profuse vomit, palpebral oedema and pruriginous urticaria-like cutaneous rash involving the torso and the neck region. Patient clinical features suggested, a scombroid syndrome (Cattaneo, 2011), related to histamine poisoning caused by seafood consumption. Anamnesis consented to relate the symptoms to consumption of marinated fresh anchovies, that hadn't been frost before raw consumption, on the previous evening.

The local Veterinary Service and the SIAN of ASL CN1 Dipartimento di Prevenzione were promptly informed and the remaining marinated anchovies were sampled to be analyzed. Since preliminary examination suggested presence of parasites in the fillets, anchovies samples were sent to the Controllo Alimenti Laboratory of Sezione di Cuneo (Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta - IZSPLV) for further analysis.

Inspection of anchovies was performed by examination of fillets put in a Petri dish under a table light at room temperature. A Trichomatic system was employed to digest anchovy fillets and membrane filters were examined by optical and stereo-microscope.

Histamine detection was performed by HPLC-DAD at Laboratorio Chimico of Sezione di Genova (IZSPLV).

Inspection revealed presence of numerous vital parasite larvae belonging to pathogen *Anisakis* (3) and to innocuous *Hysterothylacium* (4) genera. Digestion of anchovy fillets by Tricomatic system didn't reveal instead any parasite. Histamine level didn't exceed limits set by EC Regulation 2073/05, thus excluding scombroid syndrome diagnosis.

Parasites belonging to *Anisakis* genus are human pathogen since ingestion of vital larvae can determine gastroenteric syndromes characterized by acute pain mimicking the clinical features of gastric peptic ulcer or other inflammatory abdominal disorders. Furthermore, ingestion of *Anisakis* larvae has recently been related to acute allergic disorders (Hoshino and Narita, 2011).

In this case, patient clinical features and timing of symptoms onset (a few hours after larvae ingestion) are strongly suggestive of *Anisakis* -induced anaphylaxis.

As far as catering industry is concerned, EC Regulation 853/04 disposes precautionary freezing of seafood to be consumed raw or submitted to mild marinating and salting treatment in order to kill eventual *Anisakis* larvae. Furthermore, regarding domestic consumption, DM 17/07/13 imposes to retailers to affix specific warning to educate consumer to proper precautionary freezing practices of raw seafood.

Occurrence of clinical cases reveals an insufficient consumer information and the need for further specific education. Furthermore, since adoption of proper freezing practices is not effective in preventing *Anisakis* -induced anaphylaxis, a prompt and correct diagnosis and collaboration in a "one health" perspective between all the involved professional figures is necessary for an effective consumer protection.

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EC Regulation 2073/05

Hoshino C, Narita M (2011). *Anisakis simplex*-induced anaphylaxis. J Infect Chemother (2011) 17:544–546

EC Regulation 853/04

Decreto del Ministero della Salute 17/07/13

Sanità pubblica

Anisakis, Inspection, Histamine

MANAGEMENT OF A SALMONELLOSIS OUTBREAK IN SUCKLING BEEF CALVES.

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Collaboration between field veterinarians, Veterinary Service and Istituti Zooprofilattici Sperimentali (IZS) net is capital for public health protection. This study describes the joint management of a salmonellosis outbreak in suckling calves from a farm breeding both beef and dairy cattle in two separate stables.

The first case, diagnosed in a beef calf by the Patologia e Benessere Animale Laboratory of Sezione di Cuneo (IZS), was promptly reported to the competent Veterinary Service and appropriate monitoring and management measures were immediately set up.

Dead animals (4) were necropsied and calves faeces (10) were analyzed to detect *Salmonella* spp presence. Particularly, specific enrichment broth was inoculated and suspect colonies cultured on selective media [1]. Biochemical confirmation was performed by API 20E system (Biomeriaux). Antimicrobial susceptibility was assessed by Kirby-Bauer disk diffusion test [2]. Isolated *Salmonella* spp strains were then serotyped according to the Kauffmann-White scheme.

After *Salmonella* spp isolation, competent Veterinary Service and field veterinarian were immediately alerted and appropriate biosecurity measures (separation of clinical and asymptomatic animals, pens disinfection, immediate and strict control of access to dairy cattle stable) were set up. Animals were then treated with antimicrobials, according to antibiogram results. Particularly, a parenteral administration of gentamicin (5 days) and enrofloxacin (5 days) was performed. After antimicrobial treatment a second inspection was performed and animals faeces sampled to monitor therapy effectiveness.

A total of 4 calves died in the outbreak showing peritonitis and various degree of enteritis. *Salmonella* spp was isolated from the liver of all animals. As far as calves faeces are concerned, *Salmonella* spp was detected in 3 samples. Antibiogram revealed susceptibility to all the major antimicrobial classes and isolated strains were identified as *Salmonella* Typhimurium by serotyping analysis. All faeces samples collected after antimicrobial treatment resulted negative for *Salmonella* spp and all calves were asymptomatic.

This study describes management of a salmonellosis outbreak in suckling beef calves.

Collaboration between field veterinarians, Veterinary Service and IZS allowed an adequate outbreak management. Implementation of appropriate biosecurity measures and integration of specific expertise of all the professional figures involved resulted in a rapid and effective outbreak solution that both protected consumer health and limited farmer economical losses. Furthermore, appropriate salmonellosis outbreak management and implementation of biosecurity measures resulted in an improvement of animal production efficiency.

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[2] CLSI (2008). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved Standard-3rd Edition. CLSI Document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute

sanità animale

Salmonella spp, outbreak, management

A NEUROCHEMICAL STUDY OF THE POSTNATAL REELER MOUSE CEREBELLUM

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The reelin protein is absent in the Reeler mouse that harbours a homozygous mutation of the *reln* gene. Due to the lack of this glycoprotein, neuronal ectopia and defection in layered structures of the brain is observed, among which the cerebellar cortex (D'Arcangelo and Currant, 1998; Tissir and Goffinet, 2003; Folsom and Fatemi, 2013).

We here aimed to better characterize the neurochemical cerebellar phenotype of postnatal and adult *reln*^{-/-} mice and establish whether the absence of reelin can be responsible of an imbalance in cell proliferation and apoptosis in the cerebellum of these mutants.

Homozygous *reln*^{-/-} and wild type *reln*^{+/+} cerebella were harvested at postnatal ages P0, P5, P10, P15, P30, P60. BrdU was injected intraperitoneally 2 hours before the sacrifice. Apoptosis were detected by the TUNEL assay and proliferating cells with a monoclonal antibody against BrdU. Standard immunohistochemical procedures with antibodies against NeuN, calbindin, GFAP, vimentin, SMI32, GAD67 were performed. Semaphorin 3A and GAD65 were analyzed by Western blot and SDS-page.

A preliminary analysis indicated that apoptosis was significantly higher in P15 *reln*^{-/-} compared to *reln*^{+/+} mice and cellular proliferation was firstly reduced at P5 and then increased from P10 onwards in mutants. Temporal and cellular patterns of expression of neuronal/glial markers were the same between Reeler and wild type. All the markers considered are expressed from P5 onwards in both genotypes with the exception of calbindin and vimentin that were already at P0. Western blots showed a reduction in GAD65 and an elevation in semaphorin 3A expression in Reeler cerebella.

In mutants there are no obvious differences that may be indicative of altered neurochemical maturation, rather the profound structural alterations in Reeler may be related to an imbalance of cell proliferation/death in early postnatal life.

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NEUROSCIENZE/NEUROSVILUPPO
REELIN, CEREBELLUM, DEVELOPMENT

BACTERIOLOGICAL SURVEY ON THE PRESENCE OF ZONOTIC PATHOGENS IN FECES OF BIRDS**Bertelloni F.*^[1], Ebani V.V.^[1], Mani P.^[1]**^[1]*Department of Veterinary Science, University of Pisa, Italy ~ Pisa*

Zoonoses are diseases transmissible directly or indirectly between animals and humans. In many cases animals represent asymptomatic carriers. The most important zoonoses reported in Europe in the last years are campylobacteriosis, salmonellosis, listeriosis, verotoxigenic *Escherichia coli* (VTEC) infections and yersiniosis (1). The aim of this study was to screen fecal samples collected from birds belonging to different species for some of the most important zoonotic pathogens, in particular *Salmonella* spp., *Yersinia* spp., VTEC, *Campylobacter* spp., and *Listeria monocytogenes*. Comparison between farm, pet and wild birds was carried out to evaluate their role in spreading of these bacteria.

From January to December 2013, 409 avian fecal samples were collected. One hundred and sixty-nine samples were from laying hens, 107 from broilers, 52 from pigeons, 35 from raptors, 20, 16 and 10 from water birds, pheasants and pet birds respectively. Bacteriological examinations to detect *Salmonella* spp., *Yersinia* spp., VTEC, *Campylobacter* spp. and *Listeria monocytogenes* were carried out as previously described (2, 3, 4, 5, 6).

Among the 409 samples tested, 9 (2.2%) resulted positive for *Yersinia* spp., 3 (0.73%) for *Salmonella* spp. and 3 (0.73%) for VTEC. In particular, 3 *Yersinia* spp. isolates were obtained from laying hens feces, 2 from raptors (*Athene noctua*, *Falco peregrinus*) and 4 from seagulls (*Larus michaellis*). One *Salmonella* spp. isolate was detected in a laying hen and 2 isolates in two raptors (*Falco peregrinus*, *Bubo bubo*). Three VTEC isolates were found in fecal samples collected from one raptor (*Athene noctua*) and 2 pheasants (*Phasianus colchicus*). Two of the fifty-two samples collected from pigeons resulted positive for *L. monocytogenes* and 1 for *C. jejuni*.

We reported the results of one year survey on different categories of breeding, pet and wild birds for some bacterial pathogens. Low contamination was registered in farm broiler and laying hens (1.4%), and in pet birds (0%). On the basis of our results, with 8.9% of positive wild birds, it appears that these animals may be a relevant source of pathogen bacteria for other animal species and humans.

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Malattie Infettive

Birds, Pathogenic bacteria, Zoonoses

EVALUATION OF LAMB-GROWING IN A SHEEP ITALIAN INTENSIVE FARM

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The aim of the present study was to evaluate lamb-growing in an intensive sheep farm in Tuscany.

55 Massese' lambs were included in this study. Lambs were housed with their ewes in the same barn, but in different pens. Adults were allowed to stay outside during the day, spending only the night inside the barn, while lambs pen had only an inside area. The sheep were fed with total mixed ration composed by grass hay (700 g/sheep), mais silage (150 g/ sheep) and concentrate (200 g/sheep) (Cereal del Sol® Italy). Each sheep received 4 hg/day of concentrate supplement during milking (OVIPLUS 21®, Italy).

Sheep were evaluated for maternal behavior and lambs caring. Data collected for lambs at birth (T0) and every 5 days until 25 days (T1, T2, T3, T4, T5) were: 1) body weight (BW); 2) thoracic circumference (TC); 3) metacarpal circumference (MC); 4) withers height (WH). Statistical analysis: data collected were expressed as mean $\pm$ standard deviation. Distribution was evaluated by Komolgorov-Smirnov test. Distribution was not normal, thus KW test and Dunn's test as post hoc were performed to evaluate differences in BW, TC, MC and WH relating to time. Statistical significance was set at $P < 0.05$.

Data for BW, TC, MC and WH at T0, T1, T2, T3, T4 and T5 were reported in Table 1. 24/25 lambs were nursing by their own ewe, while 1/25 was feeding by the owner with a milk replacer due to polyarthritis. Statistical analysis showed differences for all the measures in relation to time.

	BW (kg)	TC (cm)	MC (cm)	WH (cm)
T0	4.82 $\pm$ 0.95	38.36 $\pm$ 3.19	8.38 $\pm$ 0.78	41.66 $\pm$ 2.98
T1	5.98 $\pm$ 1.16	42.72 $\pm$ 3.55	8.88 $\pm$ 0.65	45.2 $\pm$ 2.14
T2	7.45 $\pm$ 1.53	45.74 $\pm$ 4.22	9.32 $\pm$ 0.77	47.12 $\pm$ 2.81
T3	8.42 $\pm$ 1.73	47.98 $\pm$ 4.62	9.58 $\pm$ 0.83	48.44 $\pm$ 2.71
T4	9.55 $\pm$ 2.07	49.77 $\pm$ 4.80	9.94 $\pm$ 0.75	50.17 $\pm$ 2.94
T5	10.39 $\pm$ 2.54	52.93 $\pm$ 4.54	10.20 $\pm$ 0.73	51.63 $\pm$ 3.97

Table 1. Data concerning BW, TC, MC and WH evaluation at T0, T1, T2, T3, T4 and T5. Legend: BW: body weight; TC: thoracic circumference; MC: metacarpal circumference; WH: withers height

All the sheep showed an optimal maternal behavior. BW was similar to those reported for Massese lambs breeding under traditional conditions (1), while was slightly higher if compared to other breeds managed traditionally (2). The lack of differences between our Massese lambs the ones breed under traditional conditions could be due to the short observation period of this study. In fact, other studies (2) showed a higher lamb-growing in animals breeding in an intensive farm than other breed in field, but the statistically differences started from 50, or more, days of life. To the authors knowledge there are no data on TC, MC, and WH in lambs during the first month of life, thus our results cannot be compared to literature. Statistical analysis showed that our lamb population starts to increase in size from 10 days of life.

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zootechnia

Massese sheep, lamb, intensive farm

WALNUT DETECTION IN FOOD: IMPACT OF THERMAL TREATMENT ON ELISA DETECTION

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Food allergies represent an increasing food safety problem and 1–2% of adults and up to 5–7% of children suffer from allergy caused by some food products, such as peanuts and tree nuts (1). The beneficial effects of walnut, combined with its pleasant taste, have determined its inclusion in several pastry products (2). However, thermal exposure could determine changing of the protein structure, influencing both allergenicity and performance of methods aimed to identify specific antigens (3). The aim of our study was to evaluate the effects of thermal treatment both on walnut allergenicity that on ability of ELISA kits to detect the presence of this food allergen.

Walnut were exposed to the follows treatments: 1) boiling, 2) roasting at 80 °C (10 minutes), 3) roasting at 180 °C (10 minutes), 4) roasting at 180 °C (30 minutes). Forty backed dry biscuits and 10 dry pastry products were spiked (10 mg/kg) with raw and previously treated walnuts. ELISA analysis were performed. Data were elaborated by one-way ANOVA (Prism graphPad 5.03). A $p \leq 0.05$ was considered statistically significant. Moreover, treated and raw walnut were used to performed prick test in nine walnut-allergic patient.

Boiling ($P < 0.05$), intense and prolonged roasting ($P < 0.0001$) showed high influence the both kit sensitivity. Intense roasting caused significant effect only on kit A, while mild roasting did not significantly influence the detection ability ($P > 0.05$) of both kit. These results suggest that kit B is lower sensitive to a protein denaturation than A. Concerning the prick test, unlike of previous data (4), it showed a decrease of the walnut immunogenicity after thermal treatment. In particular, prolonged roasting determined a reduction of walnut immunogenicity (7/9 patients); However, only two subjects were characterized by total loss of reaction. These results associated with ELISIA test results after prolonged roasting highlight the unsuitability of these kits for walnut detection on some processed food.

Our results evidence the importance to evaluate the most suitable allergen contaminant detection method in food, also considering the effect of thermic treatments, in order to ensure the consumer safety.

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3-Besler et al., 2001. "Stability of food allergens and allergenicity of processed foods." *J Chromatogr B Biomed Sci Appl*. 756:207-28.

4-Masthoff et al., 2013. "A systematic review of the effect of thermal processing on the allergenicity of tree nuts." *Allergy*. 68:983-93.

Food Control

allergenic food, ELISA, thermal processes

PARASITES COMMUNITY OF AN ISOLATED POPULATION OF SYLVILAGUS FLORIDANUS FROM BOLOGNA PROVINCE

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The use of alien species for hunting purposes represents the 21% of invasive mammals' sources (Genovesi et al. 2008). They can represent an enormous biological and economic problem, as the case of eastern cottontail (*Sylvilagus floridanus*). Native to the American continent was introduced into some European countries, but wild population have only survived in Italy (DAISIE, 2008). Eastern cottontails have been vectors of allochthonous pathogens, in particular gastroenteric nematodes such as *Obeliscoides cuniculi*, *Trichostrongylus calcaratus*, *T. affinis* and *Passalurus nonannulatus* (Tizzani et al, 2002). Parasitological investigation on eastern cottontail in Italy are focused especially on the main population in Northwestern Piedmont. The aim of our study was to investigate the parasite community of this species in an isolated population located in Bologna province.

The population considered was created with the introduction of specimens in a private hunting estate close to Castel San Pietro Terme (BO) thirty years ago. Now the population is strongly rooted on around 70 kmq between 50 and 350 m of altitude. Ten specimens were collected during hunting season. From carcasses the whole gut was frozen at -20°C until the analysis. Standard laboratory techniques were used to recover, collect, store the parasites (MAFF, 1986). Parasites were identified using a morphometric approach and through the use of dichotomous keys (Skrjabin et al, 1954).

Five nematode species were reported: *O. cuniculi*, *T. calcaratus*, *T. affinis*, *P. nonannulatus*, *Protostrongylus* sp. and one cestode. Prevalence, Intensity and Abundance of the parasites are reported in table 1.

O. Cuniculi was the most common parasites while *T. affinis* was reported only in one specimen. The report of *P. nonannulatus* is very interesting as it is only occasionally described in *S. floridanus*. Regarding *Protostrongylus* sp. this is the first report for Italy.

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Parassitology

Eastern cottontail, Exotic parasites, Bologna

	P	CI_L_95	CI_U_95	I	SD	A	min	max
<i>O. cuniculi</i>	80%	0.55	1.05	10.13	0.77	8.1	3	20
<i>T. calcaratus</i>	50%	0.19	0.81	98	7.16	49	30	220
<i>T. affinis</i>	10%	-0.08	0.28	0.16	0.28	0.1	1	1
<i>P. nonannulatus</i>	70%	0.41	0.98	249. 14	73. 21	174. 4	30	750
Cestode	30%	0.02	0.58	1.67	0.35	0.5	1	3
<i>Protostrongylus</i> <i>sp.</i>	20%	-0.05	0.45	2,5	2.12	0.5	1	4

Table 1. Prevalence (P), Confidence Interval (CI), Intensity (I), Standard Deviation (SD), Abundance (A) and Range (min-max) of the parasites reported

EVALUATION OF BLOOD LACTATE CONCENTRATION IN DOGS BEFORE AND AFTER AGILITY COMPETITIONS

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Literature shows few reports about the effect of agility exercise on the metabolism of the dog. Aim of the study was to contribute to the information available, providing data about the changes in blood lactate levels in dogs before and after agility competitions.

Blood samples were collected before and after exercise from the radial vein of 23 dogs competing in agility shows (3 small, 15 large, 5 medium). Blood lactate was measured using a point-of-care system (Accutrend Plus, Roche). The results were compared based on the size of the dog and statistical analysis was made (mean, standard deviation).

In the small dogs, mean blood lactate was 4.07mmol/l (standard deviation 2,47mmol/l) before exercise and 4,57mmol/l (s.d. 1.65mmol/l) afterwards. In the medium dogs, before the race the mean lactate was 1,66mmol/l (s.d. 0.85) and 4.10mmol/l (s.d. 3.74) after. In the large dogs, the values were 2.31mmol/l (s.d. 0.81) and 5.89mmol/l (s.d. 3.06) before and after exercise respectively.

Blood lactate is a waste product of anaerobic metabolism, its value in the blood defines the type of exercise the body is doing: above 4mmol/l the exercise is considered anaerobic. Agility competitions are dog shows in which the animal has to clear an obstacle course in a predetermined order: the winner is the fastest dog, and the one that makes less mistakes. The results shows that in all three categories, the exercise was anerobic: this is especially true for the large dogs, that tend to be faster than the others. Comparing the lactate levels with the speed of the animals, the faster the dog, the higher the lactate: in particular, dogs that ran faster than the mean speed for their category tended to show higher lactate concentrations. Also, the training levels seems to be important: the maximum lactate concentration was found in animals that were less trained. These results are consistent with the type of exercise and also with the data shown in horses and human, where exercise metabolism is been extensively studied.

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Sports Medicine

agility, dog, blood lactate

EFFECT OF TURMERIC POWDER (CURCUMA LONGA) ON MEAT QUALITY OF CINTA SENESE PIGS REARED OUTDOORS

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Cinta Senese pig is an autochthonous breed of Tuscany, usually reared outdoors (1). Recently Turmeric powder has been widely studied for its healthy properties (2) and antioxidant activity in different species (3, 4). This study evaluates the effect of the Turmeric powder diet supplementation on Cinta Senese pig meat quality.

Twenty Cinta Senese pigs, divided in two groups (Control, C and Treated, T) were reared in pasture and fed with natural diet supplemented with wheat and barley flours. During one month before slaughter to T group diet was added Turmeric powder (30 mg/Kg LW per die). Pigs were slaughtered at 150±10 Kg and from each right half carcass Longissimus dorsi was taken. One day after slaughter, physical and chemical meat traits were assessed: pH was measured using a HANNA pH 2011 pHmeter; meat color was evaluated on a 2.5 cm thick slice of meat, using a Minolta CR300 colorimeter (Illuminant D 65), which measures the Lightness, redness, yellowness, Hue and Chroma values (5). Before color evaluation, each sample was oxygenated at 4°C for 1h. The water holding capacity was determined as drip loss, measured as percentage of weight loss of a meat sample kept at +4°C for 24h (6) and as cooking loss, measured as percentage of weight loss of a meat sample cooked in an oven at 163°C to reach an internal temperature of 71°C (7). Tenderness was measured as shear force (kg) using Warner-Bratzler Shear applied to an Instron 1011. Meat chemical composition was determined according to AOAC methods (8). Data underwent a variance analysis (9).

Table 1 showed the effects of the diets C and T on physical and chemical characteristics of meat. No significant differences were observed for pH, Lightness, water holding capacity, tenderness and chemical composition. The diets had a significant effect on colour parameters: meat derived from pigs fed with Turmeric powder showed lower redness and yellowness indexes ($P < 0.001$). However considering Hue and Chroma values the meat derived from pigs fed with Turmeric appeared more red with less intense colour. The supplementation of diets with Turmeric powder might determine less oxidation of myoglobin with consequent lower levels of met-myoglobin.

In conclusion, the supplementation of Turmeric powder in pig diet seems to modify the colour parameters of meat, producing a better impact on consumers at the time of purchase. Further studies will be assessed to evaluate the effect of Turmeric powder supplementation on shelf life of pig meat.

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Nutraceutica

Turmeric, Meat quality, Pig

Table 1. The effects of the diets on physical and chemical characteristics of meat.

		Control group	Treated group	<i>P value</i>	RMSE ¹
Animals	n	10	10		
pH		5.53	5.41	ns	0.229
Lightness		47.03	46.18	ns	3.726
redness		11.60	10.55	***	1.476
yellowness		5.46	4.46	***	1.234
Hue		24.93	22.81	**	4.058
Chroma		12.86	11.47	***	1.720
Drip loss	%	1.66	1.98	ns	0.008
Cooking loss	%	21.00	21.34	ns	0.039
Tenderness	Kg/cm ²	11.40	11.51	ns	2.781
Moisture	%	71.70	72.35	ns	0.016
Ether extract	%	2.00	1.90	ns	0.010
Protein	%	25.27	24.72	ns	0.012
Ash	%	1.00	1.01	ns	0.000

ns: not significant; ** : $P < 0.01$; ***: $P < 0.001$; ¹: residual means standard error

EFFECT OF TURMERIC POWDER AND ASCORBIC ACID ON QUALITY AND OXIDATIVE STABILITY OF RABBIT BURGERS

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Rabbit meat has excellent nutritive and dietetic properties and may be considered functional food since is a good source of mineral, vitamin and unsaturated fatty acids that are favourable for human health (1). However the high content of n-3 fatty acids might reduce the meat products shelf life and consequently the beneficial effects. For this reason the supplementation of natural additives with antioxidant properties might constitute the strategy to achieve more functional meat product (2 and 3). The aim of this study was to evaluate the effect of Turmeric powder (*Curcuma longa*) on rabbit burger quality in comparison with a synthetic antioxidant as acid ascorbic.

The hind legs of hybrids rabbits, derived from the same rearing system, were used. The meat was minced in a grinder until a homogeneous raw batter and three different types of meat formulations (F) were made: meat with no additives (Control), meat with Turmeric powder (3.5 g/100 g meat) and meat with ascorbic acid (1 g /100 g meat). Burgers of 50 g each were formed in a Petri dish (six per formulation). To evaluate the effect of *Curcuma longa* and ascorbic acid on meat quality, the following analysis were made: pH was measured using a HANNA pH 2011 pHmeter ; meat colour was evaluated using a Minolta CR300 colorimeter (Illuminant D 65), which measures Lightness, redness, yellowness, Hue and Chroma values (4). Water holding capacity (5, 6) was determined as drip loss (percentage of weight loss of a burger kept at +4°C for 7 days) and as cooking loss (percentage of weight loss of a burger cooked in an oven at 163°C to an internal temperature of 71°C). Meat chemical composition was determined according to AOAC methods (7). Data underwent a variance analysis and the statistically significance of the differences was assessed with the Tukey test (8).

Table 1 showed the effects of Turmeric powder or ascorbic acid on rabbit burgers quality. The formulations significantly affected all parameters except for Lightness, Drip loss, ether extract and protein. The addition of Turmeric powder increased pH value, ash and decreased moisture. Natural color of Turmeric influenced significantly burger color parameters: the patties added with Turmeric powder presented the highest redness and yellowness indexes, consequently they showed a more intense yellow color, as revealed by Hue and Chroma indexes ($P < 0.001$). As regard water holding capacity, the drip loss didn't show any significant difference among formulations, even if the burger added with Turmeric and ascorbic acid tended to low values; cooking loss was significantly lower in Turmeric burgers ($P < 0.001$). This last result might be considered positively by the consumer.

In conclusion, ascorbic acid didn't affect significantly burger quality while Turmeric powder influenced some quality parameters: further studies will be carried out to verify antioxidant effect of Turmeric on meat product shelf life.

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Nutraceutica

Turmeric, Burger, Rabbit

Table 1. The effects of Turmeric powder and ascorbic acid on physical and chemical characteristics of rabbit burgers.

		Control	Turmeric	Ascorbic acid	<i>P value</i>	RMSE ¹
Burger	n	6	6	6		
pH		5.77 ^b	5.84 ^a	5.78 ^b	***	0.015
Lightness		55.51	54.16	54.30	ns	1.248
redness		8.94 ^b	13.18 ^a	9.15 ^b	***	0.608
yellowness		6.29 ^b	53.18 ^a	6.23 ^b	***	0.520
Hue		35.09 ^b	76.12 ^a	34.40 ^b	***	2.219
Chroma		10.94 ^b	54.79 ^a	11.08 ^b	***	0.619
Drip loss	%	10.40	7.20	7.60	ns	0.039
Cooking loss	%	25.60 ^a	18.40 ^b	26.00 ^a	***	0.018
Moisture	%	74.79 ^a	72.56 ^b	74.72 ^a	***	0.003
Ether extract	%	2.75	2.61	2.82	ns	0.006
Protein	%	20.79	21.43	20.81	ns	0.005
Ash	%	1.67 ^b	3.40 ^a	1.65 ^b	***	0.005

ns: not significant; ***: $P < 0.001$; different letters in the same row indicate significant differences; ¹: residual means standard error.

MUSCARINIC M2 RECEPTORS INTERACT WITH NEUROKININERGIC NK1 AND NK2 RECEPTORS IN THE CONTRACTIONS OF ISOLATED BRONCHI IN THE HORSE

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Acetylcholine and neurokinins, known as neurotransmitters that induce bronchoconstriction in horses and other species, are involved in the pathogenesis of obstructive respiratory diseases. However, the knowledge about the role of muscarinic receptors in the control of airway contraction in equines is not fully elucidated and that of neurokininergic receptors is limited.

Bronchial smooth muscle rings of slaughtered horses were obtained from healthy lungs, or from lungs with macroscopic signs of inflammation, put into organ baths and connected to isotonic transducers. Electrical field stimulation was applied (50 Hz, 1 ms, 300 mA, 50 V every 120 s), and phasic contractions of bronchial smooth muscle were evoked. Previous experiments showed the neurogenic cholinergic nature of these contractions and a central role of muscarinic M3 receptors [1]. The effects of drugs were expressed as variation of the pre-drug contraction amplitude, assumed as 100%.

Bronchial contractions were partially inhibited by selective M3 receptor antagonist (pFHHSiD) up to 10⁻⁶ M, and totally abolished at 10⁻⁵ M in normal and inflamed tissues. Methoctramine, selective M2 receptor antagonist, was able to reduce the contractions only in healthy bronchial rings, whereas selective M1 receptor antagonist, VU0255035, reduced the contractions only in pathologic ones.

L-732,138 and GR159897, selective NK1- and NK2-receptor antagonist respectively, slightly reduced the contractions in normal and pathologic tissues, whereas SB218795, selective NK3-receptor antagonist, was ineffective. In presence of either NK1, NK2, but not NK3 receptor block, M2 antagonist, methoctramine, was able to reduce the contractions of the pathologic bronchi, while the response in normal tissue was unchanged.

Muscarinic M1, M2, and M3 receptors are involved in the contractions of horse bronchi, M3 receptors playing a major role. Present results suggest an interference between cholinergic and neurokininergic systems involving M2, NK1 and NK2 receptor subtypes. The influence of the excitatory peptidergic system seems to be more evident in pathologic tissue, and indeed up-regulation of NK2 receptors in horses with RAO was detected [2]. A localization of inhibitory M2 receptors on neurokininergic neurons releasing substance P and/or neurokinin A could be hypothesized, as already observed in other species [3]. A better knowledge of the interactions between cholinergic and neurokininergic systems could help the development of more effective drugs for the treatment of bronchial hyperactivity in horses and, possibly, in humans.

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Veterinary Pharmacology
Horse, Bronchi, Neurokinins

PRESENCE OF D. IMMITIS IN DOGS OF RURAL COMMUNITIES LOCATED IN SOUTH EASTERN BOLIVIA

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Dirofilariasis is a worldwide-distributed disease caused by nematodes of the genus *Dirofilaria* superfamily Filarioidea. These nematodes may infest wild and domestic mammals. The principal agent of canine dirofilariasis in the Americas is *Dirofilaria immitis*, which may also occasionally infest humans. Human cases of dirofilariasis by *D. immitis* are relatively frequent in the Americas while only few cases have been recorded in Europe. In Bolivia dirofilariasis has been found in dogs and in wild canids (Bronson et al., 2008; Fiorello et al., 2004). We refer on the results of a preliminary screening carried out in 2013 on the presence of *D. immitis* in dogs of two rural communities (Ivampirapinta and Bartolo) located in south eastern Bolivia. Blood samples were collected from 50 dogs in the community of Bartolo (municipality of Monteagudo) and 50 in the community of Ivampirapinta (municipality of Gutierrez). Serum samples was subjected to an ELISA test for the detection of antigens of *D. immitis* (DiroCHEK®, Synbiotics).

All dogs in the community of Bartolo tested negative while 3 dogs in the community of Ivampirapinta resulted positive. This preliminary screening confirms the circulation of *D. immitis* in the community of Ivampirapinta.

Further studies will be carried out by direct diagnostic methods to further characterize the filarioid parasites infesting dogs and to evaluate the risk of human infections.

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Parasitology

Dirofilaria immitis, dogs, Bolivia

V.A.C.TM THERAPY IN AN ITALIAN FRIESIAN CALF

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Vacuum Assisted Closure (V.A.C.) is a non-invasive therapy based on the application of negative pressure by controlled suction to the wound surface¹. V.A.C. has been widely employed in human medicine for over a decade²; its use in animals is limited in pets and horses and, to our knowledge, it has not been described in cattle yet. The principles of this therapy are based on the delivery of subatmospheric pressure to the wound bed through an open pore sponge dressing, that is placed in the wound and covered with plastic adhesive drape. The application of negative pressure to the foam has been proved to be effective in improving the development of granulation tissue, decreasing of tissue edema, reducing bacterial colonization of tissues and decreasing rapidly the wound area².

The aim of the paper is to describe the first utilization of V.A.C. in a calf with a large infected wound on the left hindlimb.

A one-month-old female Italian Friesian calf was admitted to our clinic for a large wound on the left hindlimb in October 2011. History reported that the calf presented a grade 2 congenital arthrogryposis of both forelimbs. The prolonged recumbency caused by this pathology induced the compression of the skin of the left hindlimb resulting in skin necrosis and consequently in an infected large plague that extended from the dorsal surface of the knee area up to the tibio-tarsal area. Traditional methods would have likely failed with a such severe wound, therefore the use of V.A.C.^{1,2} was preferred. The patient underwent surgical debridement and application of the V.A.C. The skin area around the wound was cleaned, clipped and aseptically prepared. A reticulated 400-600 µm pore polyurethane foam dressing was applicated on the wound. It was then covered with an adhesive plastic drape, where a 1 cm hole was created in order to apply a trackpad connected with a computer-controlled therapy unit that allows continuous, controlled application of subatmospheric pressure of 125 mmHg. The area surrounding the wound was protected with a classic bandage.

The calf was subjected to a long antibiotic therapy till the almost complete healing of the wound. Every 3 days the wound was medicated.

The present report describes the first use of the V.A.C. in cattle. The clinical outcome was excellent although we recommend the V.A.C. therapy only in selected high value patients.

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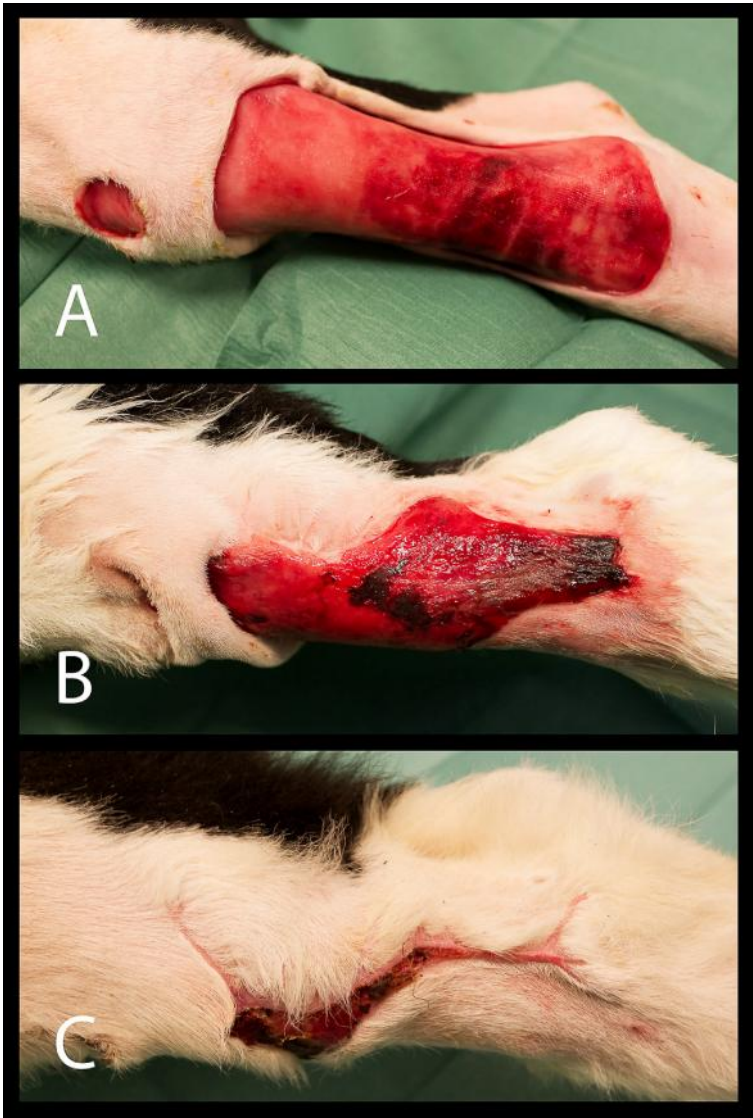
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ANNEX

Aspect of the wound one month (a), 3 months (b) and 5 months (c) after the beginning of the V.A.C. therapy

vet 08- clinica medica veterinaria

V.A.C. THERAPY, CALF, WOUND



MOLECULAR DISCRIMINATION OF CULICOIDES OBSOLETUS COMPLEX MEMBERS COLLECTED IN SICILY DURING 2012

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Culicoides genus (Diptera: Ceratopogonidae) contains important vectors of pathogens responsible for several diseases with public health significance, including bluetongue (BT), Schmallenberg virus and filarial diseases. Members of the C.obsoletus complex have an important role and their occurrence correlates well with previous BT outbreaks in Europe (a). C.obsoletus complex is composed by three species: C.obsoletus, C.scoticus and C.chiopterus. C.dewulfi, considered for a long time a this complex member, is nowadays treated as a separate taxonomic group. Culicoides taxonomy is based on morphological differences and requires a specialized knowledge of insect morphology, often overlapping among the species.

The study is aimed to provide a deep picture of C.obsoletus complex distribution in Sicily integrating data obtained by morphological and molecular analyses.

During 2012, 61501 Culicoides were collected in Sicily from January to December using Blacklight traps. Catches were carried out in 25 sheep, goat and cattle farms weekly monitored. Culicoides were identified morphologically (b) and 38123 (62%) belonged to the C.obsoletus complex. Out of them, 1956 midges (5%) were analysed by molecular tools. Total DNA was extracted from single midges using Chelex 100® (c), quantified by Nanodrop and used as template for a multiplex PCR targeting the Mitochondrial Cytochrome C Oxidase I Gene (d). PCR products were randomly selected for sequencing.

Culicoides seasonal distribution was analysed, showing a higher presence of the C.obsoletus complex specimens during the cold season. Multiplex PCR performed on C. obsoletus complex midges showed that 872 belonged to C.obsoletus sensu strictu and 1084 to C.scoticus. No one belonged to the species C.chiopterus and C.dewulfi. Samples sequencing confirmed PCR results. Seasonal distribution showed a higher abundance of C. scoticus with respect to the C.obsoletus sensu strictu from January to May, while in the warm season the opposite condition was observed.

Molecular techniques allowed to easily distinguish C.obsoletus complex species composition in Sicily. The four species have different behaviours and are differently involved in pathogen transmission, for example, C.dewulfi was incriminated as a vector of BTV serotype 8 in the outbreak in central Europe. In Sicily, probably other species of the C.obsoletus complex can be involved in virus maintenance, and further studies have to be performed to well understand the vector capacity and competence of Culicoides species for the different BT virus serotypes.

Research funded by Italian Ministry of Health (IZSSI 01/13).

Acknowledgements: Thanks to Rosa Filippi and Pippo Bono for their technical support.

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(b) Wirth and Hubert. Mem. Amer. Entomol. Inst 1989, 44: 1-508

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Entomology

Culicoides, C. obsoletus complex, Multiplex PCR

THE REGIONAL CENTER FOR MONITORING PARASITIC INFECTIONS (CREMOPAR): A SERVICE FOR LIVESTOCK IN SOUTHERN ITALY

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The Regional Center for Monitoring Parasitic Infections of livestock (Centro Regionale per il Monitoraggio delle Parassitosi - CReMoPAR) was born 15 years ago through an agreement between the Department of Veterinary Medicine and Animal Productions (University of Napoli Federico II) and the Agricultural Department of the Campania Region, southern Italy.

The aim of this paper is to show the importance to guarantee a diagnostic service on livestock for veterinarians and farmers.

Highly sensitive and accurate diagnostic techniques are used at CREMOPAR, namely FLOTAC, Mini-FLOTAC, Fill-FLOTAC (Cringoli et al., 2013), and field trials are conducted to study the strategic and economic efficacy of different control strategies of parasites in sheep, goats, cattle and buffaloes. Furthermore, spatial epidemiology is used to map and model the distribution of parasites in livestock through the use of modern and powerful resources provided by geographical information systems (GIS) and other geospatial tools. Dissemination activities are also conducted to increase the awareness of veterinarians and farmers regarding the impact of parasitic infections on livestock productivity.

The results of the diagnostic service at CREMOPAR showed that parasites are widespread in livestock in southern Italy and that the knowledge of the parasitological scenario, using parasitological maps produced by GIS, is fundamental to plan sustainable control strategies and to increase the awareness of stakeholders.

CReMoPAR is an example of service for livestock that allows academics, veterinarians, and field researchers, to “touch” the real problems of the farmers, detecting new areas of applied research and in turn to increase the farmer’s awareness on the importance of diagnosis and control of parasites in livestock. CReMoPAR is useful for the development of knowledge in the fields of parasitology, animal production and veterinary public health.

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Diagnostic methods

CREMOPAR, livestock, parasites

SARCOCYSTIS SPP. INFECTION IN DOMESTIC TURKEY.

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Sarcocystis spp. has a 2-hosts cycle where carnivores are definitive hosts, while herbivores and birds are intermediate hosts. This parasitic disease has been described in wild and domestic avian species, but little is known regarding ethiology, pathogenesis and epidemiology in birds. *S. falcatula* is highly pathogenic and has been reported in canaries, pigeons and budgerigar (Smith, 1990), *S. calchasi* is responsible for a lethal neurological disease in domestic and wild pigeons, and psittacine (Olias, 2011; Rimoldi, 2013) and also chickens may show neurological signs related to *Sarcocystis* spp. infection (Mutalib, 1995). Only 2 case reports describe *Sarcocystis* spp. infection in wild turkey (Teglas, 1998; Dubey, 2000). To our best knowledge this is the first report of *Sarcocystis* spp. infection in domestic turkey.

Thirty-six turkeys were regularly slaughtered, hearts were collected, and a macroscopic examination was performed. Tissue specimens were fixed in 10% buffered neutral formalin, paraffin embedded sections were stained with hematoxylin and eosin, and examined microscopically.

At gross examination no abnormalities were detected. Histologically in 4 out of 36 hearts *Sarcocystis* have been identified in myocardial fibers and in the heart conduction tissue. No inflammatory infiltration around parasites was detected.

Little is known about ethiology and pathogenesis of *Sarcocystosis* in birds, and no cases of infection in domestic turkey have been reported. In contrast with previous investigations (Teglas, 1998; Dubey, 2000), inflammatory response and necrosis surrounding the cysts have never been found. The presence of *Sarcocystis* spp. in poultry is a matter of concern considering the health implications, given that consumers may contract the parasite eating not properly cooked meat. It will be of interest to investigate which species of *Sarcocystis* is responsible for these infections, how turkeys may contract this parasite, and how to control infection spread.

Acknowledgements.

We thank the Centro di Referenza di Patologia Comparata 'Bruno Maria Zaini,' Italy.

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Anatomia patologica

SARCOCYSTIS, heart, DOMESTIC TURKEY

OXIDATIVE STRESS AND SURVIVAL PREDICTION INDEX (SPI2) IN CRITICALLY ILL DOGS

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In the last years oxidative status has been investigated in human and canine critically ill patients. Oxidative stress has been observed with both an increase of oxidant reactive species and an antioxidant capacity reduction^{1,2}.

The aim of this study was to compare the plasmatic redox capacity of critically ill patients at admission with that measured at the time of discharge, after hospitalization. In addition, we looked for a correlation between the values of oxidative status and indices of severity of the patients.

34 dogs, arrived at the Emergency Care Unit with yellow code, were included. Oxidative status of all the subjects was evaluated with d-ROMs (Reactive Oxygen Metabolites-derivatives) and BAP (Biological Antioxidant Potential) spectrophotometric tests (Diacron International, Grosseto, It) ³. The Survival Prediction Index (SPI2), a scoring system that weights readily available clinical data points to objectively determine the patient's probability of survival as a percentage, was determined for each patient. Data points included in the SPI2 are mean arterial pressure, respiratory rate, creatinine, PCV, albumin, age, and medical versus surgical status. The higher SPI2 score, the greater the likelihood is that the dog will survive⁴. T Student test for paired data, one-way ANOVA and Pearson multiple correlation test were applied.

The results show that all hospitalized subjects are affected by oxidative stress at admission (reference ranges are 56,4-91,4 U. CARR and 2069-2554 mmoli/L for d-ROMs and BAP respectively)³; oxidative values change significantly during hospitalization with an increase both in d-ROMs ($172,40 \pm 58,27$ vs $266,77 \pm 51,77$ U.CARR.) and BAP ($1778,45 \pm 210,39$ vs $2096 \pm 349,57$ mmoli/L). These observations agree with those reported both in human and dogs; the significant pro-oxidants increase may be due to the marker used, and to stress related to hospitalization^{1,2}. The differences between the input and output d-ROMs values ($42,48 \pm 34,45$ vs $111,82 \pm 61,14$ U. CARR.) are affected by antioxidants administration, N-acetylcysteine (NAC) in particular, during hospitalization; there are many studies that confirm the utility of NAC as antioxidant with multiple actions decreasing oxidant damage^{2,5}. An highly significant positive correlation between the BAP and SPI2 values at admission ($r=0,41$) has been observed, confirming the relationship between antioxidant capacity and clinical status^{1,2}.

The study confirms the opportunity of assessing the oxidative status of hospitalized dogs and setting the antioxidants administration, in order to improve their prognosis and outcome.

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internal medicine

dog, antioxidants, critically ill

HISTOGENESIS OF LAMBL'S EXCRESCENCES IN TUNA FISH (THUNNUS THYNNUS)

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Lambl's excrescences have been described in humans and in horses. Are very rare in dogs and pigs and have been found for the first time by Salogni et al. in tuna fish and by Mignone et al. in swordfish.

The histogenesis of the lesions is still under discussion: according to some authors, they derive from fibrin deposition on the upper surface of the aortic or mitral valves, which organizes into connective tissue covered by endothelium. According to others authors, a rupture of the valve causes evagination of the tissue and subsequent formation of excrescences.

Sixteen hearts of bluefin tuna fish (*Thunnus thynnus*) caught in the Ligurian Sea, ranging in age from 3 to 12 years, were macroscopically examined and fixed in 10% buffered formalin, and referred to the Department of Veterinary Science of the University of Turin for histological and histochemical investigations.

No gross lesions were observed. Histological findings were represented by Lambl's excrescences in four subjects at different developmental stages. In all cases excrescences were observed on both surfaces of the valve, at the opposite of what occurs in mammals and man, and were accompanied by endocardial or valvular inflammation or endocardiosis.

The first stage of the Lambl's excrescences was represented by thrombi on an inflamed valvular tissue, followed by the deposition of clusters of inflammatory cells, mostly lymphocytes, covered by a few endothelial cells. The lesions subsequently enlarged, with deposition of inflammatory and mesenchymal cells, to form a granulation tissue covered by endothelium. In the last stage the granulation tissue is transformed into fibrous connective tissue with formation of the Lambl's excrescences.

Occasionally, during the organization of the lesion, thin argentofilic elastic fibers were observed, accompanied by fibroblasts, and fibrocytes and connective fibers.

Finally on the endocardial surface, in promimity of the valves, regular, elongated, slightly curved, smooth-walled, always covered with endothelial projections, probably due to abnormal growths of Lambl excrescences, were observed.

These findings, to the authors' best knowledge, have never been observed in fish or mammals, and are difficult to detect, even if their structure is very similar to that of Lambl's excrescences, and therefore could be interpreted as an abnormal forms.

The study of the development of Lambl's excrescences is interesting for comparative pathology to better understand the pathyogenesis of these lesions in humans.

Acknowledgements. We thank the Centro di Referenza di Patologia Comparata 'Bruno Maria Zaini,' Italy.

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Anatomia patologica

LAMBL'S EXCRESCENCES, TUNA FISH, THUNNUS THYNNUS

HISTOLOGICAL AND BIOCHEMICAL VARIABILITY IN THE MUSCLE FIBERS OF CONGENITAL PSEUDOMYOTONIA AFFECTED CATTLE

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Cattle congenital Pseudomyotonia (PMT) is an inherited muscular disease characterized by an exercise-induced muscle contraction. The symptoms are caused by a prolonged high level of cytosolic free Ca²⁺ ions in muscle fibers. Genetic analysis has provided evidence of mutations in ATP2A1 gene coding for Sarco(endo)plasmic Reticulum Ca²⁺-ATPase isoform1 (SERCA1), a sarcoplasmic reticulum (SR) membrane protein involved in re-uptake of calcium from cytosol into SR lumen[1].

So far different mutations in ATP2A1 gene were described in Chianina, Romagnola, Belgian Blue cattle breeds and a Dutch crossbred calf. The clinical symptoms and genetic correlations make bovine PMT the true counterpart of human Brody's disease[2,3,4,5].

SERCA1 has been largely investigated in structure, implication of the different domains, functional mechanisms and its alterations[6,7,8,9].

The aim of this study was to obtain a complementary picture to the clinical aspect of the disease.

We performed histological (H&E, COX, SDH), immunohistochemical (TOM20, SERCA1, SERCA2, PLB) and biochemical (Western, Immunoblot, Ca²⁺-ATPase activity assay) analysis on the pathological cases: 3 full-blood Romagnola calves and 1 Dutch crossbred calf.

We found a variability in severity of alterations, in protein expression and functionality. We also described a fiber type-specific hypertrophy and an increase in mitochondria presence.

Our preliminary conclusions are that the disease shows a subject-dependent alterations and severity grade. Moreover, some of the alterations could suggest compensatory mechanisms.

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Inherited muscle disease in cattle

PSEUDOMYOTONIA, MUTATION, CATTLE

ILLICIT TREATMENT IN BEEF CATTLE. CAN A STRESS OXIDATIVE MARKER GIVE A PERSPECTIVE?

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The identification of biomarkers for the detection of the illegal treatment of animals is necessary to the development of new tools and new screening strategies for the detection of residues, particularly in beef cattle.

The oxidant chemical species are a group of molecules able to remove one or more reducing equivalents (hydrogen atoms and/or electrons); in all living organisms there is a delicate balance between production and elimination.

Inside the blood, the defense against noxious stimuli of reactive species, free radicals, is guaranteed by antioxidant barrier power (PAO), moreover the reactive oxygen metabolites (ROMs) are considered valid biomarkers for oxidative stress in both humans and animals.

On the basis of these premises, the aim of this study was to assess whether it is possible to use a combination of oxidative stress indicators as biomarkers of chronic stress situations occurring in illicit treatments.

47 male veal calves aged between 15 and 35 days were divided in 2 groups (20 calves) that received experimental therapies simulating protocols of illicit treatments. During the sixth month of the study period the groups were subjected to different protocols of illicit treatments: Group A received 19-nortestosterone (50 mg), once a week for four weeks; Group B received a cocktail of 19-nortestosterone (50mg) and 17 beta-estradiol valerate (5 mg) once a week for four weeks, Group K (7 calves) was kept as control. The antioxidant barrier power was valued in this study by measuring the ability to oppose the massive oxidant action of hypochlorous acid. The reactive oxygen metabolites of samples (hydroperoxides), in presence of iron were able to generate alkoxyl and peroxy radicals, according to the Fenton's reaction, and photometrically quantified. The procedures were carried out according to indications of OXY Adsorbent & d-ROMs Test, Diacron srl and the tests were done in triplicate.

In this study PAO and ROMs were analyzed. The results of the preliminary study showed that there is a statistically significant difference between both treatment groups compared to the control group. However, the results showed no significant differences between the two treatment groups.

The decrease of the PAO values show a "reduced thickness" of the antioxidant haematic barrier, signal of oxidative stress, and it is directly correlated with the degree impairment of them. Otherwise, the increased levels of ROMs values, indicates the progressively increasing of oxidative stress.

The results, though still preliminaries, showed that the ROMs and PAO could be used as biomarker-based approach to highlight illegal treatments with molecules with anabolic action; however, we were not able to differentiate the various treatments. Finally, the oxidative stress biomarkers can play a key role in the animal welfare assessment through the detection of stress level to which the animals are subjected.

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animal welfare, Illicit treatments, food safety

Illicit treatments, animal welfare, food safety

EQUESTRIAN REHABILITATION - INDICATORS OF WELFARE IN THE HORSE

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The present study is part of a series of studies involving the assessment of indicators of welfare in the horse. These physiological indicators are, in particular, the heart rate and the respiratory rate.

The equestrian rehabilitation is a therapeutic practice that takes care of people with psycho-physical problems aimed at improving the quality of their lives through a strong interaction with the horse.

The equestrian rehabilitation consists of three different methods :

- Hippotherapy;
- Equestrian Rehabilitation;
- Riding and pre- sports [1]

Four different horses of different age and sex have been used and led by eleven children suffering from various diseases. The sessions had weekly basis and those examined were a total of ten.

The heart rate and the respiratory rate have been monitored at specific times of the day:

- at rest;
- during the sessions, 25 minutes after the start of therapy.

The data were expressed as average. The student t-test was applied to compare the value of heart rate and brief rate at rest and during the session. The values are considered significant for $P < 0.05$.

When all the surveys came to an end, it was calculated the arithmetic mean of P0 (heart rate at rest), of P1 (lower heart rate of the day), and P2 (last measured heart rate) of each horse.

A similar situation has been made to R0 (respiratory rate at rest) R1 (lower respiratory rate of the day) and R2 (last respiratory rate).

All the average results of P0, P1 and P2 as well as all the average results of R0, R1 and R2 of the four horses examined were added together. It can be said that:

- a total reduction of P1 out of P0 is 16%;
- a total reduction of P2 out of P0 is 14%;
- a total reduction of R1 out of R0 is 22.3%;
- a total reduction of R2 out of R0 is 19.6 %.

Comparing the heart rate at rest and during the exercise, it was demonstrated that $P < 0,05$, so we can assert that the results regarding the heart rate are higher significant . No significant the brief rate.

From the analysis of the average cardiac and respiratory frequencies, there is a reduction of these indicators. As a result, we think that in the future this “sweet” therapy can be a valid rehabilitative support for certain types of horses. In the Equestrian Rehabilitation both the component, the horse and the disabled person, can gain benefit from this extraordinary horse-rider combination, establishing a dialogue through their bodies communicating without words. It is a path of change and growth that involves both. We think that we can speak of reversed pet therapy. [2]

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rehabilitation, hyppotherapy, welfare

CAVALLI	P ₀	P ₁	P ₂	P ₀ /P ₁	P ₀ /P ₂	R ₀	R ₁	R ₂	R ₀ /R ₁	R ₀ /R ₂
Zietto	33,6 b.p.m.	28,6 b.p.m.	30,6 b.p.m.	-14,9%	- 8,9%	15,2 breaths /min	9,8 breaths /min	11 breaths /min	- 35,5 %	- 27,6%
Muffin	36,3 b.p.m.	29,7 b.p.m.	30,3 b.p.m.	- 18,2%	- 16,5%	13,2 breaths /min	9 breaths /min	9,7 breaths /min	- 31,8%	- 26,5%
Nency	35 b.p.m.	28 b.p.m.	28 b.p.m.	- 20%	-20%	13breath s /min	10 breaths /min	10 breaths /min	- 23,1%	-23,1%
Janessa	32,7 b.p.m.	29,3b. p.m.	29,3 b.p.m.	-10,4%	-10,4%	17,7 breaths /min	17 breaths /min	17 breaths /min	- 3,9%	- 3,9%

A COOPERATION PROGRAMME IN HIGHER EDUCATION TO IMPROVE LIVESTOCK PRODUCTIONS, FOOD SECURITY AND ENVIRONMENTAL SUSTAINABILITY IN THE SAHEL

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In order to increase agriculture, driver for economic growth in Sub-Saharan Africa, a trained cadre who is technologically competent and able to manage natural resources and improve food security with a multidisciplinary and sustainable approach should be produced. To reach this aim, the CISAO of the University of Turin, established a network with three Sahelian Institutions of Higher Education (HEIs) of Niger, Burkina Faso and Tchad, to lead the project "Réseau des Universités Sahéliennes pour la Sécurité Alimentaire et la Durabilité Environnementale (RUSSADE)" financed by the ACP-EU Cooperation Programme in Higher Education (EDULINK II).

The project foresees the organization of Master courses to give technical, scientific and methodological bases to the students, allowing them to manage natural resources and to improve agriculture, livestock and food security with a multidisciplinary and sustainable approach, considering both the socio-economic perspectives and the environmental risks.

Innovative teaching strategies will be prepared and proposed in order to connect different themes. Thanks to the innovative procedures of this kind of training, the project recommends measures designed to strengthen the capacity and effectiveness of higher education, to promote basic and applied scientific research and to improve educational quality through updated teaching methodologies.

The first step of the project allows the strengthening of links between HEIs, encouraging the exchange of academic staff and creating a more favourable environment for debate and innovative research to promote a greater awareness of the complex connections between human choices and natural processes. During the Master course, students will not receive technical training but the required expertise to be aware of local issues in agriculture, farming and environmental protection and to suggest and communicate appropriate and sustainable solutions, allowing them to work in key sectors of the region.

An action of this kind may cause a change in mentality: for example, awareness of the links between development and modernization of the rural sector, environmental degradation and poverty should lead to changes in individual behaviour for the maintenance and respect of natural resources.

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Cooperation in higher education and food security

Academic cooperation, Animal production, Sustainability

EFFICACY OF ATTENUATED SALMONELLA TYPHIMURIUM DELTAZNUABC VACCINE AGAINST SALMONELLA CHOLERAESUIS INFECTION IN PIGLETS. A COMPARISON WITH SALMONELLA CHOLERAESUIS KILLED VACCINE.

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Recently, we produced a mutant strain of Salmonella Typhimurium, deleted of ZnuABC genes (S.Typhimurium ΔznuABC) and we proved its safety and efficacy either in mouse or pig model infection with virulent S.Typhimurium.

In this study we assess the efficacy of attenuated S.Typhimurium ΔznuABC vaccine and killed S. choleraesuis vaccine during S. choleraesuis infection.

Clinical symptoms of S.choleraesuis infection in pigs are septicemia, pneumonia, enterocolitis, hepatitis, meningo-encephalitis and abortion. Animals are routinely treated with antibiotics to prevent infection. This practice can lead to an increase of multi-drug-resistant strains. Bio-safety programs and good management practices, are applied for infection control. However, vaccination could represent a complementary solution to reduce prevalence.

18 weaned piglets, born in Salmonella–SPF farm were divided in 3 groups. 6 piglets were vaccinated by a gavage with 5x10⁷ CFU of S.Typhimurium ΔznuABC (group A), 6 piglets were intramuscularly vaccinated by killed S. choleraesuis vaccine (group B) and 6 piglets were naïve (group C). Groups were challenged with 5x10⁸ CFU of virulent S. choleraesuis day 25 post vaccination.

Animals were weighted during vaccination and killing. After challenge, temperature was recorded day 3, 4, 5, 7, 11 and fecal samples were collected day 3, 7, 11. Tonsils, lymph nodes, intestinal content of caecum and colon were collected for microbiological analysis during necropsy.

The results reported show that group A presents a temperature 1°C lower than groups B and C until 7 days after infection. Afterwards mean temperature of each group is range among 39.5°C and 40 °C. Any difference in weight gain is recorded among vaccinated and naïve groups.

However, microbiological results are significant. S. choleraesuis is not detectable in faeces of piglets vaccinated with attenuated strain of S. Typhimurium (group A) day 11 post infection. Furthermore, S. choleraesuis colonization is limited in ileocaecal lymph nodes, caecum and colon of group A piglets.

In conclusion, these findings extend the validity of attenuated S. Typhimurium ΔznuABC strain as a useful mucosal vaccine either in S. Typhimurium or S. choleraesuis pigs infection. Furthermore, these data corroborate the greater competency of attenuated vaccine than killed one to reduce intestinal colonization. Schwarz P1, Kich JD, Kolb J, Cardoso M. Use of an avirulent live Salmonella Choleraesuis vaccine to reduce the prevalence of Salmonella carrier pigs at slaughter. Vet Rec. 2011 Nov 19;169(21):553.

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VACCINATION, SALMONELLA INFECTION IN PIGLETS
VACCINATION, SALMONELLA, PIGLETS

REFERENCE VALUES OF BLOOD PARAMETERS IN CLINICALLY HEALTHY CROSSBREED DONKEYS OF DIFFERENT AGES

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The number of donkeys herds has increased in recent years in Piedmont, (North-Western Italy) due to the growing demand of milk, mainly as a substitute food for feeding of pediatric patients intolerant to cow's milk proteins.

The assessment of the health status of donkeys bred for milk production is difficult, both due to the lack of experience on this type of breeding, and to the lack of studies conducted to establish the normal reference values of serum biochemical profiles in females donkeys. Therefore, the present study, was designed to evaluate the reference values of specific haematological and serum biochemical parameters of the healthy lactating jenny in its own environmental adaptation.

Blood samples were obtained from the jugular vein from a total of 57 healthy crossbreed females donkeys. Samples were analyzed with a cell counter (Melet-Schloesing®-MS4) the same day of the collection, to determine hematological parameters. The serum biochemical parameters of clinical chemistry were analyzed using Screen Master Touch automated instrument.

For statistical analysis (ANOVA), the animals were divided into the following 3 groups based on age: $\leq$ 3-year-old (n=19), from 3 to 10-year-old (n=23), $\geq$ 10 year-old (n=14) groups. Mean and standard deviation [SD] were calculated for each hematological and biochemical parameter in all tested groups.

All animals involved in this study were clinically healthy and the parameters reported represent physiological values. The result indicated that $\leq$ 3-year-old animals have a lower serum ALT/GPT concentration ($7,62 \pm 2,03$ U/L) than those in 3 to 10-year-old ($13,88 \pm 7,35$ U/L) ($P < 0,001$) and $\geq$ 10-year-old groups ($12,32 \pm 6,27$ U/L) ($P < 0,05$). The serum creatinine concentration of $\leq$ 3-year-old group ($1,23 \pm 0,19$ mg/dL) was higher than that of $\geq$ 10-year-old group ($1,11 \pm 0,14$ mg/dL) ($P < 0,05$). A significantly lower serum WBC concentration was found for $\geq$ 3-year-old animals ($14,71 \pm 3,77$ m/mm³) than for $\geq$ 10-year-old animals ($11,46 \pm 2,00$ m/mm³) ($P < 0,05$). The result indicated that $\leq$ 3-year-old animals have a higher serum RBC concentration ($6,26 \pm 0,97$ M/mm³) than those in 3 to 10-year-old ($5,66 \pm 0,92$ M/mm³) ($P < 0,05$) and $\geq$ 10-year-old groups ($5,53 \pm 0,81$ M/mm³) ($P < 0,05$).

No significant differences were observed in the concentrations of total protein, AST/GOT, urea, BUN, Hct and MCHC.

These preliminary data could be useful in clinical practice to assess hematobiochemical profile of healthy donkeys. The reported values obtained on a large number of donkeys, could be useful as clinical guides and a basis for further research.

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Animal Welfare and Laboratory Analysis
Animal Welfare, Laboratory analysis, Donkey

KETAMINE-DEXMEDETOMIDINE-METHADONE OR TILETAMINE-ZOLAZEPAM-METHADONE FOR IMMOBILIZATION OF DOMESTIC PIGS

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The aim of this study is to compare the cardio-respiratory effects and the quality of induction (Qi), immobilization (QI) and recovery (QR) in pigs anesthetized IM with ketamine, dexmedetomidine, methadone or tiletamine-zolazepam, methadone for cutaneous and mucosal biopsies.

Anesthesia was induced with tiletamine-zolazepam 8 mg/kg and methadone 0.2 mg/kg in 9 pigs (TzM group) and with ketamine 8 mg/kg, dexmedetomidine 20 µg/kg and methadone 0.2 mg/kg in 18 pigs (KDM and KDMA groups). Anesthesia was partially reversed with atipamezole (0.2 mg/kg, IM) in 9 pigs (KDMA group). Times from drugs injection (DI) to the first signs of sedation and to sternal (ST), lateral recumbency (LR) and animal approach (AA) were recorded. The Qi was assessed by a descriptive score ranging from 1(excellent) to 4 (poor). QI was assessed using a descriptive score ranging from 1(light sedation) to 6 (excessive sedation). Physiological parameters and the QI were recorded at the time of the AA and at 10, 20, and 30'. A blood gas analysis was made at T20 and the P(A-a)O₂, the PaO₂/FiO₂ and the Fshunt were calculated.

Recovery from anesthesia was recorded as: times between the DI and the first head movements, ST, LR and standing. QR was assessed using a descriptive score ranging from 1 (excellent) to 4 (poor). For all data the mean, SD, and range (parametric data) or median and range (non-parametric data) were calculated. Parametric physiological data were compared among study times (T0, T10, T20, T30) using one-way ANOVA for repeated measures.

The first signs of sedation, ST and LR were observed earlier in TzM as compared to the other groups. The median induction score was similar in all groups.

The mean time required to the AA was shorter in the TzM group as compared to the other groups. The mean values of HR (heart rate), RR (respiratory rate), NIBP (non invasive blood pressure) were similar in all groups. The mean values of T° were lower in the TzM group as compared to KDM and KDMA. SpO₂ was lower in the TzM group as compared to KDM and KDMA at T10,T20,T30. The PaO₂, SaO₂ and PaO₂/FiO₂ were lower and the P(A-a)O₂ and Fshunt were higher in the TzM as compared to KDM and KDMA. The times from DI to first head movements, ST and deambulation were shorter in the KDM group as compared to the TzM group. The median recovery score was higher in the TzM group.

These combinations provide an adequate anesthesia for minor procedures lasting about 30', but the TzM combination causes a worse oxygenation.

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Anesthesia

Anesthesia, Ketamine, Pigs

SEARCH FOR PARASITES IN THE FISHES IN THE MASSACIUCCOLI BASIN (TUSCANY, CENTRAL ITALY)

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The water district Massaciuccoli is an eutrophic coastal basin located in the provinces of Lucca and Pisa (Tuscany) and includes the lake, the marsh and the reclaimed areas with their network of drainage channels. The fish fauna has shown a progressive increase of introduced species to the disadvantage of the species evolutionarily originated in the basin (Chelucci, 2005).

The aim of this study, which was granted as a PRIN (2008), was to detect the parasites of the fishes in the water district Massaciuccoli, an area never investigated so far, and the eventual presence of *Opisthorchis felinus* (Rivolta, 1884).

In the years 2010-2012, 381 fishes were captured in the district of Massaciuccoli, 147 of which were carps, 103 goldfishes, 32 rudds, 31 black catfishes, 20 triottos, 15 pumpkinseeds, 13 stone morokos, 9 black basses, 7 grey mullets and 4 Italian bleaks. Skin, eyes, gills, digestive system, liver, bladder and kidneys of each fish were examined with optical and stereo microscope for the presence of ecto and endoparasites, which were classified according to keys (Moravec, 1994). The research of *O. felinus* was carried out by submitting epiassial and hypoassial muscles of the fish to a compression technique and a digestion technique with pepsin and hydrochloric acid.

192 fishes (prevalence 50.4%, 95% Confidence Interval: 45.4-55.4%) were infected. 91 (23.9%) among them presented multiple infections. 132 fishes (34.6%) presented only ectoparasites on skin and gills, 20 (5.2%) only endoparasites in the intestine, liver or kidneys and 40 fishes (10.4%) presented mixed infections. The most frequent parasites were *Trichodina* spp (prevalence 18.6%), *Trichodinella* spp (15.0%) and *Dactylogirus vastator* (18.9%). *O. felinus* was not found in any examined fish.

The fishes of the basin, especially carps, goldfishes and catfishes, are often used for aquaculture and for sportive fishing and therefore their parasites may be introduced into other water districts. Some times these fishes are eaten in spite of prohibitions, so that more attention to the parasitological fauna of the fishes in Massaciuccoli could be important.

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parasitology

Massaciuccoli basin, fishes, parasites

TOTAL ANTIOXIDANT CAPACITY OF FOLLICULAR FLUID INFLUENCES OOCYTES MATURATION RATES IN PIG**Addis D.*^[1], Pasciu V.^[1], Succu S.^[1], Leoni G.G.^[1], Berlinguer F.^[1], Naitana S.^[1]**^[1]~ *Sassari*

Porcine Follicular Fluid (PFF) is a key component in in vitro maturation (IVM) of pig oocytes, being added to the extent of 10% (v/v) at culture medium NCSU-37. The aim of this study was to investigate whether there are qualitative differences in follicular fluids collected from follicles at different times of the year, and whether these differences affect the quality of the maturation medium and hence oocyte maturation rates. Oocytes maturation medium was composed of NCSU-37, supplemented with insulin (5µg/ml), beta-mercaptoethanol (50µM) and cysteine (0.57 mM), and 10% PFF (v/v). We evaluated PFF collected in June, July, September, November and December. PFF was aspirated from follicles 3-6 mm in diameter from the ovaries of prepubertal gilts, using a 18 gauge needle placed on a 10 mL syringe. After withdrawal, the PFF was centrifuged at 4000 rpm for 30 minutes, at 4 ° C. Samples were processed rapidly and kept frozen at – 80 °C until assayed. TEAC of PFF was determined using the TEAC assay as described by Re et al. (1999). Briefly, the TEAC assay measures the relative ability of circulating antioxidants to scavenge 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cations (ABTS⁺) in comparison with the antioxidant capacity of Trolox standards. The absorbance was measured at 734 nm and the antioxidant activity was expressed as Trolox equivalent.

Obtained results showed that TEAC reached its highest values in November and December's PFF, while the lowest values were found in July (P<0.0001). The highest oocyte maturation rates, i.e. percentage of MII stage oocytes after 48 hrs of incubation, were reached when November's PFF was added to the medium, while the lowest rates were obtained when July's PFF was used (p<0.01).

Considering the two different periods of the year, our results suggest that the quality of the follicular fluid and the ability to increase the IVM may be linked to its oxidative state. Further studies are needed to characterize the concentration of the full spectrum of possible anti-oxidants in PFF. This finding can be applied to develop protocol to optimize IVM in pig.

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Riproduzione animale

IVM, Follicular fluide, Antioxidant

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Porcine Follicular Fluid (PFF) is a key component in in vitro maturation (IVM) of pig oocytes, being added to the extent of 10% (v/v) at culture medium NCSU-37. The aim of this study was to investigate whether there are qualitative differences in follicular fluids collected from follicles at different times of the year, and whether these differences affect the quality of the maturation medium and hence oocyte maturation rates. Oocytes maturation medium was composed of NCSU-37, supplemented with insulin (5µg/ml), beta-mercaptoethanol (50µM) and cysteine (0.57 mM), and 10% PFF (v/v). We evaluated PFF collected in June, July, September, November and December. PFF was aspirated from follicles 3-6 mm in diameter from the ovaries of prepubertal gilts, using a 18 gauge needle placed on a 10 mL syringe. After withdrawal, the PFF was centrifuged at 4000 rpm for 30 minutes, at 4 ° C. Samples were processed rapidly and kept frozen at - 80 °C until assayed. TEAC of PFF was determined using the TEAC assay as described by Re et al. (1999). Briefly, the TEAC assay measures the relative ability of circulating antioxidants to scavenge 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cations (ABTS⁺) in comparison with the antioxidant capacity of Trolox standards. The absorbance was measured at 734 nm and the antioxidant activity was expressed as Trolox equivalent. Obtained results showed that TEAC reached its highest values in November and December's PFF, while the lowest values were found in July ($P < 0.0001$). The highest oocyte maturation rates, i.e. percentage of MII stage oocytes after 48 hrs of incubation, were reached when November's PFF was added to the medium, while the lowest rates were obtained when July's PFF was used ($p < 0.01$). Considering the two different periods of the year, our results suggest that the quality of the follicular fluid and the ability to increase the IVM may be linked to its oxidative state. Further studies are needed to characterize the concentration of the full spectrum of possible anti-oxidants in PFF. This finding can be applied to develop protocol to optimize IVM in pig.

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FIRST REPORT OF *EUCOLEUS BÖHMI* AND *PEARSONEMA PLICA* INFECTIONS IN WOLVES (*CANIS LUPUS*) FROM ITALY

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Worldwide, studies on *E. böhmi* and *P. plica* infections among wolves are scanty. The aim of the present study was to evaluate *E. böhmi* and *P. plica* infections and associated lesions in wolves from Italy.

Between January and May 2014, 5 wolves (*Canis lupus*) from Central Italy (W1-4 from Tuscany, W5 from Abruzzo) were necropsied. From all animals the bladder was opened, examined for gross lesions and washed with 70% ethanol. The lavage fluid was microscopically observed under stereomicroscopy for presence and collection of trichuroid adult nematodes and eggs. From W3, W4 and W5 the urinary bladder was removed, formalin fixed, embedded in paraffin and processed for histopathological lesions. The skull from W5 was sawn and nasal cavities and sinuses were microscopically examined for trichuroid nematodes. By light microscopy, isolated adults and eggs were identified at the species level on the basis of their morpho-metrical features [1,2].

From the urinary bladder of W1 and W5, *P. plica* adults and eggs were identified. Gross lesions, characterised by 1-2 mm multiple foci of mucosal pigmentation, were observed in W5. At histological examination, nematodes were found threaded in the bladder mucosa. From W5, a large number of *E. böhmi* nematodes threaded through the mucosa of nasal turbinates were isolated.

P. plica (syn. *Capillaria plica*) infects the urinary tract of domestic and wild carnivores worldwide [3] and may be responsible for urinary lesions and signs in pets [4,5,6] and foxes [7,8,9]. Although previously reported in European wolves [10,11,12], pathological lesions were never evaluated before. *E. böhmi* (syn. *Capillaria böhmi*) is a neglected and poorly known nematode infecting nasal turbinates, frontal and paranasal sinuses of canids [3], recently reported as a cause of respiratory signs with varying degrees of severity in dogs [3]. In the wolf, this species has been previously reported only in a single study in Poland [13].

To our knowledge the present study is the first report of *E. böhmi* and *P. plica* infections in wolves from Italy.

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THE EU REGULATION NR. 1169/2011 ON THE FOOD LABELLING: DOES IT REALLY PROTECTION OF CONSUMER GUARANTEE THE CONSUMER, THE TRACEABILITY AND THE FOOD SAFETY?

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Parole chiavi: Sicurezza Alimentare, Legislazione Europea, etichettatura, tutela del consumatore

Key words: Safety food, EU Legislation, food labelling, protection of final consumer

SUMMARY.

As a result of the cases occurred in 2013 some EU Member States (Ireland, Great Britain and France) regarding the contamination of horse meat in the stew beef in processed foods is not declared on the label, the authors have analyzed the decisions contained in the recent EU Regulation no. 1169/2011 in order to examine whether we can actually talk about protection of the consumer in terms of correct and clear information of food products, in terms of proper education, communication in terms of knowledge of the food by the consumer and for the prevention and traceability of foods of animal origin 'not comply'. We analyzed the EU Reg. 1169/2011, we highlight the problems of proper labeling of food products of animal origin in relation to the role played by the veterinary as a operator of food safety

Metastrongyloid lungworms: epidemiological updates on cats of Sardinia (Italy)

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Aim of this study was investigate the presence of *A. abstrusus* and *Troglostrongylus* spp. in cats from Sardinia by combining copro-microscopical and biomolecular techniques.

An epidemiological survey was carried out between July 2011 and March 2014 in Sardinia (Italy), in order to achieve epidemiological data on parasites infesting broncho-pulmonary system of cats. One hundred and fifty-four (154) individual faecal samples were recovered and examined with qualitative Baermann technique. First stage larvae obtained with this method were subsequently identified using morphometric keys (Sloss et al., 1994, Veterinary clinical parasitology, 6th edn., 17-44; Gerichter, 1949, Parasitology, 39:251-262). An aliquot of the Baermann sediment of each positive sample was examined with biomolecular techniques amplifying *cox1* and 18S genes using previously described protocols.

Based on Baermann results, 29.9% (46/154) of examined cats were infected by broncho pulmonary nematodes. *A. abstrusus* was the most frequent specie 26% (40/154), whereas larvae of *Troglostrongylus* spp. were found in 5.2% (8/154) of positive samples. Frequencies of infestation were slightly higher in female cats (33.3%) than in males (25.7%) ($p > 0.05$). The mean age of positive animals was of 20.1 months (SD: ± 26.9), more specifically the mean age of positive for *A. abstrusus* was 22.1 months (SD: ± 28.2), and for those positives for *T. brevior* was 7.5 months (SD: ± 7.6); the difference is not statistically significant (t -test=2.81; $p=0.008$), probably due to the small number of positive samples to *T. brevior*. Biomolecular investigation confirmed the morphological diagnosis for all the *A. abstrusus* positive samples while *Troglostrongylus* spp. positive samples were molecularly identified as *Troglostrongylus brevior*.

The overall prevalence of metastrongyloids infection here detected (29.9%) indicates that metastrongyloid lungworms are frequent parasitic agents in the studied cat population with a prevalence of *A. abstrusus* (26%) higher than those reported in other European Countries (Barutzki & Schaper, 2012, Parasitol. Res., 112:855-861).

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Study of the therapeutic efficacy of Oxyclozanide + Levamisole preparation against Paramphistomidae in sheep

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The goal of the present survey was to evaluate the efficacy of a Oxyclozanide + Levamisole preparation (Toloxan[®], Fatro spa) against Paramphistomidae in sheep, administered in two different dosages. The trial was carried out in November 2012 in a Comisana breed sheep farm naturally infected with Paramphistomidae in the province of L'Aquila (central Italy). Faecal samples were collected from 200 sheep (> 3 years of age) at D-7, that were then examined with FLOTAC[®], a quali-quantitative technique for the evaluation of eggs per gram (EPG) of Paramphistomidae. Considering the parasitary burden, 120 sheep were enrolled in the trial, that were then divided into three groups of forty animals each, uniform for parasitary charge. The first group was treated with 0.5 ml/kg b.w. of Toloxan in a single dose (15 mg/kg b.w. of Oxyclozanide + 7.5 mg/kg b.w. of Levamisole), the second group was treated with 0.75 ml/kg b.w. of Toloxan in a single dose (22.5 mg/kg b.w. of Oxyclozanide + 11.25 mg/kg b.w. of Levamisole), while the third group was not treated (control). The anthelmintic efficacy was evaluated according to Coles et al. (1992), sampling animals at D0 (Treatment), D14 and D28. The Mann Whitney test did not show any statistical difference between EPG means of the three groups at D0 and also between the two groups treated (1 and 2) at D14 and D28. Results have highlighted a better efficacy of the drug at the posology of 0.75 ml/kg b.w. (99.3% at D7) compared with that recommended by the Manufacturer (0.5 ml/kg b.w.), that have never reach 90% of efficacy during all the trial (Maximum efficacy: 89.7% at D7). This survey confirmed what reported in the past by Georgiev and Gruev (1977), that found an efficacy between 85.1% - 89.1% of the same drug with a dosage of 20 mg/kg b.w. against sheep Paramphistomidae. In our experience, the low efficacy found at the recommended dosage (clearly under-dosed) could be a predisposing factor for the future development of anthelmintic resistance.

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Comunicazioni Orali Aipvet

MDM2 EXPRESSION IN CANINE LIPOSARCOMA

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Canine liposarcoma (LP) is an uncommon soft tissue sarcoma (STS) that arises more frequently in the subcutis. Three variants of LP have been described: well differentiated (WDLP), myxoid (MLP) and pleomorphic (PLP). In man, LP is the most common STS and is classified in WDLP/atypical lipomatous tumor (ATL), dedifferentiated LP (DDLp), MLP and PLP. WDLP/ALT and DDLp are considered different morphological presentations of the same biological entity bearing the amplification of the genes encoding for mdm2 and CDK4, and overexpressing these proteins. The aim of this study is to assess by immunohistochemistry (IHC) the expression of mdm2 in the different subtypes of canine liposarcoma.

Cases were selected from the pathology archives of the Universities of Bologna, Milano and Perugia. Hematoxylin and Eosin stained sections were evaluated and cases were classified according with the WHO classification of tumors of domestic animals when possible. IHC was performed with anti-human mdm2 mouse monoclonal antibody (clone 2A10). Cross reactivity of the antibody was assessed by western blot analysis. According with the guidelines applied in human medicine, cases were scored as positive if at least 10% of neoplastic cells had nuclear staining.

A total of 47 canine liposarcoma were collected: 19 were WDLP, 18 were PLP, and 7 were MLP. Three cases did not fit in any of the categories and had histological features consistent with DDLp. A total of 15/47 cases were mdm2 positive. WDLPs expressed mdm2 in 12/19 cases (63.2%), MLPs in 1/7 cases (12%), PLPs in 0/18 cases (0%) and DDLps in 2/3 cases (75%). Taken together WDLPs and DDLps expressed mdm2 in 14/22 cases (63.6%).

These preliminary results suggest that WDLP/DDLP can represent a biological entity characterized by mdm2 overexpression and distinct from MLP and PLP, paralleling what is reported in human medicine. Nevertheless, several WDLP were mdm2 negative. This result may be related to a low sensitivity of IHC. Since a greater sensitivity and specificity of fluorescent in situ hybridization (FISH) has been reported compared to IHC in evaluating mdm2 expression in human LP, further studies may clarify the level of mdm2 gene amplification in canine LP.

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oncology

canine, liposarcoma, mdm2

CYTOLOGIC GRADING OF CANINE AND FELINE SPINDLE-CELL SARCOMAS OF SOFT TISSUES AND ITS CORRELATION WITH HISTOLOGIC GRADING

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The grading of soft tissue spindle cell sarcomas (STSCS) is considered more useful than histologic histotype to assign a specific therapy and determine the prognosis in humans (Enzinger et al., 2001). Histologic grading is considered an important prognostic factor in canine STSCS as it may predict the onset of local recurrence and metastatic potential. In human pathology and in the veterinary practice, fine needle aspiration cytology (FNAC) of soft tissue mass lesions can be an accurate and minimally invasive method for the initial pathologic diagnosis of soft tissue masses. The aim of our study was to assess the utility and the accuracy of the cytologic grading on FNAC smears, in comparison to the histologic grading in STSCS

Over a period of 2 years (2009-2011) 33 cases of cytologically diagnosed STSCS (20 canine and 13 feline) with a following histological diagnosis were retrospectively separately reviewed. The FNAC smears were graded without prior knowledge of the histologic grade, using the scheme proposed by Weir et al., (1999), which is a three-tier system based on nuclear atypia, nuclear overlap, mitosis, and necrosis. The corresponding histological sections were graded using the Coindre et al., (1988) criteria in a blind fashion. The degree of concordance was established using the Cohen's K coefficient. (Palmer et al., 2001).

In the canine STSCS there was an overall cytologic and histologic concordance for grading in 12/20 cases (60%). The concordance was observed in 4/8 (50%) cases of histologic grade 1, in 8/12 (67%) cases of grade 2, and in 0 cases of grade 3. In the feline species the concordance was 11/13 cases (85%). The concordance was observed in 5/6 (83%) tumors of histologic grade 1, in 4/4 (100%) tumors of grade 2, and in 2/3 (66.6%) cases of grade 3. The overall concordance in the entire study population of canine and feline STSCS was 70%. The gradewise concordance was 65% in grade 1 cases, 75% in grade 2 cases, and 66% in grade 3 cases.

The overall concordance is quite similar to that reported in human literature. Although a wider population is required to strengthen our findings, these results suggest that cytologic grading of STSCS obtained by FNA may be a useful tool for therapeutic approaches and as a prognostic indicator.

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diagnostic cytopathology, small animal oncology
cytologic grading, soft tissue sarcoma, dog and cat

EFFECTS OF 17-AAG TREATMENT IN CANINE OSTEOSARCOMA CELL LINES: APOPTOSIS, AUTOPHAGY AND MITOPHAGY EVALUATION.

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Canine osteosarcoma is highly resistant to current chemotherapy so we wanted to research how tumour cells resist therapy. We tested a geldanamycin derivative prototype of an Hsp90 inhibitor, 17-AAG, on canine osteosarcoma cell lines D22 and D17, derived from primary tumour and secondary tumours respectively, with the aim of understanding the interplay between apoptosis, autophagy and mitophagy, given the dual effect of this process in regulating cancer cell viability.

Canine osteosarcoma cell lines D22 and D17 were treated with different 17-AAG concentration, 0.5µM and 1.0 µM, for 24 and 48 hours.

For ultrastructural analysis of cell lines, cell pellets were fixed and embedded in epoxy resin; ultrathin sections were obtained and double stained with uranyl acetate followed with lead citrate to be examined by means of an EM 900-ZEISS electron microscope. For apoptosis quantification cells were stained with AnnexinV-FITC Kit and analyzed by flow cytometry. For autophagy quantification cell culture were fixed and probed with rabbit polyclonal anti-LC3 for autophagosome count. Mitophagy were evaluated with colocalization of LC3 dots and mitochondria. Images were acquired with confocal microscope.

Results were normalized to DMSO-treated cells and data analyzed using the odds ratio. In the D22 cell line, growing dose and time of 17-AAG treatment caused apoptosis increase; in D17 cell line tendency was the same except for apoptosis decrease after 48h of treatment at maximum dosage 1.0µM. In between cell lines, in terms of autophagy and mitophagy, number of cytoplasmic dots per cell increased in presence of drug, especially after 48h of treatment and difference between cell lines were significant.

Further investigation is necessary to understand if induction of autophagy and mitophagy at high dosage treatment have a protective role in D17 cell survival. Our results highlight important differences between cell tumour type in resistance to Hsp90 inhibitors, underlining the potential of inducing apoptosis and simultaneously enhancing or inhibiting autophagy thus improving the efficacy of Hsp90 inhibitors in treatment.

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disciplinare

osteosarcoma, 17-AAG, HSP90

THE EXPRESSION OF MATRIX METALLOPROTEINASE 2 (MMP-2) AND 9 (MMP-9) AND THEIR POSSIBLE PROGNOSTIC ROLE IN FELINE INJECTION-SITE SARCOMAS (FISS)

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Aggressive cancer is often characterized by high expression of gelatinases, namely matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9). These enzymes play a pivotal role in tumor invasion and metastasis by degradation of extracellular matrix (ECM) leading to cancer spread both to contiguous tissues and distant organs. Feline injection-site sarcoma (FISS) is an aggressive subcutaneous tumor that develops a variable period after vaccination. It is believed to arise as a result of fibroblasts and myofibroblasts proliferation at sites of chronic inflammation induced by the vaccine's adjuvants, its antigens, or both. Histologically, ISS are characterized by inflammatory peritumoral infiltration, proliferation of atypical spindle cells and multinucleated giant cells. Aim of this study was to investigate the expression of MMP-2 and MMP-9 in FISS and assess their possible role as prognostic markers to predict tumor recurrence or metastasis after excisional surgery.

24 cases of FISS were selected from the archive of the Department of Veterinary Medicine of Perugia. Tumor dimension, completeness of excision, mitotic index, inflammation, presence of lymphoid follicles, necrosis and the presence of multinucleated giant cells were evaluated. Immunohistochemistry was performed for MMP-2 and MMP-9. Moreover, veterinarians were contacted to get additional information about the patients' follow-up after tumor excision.

MMP-2 showed a variable expression in FISS, predominantly in spindle mononucleated cells and its expression was higher in recurrent tumours, even if histological margins were not infiltrated. On the other hand, positivity for MMP-9 was seen more frequently in giant cells of the tumors and in a lower percentage of mononucleated spindle cells. As for MMP-2, the expression of this gelatinase was higher in the group of tumors that recurred. Recurrent FISS were also associated with a larger size at the moment of first excision, with a mean higher degree of necrosis and a higher mitotic index, whereas inflammation and lymphocytic follicles were not significantly related to the lesions' follow-up.

Taken together, the expression of MMP-2 and MMP-9 seems to be associated with a higher risk of tumor recurrence. The size of the tumor at the moment of excisional surgery seems to be another important factor, all along with mitotic index and tumor necrosis. The results from our study would suggest that gelatinases can be used as useful prognostic immunohistochemical markers in FISS.

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Oncologia

feline, sarcoma, metalloproteinases

IMMUNOHISTOCHEMICAL DETECTION OF CD25+ LYMPHOCYTES IN FELINE INJECTION-SITE SARCOMA

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CD25 (IL-2Ra) is the α -subunit of the membrane-bound interleukin-2 receptor (IL-2R), which is mainly expressed by regulatory T cells (Tregs). Tregs play a pivotal role in the homeostasis of the immune system and in the modulation of the immune response (1,2). Two type of Tregs are known: natural Treg and adaptive Treg. Normally these cells regulate immune system activity and prevent autoimmunity. Imbalanced function or number of these cells, either enhanced or decreased, might lead to tumor development and autoimmunity, respectively (1,2,3,4). In the present study we hypothesized the presence of CD25+ cells among tumor infiltrating lymphocytes (TILs) that are normally associated with feline injection-site sarcoma (FISS), a type of tumor considered a good model to study a link between inflammation and neoplasia. To this end the CD25 protein expression levels were analyzed by immunohistochemistry in eighteen cases of FISS.

4 μ m sections were immunostained by avidin-biotin technique for CD25 (Thermo Scientific, mouse monoclonal antibody). Staining was evaluated semi-quantitatively for percentage of positivity (10 fields at 40X) and the expression was considered as follows: percentage <20% = weak positivity; 21-50% = moderate positivity; >50% = strong positivity.

All the cases studied (18/18) had TILs positive for CD25. The immunolabeling appeared as distinct membrane staining or as diffuse cytoplasmic expression. 4 cases weakly expressed CD25 protein, 5 cases had moderate expression and 9 cases strongly expressed CD25. The medium expression percentage for all the 18 cases was of 55%.

The present study identified large proportions of CD25+ cells among tumor-infiltrating lymphocytes in FISS. The exact function and molecular mechanism by which CD25 is associated with carcinogenesis is still unclear. As observed in experimental animal model, CD25+ lymphocytes inhibit Th1 and CD8+ immune response and consequently they could represent a permissive and stimulatory factor for tumor development. The knowledge on their induction, activation and function opens the possibility for their selective in vivo manipulation as an attractive immunotherapeutic approach in a tumor such as FISS.

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VETERINARY ONCOLOGY

CD25, sarcoma, immunohistochemistry

CHARACTERIZATION OF A NOVEL AUTOPHAGIC VACUOLAR MYOPATHY IN MICE

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The aim of this study was to describe histopathological and molecular findings in a group of 50 crosses of C57/BL6 mice strain that shows many similarities with X-linked myopathy with excessive autophagy (X-MEA) in humans. X-MEA is a congenital lysosomal myopathy characterized by progressive vacuolation and atrophy of skeletal muscle. In humans, X-MEA has been associated to a VMA21 gene hypomorphic alleles (1,2), that encodes for an important constituent of V-ATPase proton pump complex. Vma21p deficiency impairs autophagic acidification and consequently its degradative functions.

Skeletal muscle biopsies from 50 mice crosses of C57/BL6 strain and 10 control mice of different strains were collected and snap frozen in liquid nitrogen. Cryosection were subjected to a panel of different histological and histoenzymatic stains; immunohistochemical (HRP method) or immunofluorescence detection of sarcolemmal (β spectrin, β dystroglycan, dystrophin) and autophagic (LAMP2, LC3, Beclin1, p62, BAG3) markers was carried out. Immunofluorescence analysis for Calsequestrin and RT-PCR for VMA21 mRNA were performed as well. Part of the samples was fixed in glutaraldehyde for ultrastructural examination.

Hematoxylin-eosin stained cryo-sections from only male C57/BL6 crosses mice showed marked increase in fiber size variation, and massive presence of large intracellular vacuoles containing basophilic material which was reddish at Engel's trichrome stain. The vacuoles were positive for Esterase and Alizarin Red stain. Immunostaining with antibodies directed against β spectrin, β dystroglycan, and dystrophin showed strong vacuolar membrane positivity. Some vacuoles were positive for the LAMP-2 lysosomal membrane, for autophagic markers LC3, Beclin1 and P62. Aggregates resulted negative at immunofluorescence staining for the tubular associated protein Calsequestrin, allowing us to rule out tubular myopathy. Immunofluorescence examination showed also a co-localization of LC3 and BAG3, suggesting that also chaperone-mediated autophagy could be implicated in the worsening of the myopathy, especially in oldest animals. At the ultrastructural investigation two types of vacuoles were found, corresponding to pre-autophagosomes and autophagolysosomes accumulations. Lastly, Q-RT-PCR analysis revealed a significant decrease of VMA21 mRNA rate in muscle tissue from old male mice compared to younger male mice and in controls from different strains.

Our data suggest that this mouse could be a novel spontaneous mouse model for X-MEA.

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Lab animal Veterinary Pathology
myopathy, autophagy, mouse

GENETIC AND PATHOLOGICAL FOLLOW-UP STUDY IN GOATS EXPERIMENTALLY AND NATURALLY EXPOSED TO A SHEEP SCRAPIE ISOLATE

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Scrapie is a slowly progressive and fatal neurodegenerative disease affecting sheep, goat and mouflon and is one of the family of transmissible spongiform encephalopathies (TSE)[1]. Transmission of scrapie from sheep to goat has extensively proved[2], however an adequate knowledge on the genetic and pathological aspect characterizing this interspecies transmission lacks.

Here we studied the dynamic of scrapie occurrence, with particular emphasis on genetic and phenotypical aspects, throughout 2 generations (F1 and F2) in a goat herd in which the parental generation was experimentally challenged with a natural sheep scrapie isolate by oral infection.

Thirty-two goats were orally infected with a scrapie brain homogenate from ARQ/ARQwildtype. The goats were mated to obtain 2 additional generations of offspring which were kept in the same environment to be naturally exposed to scrapie agent.

Clinical monitoring of the experimental group was performed daily. At necropsy from all these animals included in the experiment the brain, the spinal cord and the gut-associated lymphoid tissue (GALT) were also promptly collected. One half was fixed in formaldehyde for immunohistochemical (IHC) investigations, while the other one was frozen at -20°C for immunobiochemical analysis.

All the wildtype or G37V, Q168Q-P240P, and P/S240P goats had neurological signs indicative of scrapie after an incubation time ranging from 678 to 796 days, while goats with R154H, H154H, R211Q or P168Q-P240P dimorphisms became sick after a longer average incubation time (1,271 days). At 1,912 and 2,066 days post infection, the goats with D145D or Q222K dimorphisms were clinically healthy and without pathological prion protein in nervous and lymphoid tissues, respectively.

Interestingly, all but 2 of the wildtype, G37V and P/S240P goats belonging to the F1 offspring developed clinical scrapie at an average age of 746 days.

Our results demonstrate that 222K genetic variant is associated with scrapie resistance in goat even against scrapie of ovine source. Differently, 211Q and 154H did not provide a similar level of resistance as following infection with specie homologous isolates[3]. In addition, we observed for the first time a protective effect of the 145D goat variant against scrapie. Finally, our preliminary results demonstrate that immunohistochemical and molecular phenotypes of our natural ARQ/ARQwildtype scrapie ovine source was substantially preserved in goat carrying different susceptible PRNP variants.

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PATHOLOGY

Scrapie, Goat, Genetic

ELLIS-VAN CREVELD (EVC-2) SYNDROME IN TYROLEAN GREY CATTLE: MORPHOLOGICAL STUDY OF A TYPE OF CHONDRODYSPLASTIC DWARFISM.

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Ellis-van Creveld (EVC2) syndrome has been first described in children and is a complex clinical syndrome that presents with short limbs, retarded growth, polydactyly, ectodermal and heart defects and ciliopathies. This syndrome is due to a mutation of EVC2 gene (1). The aim of this study was to describe the gross and histological features of bovine chondrodysplastic dwarfism (CD) with deletion of EVC2 gene.

Four calves (3 female and one male) aged 2 to 5 months, with a clinical diagnosis of CD, were subjected to necropsy. The same cases were included in a whole genomic re-sequencing study that confirmed the deletion of the EVC2 gene (2). Bones, ligaments, heart and genital tract were routinely processed. Sections were stained with H&E.

At necropsy, the limbs of all the subjects were disproportionately short and bulky, rotated and arched in a “dumbbell-like” position. The long bones were severely reduced in length, with a very short diaphysis. In the 3 female calves, despite the young age, the genital tract was fully developed. In one case endocardiosis of the atrioventricular valves was observed. Histologically, the growth plates were irregular and closed prematurely. The reserve zone was variably thickened at the expense of proliferative and hypertrophic zones. Chondrocytes in the latter zones were disorganized, had multifocal loss of normal columnar arrangement, and were haphazardly arranged individually or in nests. The metaphysis was reduced in length, and the trabeculae in the primary spongiosa were shortened. Ovaries had follicles and corpora lutea; ligaments occasionally had multifocal lymphoplasmacytic inflammation.

CD due to an autosomal recessive mutation of the Limbin gene was described for the first time in the Japanese brown breed (3). A genetic study on inherited chondrodysplasia due to EVC2 deletion was recently reported in Tyrolean grey cattle (2), and now we describe the pathological aspects of EVC2 in this breed. As in humans, where EVC2 involves multiple organs, one of our cases showed endocardiosis. No genital lesions have been reported so far in human EVC2. EVC2 syndrome in Tyrolean Grey cattle is characterized by CD, genital and heart defects and could be a useful model for human medicine.

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ANATOMIA PATOLOGICA

Calf , EVC2 syndrome , chondrodysplasia

GROWTH PROMOTERS REGULATION OF REGUCALCIN GENE EXPRESSION IN SEX ACCESSORY GLANDS OF BEEF CATTLE

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Regucalcin (RGN) is a calcium (Ca²⁺)-binding protein regulating the intracellular Ca²⁺ homeostasis and the activity of several enzymes and proteins involved in intracellular signalling pathways (1). Recently, RGN was identified as a sex steroid-regulated gene in prostate and testis of rat and human (2, 3) and in sex accessory glands of veal calves (4).

This study investigated RGN expression in the sex accessory glands of beef cattle experimentally treated with growth promoters (GPs) to establish whether the RGN gene can be considered as a novel biomarker for the detection of GPs abuse in beef cattle.

In trial 1, 18 Charolaise beef cattle, 17-22 months old, were divided as follows: group A (n=6) treated weekly with 20 mg/animal of 17 β -estradiol for 5 weeks; group B (n=6) treated daily with 0.7 mg/animal of dexamethasone 21-phosphate disodium salt for 40 days; group K1 (n=6) was the control. The animals were euthanized six days after the last treatment. In trial 2, 24 Friesian beef cattle, 13-20 months old, were divided as follows: group C (n=8) was administered 200 mg of trenbolone acetate and 20 mg of 17 β -estradiol (Revalor-200®) as a subcutaneous implant for 89 days; group D (n=8) was administered 200 mg of trenbolone acetate (Finaplix®-H) as a subcutaneous implant for 89 days; group K2 (n=8) was the control. The implants remained in place until slaughtering. Samples of the testis, prostate and bulbo-urethral glands were collected from each animal and preserved for further analyses. Quantitative PCR (qPCR) of RGN mRNA, immunoistochemistry (IHC) and western blotting (WB) of RGN protein were performed. Statistical differences were determined by ANOVA, followed by Dunnett's post test.

In trial 1, high doses of 17 β -estradiol (group A) significantly down-regulated the RGN mRNA expression in testis (P<0.01), whereas no effect has been detected in bulbo-urethral glands and prostate. In trial 2, low doses of 17 β -estradiol in combination with trenbolone acetate (group C) significantly down-regulated RGN mRNA expression in prostate (P<0.05) and testis (P<0.05), whereas trenbolone acetate alone (group D) caused a decrease of RGN expression only in testis (P<0.05). The IHC and WB analyses confirm the results obtained in qPCR.

Contrary to data reported in rat (2) and veal calves (4), the estrogen regulation of RGN expression in prostate and bulbo-urethral glands of beef cattle was less pronounced, maybe due to beginning of puberty. On the other hand, testis response was similar to the data previously reported in veal calves (4).

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Patologia Animale

regucalcin, growth promotes, beef cattle

NECROPSY FINDINGS OF ANIMAL CRUELTY IN WATER BUFFALO CALVES

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Rearing of male water buffalo (Italian Mediterranean Buffalo) calves has never concerned farmers because of the lack of economic profits; in order to reduce the cost of management, male buffalo calves are usually sent to the slaughter-house too early and the animal welfare is too often a minor detail for the farmers. The aim of this work is to describe the macroscopic and microscopic findings in young buffaloes died during transportation or after their arrive at the slaughterhouse and, contextually, provide the veterinarians an useful guide for monitoring animal welfare.

Fifty water buffalo calves, farmed in Campania region (Southern Italy), aged between 7 and 20 days underwent necropsy and samples from all the major organs (lung, liver, heart, thymus, kidney, spleen, adrenal glands, gut, abomasum and skeletal muscle) were collected for histopathological evaluation in order to determine the cause of death and check for the presence of lesions suggestive of animal cruelty and stress.

The cause of death was suggestive for respiratory or heart failure or neurogenic shock occurring for: severe enteritis (55%), acute pneumonia (30%) and trauma (15%). Moreover, in every cases stress induced lesions were observed such as: secondary thymic atrophy, serous fat atrophy, atrophy or hypertrophy of the zona fasciculata of the adrenal cortex^{1,2,3}. Severe muscle atrophy was observed in 30% percent of cases caused by malnutrition.

The crime of animal cruelty is underreported and often goes without investigation especially in farm animals. Necropsy is the unique valuable tool for defining the cause of death of animals and to highlight stress-induced lesions and cruelty⁴.

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Veterinary Pathology and Animal Welfare
animal cruelty, necropsy, water buffalo

EXPRESSION OF AUTOPHAGY MARKERS IN AGED BOVINE BRAINS

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Autophagy is a highly regulated process involving the bulk degradation of cytoplasmic macromolecules and organelles in mammalian cells via the lysosomal system. The mechanisms for delivery of cargo to lysosomes label three different autophagic pathways: macroautophagy (herein referred as autophagy), microautophagy and chaperone mediated autophagy (CMA). Dysregulation of autophagy is implicated in the pathogenesis of many neurodegenerative diseases (Fields et al.) and integrity of the autophagosomal-lysosomal network appears to be critical in the progression of aging (Cuervo et al.). Here, we survey autophagy markers in aged bovine brains describing its possible role in neurodegeneration.

Samples of frontal cortex and hippocampus were collected from the brain of 20 aged cows (10-15 years old) and 5 normal controls (1-3 years old). Formalin-fixed and paraffin embedded 4µm sections were stained with Hematoxylin and Eosin and Periodic Acid-Schiff (PAS). Immunohistochemical stains were performed on selected sections using primary antibodies such as Beclin 1, LC3, LAMP-2, Ubiquitin and beta-amyloid 1-16. Antigen-antibody binding were detected by a horseradish-peroxidase (HRP) method and the slides were counterstained with hematoxylin.

We observed a mild to severe satellitosis, neuronal necrosis and a diffuse accumulation of PAS positive, granular deposits within neurons attributable to lipofuscin storage. An intraneuronal strongly positive immunoreaction was detected for Beclin-1, LC3, Ubiquitin and beta-amyloid 1-16 while immunoreaction for LAMP2 was weak or absent.

An increased expression of Beclin 1 and LC3 proves an excessive activation of autophagy and immunoreactivity for Ubiquitin indicates an intraneuronal accumulation of ubiquitinated proteins. A decrease of LAMP-2 expression suggests a primary defect in CMA activity that is related to the accumulation of pathogenic proteins such as beta-amyloid in the cytoplasm of the neurons (Rajawat et al.). Our data have showed an increase of autophagy in aged bovine brains that may be responsible for neurodegenerative conditions, including aging and neuronal death. Furthermore, progressive increase in intralysosomal concentration of lipofuscin may be responsible for a diminished efficiency of lysosomal degradation of proteins promoting their accumulation.

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neuropatology

autophagy, aging, neurodegeneration

OXYTOCIN AND OXYTOCIN RECEPTOR EXPRESSION IN C2C12 MYOBLASTS TREATED WITH 17-BETA-ESTRADIOL: PRELIMINARY STUDY

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Sex steroid administration induces muscular hypertrophy by enhanced protein synthesis and satellite cells recruitment (Kadi 2008; Rhoads 2009). Recently, skeletal muscle has been identified as a secretory organ (Bertoluzzi et al., 2006) and our preliminary results confirm the hypothesis that skeletal muscle is able to synthesize and secrete oxytocin (Oxt) in cattle treated with 17-beta-estradiol (E2), inducing an increase of muscle mass (Divari et al., 2013). Moreover it is known that estrogen treatment induces murine myoblasts proliferation (Kahlert et al., 1997).

Aim of our study is to investigate the relationship between E2 treatment and Oxt pathway in murine C2C12 culture and to detect and quantify cell proliferation.

Murine C2C12 myoblasts were seeded in DMEM high-glucose medium supplemented with 10% FCS, 1% glutamine and 1% antibiotic-antimycotic. Twenty-four hours before the treatments, cells were starved in a serum-free medium. Then, 10-8M E2 was administered for further 24 hours (pretreatment). Finally, myoblasts were challenged with 10-8M E2, alone or in association with 10-5M oxytocin, for different short period of time: 30 min, 1 hour, 2 hours, 4 hours. Real-Time PCR to evaluate the expression of Oxt prepropeptide and its receptor (OxtR) and Western blot to confirm the relative protein expression were performed. Flow cytometry immunodetection of bromodeoxyuridine to detect and quantify cells proliferation on 24 h 10-8M E2 treated myoblasts was carried out.

In myoblasts treated 24h+1h, 24h+2h with E2, Oxt gene expression was significantly ($p < 0.01$) higher than in controls. A similar but slighter increase in Oxt expression was recorded in cells without pretreatment of E2. OxtR gene expression was strongly up-regulated after 24 hours of E2 treatment ($p = 0.0016$). The presence of Oxt in medium did not induce any gene regulation.

Proliferation assay did not show any significant difference between treated and untreated cultures.

Results show the role of E2 in gene regulation of C2C12 myoblasts, in particular on the oxytocin pathway. These data, obtained in a murine model, confirm previous results achieved in cattle treated with E2 (De Jager 2011, Divari 2013), but further investigations are needed to better understand the oxytocin regulation of distinct skeletal muscle processes.

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pathology

murine myoblasts, oxytocin, estrogen

NON-DIAGNOSTIC FINE NEEDLE ASPIRATION BIOPSY (FNAB) OF CUTANEOUS MASSES IN DOGS AND CATS

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The management of cutaneous masses in which cytology is non-diagnostic remains controversial and studies addressing this problem seem not available. In this report we review the causes of non-diagnostic FNAB cytological samples in a 12 year operational span.

Cytological records of all non-diagnostic FNAB from cutaneous masses in dogs and cats (January 2002-October 2013) were retrospectively reviewed. Only cases with concurrent histology (considered as the goldstandard) were included. Cytological diagnostic samples of 100 cutaneous masses served as controls. Signalment, site, size and type of lesion (neoplastic vs non-neoplastic and benign vs malignant in case of neoplasia) were included and the number of slides submitted per case was recorded. A chi-squared test was used and p values ≤ 0.05 were considered significant.

A total of 138 cases was included. On histology 19 were classified as inflammation, 95 as neoplastic and 24 were non-neoplastic/non-inflammatory lesions. Non-diagnostic results were associated with the exclusive presence of erythrocytes (96 cases), by poor cellularity (39 cases) and by artifacts (3 cases). Site of lesions was trunk (52 cases), limbs (38 cases), head and neck (33 cases), mammary gland (14 cases) and non-specified in 1 case. Although not statistically significant, lesions on the head provided more non-diagnostic samples. Size of lesions was less than 2 cm in 39 cases, between 2-5 cm in 33 cases and above 5 cm in 19 cases. Lesions larger than 5 cm were significantly less diagnostic. Cytology was a good technique for round cell tumor diagnosis whereas, mesenchymal tumors were a frequent cause of a non-diagnostic results. Cytology diagnosed more easily benign than malignant neoplasms when lipomas were included. Compared to the control group, the number of slides obtained was higher in inconclusive cases for the category "4-6 slides".

Size and type of tumor were statistically correlated with reduced diagnostic power of FNAB cytology. Tumor type (mesenchymal versus epithelial or round) confirmed to have a role in the adequacy of samples.¹ The larger size of lesions typical of mesenchymal tumors and their poor cell yield represented one of the major causes of non-diagnostic results. Lesions on the head may provide more non-diagnostic cytology compared to other sites probably due to the patient compliance. Blood contamination is a common cause of non-diagnostic samples since poorly cellular samples not contaminated by blood can be easily esteemed grossly. The higher number of slides collected in non-diagnostic cases suggests that the operator is aware of the poor quality of the sampling. Despite the limitations of this retrospective study, the evaluation of non-diagnostic cytological samples may provide valuable information and improve the understanding of the limitations of diagnostic cytology.

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CITOLOGIA

Cytology, non-diagnostic FNAB, Skin

TP53 EXPRESSION AND ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) IN FELINE SQUAMOUS CARCINOMAS

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Feline cutaneous squamous cell carcinoma (FC-SCC) is the most common skin malignant tumour in cats (15–50% of all skin neoplasms) and the white-coated cats have a 5–13 times higher incidence. Development of FC-SCC has been associated with chronic sunlight exposure and TP53 protein represents one of the most important tumor suppressor over-expressed and mutated in response to UV light exposure. In FC-SCC TP53 expression has been demonstrated but mutation analysis in hot spot of TP53 gene have not been investigated yet. The aim of the present study was to evaluate the expression and presence of mutations of TP53 in FC-SCC.

Immunohistochemistry against feline TP53 was performed on formalin-fixed paraffin-embedded FC-SCC from 25 white-coated domestic shorthair. IHC expression score was evaluated considering the percentage of positive nuclei in keratinocytes as follows: 0 ($\leq 10\%$), 1+ ($>10-30\%$), 2+ ($>30-60\%$), 3+ ($>60\%$). Eight samples overexpressing TP53 (score +3) were submitted to genomic DNA extraction and mutation analysis by direct sequencing was performed from exon 4 to exon 8 of feline TP53 gene.

Immunohistochemical results for TP53 showed that 16 FC-SCC (64%) were positive (32% score +3, 16% score +2 and 16% score +1) while 9 cases (36%) were negative. DNA sequencing analysis revealed two Single Nucleotide Polymorphism (SNP) in the intron 8 in 6 samples and a missense mutation (C>T) in the exon 5 in one sample coding for aminoacid tyrosine

In this study we found that TP53 is expressed in 64% of FC-SCC and this expression can be correlated to UV injury since TP53 is one of the first tumor suppressor activated by UV. The presence of SNPs in intron 8 has been already described as a feline genotypic variation and cannot be considered correlated to the disease. The identification of a missense mutation in exon 5 has not been previously reported in FC-SCC. If confirmed in a wider number of cases, the C>T nucleotide change (substitution UV-dependent) may indicate a possible role of UV in causing this genomic injury in FC-SCC.

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anatomia patologica - oncologia

TP53, feline SCC, oncology

SOX9: IMMUNOHISTOCHEMICAL STUDY OF NORMAL AND NEOPLASTIC CANINE SERTOLI CELLS**Banco B.*^[1], Sironi G.^[1], Martignoni B.^[1], Giudice C.^[1], Grieco V.^[1]**^[1]*Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano*

SOX9 protein plays a pivotal role for male sexual development. Among its numerous functions, it regulates the transcription of the anti-Müllerian hormone (AMH) gene and interacts with other genes to promote the development of testis cords, the multiplication and maturation of Sertoli cells (SCs) and the maintenance of spermatogenesis in the adult testis. The expression of SOX9 in normal testes has been demonstrated in humans, mice and rats. In canine species SOX9 expression in normal SCs has never been investigated and no data are available about neoplastic canine SCs (Sertoli Cell Tumours, SCTs).

The present study aimed to investigate the expression of SOX9 in canine SCs during testicular maturation and neoplastic transformation.

Testicular samples derived from 1 foetus, 4 newborns, 2 prepuberal puppies, 3 adult dogs, 28 SCTs (1 of them metastasizing) and 3 Leydig cell tumors (LCTs) were selected from the archive and tested immunohistochemically with a polyclonal antibody against SOX9 (1:150).

Histologically, 18/28 SCTs were typical SCTs, 10/28 were classified as “lipid rich” SCTs, and 6/28, including the metastasizing one, characterized by capsular invasion, solid growth, severe anisocytosis and anisocytosis, large areas of necrosis and hemorrhages were considered undifferentiated SCTs.

Immunohistochemically, all SCs from foetal, neonatal and adult testes had a strong, diffuse and exclusively nuclear labelling for SOX9. In prepuberal testes, SOX9 stained exclusively SCs nucleus in one puppy and both nucleus and cytoplasm in the other one. Leydig cells (LCs) were constantly negative in all samples. Similarly negative were all the LCTs.

Concerning the 28 Sertoli cell tumors, 2 were negative for SOX9 and were reclassified as LCTs, while in the remaining 26 SCTs, SOX9 was diffusely expressed confirming the diagnosis of SCTs. In these tumours, the expression of SOX 9 was nuclear, nuclear and cytoplasmic or exclusively cytoplasmic in 14/26, 10/26 and 2/26 SCTs respectively. Moreover, all the undifferentiated SCTs and “lipid rich” cases were characterized by less intense staining.

This is the first report on immunohistochemical SOX9 expression in canine testes and demonstrates that in normal SCs from foetal, neonatal and adult testes, SOX9 labelled the nucleus as in human species. On the other hand, the cytoplasmic labelling observed in one puppy might parallel with prepuberal rat testes staining. Considering this datum, the cytoplasmic expression of SOX9 described in numerous canine SCTs could reflect cellular immaturity/dedifferentiation. In addition, the expression of SOX9 in SCTs and its absence in LCTs suggests that SOX9 is a reliable marker in the differential diagnosis between canine SCTs and LCTs.

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Canine testes- oncology- pathology

canine Sertoli cells, immunohistochemistry, SOX9

GENETIC HETEROGENEITY OF CANINE DLBCL BY OLIGONUCLEOTIDE ARRAY CGH

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The role of genomic alterations (GA) at high resolution by oligonucleotide Array CGH (oACGH) in Diffuse Large B-cell Lymphoma (DLBCL) has been scarcely investigated in dogs. The first aim of this work was to analyse canine DLBCL in order to find genomic regions or even gene-specific GA at a higher resolution than conventional cytogenetic. We also compared genomic imbalances by oACGH in a reduced number of biopsy specimens of DLBCL at diagnosis and at their clonally related relapses.

We analysed 12 newly-diagnosed multicentric DLBCLs using an 180,000 oACGH on lymph nodes (LN) samples. oACGH was also repeated in LNs of 3 relapsing dogs and 4 dogs in remission after chemotherapy. All LNs were matched with corresponding skin biopsies. Recurrent aberrations were defined as gains or losses occurred at a frequency greater than 30%. Data analysis was performed using bioinformatic resources: Ensemble Genome Browser, Functional Annotation Tool Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources and CGHTools.

In pre-treatment DLBCLs, the pattern of GA consisted of 90 different genomic imbalances (mean per tumour, 17), 46 gains and 44 losses. Two gains in Chr13 were significantly correlated to stage III-IV of the disease ($p=0.002$). Statistical analysis identified gains ($n=6$) and losses ($n=8$) significantly associated with time of remission ($FDR<0.001$). Functional annotation obtained by DAVID showed enriched pathways related to nucleotide biosynthesis, ascorbate and aldarate metabolism considering gained regions; in loss intervals immune response was the most enriched pathway. In the LNs of 4 dogs in remission after chemotherapy, 4 new GA were found, whereas 3 new GA were observed in relapsing dogs, compared with the sample at diagnosis.

In pre-treatment DLBCLs, individual variability in the number of GA was found, however 14 recurrent aberrations were identified. Recurrent losses involving IGK, IGL and IGH were observed in all tumours and in 2 dogs in clinical remission. The recurrent gains along the length of Chr13 were found in more than 50% of cases and were associated with a longer time of remission. In these segments, some genes significantly involved in several human and canine tumours, such as MYC, LDHB, HSF1, KIT and PDGFRA, were annotated. The recurrent gains along the length of Chr31 (>41% of cases) were associated with a shorter time of remission. One ex novo GA, involving TCR, was present in dogs in remission after chemotherapy. A reduced number of chromosomal rearrangements were found in relapsed ($n=17$) DLBCLs when compared with pre-treatment DLBCLs ($n=90$). To our knowledge this is the first time where regions of GA are associated with response to therapy and outcome of lymphoma in dogs. Further studies are needed with a larger number of cases and to identify correlations with gene expression and protein transduction.

oncologia

DLBCL, Dog, Oligo Array CGH

HYSTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF FELINE RENAL CELL CARCINOMA: A CASE SERIES

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Renal cell carcinomas (RCCs) are rare neoplasms that occur mainly in middle-aged to old cats. To the authors' knowledge few studies are focused on the morphology, however a immunophenotypical characterization of RCC has never been performed.

In this study, 4 cases of RCCs were retrieved from the archive of the Histopathology Service of the Dept. of Comparative Biomedicine and Food Science in a spanning period of 9 years. Tumours were obtained by nephrectomy. Specimens were classified by predominant histological pattern according to WHO criteria. The presence or absence of necrosis, nuclear pleomorphism and mitotic index (MI) were also noted. Furthermore, a panel of antibodies including cytokeratin (CK), vimentin (VIM), E-cadherin (E-CAD), β -catenin (β -CAT), CD10 and c-KIT was selected to characterize the tumors. The angiogenic activity was analysed by VEGF and VEGF-R2. A semiquantitative analysis was assessed for all antibodies. Normal renal tissue was used as control.

The cats were all male, 3 domestic shorthair and 1 Persian, averaging 10.5 years in age. The tumors were classified as tubular RCCs (3) and papillary RCC (1). The MI was <1 per HPF associated with a slight nuclear pleomorphism.

Epithelial cells (ECs) in the tumors were CK, VIM, E-CAD, VEGF-R2 positive and c-KIT negative; 3 cases were β -CAT positive whereas only 2 tumors were CD10 and VEGF positive. All the markers were detected both at cytoplasmatic and membranous level, except for VEGF, VEGF-R2 and VIM that were only cytoplasmatic.

In humans, CK and VIM co-expression is used to confirm the diagnosis of RCC. Despite the low number of cases, feline RCCs seem to have a similar profile for CK and VIM. This observation might be helpful to exclude possible form of carcinoma metastasis in kidney and the panel of the two antibodies included in a routine diagnostic assay for suspects of RCCs. The absence of c-KIT expression in ECs suggests that this tyrosine kinase receptor is not involved in the pathogenesis of feline RCCs. This data is different from previous results in human and canine RCCs where specific histotypes are associated with c-KIT expression.

In human, E-CAD and CD10 are useful markers to differentiate distal or proximal origin of RCCs. E-CAD expression in ECs associated to absence of CD10 immunoreactivity suggests the distal origin in two tumors in our study. So far, the double positive expression of both markers in ECs might be related either proximal or distal tubular origin in the other 2 tumors, but aberrant expressions cannot be rule out.

VEGF-R2 expression in the neoplastic ECs didn't differ from control ECs whereas VEGF results might be compatible with a variable angiogenic activity intrinsic to the tumour.

Our results confirm the low frequency of RCCs in cats and also reveal a peculiar immunohistochemical profile different from the canine RCCs.

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cat, immunohistochemistry, renal cell carcinoma

AN IMMUNOHISTOCHEMICAL STUDY OF THE PTEN/AKT PATHWAY INVOLVEMENT IN CANINE AND FELINE MAMMARY TUMOURS

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The PTEN/AKT pathway is strongly involved in cell metabolism, proliferation and survival. Its dysregulation is implicated in several human cancers. The aim of this study was to investigate the role of this pathway in canine (CMTs) and feline mammary tumors (FMTs) by immunohistochemistry (IHC).

Fifty CMTs (10 adenomas and 40 carcinomas) and 30 FMTs (30 carcinomas) were submitted to IHC to evaluate PTEN, phospho-AKT and Rictor expression.

All the canine adenomas (100%), 25 of 40 (63%) canine carcinomas, and 7 of 30 (23%) FMTs were PTEN-positive. In dogs, no adenomas and 15 of 25 (37%) carcinomas expressed phospho-AKT, while 24 of 30 FMTs (82%) were phospho-AKT-positive. One of 10 (10%) canine adenomas (100%), 24 of 40 (60%) canine carcinomas, and 20 of 30 (67%) FMTs were Rictor-positive. In dogs, PTEN correlated with complex carcinomas, lower mitotic index, absence of lymphatic invasion and longer survival; phospho-AKT with simple carcinomas, lymphatic invasion and poorer survival and Rictor with lymphatic invasion. In cats, PTEN correlated with tubulopapillary carcinomas, lower mitotic index, absence of lymphatic invasion and better survival, while phospho-AKT and Rictor with poorer survival. In CMTs, phospho-AKT inversely correlated with PTEN expression and positively with Rictor. In FMTs, PTEN inversely correlated with phospho-AKT and Rictor while phospho-AKT positively correlated with Rictor expression.

Our data show a strong PTEN/AKT pathway involvement in behavior worsening of CMT and FMTs. This data could provide a rationale for further studies of this system in veterinary oncology to due prognostic and therapeutic implications.

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Maniscalco L, Iussich S, de Las Mulas JM, et al. Activation of AKT in feline mammary carcinoma: a new prognostic factor for feline mammary tumours. *Vet J.* 2012;191(1):65-71.

Ressel L, Millanta F, Caleri E, Innocenti VM, Poli A. Reduced PTEN protein expression and its prognostic implications in canine and feline mammary tumors. *Vet Pathol.* 2009;46(5):860-868.

Patologia veterinaria/Oncologia comparata

Dog and cat, Mammary tumours, PTEN/AKT pathway

EXPRESSION OF BECLIN-1 IN CANINE MAMMARY TUMOURS. PRELIMINARY INVESTIGATIONS**Russo V.*^[1], Martano M.^[1], Maiolino P.^[1], Ceccarelli D.M.^[1], Restucci B.^[1]**^[1] *DIPARTIMENTO DI MEDICINA VETERINARIA E PRODUZIONI ANIMALI ~ NAPOLI*

Autophagy is a self-catabolic process consisting in the degradation of intracellular structures and organelles by lysosomal enzymes and it is involved in development, homeostasis, and cell survival. Its dysfunction is related to several pathologic processes, such as infections, metabolic disorders, neurodegeneration, and tumorigenesis. Autophagy is a multistep process characterized by nucleation, elongation, and autolysosome formation and it is carried out by a group of proteins called autophagy-related proteins (Atg). Among these, Beclin-1 is required for the nucleation of the phagophore and maturation of the autolysosome representing key moments in the autophagy process; disruption of this protein has been associated with cancerogenesis in many human neoplasms. Since relatively little is known in veterinary medicine on the subject, the aim of this study was to evaluate by immunohistochemistry the Beclin-1 expression in normal and neoplastic canine mammary glands and to correlate the results with the histopathologic features of these tissues.

5 normal and 25 neoplastic canine mammary glands were examined. Neoplastic samples were classified according to WHO criteria and graded into grades I to III tumours, applying Elston Ellis parameters, immunohistochemistry was performed by using rabbit polyclonal human anti-Beclin 1 antibody (Santa Cruz Biotechnology); immunoreactivity was evaluated by counting immunostained cells

In normal mammary glands strong cytoplasmic expression of Beclin 1 in many epithelial ductal cells was observed. In malignant tumours Beclin-1 expression was significantly weaker than in normal mammary glands and the number of immunolabelled neoplastic cells was decreased. Low expression was associated with all parameters of malignancy such as lower degree tubule formation, nuclear pleomorphism, number of mitoses and presence of necrosis. In contrast to results previously reported by others¹, we did not observe Beclin-1 nuclear positivity in any canine mammary gland sample, neither in normal tissues nor in malignant tumours

The decrease of Beclin-1 expression in malignant tumours and its correlation with all parameters of malignancy, supports the hypothesis that Beclin-1 functions as a tumor suppressor protein and its disruption may be involved in neoplastic transformation and progression. This suggests a potential involvement of dysfunction or suppression of autophagy in canine mammary gland cancerogenesis.

¹Liu J-L. et al. BMC Veterinary Research 9:75-84, 2013.

VETERINARY ONCOLOGY

AUTOPHAGY, BECLIN1, MAMMARY TUMOURS

PAPILLARY MENINGIOMA IN THE DOG: A CLINICOPATHOLOGICAL CASE SERIES STUDY.

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Papillary meningioma (PM) is considered one of the most aggressive variants of meningioma in humans and classified as grade III by WHO classification system. To date, the biological behavior of this tumour is still unclear in dogs. In this study we investigated the clinicopathological correlations of canine PMs in order to define if PM might be considered of grade III in dogs as in humans, despite its benign histological pattern. To provide more information regarding the biological behavior of this meningioma subtype in dogs, we also investigated doublecortin (DCX), E-cadherin, and N-cadherin expression by IHC.

FFPE tissue from 16 canine PMs, obtained by surgery and necropsy, was included in the present retrospective study. Clinical and follow up data were obtained from medical records. 5 µm sections of the selected PMs were stained with H&E, and the tumors were graded according to the criteria of the human WHO international histological classification of CNS tumors. To investigate the expression of DCX, E-cadherin and N-cadherin, additional 4 µm sections were used for IHC, performed with avidin-biotin-peroxidase complex method (ABC, Dako, Milan, Italy).

PMs accounted for 18% of meningiomas archived in our lab. PM tended to occur in animals older than 7 years with a male/female ratio of 1.7, and the supratentorial compartment was the most common affected site. Based on human WHO classification system, seven tumors (43,8%) and nine tumors (56,2%) were classified as grade I and as grade II, respectively. Grade III was not identified. As for surgical cases recurrence was observed in 87.5% and the mean survival time was 10.8 months. Five recurrent surgical meningiomas showed necrosis up to 50% of the tumor. In the non-surgical cases a mean survival time of 24 days was observed. The IHC results were not related neither with histological grade of malignancy, nor clinical behavior. However, an apparent negative correlation between E-cadherin and N-cadherin expression was found in tumors showing a low survival time.

Despite benign histological findings, we confirmed the aggressive behavior of PM also in dogs. This was especially true for untreated animals that showed a significantly lower survival time if compared with that reported for canine meningiomas (Bilderback et al., 2006). As for surgical cases, the post-surgery survival time was higher than that observed in canine meningioma (Axlund et al., 2002), probably due to the improvement of surgical techniques occurred in the last ten years. As in humans, the biological malignancy of canine PM seems to be correlated to intratumoral necrosis more than effective brain invasion. Although the absence of conclusive data, we might suppose that a "cadherin-switch" is involved in the biological progression of canine PM.

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Axlund TW, McGlasson ML, Smith AN (2002) Surgery alone or in combination with radiation therapy for treatment of intracranial meningiomas in dogs: 31 cases (1989-2002). J Am Vet Med Assoc 221:1597-600
Oncologia

Papillary meningioma, Biological behaviour, Dog

A BIOCOMPATIBLE SYSTEM AS CARRIER OF ANTINEOPLASTIC DRUGS IN GLIOBLASTOMA TREATMENT: PRELIMINARY DATA

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Aim of the present study is to report the in vitro and in vivo effects of Solid Lipid Nanoparticles (SLN), free drug and SLN-loaded drug in glioblastoma treatment. SLN are biocompatible systems made of physiological lipids proposed to be used as antineoplastic drug carriers (Battaglia et al., 2010, 2012). The difficulty of classical cytotoxic drugs to overcome the blood brain barrier (BBB), which have high in vitro efficacy against glioblastoma, is a major limitation to drug therapy. Anticancer drug delivery through SLN to the brain parenchyma for the treatment of glioblastoma may represent a valid strategy: aiming to overcome the BBB, SLN should be modified on the surface and functionalised with ligands to the receptors, variously expressed on endothelial cells, according to central nervous system metabolic requirements. Different lipophilic and hydrophilic antineoplastic drugs can be entrapped in SLN.

Blank and Paclitaxel-loaded SLN were tested. Glioblastoma cell lines were used to test the effects in vitro. The cytotoxicity's evaluation of drug-loaded SLN was performed by Trypan blue exclusion test and MTT method at different times and concentrations. Ultrastructural investigations were also made.

In vivo biocompatibility was evaluated on male Wistar rats (n = 8) after i.v. administration of blank and Paclitaxel-loaded SLN. Rats were sacrificed and submitted to a complete necropsy, in order to evaluate signs of toxicity in different organs. Liver, kidney, heart, lung and brain samples were collected to perform histological investigations. Selected tissue portions were paraffin embedded, sectioned at 3µm and stained with haematoxylin and eosin, Masson's trichrome and Sudan stains.

In vitro Paclitaxel loaded SLN produced a significant cell death on cell lines similar to or slightly increased in comparison with the drug alone. Ultrastructurally the main lesions were vacuoles in mitochondria, lysis and glycogen accumulation.

In vivo rats treated with blank SLN didn't show signs of systemic toxicity. In rats treated with Paclitaxel loaded SLN not significant lesions were detected in liver, kidney, heart and brain. Pulmonary granulomatous and adaptative changes, whose aetiopathogenetic mechanism remains to be clarified, were observed.

Despite the encouraging data regarding the in vitro cytological studies, further in vivo biodistribution studies on male Wistar rats are needed to confirm the suitability of SLN as drug delivery systems to the brain.

Further investigations will lead to better understand the mechanism of the pulmonary reported changes associated with the administration of SLN loaded paclitaxel drug.

Battaglia L e Gallarate M 2012 Expert Opinion on Drug Delivery 2012, 9(5): 497-508

Battaglia L et al., 2010. J Microencaps 27, 78–85

Neuropathology

Glioblastoma, Animal model, Nanoparticles

CONTRIBUTION OF CELL MARKERS TO THE STUDY OF RMS PATHOGENESIS

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Red mark syndrome (RMS) is a chronic non-lethal skin disease affecting farmed rainbow trout (*O. mykiss*) in U.K., Austria, Germany, Italy (Galeotti et al., 2011), Serbia and U.S.A. Histology shows a lymphocyte/macrophage infiltration in scale pockets, dermis and ipodermis. Aiming at the full comprehension of RMS aetio-pathogenesis, we focused on the mechanisms of cell recruitment/activation in the skin lesions, in order to elucidate if and how an hypothetical microbial agent might trigger the host inflammatory response.

Samples of skin from infected fish were evaluated by histology, immunohistochemistry and electron microscopy. The following markers were used: rabbit to human CD3 (A-0452, Dako); rabbit to rainbow trout IgT/IgM (Prof. Sunyer); rabbit to salmonid HSP70 (AS05061A, Agrisera); rabbit to human GM-CSFR α (sc-690, Santa Cruz Biotech.); mouse to PCNA (2586, Cell Signaling Technology); mouse to AE1/AE3 Cytokeratin (M3515, Dako); mouse to E Cadherin (M3612, Dako).

Anti trout IgT and IgM marked a limited number of scattered cells in the dermis and ipodermis. Anti CD3 marked a relevant number of cells composing the skin infiltrate. HSP70 marked monocyte/macrophages, dendritic like-cells and endothelial cells, within the scale pockets involved by inflammation. GM-CSFR α positive monocyte-macrophage were scattered in the derma, surrounding the scales. Anti-Cytokeratin and E Cadherin marked the epithelial cells. PCNA positive cells have been detected in epidermis, dermis and hypodermis, as well among infiltrating lymphocytes, stromal fibroblasts and vascular endothelial cells.

HSP70 are considered not only as acute phase proteins, but also as molecules able to mediate immunity and inflammation (Pockley, 2003); they can be released by several cell populations in response to various stimuli. Briefly HSP70 could act as an “antigen” inducing a severe T lymphocyte response, leading to an auto-immune like reaction. Dendritic cells and APCs are stimulated by HSP70 to release TNF- α , IL1- β and GM-csf. We might speculate that a microbial agent promotes HSP70 expression by macrophages/endothelial cells, within scale pockets. HSP70 might be also internalized by skin APCs. The pro-inflammatory cytokines released could then trigger the local inflammatory process. The GM-csf stimulates the development of osteoclasts. Skin APCs could express HSP70 and therefore stimulate T cell proliferation. These findings might justify the severe cell infiltration detectable in the skin of RMS affected fish.

Pockley, 2003. The Lancet, 362.

Galeotti et al., 2011. Proceedings 15th EAFP Conference, Split, Croatia.

ANATOMIA PATOLOGICA

Red mark syndrome, rainbow trout, cell markers

AN OUTBREAK OF EQUINE GRASS SICKNESS IN ITALY

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Equine grass sickness (EGS) is a debilitating, often fatal, neurodegenerative disease, that affects almost exclusively grazing horses (1,2). As the name suggests, EGS has a strong association with grazing, with only a few cases reported with no exposure to pasture (1). The precise aetiology of the disease is unknown, although several causes and risk factors have been proposed. Clinical signs result from neurogenic obstruction of the alimentary tract due to neuronal degeneration in the autonomic and enteric nervous systems (1,3). Although cases of EGS have been occasionally described in other countries, the disease is almost exclusively reported in Great Britain (1). The current study describes an outbreak of EGS in Italy, including gross, histological, bacteriological and immunohistochemical findings.

The outbreak occurred in a horse farm, located in the North of Italy, with approximately 20 horses living on the pasture. Most of them showed severe weight loss, progressive weakness, anorexia, poor body condition, decreased appetite, and dysphagia.

Three horses were found dead and submitted for post-mortem examination. The horses were in extremely poor body condition with severe diffuse muscular atrophy and moderate ventral oedema. All the three horses had distension of the stomach and small intestine with impaction of the large colon and caecum and hard dry faeces in colon and rectum. In addition, all displayed severe diffuse oesophageal muscular hypertrophy, mainly in the distal third of the organ. Histological findings were restricted to intestinal plexuses and ganglia that revealed marked neuronal degeneration, neuronal vacuolations, and decreased number of neurons. Immunohistochemistry demonstrated intracytoplasmic accumulation of synaptophysin within neurons of the enteric plexuses and cervical and coeliac ganglia. Bacteriology from several organs and enteric content was negative. Grass clippings, gastrointestinal contents and faeces were negative for *C. botulinum* toxins.

Gross, histological and immunohistochemical examinations were suggestive of a chronic form of EGS. Equine grass sickness is an extremely rare condition in Italy, however it should be considered as a differential diagnosis for horses with severe weight loss and anorexia.

1. Pirie RS, Jago RC, Hudson NPH. Equine grass sickness. *Equine Vet J.* 2014. [Epub ahead of print]
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Patologia equina

Grass sickness, ganglia, intestinal plexuses

STUDY OF THE PTX3 ACTIVITY IN THE PULMONARY INFLAMMATORY RESPONSE IN A MURINE MODEL

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Innate immunity plays key roles in activation and orientation of adaptive immunity and consists of a cellular and a humoral arm. The humoral arm is composed by a heterogeneous collection of weird molecules that represent functional ancestors of antibodies and form an integrated system of diverse molecules, including collectins, ficolins, and pentraxins (1). Pentraxins constitute a superfamily of multifunctional multimeric proteins and Pentraxin-3 (PTX3) is the first member of the long pentraxin subfamily. It is released from dendritic cells, mononuclear phagocytes, fibroblasts, endothelial and epithelial cells upon exposure to inflammatory signals such as cytokines (e.g. IL-1 β , TNF- α), TLR agonists, microbial moieties (e.g. LPS, OmpA) or microorganisms (1,2). PTX3 acts as an opsonin, binding the bacteria to facilitate their phagocytosis by the DCs and macrophages. On these bases, we investigated the role of PTX3 during a pulmonary infection by *Shigella flexneri*, in mouse.

For this purpose, C57Bl/6 mice were inoculated intranasally with 20 μ l of 0.9% NaCl suspensions containing 3×10^8 CFU of the strain. Control mice were similarly inoculated with 20 μ l of PBS. At the same time, PTX3 were administered via ip route once per day for three consecutive days following challenge. We monitored animals for mortality and clinical signs. After 72 h post-infection, mice were euthanatized and lungs and BALF were collected and processed for histopathological studies, and macrophage analysis. We analyzed the inflammatory response and tissue damage in lungs through histological evaluation and immunohistochemistry analysis. In order to characterize the cell-mediated response in T-cell population, tissue were tested with different primary antibody by immunohistochemistry staining. Moreover we performed in vitro macrophage activity assays, following primary culture by BALF, evaluating phagocytosis and respiratory burst.

The intranasal infection resulted in bacterial invasion of bronchial and alveolar epithelia with concomitant development of acute suppurative bronchiolitis and lethal pneumonia. Infected mice lungs showed acute bronchiolitis with diffuse alveolar damage, exuberant neutrophilic exudate and peribronchiolar and interstitial inflammatory infiltrate. Mice treated with PTX3 showed particularly activation of BALT and increased macrophage activity.

Our results show that in the mouse, PTX3 modulates the inflammatory response by reducing the acute phase, stimulating activation of the BALT and then leading a cell-mediated response.

1- Bottazzi et al., 2009.

2- Garlanda et al. 2005.

general pathology e immunopathology

PTX3, Pneumonitis, Immunomodulation

THE ROLE OF GROWTH FACTORS IN CANINE X-LINKED HEREDITARY NEPHROPATHY: AN ANIMAL MODEL OF PROGRESSIVE RENAL FAILURE

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To investigate the role of several growth factors involved in tubulointerstitial (TI) damage progression, extracellular matrix remodeling and Epithelial Mesenchymal Transition in a dog model of progressive renal failure.

10 male dogs with X-Linked Hereditary Nephropathy (XLHN) and 5 unaffected male littermates (controls) were studied. Clinical data and renal samples were obtained at 4 (T0), 6 (T1), 9 (T2) months of age and at necropsy (T3) performed at a standardized clinical endpoint. Glomerular and TI lesions were scored by light microscopy, and 21 genes known to be involved in TI damage were selected and investigated by real-time quantitative reverse transcription PCR. Morphologic, clinic pathologic and molecular data were analyzed with GLM procedure (SAS institute).

XLHN dogs had proteinuria at all time-points; controls had none. No histological lesions were identified in controls at any time-point or in XLHN dogs at T0. At T1, XLHN dogs had a significant ($p < 0.05$) increase of mesangial cellularity and matrix deposition, cystic glomerular atrophy (CGA), tubular necrosis and interstitial fibrosis. At T2, up to 88% of glomeruli were nonfunctional and CGA was prevalent (50%). Multifocal TI inflammation, fibrosis, tubular atrophy and dilation were present. At T3, CGA was even more prevalent (60% of all glomeruli), and TI inflammation and fibrosis were severe.

Glomerular obsolescence, CGA, mesangial hypercellularity, matrix increase, fibrosis, tubular dilation-atrophy, and inflammation were positively correlated ($p < 0.01$).

At T0, Transforming Growth Factor- β (TGF- β), Connective Tissue Growth Factor (CTGF), Matrix Metalloproteinase (MMP)-2, Platelet-Derived Growth Factor-D (PDGF-D) and PDGF receptor α (PDGFR α) were overexpressed in XLHN dogs compared with controls ($p < 0.05$). At T1 and T2, Clusterin (CLUST) and Tissue inhibitor of MMP-1 (TIMP-1) expression was increased; however, Epidermal Growth Factor Receptor (EGFR) mRNA was reduced ($p < 0.05$).

TGF- β and CTGF mRNA levels were positively correlated at all time-points. Expression of CTGF was positively correlated with PDGFR α , MMP-2 and MMP-9. TIMP-1, CLUST and MMP-2 mRNA levels were positively correlated with fibrosis, tubular atrophy and dilation ($p < 0.05$).

Histologic and clinic pathologic data showed canine XLHN to be a good model of chronic progressive renal failure demonstrating progressive and irreversible glomerular and TI lesions including fibrosis. Onset of proteinuria before histological changes occur (ie, at T0) suggests a key role for proteinuria in initiating progressive TI damage. TGF- β , CTGF, MMP-2, PDGF-D and PDGFRA were identified as potential key players in the progression of chronic renal damage. Increased expression of CLUST and TIMP-1 might function as a protective mechanism against progressive injury. The study investigates the expression of several molecules related with tissue injury and repair in dogs with an hereditary nephropathy that is frequently used as a model of chronic renal failure. Examined molecules are potentially involved in the pathogenesis of this condition that may be future possible target of drugs or biomarkers of early renal injury.

Malattie degenerative
renal failure, dog, gene expression

Poster Aipvet

A CASE OF CANINE THYROID CARCINOMA WITH HETEROTOPIC OSSIFICATION AND EXTRAMEDULLARY HEMOPOIESIS

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The present report describes a case of canine thyroid carcinoma with heterotopic ossification and extramedullary hemopoiesis in a 10 years old mongrel dog. Few anamnestic data were available: an apparently healthy dog was referred with the only symptom of polyphagia. A solid mass, not adherent to surrounding tissue, was palpable on the left side of the neck. The mass was surgically removed, fixed in 10% buffered formalin and send to the pathology division of our Department to be histologically examined. Grossly neoplastic mass was about 3x5 cm, well circumscribed, defined by a thin fibrous capsule. On cross section the mass had a brow-tan colour with a greyish, firm to hard, central area, grossly consistent with bone tissue.

Before trimming, the mass was therefore immersed for 1 week in a decalcifying acid. Then, samples were collected, passed trough graded alcohols, clarified in xilene and paraffin embedded. From paraffin blocks, sections were obtained and stained with Haematoxylin and Eosin.

Histologically, the tumor was composed of irregular, small follicular structures, nests and solid lobules of polygonal cells sustained by a variable amount of collagenous stroma. Neoplastic cells had poorly defined cells borders, moderate amount of eosinophilic cytoplasm and round to oval vescicolous nucleus with prominent nucleolus. Anisocytosis and anisokaryosis were moderate and mitotic figures ranged from 0 to 1 X HPF. Small multifocal necrotic foci and focal haemorrhages were also present scattered throughout the tumor. The central area of the tumor was composed of mature trabecular bone. Intertrabecular spaces were filled by both adipose cells and hemopoietic cells. A histological diagnosis of thyroid carcinoma with heterotopic bone formation and extramedullary hemopoiesis was posed.

Heterotopic ossification is a well recognized phenomenon involving organs and tissues affected by various pathologic processes, i.e. ischemia, hematomas, degenerative changes, chronic inflammation and, less frequently, tumors. Few cases of thyroid tumors with heterotopic ossification and extramedullary hemopoiesis have been described in human species. In the canine species, the presence of focal mineralization or scattered bone formation within thyroid carcinomas has been reported but poorly documented. Scattered calcifications have been described in normal thyroid gland while ossification has been reported in a single case of thyroidal carcinosarcoma. The present report represents the first description of a canine thyroid carcinoma with wide areas of mature bone formation (heterotopic ossification) and extramedullary hemopoiesis. Mechanisms of heterotopic ossification are still unknown, however the presence of inducible osteoprogenitor cells, of heterotopic environment conducive to osteogenesis and of inductive signalling such as bone morphogenetic proteins has all been evocated in the pathogenesis of this condition.

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Pathology oncology canine

thyroid carcinoma, dog, heterotopic

A CASE OF PAPILLOMAVIRUS-ASSOCIATED TONGUE ADENOCARCINOMA IN A CAPTIVE WHITE RHINOCEROS

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Papillomaviruses induce hyperplastic and tumoral lesions in different animal species⁴. We describe a case of Papillomavirus-Associated adenocarcinoma which occurred in a 41-year-old male white rhinoceros, maintained at the Zoological Garden of Pistoia, Tuscany^{3,5}.

The rhinoceros showed weight loss and difficulty in chewing the hay for three months before death². At necroscopy, the most relevant finding was a lesion that involved the tongue, characterized by erosion of the mucosa with increased involvement of the dorsal surface. The cut surface showed lardaceous appearance with escape of purulent material.

Histologically, the mass predominantly involved the submucosa and muscular portion of the tongue, resulting in closely packed large, medium, and small glands consisted of columnar cells without mucous cells. Tumor cells exhibited amphophilic to pale eosinophilic cytoplasm, high nucleo-cytoplasmic ratio and medium mitotic rate. No squamous differentiation was noted, and the squamous epithelium immediately adjacent to the adenocarcinoma showed slight perinuclear halos suggestive of PV-related changes.

The tumor cells showed diffuse staining for A1-A3 pan-keratins, but staining for keratin 7 (CK7) clearly separated the adenocarcinoma from the adjacent non-neoplastic squamous epithelium. Keratin 19, keratin 20 and MUC-5AC were negative. Immunohistochemical staining performed with a monoclonal antibody against papillomaviruses evidenced a strong nuclear immunoreactivity only in glandular epithelium similarly to CK7. DNA extracted from paraffin-embedded tissue was tested by PCR using degenerated primers (FAP 59-64), amplifying common gene region of papillomaviral L1. The PCR result was an amplicon of 480 bp. Sequence similarity analysis with the BLAST tool of the National Center for Biotechnology Information and Papillomavirus Episteme tool showed that this fragments belongs to new putative PVs, not yet characterized for rhinoceros.

To our knowledge this is the first case of Papillomavirus-Associated non-salivary gland-type adenocarcinoma arising in the base of the tongue in a captive white rhinoceros.

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ONCOLOGIA ANIMALI ESOTICI

PAPILLOMAVIRUS, WHITE RHINOCEROS, ADENOCARCINOMA

A CASE OF SYSTEMIC CORONAVIRUS-ASSOCIATED DISEASE IN A DOMESTIC FERRET IN ITALY.**Petrini D.^[1], Rondena M.^[2], Binanti D.*^[3]**^[1]*Libero professionista ~ Pisa*, ^[2]*Libero professionista, patologo veterinario ~ Padova*, ^[3]*Libero professionista, patologo veterinario ~ Ameglia*

Since 2006 a Coronavirus-associated visceral disease with clinicopathologic features resembling the “dry form” of Feline Infectious Peritonitis, has been recognized in ferrets (1,2). Confirmed cases have been reported in Spain, USA, UK and Japan (3,4,5). The present work describe the first confirmed case of Systemic Coronavirus-associated disease in a domestic ferret in Italy, with description of clinicopathologic findings.

An 8 months-old, entire male ferret (*Mustela putorius furo*) was referred for weakness and coughing. Clinical examinations showed fever, enlarged retropharyngeal lymph nodes and heart murmur. Hemato-biochemical analysis demonstrated anemia, leucocytosis, hyperproteinemia and hyperglobulinaemia (polyclonal gammopathy). Imaging revealed generalized lymphadenomegaly, severe splenomegaly and multifocal nodular renal lesions. Kidney cytological smears were compatible with granulomatous disease. Histology from spleen, mesenteric lymph node and kidney biopsies was consistent with pyogranulomatous inflammation. Due to deterioration of the condition, the ferret was euthanized five months after the first presentation and post-mortem examination revealed disseminated nodular lesions, mainly localized in kidney, spleen, mesenteric and mediastinal lymph nodes, diaphragm and lung. Histology confirmed a systemic pyogranulomatous disease. Immunohistochemistry was performed using anti-FCoV monoclonal antibody and positive staining for Coronavirus antigen was detected in the cytoplasm of macrophages in the pyogranulomas, providing a definitive diagnosis of ferret systemic coronavirus-associated disease. In conclusion ferret systemic Coronavirus-associated disease should be considered in the differential diagnosis of young ferrets presenting with enlarged lymph nodes, hyperproteinemia and hyperglobulinaemia and histology and immunohistochemistry represents the gold standard for a definitive diagnosis.

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Patologia del furetto

Ferret, Coronavirus, granulomatous diseases

COMPARISON OF DIFFERENT PROCEDURES TO ISOLATE FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) FROM SMALL VOLUMES OF BLOOD

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Studies on leukocytes isolated from feline blood do not provide details on the performances of isolation techniques.¹ From the few numerical data available, however, it can be assumed that the purity of isolated cell populations is high but their recovery rate is low.² Therefore, large volumes of blood (difficult to collect from cats with spontaneous diseases) are required to obtain enough leukocytes for in vitro studies. The aim of this study was to assess the performances of isolation techniques on small volumes of feline blood.

Blood samples (1 to 5 mls) were drawn from clinically healthy cats and placed in EDTA-coated tubes. Fifteen session of tests (10 using Ficoll, 5 using Percoll) were performed. In 9 cases cells were further separated by adherence on Petri dishes (PD) and in 5 cases using iron-labelled monoclonal antibodies against leukocyte antigens followed by magnetic sorting (MS). Cell purity (i.e. the percentage of each population) and recovery (i.e. the percentage of cells of each population recorded after isolation compared with blood) were then calculated.

The purity of lymphocytes was significantly higher ($P=0.015$) with Ficoll (79.6 ± 3.3) than with Percoll (61.0 ± 12.0); the purity of monocytes was low, and significantly higher ($P=0.015$) with Percoll (32.7 ± 13.5) than with Ficoll (9.0 ± 1.6). The recovery rate of lymphocytes was low, and significantly higher ($P<0.001$) with Ficoll (54.7 ± 27.5) than with Percoll (12.0 ± 4.2). The recovery rates of monocytes recorded with Ficoll (59.4 ± 34.7) or Percoll (38.5 ± 9.38) were not significantly different. The purity of cell types in PD was not morphologically determinable. However, assuming that at least 90% of adherent and non adherent cells were monocytes and lymphocytes, respectively, the recovery rate for both the populations was lower than 10% in most cats. The purity after MS was generally high, but the recovery rate was variable and the number of yielded cells very low.

In conclusion, none of the techniques applied in this study provides good performances in terms of number of cells, purity and recovery rate, when applied to small volumes of blood. This suggest that the isolated cells could not be representative of the population in blood, and limits the use of these 3 techniques in cats with spontaneous diseases. Therefore, cell functions in spontaneous feline disease should be investigated in whole blood rather than on isolated cell populations.

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Veterinary clinical pathology
Leukocyte isolation, Feline, in vitro studies

COMPREHENSIVE MANAGEMENT OF A PITUITARY ADENOMA WITH MALIGNANT FEATURES IN A MALTESE DOG

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The aim of the study is to describe the long-term survival of a dog affected by an ACTH producing-pituitary adenoma treated by surgery, medication and radiation therapy.

A 7-yr-old old male Maltese dog was referred to DIMEVET for epileptic seizures, polyuria, polydipsia, polyphagia and symmetrical alopecia. Pituitary-dependent hypercortisolism was diagnosed by endocrine tests. Computed tomography (CT) revealed a pituitary mass with a P/B ratio 0.75, reference <0.311 that was treated with transsphenoidal hypophysectomy; Immunohistochemistry of the surgical specimen confirmed an ACTH-producing adenoma. Hypercortisolism went into remission for three years but then polyuria/polydipsia and alopecia reappeared and CT scan revealed a small sellar abnormal structure (interpreted as pituitary tissue regrowth). Hypercortisolism was confirmed by endocrine tests and medical treatment with trilostane was started, with good control of the clinical signs for one year, until the occurrence of obtundation, seizures and stupor. CT scan showed a sellar mass and radiation therapy was performed with a protocol of 20 fractions of 2.25 Gy. Neurological signs regressed and trilostane treatment was continued with good control of the disease. Six years after the first admission, the dog developed lumbosacral pain and inability to walk and was euthanatized for animal welfare reasons. Histological sections were stained with H&E and PAS and immunostained with antibodies against ACTH, MSH, GH, C-erb-B2 and Ki67.

Macroscopically the formalin fixed brain revealed grey tissue in the pituitary region extending caudally to the mesencephalon. Histologically, unencapsulated neoplastic tissue in contiguity with the meninges and infiltrating the neuropil was detected; neoplastic cells arranged in islands and cords were embedded in a rich amorphous eosinophilic extracellular matrix; neoplastic cells showed an intensely eosinophilic cytoplasm. The nucleus was vesicular, often two or three nuclei were seen, with chromatin margination and a prominent nucleolus. Anisocytosis and anisokaryosis were moderate. Mitotic figures were three in ten random selected fields at 400x magnification. A diagnosis of a recurring infiltrative ACTH-adenoma was made; Ki67 labelling index was 4,8%.

Pituitary tumors that recur or progress despite resection and radiotherapy are often termed “atypical” adenomas, as they do not appear overtly malignant by histological criteria, but exhibit aggressive biologic behavior, have a Ki67 labelling index >3%, p53 immunoreactivity.² Reports of invasive canine pituitary tumors are rare; this is the first report of a long-term survival of a dog affected by this type of tumor that was treated by surgery and subsequently by radiation therapy. Comprehensive management of pituitary adenomas using the various treatment modalities may significantly prolong the dog’s life.

1Kooistra et al., 1997; 2Mamelak et al., 2011.

neuropathology, endocrine pathology

pituitary adenoma, dog, survival

COORDINATED IMMUNE RESPONSE OF MEMORY AND CYTOTOXIC T CELLS TOGETHER WITH IFN- γ SECRETING CELLS AFTER PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) NATURAL INFECTION IN CONVENTIONAL PIGS.

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection in the field usually dampens the pig immune system both as innate and acquired immunity. The extent of immune dysregulation and/or depression depends on the virulence of the PRRSV isolate, the intrinsic ability to interact with the immune system and on the age of the animals. (1)

The present study aims at evaluating the antibody and cellular immune response in pigs naturally infected by PRRSV in order to highlight the immune modulation.

Twenty conventional pigs were selected from a herd with a history of PRRSV infection and monitored for 22 weeks from weaning (4 weeks of age) through the fattening phase (up to 26 weeks of age). The pigs were divided in two groups: a group was naturally exposed to PRRSV infection (N=10, PRRSV-exposed) by comingling with infected resident animals and the unexposed group was used as control (N=10, C).

Blood samples were collected 2 weeks apart and PRRSV infection was detected by quantitative PCR in serum. The humoral immune response was quantified as total serum PRRSV-specific antibodies by ELISA while the cellular response was characterized by flow cytometry and IFN- γ ELISPOT to enumerate circulating T cell subsets and PRRSV-specific IFN- γ secreting cells (SC) in PBMC. (2, 3) In this study, the distribution of cells involved in cell mediated response and IFN- γ producing cells were investigated through innovative methods (flow Cytometry and ELISpot assay) in pigs naturally infected by the PRRSV through exposure to infected animals. Clinical signs were recorded throughout the study.

The results showed that all exposed pigs became infected at 16 weeks of age and viremia lasted until 20 weeks in almost 50% of the exposed animals, whereas the C group remained negative. The PRRSV-exposed group developed an antibody response since 18 weeks of age.

In infected pigs, total CD4⁺ and CD8 α ⁺ T cells increased from 18 weeks onwards, due to a significant increase of cytotoxic T CD8 β ⁺ and memory T helper CD4⁺CD8 α ⁺low lymphocytes. An early and transient increase was observed for naïve T helper CD4⁺CD8 α ⁻ cells. Also virus-specific IFN- γ SC were significantly recalled from 18 weeks, peaking at 22 weeks. Control animals showed non-significant fluctuations in cell percentages and negligible SC levels. In this study, the IFN- γ SC response was strongly induced in parallel with the positive modulation of cytotoxic and memory T cells suggesting the potential activation of these subsets to secrete the immune cytokine.

This approach demonstrated a strong IFN- γ response but also some peculiar aspect of the immune response, likely depending on the delayed infection time of animals exposed to the virus. Overall, taking into account that PRRSV infection was more delayed compared to what is generally observed in the field, the age of pigs may have favoured a more pronounced immune response.

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Patologia generale

PRRSV, PIG, IMMUNITY

CUTANEOUS NEOSPOROSIS IN A GOLDEN RETRIEVER

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This report describes cutaneous cytological and histological lesions caused by *Neospora caninum*.

A 10 years old, intact female Golden Retriever under treatment with cyclosporine for an autoimmune disorder had sudden development of multifocal dorsal cutaneous nodules. Skin cytological specimens obtained by imprint and fine-needle aspiration were stained with May-Grünwald Giemsa. Skin punch biopsy specimens were obtained. Biopsies were fixed in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin. Unstained smears and deparaffinized sections of skin were immunochemically stained with polyclonal anti-*Toxoplasma gondii* and anti-*Neospora caninum* primary antibodies.

Cytology demonstrated a prevalence of degenerated neutrophils admixed with fewer macrophages, rare neutrophils associated with adipocytes and fibroblasts. On the background and within macrophages numerous crescent shaped, 4-6 µm microorganisms, with a light basophilic cytoplasm and a central nucleus (tachyzoites) were visible. Histology revealed diffuse and severe neutrophilic, histiocytic, eosinophilic dermatitis and panniculitis associated with necrotizing vasculitis. Elevated numbers of free and cytoplasmic tachyzoites within macrophages and keratinocytes of the epidermis and follicular infundibula were present. Immunocytochemistry and immunohistochemistry warranted a diagnosis of cutaneous Neosporosis (CN).

Clindamycin administration (11 mg/kg PO every 12 hours) and withdrawal of immunosuppressive medication resulted in prolonged clinical remission.

Cutaneous nodules are a rare manifestation of Neosporosis.¹⁻⁴ Age-related immunodeficiency and immunosuppressive therapies seem to predispose to CN.¹⁻⁴ Information on cause and prognosis are fragmentary in this instance. The current dog was alive 1 month after diagnosis.

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DERMATOPATOLOGIA

Dog, *Neospora caninum*, Skin

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON SOME HAEMATOLOGICAL PARAMETERS IN COWS.

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The aim of this study was to evaluate physiopathological responses to different environmental conditions (ambient temperature, relative humidity and temperature-humidity index) on haematological parameters. Blood samples were collected at 4 time points under different environmental conditions (T1, T2, T3 and T4) by 43 Piemontese cows aged 2–12 years and analysed for haematological parameters. For each period, ambient temperature and relative humidity were recorded by means of a data logger and the temperature-humidity index (THI) was calculated as indicator of thermal comfort for cattle. Data were then measured by one way analysis of variance (ANOVA).

The obtained results showed a statistical significant effect of time on the following parameters: RBC ($P<0.0001$), Hb ($P<0.0001$), Hct ($P<0.0001$), MCV ($P<0.0001$), MCH ($P<0.0001$), MCHC ($P<0.0001$), Plt ($P<0.0001$), WBC ($P<0.0001$), neutrophils ($P<0.0001$), lymphocytes ($P<0.0001$), monocytes ($P<0.0001$) and eosinophils ($P<0.0001$).

The majority of haematological values obtained in the present study, even though within the physiological range for cattle, showed that variations in haematological parameters are related to changes in ambient temperature, relative humidity and temperature-humidity index. These results provide insight into the physiological responses of Piemontese cow to different environmental conditions, allowing to better evaluate its ability to adapt and cope with environmental stress (1-3).

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Patologia Clinica

cows, haematology, environment

EFFECTIVENESS OF ENDOMETRIAL CYTOLOGY OBTAINED BY LOW-VOLUME UTERINE FLUSH TECHNIQUE IN POSTPARTUM ENDOMETRITIS OF DAIRY COWS

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Aim of this study was to evaluate the effectiveness of endometrial cytology obtained by low-volume uterine flush technique to identify endometritis in postpartum period of dairy cows, correlating data with bacteriological examination and clinical findings.

Endometritis, responsible of great economical losses in dairy industry, is difficult to diagnose, due to the lack of gold standard criteria accepted by practitioners (1). Subclinical endometritis (SEND), defined as uterine inflammation in the absence of clinical signs, is frequently associated with significant reduction of reproductive performance and often diagnosed through endometrial cytology (2).

The study was performed in 40 multiparous Holsteins. From October to December 2013, cows underwent clinical evaluations from the day of parturition until 40 days postpartum. Intrauterine samples were collected between 30 and 40 d postpartum by infusing 100-150 ml of sodium chloride 0.9% sterile solution and then recovering the fluid; samples were delivered to the laboratory within 12 hours. After centrifugation of the fluid, an aliquot was used for bacteriological examinations and another for cytology. According to the published literature, a cut off of 10% of polymorphonuclear leucocytes (PMNs), was used to identify cows with endometritis (3).

Between 30 and 40 d postpartum, all cows were flushed and examined: 17 cows (42%) showed a positive cytology; 6 of these cows were diagnosed with a clinical endometritis, whereas the remaining 11 were clinically healthy (SEND cows). Of the 23 remaining cows with a negative cytology (58%), 20 were healthy, whereas 3 cows showed clinical endometritis (false negative). Bacteriology allowed the isolation of several bovine uterine pathogens both in clinical endometritis cases and in clinically healthy cows. Six different groups of bacteria were isolated such as *Trueperella pyogenes* (n=6), *Escherichia coli* (n=5), *Enterococcus faecalis* (n=7), coagulase-negative staphylococci (n=8), *Bacillus* spp. (n=1), *Pseudomonas* spp. (n=3) and *Enterobacter agglomerans* (n=1). Bacteriology was negative in 9 cows.

Low-volume uterine flush cytology between 30 and 40 days postpartum has proved to be a useful technique to reveal SEND especially in cases associated with bacteria considered only potentially pathogenic for the uterus, such as *E. faecalis* or *B. licheniformis*. However, we underline the limits of the technique that can lead to obtain false negative. The results of our study emphasize the need for integration of the two diagnostic tools, namely cytology and bacteriology.

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Patologia della riproduzione

endometrial cytology, cow, uterine bacteriology

ERYSIPELOTRIX RHUSIOPATHIAE ENDOCARDITIS IN A SHEEP

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Erysipelotrix rhusiopathiae is a pathogen of a wide variety of animals. Chronic polyarthritis are reported in sheep and lambs. The authors describe one systemic erysipelas infection in an adult sheep in Sicily.

A female, cross-breed, 18 months old sheep showing clinical signs of phosphate esters suspected poisoning, was sent for diagnostic investigations to the Department of Veterinary Sciences of Messina University. A complete necropsy was made and samples of affected organs were partially fixed in 10% neutral buffered formalin to perform histological investigations and partially stored at -80°C for biomolecular researches. Total DNA was extracted from heart tissue and employed in PCR test targeted to the 23S ribosomal region. The DNA sequences were amplified by primers annealing at 23S of ribosomal genes as reported in the literature (Takeshi et al., 1999). PCR products were sequenced and the obtained data were analyzed by Wu Blast 2 sequence alignment software for strain identification.

At necropsy abundant foamy exudates in the trachea and bronchi and pleural hemorrhages were observed. Moreover, hypertrophy of the left heart showing multiple grayish white foci were present. The mitral valve showed a severe acute endocarditis characterized by irregular friable vegetations. In the kidney a voluminous necrotic area with hemorrhagic limits between cortical and medullary was detected. Histology confirmed the presence of multiple myocardial and renal septic infarcts. The flaps of the mitral valve were fibrotic, infiltrated by polymorphonuclear cells, with adherent multiple thrombi. DNA extracted and the sequence analysis showed a complete overlapping with the 23S rDNA of Erysipelothrix rhusiopathiae sequence.

Erysipelothrix rhusiopathiae is a bacterium commonly isolated in swine. In author's opinion this report describes an unusual cardiac localization in sheep previously only rarely described (Chineme et al., 1973; MacLachlan, 1978). The occurrence of this unusual localization imposes the necessity do not neglect common lesions using always regularly laboratory investigations.

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Systemic erysipelas infection

Sheep, E. rhusiopathiae, endocarditis

GLIOBLASTOMA IN AN EWE

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Tumors of neuroepithelial origin (gliomas) include astrocytomas, oligodendrogliomas, and ependymomas. In veterinary medicine, primary neoplasms of the central nervous system (CNS) are most frequently reported in dogs and less commonly in cats, whereas the same tumors are considered distinctly uncommon within the other domestic species [1].

The present report aims at describing the pathological and immunohistochemical findings observed in a Sarda breed sheep with glioblastoma.

A 6 years old Sarda breed sheep was euthanized and necropsied after showing severe and worsening neurological signs, which started 8 months before and mainly consisted of depression and head pressing. At necropsy a wide range of tissues were sampled, promptly fixed in 10% neutral buffered formalin and routinely processed for histopathological investigations (hematoxylin and eosin stain). Furthermore, selected tissue sections of the cerebral mass were subject to immunohistochemistry (IHC) using the following primary antibodies: anti-glial fibrillary acidic protein, anti-vimentin, anti-synaptophysin and olig2.

At necropsy, a large (about 5 cm in diameter) whitish to grayish mass was seen partially replacing the left cerebral hemisphere.

Microscopically, the tumor consisted of polymorphic cells, with a high number of mitotic figures and large areas of necrosis and dystrophic calcification surrounded by pseudo-palisading of cells. The neoplasm was highly vascular with glomeruloid vascular proliferation.

Foci of "oligodendroglioma-like" differentiation and few multinucleated giant cells were also occasionally seen. Mitotic figures were very frequent, up to 5 mitoses per high magnification power field (Ob. x40), and often atypical. At IHC, neoplastic cells proved to be immunoreactive (IR) for vimentin and olig2 but constantly negative for synaptophysin. Only few scattered neoplastic cells were GFAP-IR. However, IHC for GFAP demonstrated the presence of few reactive astrocytes, mainly surrounding the foci of necrosis.

Few cases of primary brain tumors have been described in sheep: medulloblastoma in lambs [2], a case of ependymoma in a Suffolk sheep aged less than 1 year [3], and more recently a case of oligodendroglioma in a 1 year old male Iranian fat-tailed sheep [4]. To the best of our knowledge, the present is the first report of glioblastoma in sheep. Even in sheep, glioblastoma is similar to anaplastic astrocytoma with the additional features of necrosis and vascular proliferation and share many of the histopathological and immunohistochemical features observed in dogs [5].

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PATHOLOGY

Glioblastoma, Sheep, Immunohistochemistry

GLYCOGEN RICH CARCINOMA OF CANINE MALE AND FEMALE MAMMARY GLAND

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Glycogen rich clear cell carcinoma (GRCC) is a rare subtype of human invasive mammary gland (MG) carcinoma, in which at least 90% of the neoplastic cells have clear cytoplasm containing glycogen(1). The aim of this study was to describe the histological, histochemical and immunohistochemical (IHC) features of GRCC of canine MG.

Serial formalin fixed paraffin embedded tissue sections of two GRCC canine mammary carcinoma, one female (case I) and one male (case II) were stained with H&E, PAS, PAS diastase (dPAS), and Alcian Blue (AB). IHC was performed with anti-ER, -PR, -cerbB2, -CK19, -CK14, -CK5/6, -p63, -vimentin, -SMA, -calponin, -S100, -EGFR, -c-KIT, -E-cad and -ki67 antibodies. Sudan III was carried out on formalin-fixed frozen tissue in case II.

Histologically, 90% of neoplastic cells showed sharply distinct borders, clear or finely granular cytoplasm and low N:C ratio. In case II, residual 10% of the neoplastic cells had lipid-like vacuolated cytoplasm. In both tumors, the cytoplasm resulted strongly positive with PAS. Treatment with diastase abolished PAS reactivity. Case I showed PAS+ and dPAS- staining also in lymph node metastasis. In case II, 10% of neoplastic cells were positive to Sudan III. No stain with AB was obtained. Case I showed positivity for CK19 and CK5/6, negativity for ER, PR and c-erbB2, resulting a basal-like phenotype in primary tumor and lymph node metastasis. They were both positive for EGFR, E-cad, c-KIT, and weakly for calponin. Case II was a basal-like phenotype, presenting CK 19, E-cad, c-KIT, weak CK14 and strong vimentin positivity. The proliferative ki67 index was 26.75% in case I and 8.2% in case II.

Based on the morphology, typical features of human MG GRCC are the “fried eggs appearance”, clear cytoplasm and small dark punctate nuclei (2). On the best of our knowledge, this is the first report regarding GRCC in canine MG. The diagnosis was confirmed by PAS+ of intracytoplasmic glycogen granules and lack of stain with dPAS. Case II was considered a GRCC with lipid rich differentiation. Both cases had a basal-like phenotype and the expression of EGFR and c-KIT was suggestive of an association of cell proliferation with signal transduction of surface molecules (3). GRCC can be considered a new rare histological subtype of canine mammary tumors, with clear cytoplasm, PAS+ and dPAS-, expressing the triple negative phenotype, a tumor with clinical aggressive behavior that should be differentiated from lipid rich carcinoma.

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ANATOMIA PATOLOGICA

Canine, Mammary carcinoma, Glycogen rich

GRANULOMATOUS DERMATITIS OF THE AURICULAR PINNA IN A HEIFER

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a unique case of granulomatous dermatitis.

A 2 y.o. Italian Friesian heifer showed a papillomatous-like lesion at the edge of the right auricular pinna. The lesion sized 9 cm x 11.5 cm, was exophytic, globular in shape, firm and with the outer surface uneven, depigmented and ulcerated. Cytological examination revealed the presence of an inflammatory population mainly composed by neutrophils and macrophages. On the basis of the cytological observation, the mass was excised and subjected both to histological staining (H&E, PAS, Gram) and molecular biology examination (PCR).

The histological examination allowed the diagnosis of a pyogranulomatous inflammation characterized by a epithelioid and giant cells, neutrophilic and macrophagic infiltrate organized around focal areas of eosinophilic homogeneous material consistent with the Splendore-Hoeppli phenomenon. PAS staining highlighted numerous coccoid formations within the piogranulomatous reaction or freely standing in the tissue. Gram stain revealed Gram-positive bacterial colonies confirmed by PCR as *Corynebacterium mucifaciens*.

Granulomatous dermatitis are caused by agents against whom the body is sensitized and react through an immunomediated response(1). Sometimes the above reactions are histologically characterized by the Splendore-Hoeppli phenomenon that may represent the deposition of antigen-antibody complexes (immunoglobulins and major basic proteins) and debris from the host inflammatory cells(2). The lesion described in this work seemed worth of description because of the etiologic agent. In fact, *Corynebacterium mucifaciens* is a newly-described species belonging to the the largest genus in the group of coryneform bacteria(3). Moreover, the seat of development was extremely atypical for the bovine species. *Corynebacterium mucifaciens* is better differentiated from closely-related species by molecular biology techniques, such as sequencing of the 16S rRNA gene and is isolated from human blood or other normally-sterile body fluids, often considered as part of the normal skin flora or contaminants. To date, literature data report the occurrence of disease due to *Corynebacterium mucifaciens* only in humans where it has been related to severe infections, lethal bacteremia (4), cavitary pneumonia (5), corneal ulcer (6), otitis and nasal polyps (7). To the author's knowledge, this is the first report of a lesion induced by *C. mucifaciens* in veterinary medicine.

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Patologia Veterinaria

C. mucifaciens, Skin granulomas, Splendore-Hoeppli

GRANULOMATOUS MIOSITIS DUE TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN A HORSE

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a case of granulomatous myositis in a horse.

A 12 years old Quarter horse mare was evaluated because of the presence of different subcutaneous nodules and masses. The lesions were cytologically diagnosed as pyogranulomas and were resistant to the given pharmacological treatment. The persistence of the lesions along with the deterioration of the clinical status suggested to surgically remove three of them. Macroscopically they were fixed to the muscles, painless and firm in consistency. On cut section, they showed a purulent exudate. Tissue samples from the lesions were fixed in 10% buffered formalin and paraffin wax embedded. Histological sections were stained with H&E, PAS, Masson trichrome, Grocott and Gram. Molecular biology assay (PCR) was performed too. Histological examination revealed, in all samples, a diffuse mixed inflammatory infiltrate characterised by polymorphonuclear granulocytes (neutrophils and mainly eosinophils), macrophages, lymphocytes, plasma cells, epithelioid and multinucleated giant cells invading the endomysium. Different sized round foci of caseous necrosis with calcification and areas of collagenolytic degeneration were present in all sections. PAS and Grocott stains didn't show fungi whereas Gram stain revealed the presence of blue pleomorphic Gram + microorganisms free or inside macrophages.

The described results indicated a severe diffuse granulomatous myositis. PCR analysis revealed the etiologic agent as *Corynebacterium pseudotuberculosis*.

Corynebacterium pseudotuberculosis infection occurs world wide as caseous lymphadenitis in small ruminants and granulomatous infection in horse and cattle.

The bacterium can survive for extended periods in the environment and in the soil. Disease transmission is thought to occur thorough a contaminated environment by direct contact between animals and insects such as house flies (*Musca domestica*), stable flies (*Stomoxys calcitrans*), and other arthropods serving as mechanical vectors (1). Clinically, the infection in horses most commonly causes external abscesses or "pigeon fever" (90% of cases), however, internal abscesses (8%) or ulcerative lymphangitis (1%) may also occur (2).

In our case, none of the known pathogenetic pathways reported in the literature seems to be completely comparable being some aspects common to the so called pigeon fever and others to the chronic form of ulcerative lymphangitis. The presented case is, therefore, very rare and interesting for the pathological findings and, overall, from the epidemiological point of view.

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Patologia Veterinaria

horse, muscle granulomas, pigeon fever

HISTOLOGICAL ASPECTS OF AN UNUSUAL COLONIC DUPLICATION CYST IN A CONSTIPATED CAT

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In animals as in humans the main anomalies of canalization of enterocolic tract are represented by the persistence of Meckel's diverticulum, intestinal duplication cyst, vitelline or omphalic duct cyst or finally, diverticula losing a direct connection with the colonic lumen^{1,3}. In these condition histology may be characterized by the indistinguishable mucosal type between normal and accessories tract, or by the presence of metaplastic epithelial types. We describe a case of 5-year-old domestic short-haired spayed female cat presenting abdominal pain and suspended defecation, in which abdominal ultrasonography revealed the presence of an oval formation connected to the colon, whose wall was similar to the colonic one. Ultrasonographically the content appeared inhomogeneous. Surgery and related histopathology allowed a most likely diagnosis of a colonic duplication cyst.

For histology analysis, samples were routinely processed and stained with hematoxylin-eosin and Alcian-PAS using different types of mucins, then characterized also immunohistochemically.

Mucosal histology and histochemistry revealed particular heterotopic tissues represented by some spotted areas of gastric metaplasia, antral/pyloric in type, and a very particular area of mucosa that resemble respiratory mucosa in type. No heterotopic pancreatic tissue, Brunner glands or biliary epithelium, frequently reported in other cases¹, were observed in this cat. Two nonreactive lymphoid aggregates were observed in the lamina propria of the cyst, in absence of elements evidencing inflammation and/or subsequent bacterial superinfection of the cystic mucosa. After surgery the cat returned to defecate normally.

Duplication cyst is an uncommon congenital abnormality of the alimentary tract. Most often the patients are asymptomatic and colonic duplication cysts remain undiagnosed for years. In this case report we present a fourth description of intestinal duplication cyst case in a cat^{1, 2, 3, 4} with a descending colon location and a mucosal characterization by histology/immunohistochemistry. The importance of description of this rare congenital malformation is finalized to the inclusion in the differential diagnosis of cystic masses of the gastrointestinal tract.

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General and Special Pathology

disontogenic cyst , histochemistry, immunohistochemistry

HSP32 AND HSP90 IMMUNOEXPRESSION, IN RELATION TO GRADING AND OTHER HISTOPATHOLOGICAL FEATURES, IN CANINE CUTANEOUS MAST CELL TUMOURS

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Literature data indicate Hsp32 and Hsp90 as interesting molecular targets in canine neoplastic mast cells. However, their immunoexpression patterns in canine mast cell tumours (MCT) have not been investigated so far. Thus, the aim of the present study was to evaluate the immunohistochemical expression of Hsp32 and Hsp90 in 22 samples of canine cutaneous MCT, in relation to histological grade and other pathological variables, such as absence/presence of epidermal ulceration or tumour necrosis, growth pattern and mitotic index

Grading was established on the basis of the systems proposed by Patnaik et al. (1984) and Kiupel et al. (2011) as follows: 13 grade II (10 low-grade and 3 high-grade) MCT and 9 grade III (all high-grade) MCT. A semi-quantitative method was used to analyse immunoreactivity. Fisher's exact test and Cramer's V were used to evaluate the associations between the examined parameters.

Hsp32 showed a variably intense and distributed cytoplasmic immunostaining, not associated with histological grade. However, the low Hsp32 immunoexpression (<50% of positive cells) detected in the majority of grade III/high-grade MCT samples suggests the need for further investigating the efficacy of the pharmacologic Hsp32-inhibitors on aggressive canine MCT. Reduced Hsp32 immunoreactivity was associated with presence of tumour necrosis ($p=0.035$; Cramer's $V=0.542$), a finding in agreement with the pro-survival functions of Hsp32 in neoplastic canine MC.

Hsp90 cytoplasmic immunosignal was variably associated with nuclear and/or membranous staining. Proportion of Hsp90 immunoexpression was not associated with histopathological features and grade.

Noteworthy was the detection of a membranous expression of Hsp90, in light of its molecular chaperone functions towards the receptor tyrosine kinase Kit. Further studies are currently underway in order to investigate the possible relationship between Hsp90 and Kit immunoexpressions in canine MCT.

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disciplinare

HSPs, Mast cell tumor, Dog

IDENTIFICATION OF REGULATORY T CELLS IN CANINE MAMMARY GLAND TUMORS

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In the present study we evaluated foxp3 expression in benign and malignant canine mammary gland tumors by immunohistochemistry and we investigated its prognostic significance. Foxp3 is a member of the forkhead /widge-helix family of transcription regulators involved in regulating immune system development and function (2). This gene plays a crucial role in the generation of CD4+/CD25+ regulatory T cells. Tregs seem to enable tumors to elude host antitumoral immune response (1, 3).

Animals: a total of 33 samples of canine mammary gland tumors were selected from the archives of Veterinary Pathology of the Department of Veterinary Medical Sciences over a period between 2006 and 2013. 15 neoplasia were diagnosed as benign and 18 as malignant, based on the WHO (1999) classification. Among the benign tumors 10 were complex adenomas, 3 papillar adenomas and 1 mixed benign tumors. Among the malignant tumors 5 were solid carcinomas, 3 complex carcinomas, 9 tubulo-papillar carcinomas and 1 in situ carcinoma.

All the samples were immunohistochemically tested with an anti Foxp3 primary antibody (purified Anti-Mouse/Rat Foxp3 clone FJK - 16s of the firm eBioscience), at a 1:200 dilution in PBS overnight 4°C.

Each slide was evaluated microscopically on 5, 20X fields and all the positive cells were counted in peritumoral and intratumoral infiltrates. Benign tumors showed fewer infiltrates than malignant neoplasias. Foxp3 expression was observed in 9 benign tumors out of 15. In 6 complex adenomas the number of tregs observed was between 1 and 12, only one showed more than 13 tregs, 2 papillar adenomas showed less than 12 tregs.

Foxp3 expression was observed in 14 malignant tumors out of 18. In solid carcinomas foxp3 was highly expressed (more than 40 tregs) while in complex carcinomas, expression was less (between 1 and 12) and in 3 tubulo-papillar carcinoma between 1 and 12 cells were positive and in 4 between 13 and 40. In the in situ carcinoma foxp3 was very highly expressed (more than 40)

In the present study the number of tregs was counted and compared with the histological grade of canine mammary tumors and we observed that foxp3 (tregs transcription factor) was mainly expressed in intratumoral and peritumoral infiltrating areas of neoplasias with poor prognosis.

These results suggest that the increase number of tregs lymphocytes in mammary neoplasias may play a role in tumor progression as they suppress the immune response against it, and allow the tumor progression.

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IMMUNOREGULATION

T LYMPHOCYTES, MAMMARY GLAND TUMORS, FOXP3

MOLECULAR DETECTION OF OVIS ARIES PAPILLOMAVIRUS TYPE 3 IN FORMALIN FIXED, PARAFFIN EMBEDDED (FFPE) SHEEP SQUAMOUS CELL CARCINOMA SAMPLES.

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Recently (Alberti et al., 2010) the prototype of a novel papillomavirus genus (OaPV3) was detected in normal skin and squamous cell carcinoma (SCC) lesions of Sardinian sheep. OaPV3 belongs to the Dyokappa genus and its genome significantly differs from the 2 species of ovine papillomaviruses previously reported in Australia that instead group with the artiodactyl Deltapapillomavirus species, and have been isolated from benign cutaneous lesions. Here we investigate the relevance of OaPV3 in SCC by screening a panel of FFPE sheep SCC samples

Based on the sequence of OaPV3 L1 and E6 two DNA probes and a set of 4 primers were designed and respectively used to develop an In situ Hybridisation test (ISH) and two RT-PCR assays. These assays were applied to a collection of 41 FFPE sheep SCCs samples obtained from cutaneous tumours (5 nasal, 6 ear, 10 periocular, 3 dorsal, and 17 mammary tumours).

Diagnosis of SCC was confirmed by histopathological examination of the 41 samples. Molecular tests, summarised in the table, revealed that 26 out of 41 (63%) samples were positive to at least one test. Different tests showed different sensitivities. Also, tumours localised in different parts of the sheep body seemed to show variable degrees of positivity to OaPV3.

Results demonstrate a high prevalence (63%) of OaPV3 in sheep SCCs. This level of prevalence is particularly important and comparable to the prevalence of HPVs in human SCC (50 to 69%, Meyer et al., 2000), and suggests that OaPV3 represent an important risk factor for the development of sheep SCC. The level of positivity of nasal and periocular lesions was greater respect to other tumour locations. This can be explained by the greater level of solar exposition and/or to traumas of these area respect to other locations. As expected, RT-PCR has a greater sensitivity than ISH. However, only combining these two tests the total number of positives can be detected, and both the presence/expression and localisation in the tumour can be investigated. Concluding, we cannot rule out the presence of unknown viral types in negative tissues. Further investigation is needed to investigate the presence of viral variants associated to different tumour locations and the presence of uncovered papillomaviruses in negative samples.

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Anatomia Patologica e Malattie Infettive
OaPV3, squamous carcinoma, RT-PCR and ISH

Tumour location	N° samples	N° of positive	positive to all tests	ISH positive	RT-PCR positive	ISHE6 positive	ISHL1 positive	RT-PCR positive E6	RT-PCR positive L1
Nose	5	5	2	4	5	4	4	4	3
Ear	6	6	1	4	5	4	3	4	4
Eye	10	4	0	4	4	3	2	2	2
Flank	3	2	0	2	2	1	2	1	1
Udder	18	9	1	5	8	3	2	4	6
Tot.	42	26	4	19	24	15	13	15	16

MYOGENIC REGULATORY FACTORS EXPRESSION IN MURINE C2C12 CELLS TREATED WITH 17BETA ESTRADIOL: PRELIMINARY DATA

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Myogenesis is controlled by a family of transcription factors known as Myogenic Regulatory Factors (MRFs) including Myogenic Differentiation 1 (MyoD1), Myogenic Factor 5 (Myf 5), Myogenin (Myog) and Myogenic Regulatory Factor 4 (MRF4), which are differently expressed throughout the myogenic process. MyoD1 and Myf5 are mainly involved in the satellite cells commitment into proliferating myoblasts, whereas Myog and MRF4 are expressed during middle and late differentiation stages. MRF4 is also involved in fibers maturation (Dedieu 2002, Ferri 2009). These factors are regulated by extrinsic signals such as extracellular matrix substances, growth factors and hormones (Rhoads, 2009).

It is known that estrogens control myogenesis and skeletal muscle fibers changes. However the hormones mechanism of action are not yet completely understood (Boland 2008, De Jager 2011). In this preliminary study we evaluated the 17 β estradiol (E2) influence on MRFs expression in C2C12 cells.

C2C12 cells were seeded and, at about 80% confluence, cells were switched into a 10% horse serum medium to induce differentiation.

Cells were treated 5 times with 10⁻⁵ M or 10⁻⁸ M E2 every 48 hours and were harvested every 48 hours to evaluate expression of MyoD, Myf5, Myog, MRF4 by Real-time PCR and Western Blot. The evaluation of Myosin heavy chain I (MHCI) gene expression was carried out to confirm myotubes formation. Data were properly analyzed using GraphPad InStat (vers. 3.05).

No difference in myoblasts MRFs gene expression was revealed, while a difference in MRF4 gene expression was detected between treated myotubes and controls culture. In particular, 10⁻⁸ M E2 administration induced a greater MRF4 expression in myotubes than in untreated culture and 10⁻⁵ M E2 administration. Western Blot analysis confirmed these data.

The study confirmed that MRFs expression varie throughout the myogenic process, as previously described by Dedieu, 2002 and Ferri, 2009. In particular MRF4 expression, which is physiologically up-regulated during the differentiation phase, is further increased on myotubes by E2 administration.

Results confirm the over expression of MRF4, already described in adult bovine skeletal muscle cultures treated with E2 (Divari, 2013), and could help to understand the estrogen pathway regulation in muscle hypertrophy

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Pathology

myogenic factors, estrogen, mouse cell culture

NECROSCOPIC FINDINGS ASSOCIATED WITH EARLY REPLACEMENT IN BREEDING DOES

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In commercial rabbit farms, mortality and culling are of great relevance from the financial viewpoint.¹ Lesions in breeding rabbits are recorded mainly on animals at the end of their reproductive life and the replacement is usually performed based on reproductive parameters without further diagnostic investigations. The aim of this study was to assess the main necroscopic lesions in does replaced from reproduction and not slaughtered.

Sixty-seven dead does were collected from a commercial rabbit farm (Centro genetico Martini s.p.a., S. Maria Codifiume, FE) housing 5000 does, and submitted for postmortem examination. During necropsy, samples for histological and microbiological examinations were also collected. Information about age, number of deliveries and number of mating were recorded. To evaluate the effect of age, does were arbitrarily divided into two categories based on the median of number of deliveries (if available).

The age of does ranged from 115 to 587 days (median 286), the number of deliveries ranged from 0 to 13 (median 2). On postmortem examination, respiratory and genital tracts were the most frequently affected (n = 40; 59.7% each), the digestive system was involved in 30 does (44.77%) and other concomitant lesions, such as traumatic fractures, abdominal effusion, splenomegaly or cardiac injuries were seen in 36 cases (53.73%). Presence of pododermatitis was also observed in 42 animals (62.69%). Genital lesions included 16 uterine torsion (40%), 12 endometritis (30%), 12 uterine retention (30%) and others, such as dystocia or rupture (n= 6; 15%). Uterine torsion was significantly more frequent in does with more than 2 deliveries (P< 0.05). No other significant differences were observed in the 2 sets of animals grouped according to the number of deliveries. Bacteriological examinations performed on randomly selected pleural effusions were positive for *Pasteurella* spp. (n = 2); *Staphylococcus* spp. were isolated from endometritis (n = 2), macerated fetus (n = 1) and pododermatitis (n = 1).

Mortality in does was highest during the first two deliveries, similar results of highest mortality risks in the first three deliveries are reported also in literature.² Respiratory and genital lesions were the most relevant, in contrast with other authors,² who reported a higher prevalence of respiratory and digestive pathologies. Moreover, in our study, uterine torsion resulted the most important lesion associated with increased number of deliveries, although the related pathogenesis is still unclear.

1 Gómez EA (2006): Producción industrial de gazapos: algun puntos críticos. Boletín de cunicultura 145:12-23.

2 Rossel JM & de la Fuente LF (2009): Culling and mortality in breeding rabbits. Preventive Veterinary Medicine, 88:120-127.

Pathology

Necropsy, Rabbit reproduction, Rabbit Pathology

PARASITIC DISEASES IN MEDITERRANEAN JOHN DORY (ZEUS FABER)

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The aim of the present paper was to identify, describe and compare tissue changes due to parasites occasionally found in different subjects belonging to a wild teleost species, *Zeus faber*.

A total of 28 John dory (*Z. faber*) were examined. 4 fish had been collected during an experimental trawl survey carried out along the southern Tyrrhenian coasts in May 2012. 24 fish were collected from the fish market of Porto Empedocle and were coming from southern Mediterranean sea (Strait of Sicily). Scientific investigations were performed following routine histopathology methods. For parasitology investigation, parasite specimens were fixed in 70% ethanol, clarified by lactophenol and finally observed under the stereoscope. The molecular investigation was performed for anisakid larvae identifications by RFLP-PCR, amplifying ITS1, ITS2 and the 5.8S subunit.

Two fish, out of a total of four coming from Tyrrhenian sea, showed macroscopic parasites on the skin. Particularly, on the external surface of one fish a marine leech (*Hirudinea*) belonging to the genus *Calliobdella* was found. In the second specimen an adult taenia, 24 cm long, was found. The parasite was identified at genus level as *Alloptychobothrium* sp. At macroscopical evaluation of internal organs, within coelomic cavity of the same fish few anisakid larvae encysted in the stomach serosa were seen.

Only a single fish, out of a total of 24 subjects coming from the Strait of Sicily, at macroscopical external examination, showed grossly evident tissue changes. This fish showed a nodular bulge, 1 cm in size, in the left jaw. Histologically, such newborn tissue, was characterized by a granulomatous reaction containing pieces of parasitic bodies surrounded by an external chitinous cuticle; these parasites were identified as copepods, likely belonging to the genus *Chondrachanthus*. At the level of the caudal and the left pectoral fin, several whitish nodules (more than 50), about 1-2 mm sized, were seen as seriated or grouped growths; histologically, such nodules resemble granulomas found in the jaw. Within the coelomic cavity of all the fish a large number of anisakid larvae, identified by PCR as *Anisakis pegreffii*, were detected. Only two fish were negative for parasites. Moreover, in two fish, adult cestoda, belonging to the same genus described above, were detected within the intestinal lumen. Finally, slight fatty liver degeneration was demonstrated in all the examined fish.

By the results here obtained and according to the literature on teleost parasitoses, it can be assumed that parasites are fully integrated in all the different aquatic ecosystem and can be found in both wild and farmed teleost fish, although generally in wild conditions parasites have a low impact and only rarely can cause mortality. Although copepods and leeches are a common finding in the John dory, no data are available on intestinal cestoda. Finally, according to Angelucci et al. (2011), the presence of anisakid larvae in a so high percentage of specimens is in contrast with data recently reported on *Z. faber* fished in Mediterranean sea (Costa et al., 2010).

1) Angelucci G., Meloni M., Merella P., Sardù F., Madeddu S., Marrosu R., Petza F., Salati F. Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. *J Food Prot.* 2011 Oct;74(10):1769-75. doi: 10.4315/0362-028X.JFP-10-482.

2) Costa A., Sciortino S., Reale S., Alio V., Cusimano M., Caracappa S. 2008. Indagine sulla presenza di Nematodi Anisakidae in specie ittiche marine. *Atti X Congresso Nazionale S.I.Di.L.V.* Available at: http://www.sidilv.org/index.php?option=com_docman&task=cat_view&gid=4&Itemid=6. Accessed 10 September 2010.

fish pathology

ZEUS FABER, MEDITERRANEAN SEA, PARASITE

PATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATIONS OF EQUINE INFECTIOUS ANEMIA INFECTION IN MULES

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Following the implementation of a National surveillance program for equine infectious anaemia (EIA), a high seroprevalence was detected among Italian mules population. EIA infection in mules has been studied limitedly. To increase the knowledge about the pathogenesis EIA virus (EIAV) in mules and to better understand their role in the epidemiology of the infection, a study was conducted.

Naturally EIA-infected mules were immunosuppressed. At the end of the observation period, the mules were euthanized, and their organs were examined for gross and microscopic lesions. Furthermore, immunohistochemistry (IHC) was conducted to investigate the distribution of the virus.

At necropsy, no relevant gross lesions were observed. Microscopic examination of the different animals revealed mild multifocal haemorrhages in several tissues, a moderate to severe hemosiderosis in spleen and lymph nodes and mild to moderate lymphoid infiltrates, mainly in kidney, and lesser in liver and in the adrenal glands. Membranous glomerulonephritis was detectable in about 40% of the kidney. In the lungs, a mild interstitial pneumonia characterized by slight hypercellularity and thickening of the alveolar septa was evident. Interestingly, mild lymphocytic myocarditis with fibres degeneration, together with a multifocal, mild to moderate, lymphocytic meningoencephalitis, with perivascular cuffings thicker in the brain stem, were observed.

IHC, performed with a monoclonal antibody anti-p26 protein, detected EIAV infected cells in different tissues, both in sites as well as in the absence of lesions; this is particularly true in the adrenal glands, where a high number of positive reacting macrophages were detected in the medulla, and in the heart, with a lower positivity in the interstitial space. In contrast to what described in horses, the amount of positive cells in spleen seem to be lower. A positive signal was revealed mainly in the periportal areas of the liver, the alveolar septa of lungs and interstitium of the kidney cortex, and generally limited to cells morphologically resembling macrophages.

Microscopic lesions and EIAV localization in mules, highly resembles the infection described in horses. However, differently from what is reported for certain strains of EIAV in horses, no virus was detected in the endothelia. The present description is a preliminary contribution for the study of pathological alterations and EIAV localization in non-horse species.

T. C. McGuire et al. Immunofluorescent localization of equine infectious anemia virus in tissue. *Am J Pathol.* Feb 1971; 62(2): 283–294. Spyrou V et al. Equine infectious anemia in mules: virus isolation and pathogenicity studies. *Vet Microbiol.* 2003 Aug 29;95(1-2):49-59. J. Lindsay Oaks et al. Equine Infectious Anemia Virus Is Found in Tissue Macrophages during Subclinical Infection. *J Virol.* Sep 1998; 72(9): 7263–7269.

Sanità animale, virologia, anatomoistopatologia
EIA virus, Mules, Immunohistochemistry

PRIMARY “AMYLOID TUMOR” OF THE MAMMARY GLAND IN A DOG**Giudice C.*^[1], Baldassarre V.^[2], Turati L.^[3], Rondena M.^[2], Grieco V.^[1]**^[1]*DIVET, Università degli Studi di Milano ~ Milano*, ^[2]*Laboratorio Privato d'Analisi Veterinarie San Marco ~ Padova*,^[3]*Ambulatorio De Amicis ~ Milano*

The present report describes an unusual mammary lesion in a dog, with gross and histological features similar to the lesion known as “amyloid tumor of the breast” in the woman.

A fourteen year-old female spayed Dalmatian dog was presented to the practitioner with a large, bilobated lump involving left and right IV mammary gland. The mass was surgically excised and submitted for histopathological examination.

Grossly, two large subcutaneous nodular, firm masses, merging on the median line, expanded left and right IV mammary glands. Histologically, masses were encapsulated and mainly composed of large, coalescing lakes of pale eosinophilic, homogenous hyaline amorphous material, that stained positive with Congo Red (amyloid). Numerous plasma cells with moderate anisocytosis and anisokariosis and rare mitoses were multifocally recognizable. Multinucleated giant cells (MNGCs) with nuclear features similar to plasma cells were occasionally present. Moreover, MNGCs with scant eosinophilic cytoplasm and up to 25 dense nuclei were visible closely associated with amyloid deposits. Moderately atrophic mammary parenchyma was present at periphery. Immunohistochemistry (ABC method) was performed to characterize MNGCs and amyloid, applying anti-Lysozyme; Lambda-light-immunoglobulin-chains and HLA antibodies. Plasma cells and MNGCs were Lysozyme and HLA negative. Lambda-chains stained plasma cells, the majority of MNGCs and amyloid (AL amyloid).

Bilateral mammary extramedullary plasmacytoma with abundant AL amyloid deposition was diagnosed. Clinical workup did not reveal systemic amyloidosis or multiple myeloma. Sixteen months later the dog was humanely killed because of cardiac failure. No signs of multiple myeloma or recurrence of the tumor were reported.

Primary amyloidosis of the breast not associated with mammary carcinoma (amyloidoma), is a rare entity that has been documented in women since 1973, affecting mostly elderly patients, with bilateral involvement and related neither to systemic amyloidosis nor to multiple myeloma. Despite similar gross and histological appearance, some cases have been described as primary amyloidosis, while others as plasmacytoma with massive amyloid deposition

In the canine species, mammary gland amyloid deposition was described in association with mammary carcinoma whereas mammary primary amyloidosis or plasmacytoma with massive amyloid deposition have never been reported so far. To the best of author's knowledge this is the first report in the dog of a bilateral mammary extramedullary plasmacytoma with features consistent with primary amyloidoma of the breast in the woman.

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Veterinary and comparative pathology
amyloid, dog, mammary gland

SKELETAL MUSCLE EXPRESSION OF MYOSTATIN, IGF-1 AND GATA-2 IN CATTLE TREATED WITH GROWTH PROMOTERS

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Hormones are key regulators of mammalian muscle metabolism both in health and disease. Myostatin (MSTN), a member of transforming growth factor-beta family is a negative regulator of skeletal muscle mass. Several authors reported a direct relationship between myostatin expression and hormones treatment (Santos et al., 2012). Moreover, insulin-like growth- factor 1 (IGF-1) as well as GATA-2 have been broadly implicated in skeletal muscle growth, hypertrophy and regeneration (Musrò et al., 1999). This study aimed to determine the effects of different growth promoters on myostatin, IGF-1 and GATA-2 expression in skeletal muscle of veal calves and beef cattle.

Trial 1: 24 Charolaise beef cattle, randomly divided into 3 groups received: group A (n=6) 5 doses of 17beta-estradiol (20 mg/week/animal, IM), group B (n=6) DEX per os (0.7 mg/day/animal) for 40 days, group C (n=6) prednisolone per os (15 mg/day/animal) for 30 days; and group K1 (n=6) was untreated. Trial 2: 24 Friesian beef cattle, divided in 4 groups were treated as follows: group D (n=8) Revalor-200 subcutaneous implant for 89 days; group E (n=8) Revalor-200 subcutaneous implant for 89 days and dexamethasone per os (0.7 mg/day/ animal) for 40 days; group F (n=8) Finaplix-H subcutaneous implant for 89 days; group K2 (n=8) was the control.

Trial 3: 18 Friesian veal calves, randomly allotted in 3 groups received: group G (n=6) 35 mg/week of 17beta-estradiol, for 6 times; group H (n=6) 175 mg/week of testosterone, for 6 times; and group K3 (n=6) was untreated.

Trial 4: 30 Friesian veal calves, divided in 4 groups received: group L (n=8) was 17beta-estradiol (5 mg/week/animal) for 6 times and brotizolam (0.25 mg/day/animal) for 31 days; group M (n=8) 17beta-estradiol (5 mg/week/animal) for 6 times and dexamethasone (0.4 mg/day/animal) for 31 days; group N (n=8) nandrosol (150 mg/biweekly/animal) for 4 times and ractopamine (80 mg/day/animal) for 31 days; and group K4 (n=6) was untreated.

Samples of the sternocleidomastoideus muscle were collected from each animal and subjected to quantitative PCR for MSTN, IGF-1 and GATA-2 genes.

No statistical difference was observed in skeletal muscle for MSTN and GATA-2 genes examined in all experimental groups compared to controls.

The IGF-1 expression was significantly up-regulated in the group A ($P<0.01$) as well as in the group F beef cattle ($P<0.01$). Also in veal calves IGF-1 gene expression was significantly up-regulated. In particular, group L and group N gene expression were respectively 2.18-fold increase ($P<0.05$) and 2.48-fold increase ($P<0.01$) compared to controls.

The growth-promoting effects of sex steroids administration in livestock are well known.

IGF-1 is a potent anabolic factor that can activate myocyte proliferation and differentiation, leading to muscle hypertrophy. This study demonstrate the up-regulation of IGF-1 in skeletal muscle of cattle treated with estrogenic and androgenic molecules, as reported in humans and rats (Gentile et al., 2010; Pöllänen et al., 2010). However, in our study the increase of IGF-1 did not induce the up-regulation of mRNA GATA-2, as previously reported by Musrò and colleagues (1999). Further studies are needed to understand the role of sex steroids in the regulation of skeletal muscle hypertrophy.

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pathology

skeletal muscle, growth promoters, cattle

SPONGY POLIOENCEPHALOPATHY IN TWO BELGIAN MALINOIS PUPPIES

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Spongy degeneration is seen in prion diseases and in a variety of progressive, invariably lethal neurodegenerative disorders. In various dog breeds it can involve the white or the grey matter. Amongst those involving the gray matter, cases are described in Bull Mastiff, Rottweiler, Saluki, Cocker Spaniel and Australian Cattle dog. A hereditary spongy degeneration has been reported in Malinois puppies, which suffer from congenital tremors with ataxia. In this report two cases of spongy degeneration in Malinois puppies are described, emphasizing the morphological and immunohistochemical features of the CNS lesions.

Two unkindred Belgian Malinois puppies were presented for mild depression, tremors of the head, cerebellar ataxia, and progressive ascending paraparesis. Complete blood count and serum biochemistry were normal. Serology for canine distemper virus, Toxoplasma spp. and Neospora spp. was negative. Cerebrospinal fluid showed no abnormalities. Because of the progressive ataxia and development of proprioceptive deficits on all limbs, and poor prognosis, the owners elected for humane euthanasia.

Brain, spinal cord, and samples of major organ were fixed in 4% buffered formaldehyde. Transverse sections of brain and spinal cord were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin, Luxol fast blue. Immunohistochemistry for 2',3'-cyclic nucleotide-3'-phosphohydrolase (CNPase), Olig2, glial fibrillary acidic protein (GFAP), phosphorylated neurofilaments, heat shock protein (HSP)70 and ubiquitin were performed on selected sections.

No lesions were detected at gross examination.

Histological lesions were restricted to the brain and were characterized by the presence of bilateral and symmetric large interneuronal vacuoles in the area of cerebellar nuclei, granular cell layer and foliate white matter of the cerebellum. Phosphorylated neurofilaments revealed numerous axonal spheroids; no ubiquitin-positive intracellular deposits were detected and a normal myelin pattern was evident with Luxol Fast Blue and CNPase immunohistochemistry. Oligodendrocytes showed a normal nuclear reaction for Olig2.

Lesions detected in these puppies are similar to previously described cases (1,2); the predominant lesions were found in cerebellar nuclei and flocculonodular cerebellar cortex, whereas spongy degeneration was not detected in the cerebral cortex. These are the first cases of spongy polioencephalopathy of Malinois dogs described in Italy, and the first time that immunohistochemical features are reported.

1.Cachin et al., 1991, 2.Kleiter et al., 2011

neuropathology

neuropathology, malinois, immunohistochemistry

STEM CELL MARKERS IN BENIGN AND MALIGNANT CANINE PROSTATE TISSUES: AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Canine prostatic carcinoma (PCa) is considered a relevant model for studying advanced, hormone refractory PCa in men. It has been proposed that cancer contains a minor population of cells that can self-renew while simultaneously giving rise to tumour cells (cancer stem cells). Survivin is an acknowledged cancer therapy-resistance factor overexpressed in several tumour types, proposed as a valid cancer biomarker for human prostatic cancer for an early screening for malignancy. Sox9 is a stem cell marker expressed in several adult tissues, required for prostate development. Accumulating evidence indicates that it contributes to the development of human PCa. No studies have been published concerning the immunolocalization of survivin and Sox9 in canine prostatic hyperplasia (BPH) and neoplasia. AIM: to evaluate the patterns and levels of expression of survivin and Sox9 in canine BPH and PCa, in order to correlate their expression with malignant histological features.

immunohistochemistry with specific antibodies in a set of canine BPH and PCa.

Survivin nuclear and rare cytoplasmic immunostaining were present among the basal/reserve cell layer of normal and hyperplastic prostatic lobules. An increase of survivin expression was observed in PCas compared with BPHs. 6/11 PCas showed rare positive nuclei, mainly among the "basaloid" neoplastic cells in the areas with a tubular-papillary arrangement. In the areas with a solid pattern the cytoplasmic immunostaining was more diffuse. Sox9 expression was absent in normal prostatic glands and in all BPHs. 6/9 cases of PCa were highly positive.

Based on the role of survivin as a stem cell marker and the main role in proliferation of nuclear survivin, the positive cells among basal cell layer in normal and HBP cases could represent transit amplifying cells maintaining some stem cell proprieties. The increased survivin expression in Pcas would indicates the molecule as a valid prognostic marker. The absence of expression of Sox9 in the normal gland and all the BPHs suggests that Sox9 is not a stem cell marker of canine adult prostatic stem cells. The high expression observed in PCas clearly suggests an important role of the molecule in canine prostatic carcinogenesis and malignant progression. Further studies should be done in order to confirm this hypothesis.

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Disciplinare

dog, stem cell, prostate cancer

STUDY OF THE HIPPO-TRANSDUCER TAZ IN CANINE AND FELINE MAMMARY TUMORS: PRELIMINARY DATA

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Among others, HIPPO signaling was recently indicated as a key regulator in both physiological and neoplastic cell growth, differentiation, and survival. One of the major output of HIPPO pathway is the inhibition of the coactivator transducer TAZ which controls cell growth, proliferation and survival but also cell differentiation, stem cell renewal, reprogramming, and patterning. TAZ activity correlates with high grade, metastasis, and Cancer Stem Cells (CSCs) content in human breast cancer. Particularly, TAZ is required to sustain self-renewal and tumor-initiation capacities in breast CSCs, moreover its biological activity is associated with epithelial-mesenchymal transition which disrupts TAZ inhibition, increasing its activity. The aim of our study was to evaluate TAZ expression in canine (CMCs) and feline mammary carcinomas (FMCs) particularly investigating correlation to grading and histopathological morphology.

We investigated TAZ expression in 60 CMCs and FMCs and surrounding non-neoplastic mammary gland by immunohistochemistry. The selected mammary tumors equally belonged to the following histopathological groups: simple tubular carcinomas grade I, simple carcinomas grade III, ductal-associated tumors grade I (cats), complex carcinoma grade I (dog), simple-carcinoma-and-myoepithelioma (SCMM) grade I (dog). Three different scoring systems were applied and compared.

TAZ was found to be diffusely overexpressed in grade III simple CMCs and FMCs. In addition, basal cells were TAZ positive both in neoplastic and hyperplastic canine glandular tissue. Interestingly, a moderate positivity was detected also in basal undifferentiated cells of ductal-associated FMCs. Canine SCMMs showed higher TAZ expression compared to complex CMCs.

These preliminary results indicate that also in CMCs and FMCs TAZ may be an important pathway transducer for aggressiveness and might regulate basal cells proliferation and fate. Further samples collection and analyses are required to give insights into its role and regulation in mammary cancers and eventually its additional prognostic information.

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Anatomia patologica veterinaria - Tumori mammari

Hippo, TAZ, Mammary tumor

THE ASSESSMENT OF FEED EFFECTS ON LIVER AND INTESTINE OF REARED FLATFISH: WHAT IS THE BEST STANDARD ANATOMOPATHOLOGIC PROTOCOL?

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This presentation highlights the importance of using standardized methods by the fish pathologists to assess the effects of feed on reared flatfish. The liver and intestine are the most important organs for the digestion and absorption of nutrients from feed, and monitoring their histological structure is of fundamental importance in assessing the effects of nutrient mixtures.1 A critical analysis of the histological methods on feed effects will be presented, aiming to suggest a standardized protocol that should be adopted, on the basis of the advantages and disadvantages that have been encountered in the course of our recent research trials.

Six feeding trials have been carried out on juvenile common sole (*Solea solea*), Senegalese sole (*Solea senegalensis*) and turbot (*Psetta maxima*) (fed diets with increasing dietary protein levels;2,3 increasing dietary plant protein in replacement of fishmeal;4 increasing lipid levels5 and increasing mussel meal6, respectively).

Routine histology (haematoxylin-eosin stain on formalin-fixed and paraffin-embedded sections) was carried out to check degenerative and/or inflammatory changes. In the liver, as well as the graduation of the lipid content, further histochemical techniques have been applied in order to characterize the type of intracellular accumulation (OilRedO, Toluidine blue, PAS) and transmission electron microscopy (TEM), while in intestine in situ techniques to assess the cellular turnover and trophism of epithelial mucosa (anti-PCNA immunohistochemistry and TUNEL method for apoptosis) evaluated by image analysis and TEM were employed.

The routine histological evaluation is helpful only to demonstrate degenerative-inflammatory changes and its usefulness increases when combined with a grading system for the lipidic content of the liver that revealed the most frequent degeneration assessed. To confirm the lipidic content, Toluidine Blue on semithin sections revealed better than OilRedO which run on frozen sections that require a more difficult management of the samples.

IHC on intestinal sections and the evaluation of cellular kinetics and mucosal turnover are necessary tools to assess the influence of a diet on mucosal trophism.

For both tissues TEM is the elective method to further characterize the type of intracellular accumulation and the early signs of cell damage.

On the basis of our experience it is necessary to adopt a standardized approach to evaluate the effects of experimental diets on liver and intestine; in particular, investigations in liver require Toluidine Blue on semithin sections and TEM, while for intestine IHC together with digital image analysis and morphometry are suggested in addition to routine histology.

1Raskovic et al, 2011; 2Gatta et al., 2011; 3Bonaldo et al., 2011; 4Mandrioli et al., 2012; 5Bonvini et al., 2014 (Proceedings of ISFNF); 6Bonaldo et al., 2014 (Proceedings of ISFNF).

fish pathology

flatfish, liver and intestine, histopathology

UTERINE LEIOMYOMA ASSOCIATED WITH CYSTIC ENDOMETRIAL HYPERPLASIA-PYOMETRA COMPLEX IN ASIAN ELEPHANT (ELEPHAS MAXIMUS).

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Leiomyoma is the most common uterine tumor in women, occurring also in many domestic animals³. Most captive female elephants are nulliparous and aged and many have endometrial diseases, factors that may hinder fertility^{1,4}. We here describe a case of uterine leiomyoma associated with cystic endometrial hyperplasia—pyometra complex occurred in a 42-year-old female Asian Elephant (*Elephas maximus*), maintained at the Rome Zoological Garden.

The Elephant, nulliparous, occasionally expelled necrotic material from uro-genital opening for nine years before death. Histologically, this material was always identified as an exudate in the process of organization, resulting positive to *Escherichia coli*. Repeated cycles of antibiotic (moxifloxacin) were made. Two days before death, the animal showed systemic malaise, weight loss and ataxia.

At necropsy, the uterus was significantly increased in volume and abundant purulent material was present in the lumen; the wall was considerably thickened, with the presence of numerous cysts mixed with nodular lesions of variable size, whitish in color and with increased compactness. Masses of necrotic material could also be seen adhering to the wall or free in the lumen. Histologically, numerous nodular lesions that showed neoplastic proliferation of smooth muscle cells in diffusion-type arrangement were observed. The mitotic index was low and there was absence of nuclear atypia. In addition, diffuse hyperplasia of the endometrium with formation of numerous cysts containing liquid amorphous eosinophilic were seen. In some areas there were extensive purulent-necrotic foci. Histological sections stained with Masson's method emphasized the presence of smooth muscle cells and, immunohistochemically, neoplastic cells were constantly positive for vimentin and for α -smooth muscle actin, while they were negative for cytokeratin and S-100. In this case report, the features of the neoplastic cells and the positivity for Masson's method, vimentin and for α -smooth muscle actin clearly express the origin of the tumor from smooth muscle². In addition, *Candida* sp., *Aeromonas* sp. and *E. coli* were isolated from the uterus.

To our knowledge, this is the first case of uterine leiomyoma associated with cystic endometrial hyperplasia-pyometra complex described in an Asian Elephant.

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ONCOLOGIA ANIMALI ESOTICI

UTERINE LEIOMYOMA, CYSTIC ENDOMETRIAL H, ELEPHAS MAXIMUS

WINTER DISEASE IN ASSOCIATION WITH INTESTINAL NON-FORMING XENOMA MICROSPORIDIA IN GILTHEAD SEABREAM (SPARUS AURATA)

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Winter disease (WD) is a multifactorial disease found primarily in sea caged gilthead sea bream (*Sparus aurata*) along the Mediterranean coast.^{1,2} An emaciative syndrome has been recently observed in Spain and *Enterospira nucleophila*, a new microsporidian species of the family Enterocytozoonidae has been described; these intracellular, non-forming xenoma microsporidia, opportunistic in nature exploit a weakened host immune status, as it could happen in WD.⁶ Teleost intestine contains Mast Cells (MCs), whose functional properties are similar to those of mammalian mast cells;⁵ recruitment of MCs to sites of persistent inflammation is a general response in parasites-affected fish. An increased number of the MCs is also reported in WD-affected fish.^{3,4} In December 2013 a disease outbreak in sea caged gilthead seabream in Italy, affecting 0+ year fish occurred. The aim of this study was to evaluate the histopathological changes related to these two conditions, to characterize the MCs by immunohistochemistry and the microsporidia by PCR.

From twenty gilthead sea bream intestinal tracts were fixed in buffered formalin at the sample site and sent to DIMEVET. Routine histological sections were obtained; Luna stain was also performed. Immunohistochemistry with CD117 antibody (1:100, Dako) was also carried out. Intestinal tissue was also subjected to molecular analysis; a fragment of the 18S rDNA was amplified and then sequenced.

Hindgut showed a moderate dilatation of the lumen in association with whitish casts, similar to the milk-like mucous casts reported in WD outbreaks.^{1,2} A severe mucosal atrophy with total folds flattening was present; within lamina propria and submucosa a mild to moderate MCs hyperplasia and mild mucous cells hyperplasia were observed. Multifocally, within enterocytes and rodlet cells, the nucleus and/or cytoplasm contained microsporidian spores, more evident with Luna stain. The sequences obtained from intestines showed 99.9% identity with *E. nucleophila*. Within perivisceral exocrine pancreatic acini, focal necrosis and MCs infiltration have been observed, as reported by other authors during WD outbreaks.

MCs are interpreted as “standing force” in particular tissues consistently exposed to pathogens, in contrast to a “mobilization force” that has been an advantage in those being exposed to noxious agents only occasionally.⁷

The severe mucosal flattening could be interpreted as an effect of a chronic insult, not only related to the microsporidia infection; other concurrent predisposing factors as those reported in WD could be then considered into the development of an overt pathology.

¹Birincioglu et al, 2013; ²Colorni, 2011; ³Contessi et al, 2006; ⁴Ibarz et al, 2010; ⁵Lauriano et al., 2012; ⁶Palenzuela et al., 2014; ⁷Reite and Evensen, 2006.

fish pathology

winter disease, *sparus aurata*, microsporidia

DILATION OF THE VERTEBRAL CANAL IN A PARETIC BOA CONSTRICTOR (BOA CONSTRICTOR IMPERATOR) AFFECTED BY PROLIFERATIVE OSTEOARTHRITIS

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Proliferative vertebral lesions are a common finding in snakes and have been classified into three distinct categories: bacterial osteoarthritis, including active septic osteomyelitis or osteoarthritis with multiple inflammatory foci, and non-bacterial degenerative ankylosing osteoarthritis. Computed tomography (CT)-guided biopsy could be especially useful to rule out active bacterial infections.

AIM. To describe a case of proliferative osteoarthritis and dilation of the vertebral canal in a paretic boa constrictor (*Boa constrictor imperator*).

Radiography and CT-guided biopsy, haematoxylin-eosin staining and histopathological examination.

A 1-year-old, male boa constrictor presented with paresis of the trunk originating cranial to the cloaca. Radiographies were consistent with proliferative bone lesions involving several vertebrae. CT demonstrated the presence of lytic lesions, characterized by widening of the spinal canal. CT-guided biopsies of the lesions were performed. Histology was consistent with bacterial osteomyelitis and osteoarthritis. Gram-negative bacteria (*Salmonella* sp.) were isolated from cultures of the biopsies. A medical treatment was attempted for several weeks without clinical or radiographical improvements. The animal was euthanized and necropsy confirmed the findings observed upon CT. The main lesion comprised 3 vertebral bodies and the vertebral foramen appeared dilated and filled with friable gray/green to brown (purulent/necrotic) material, surrounded by abnormal vertebral bone. Another lesion, which was clinically undetected, consisted in accumulation of purulent material on the ventro-lateral aspect of the vertebra, involving the left rib. Histologically, the vertebral foramen was filled with degenerated heterophils and necrotic material, with bacterial colonies, completely replacing the spinal cord, surrounded by a rim of reactive macrophages and fewer multinucleated giant cells.

Dilation of the vertebral canal is an uncommon finding in snakes presenting proliferative osteoarthritis and possibly carries poor prognosis. Considering that proliferative vertebral lesions may be secondary to bacterial or other causes, and blood culture is not one hundred per cent sensitive, CT-guided bone biopsies may be useful to reach a definitive diagnosis.

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generale

snake, *Salmonella*, osteoarthritis

A.P 02 Aipvet Poster

A CASE OF CANINE THYROID CARCINOMA WITH HETEROTOPIC OSSIFICATION AND EXTRAMEDULLARY HEMOPOIESIS

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The present report describes a case of canine thyroid carcinoma with heterotopic ossification and extramedullary hemopoiesis in a 10 years old mongrel dog. Few anamnestic data were available: an apparently healthy dog was referred with the only symptom of polyphagia. A solid mass, not adherent to surrounding tissue, was palpable on the left side of the neck. The mass was surgically removed, fixed in 10% buffered formalin and send to the pathology division of our Department to be histologically examined. Grossly neoplastic mass was about 3x5 cm, well circumscribed, defined by a thin fibrous capsule. On cross section the mass had a brown-tan colour with a greyish, firm to hard, central area, grossly consistent with bone tissue.

Before trimming, the mass was therefore immersed for 1 week in a decalcifying acid. Then, samples were collected, passed through graded alcohols, clarified in xylene and paraffin embedded. From paraffin blocks, sections were obtained and stained with Haematoxylin and Eosin.

Histologically, the tumor was composed of irregular, small follicular structures, nests and solid lobules of polygonal cells sustained by a variable amount of collagenous stroma. Neoplastic cells had poorly defined cells borders, moderate amount of eosinophilic cytoplasm and round to oval vesiculous nucleus with prominent nucleolus. Anisocytosis and anisokaryosis were moderate and mitotic figures ranged from 0 to 1 X HPF. Small multifocal necrotic foci and focal haemorrhages were also present scattered throughout the tumor. The central area of the tumor was composed of mature trabecular bone. Intertrabecular spaces were filled by both adipose cells and hemopoietic cells. A histological diagnosis of thyroid carcinoma with heterotopic bone formation and extramedullary hemopoiesis was posed.

Heterotopic ossification is a well recognized phenomenon involving organs and tissues affected by various pathologic processes, i.e. ischemia, hematomas, degenerative changes, chronic inflammation and, less frequently, tumors. Few cases of thyroid tumors with heterotopic ossification and extramedullary hemopoiesis have been described in human species. In the canine species, the presence of focal mineralization or scattered bone formation within thyroid carcinomas has been reported but poorly documented. Scattered calcifications have been described in normal thyroid gland while ossification has been reported in a single case of thyroidal carcinosarcoma. The present report represents the first description of a canine thyroid carcinoma with wide areas of mature bone formation (heterotopic ossification) and extramedullary hemopoiesis. Mechanisms of heterotopic ossification are still unknown, however the presence of inducible osteoprogenitor cells, of heterotopic environment conducive to osteogenesis and of inductive signalling such as bone morphogenetic proteins has all been evoked in the pathogenesis of this condition.

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Pathology oncology canine
thyroid carcinoma, dog, heterotopic

A CASE OF PAPILLOMAVIRUS-ASSOCIATED TONGUE ADENOCARCINOMA IN A CAPTIVE WHITE RHINOCEROS

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Papillomaviruses induce hyperplastic and tumoral lesions in different animal species⁴. We describe a case of Papillomavirus-Associated adenocarcinoma which occurred in a 41-year-old male white rhinoceros, maintained at the Zoological Garden of Pistoia, Tuscany^{3,5}.

The rhinoceros showed weight loss and difficulty in chewing the hay for three months before death². At necroscopy, the most relevant finding was a lesion that involved the tongue, characterized by erosion of the mucosa with increased involvement of the dorsal surface. The cut surface showed lardaceous appearance with escape of purulent material.

Histologically, the mass predominantly involved the submucosa and muscular portion of the tongue, resulting in closely packed large, medium, and small glands consisted of columnar cells without mucous cells. Tumor cells exhibited amphophilic to pale eosinophilic cytoplasm, high nucleo-cytoplasmic ratio and medium mitotic rate. No squamous differentiation was noted, and the squamous epithelium immediately adjacent to the adenocarcinoma showed slight perinuclear halos suggestive of PV-related changes.

The tumor cells showed diffuse staining for A1-A3 pan-keratins, but staining for keratin 7 (CK7) clearly separated the adenocarcinoma from the adjacent non-neoplastic squamous epithelium. Keratin 19, keratin 20 and MUC-5AC were negative. Immunohistochemical staining performed with a monoclonal antibody against papillomaviruses evidenced a strong nuclear immunoreactivity only in glandular epithelium similarly to CK7. DNA extracted from paraffin-embedded tissue was tested by PCR using degenerated primers (FAP 59-64), amplifying common gene region of papillomaviral L1. The PCR result was an amplicon of 480 bp. Sequence similarity analysis with the BLAST tool of the National Center for Biotechnology Information and Papillomavirus Episteme tool showed that this fragments belongs to new putative PVs, not yet characterized for rhinoceros.

To our knowledge this is the first case of Papillomavirus-Associated non-salivary gland-type adenocarcinoma arising in the base of the tongue in a captive white rhinoceros.

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ONCOLOGIA ANIMALI ESOTICI

PAPILLOMAVIRUS, WHITE RHINOCEROS, ADENOCARCINOMA

A CASE OF SYSTEMIC CORONAVIRUS-ASSOCIATED DISEASE IN A DOMESTIC FERRET IN ITALY.**Petrini D.^[1], Rondena M.^[2], Binanti D.*^[3]**^[1]*Libero professionista ~ Pisa*, ^[2]*Libero professionista, patologo veterinario ~ Padova*, ^[3]*Libero professionista, patologo veterinario ~ Ameglia*

Since 2006 a Coronavirus-associated visceral disease with clinicopathologic features resembling the “dry form” of Feline Infectious Peritonitis, has been recognized in ferrets (1,2). Confirmed cases have been reported in Spain, USA, UK and Japan (3,4,5). The present work describe the first confirmed case of Systemic Coronavirus-associated disease in a domestic ferret in Italy, with description of clinicopathologic findings.

An 8 months-old, entire male ferret (*Mustela putorius furo*) was referred for weakness and coughing. Clinical examinations showed fever, enlarged retropharyngeal lymph nodes and heart murmur. Hemato-biochemical analysis demonstrated anemia, leucocytosis, hyperproteinemia and hyperglobulinaemia (polyclonal gammopathy). Imaging revealed generalized lymphadenomegaly, severe splenomegaly and multifocal nodular renal lesions. Kidney cytological smears were compatible with granulomatous disease. Histology from spleen, mesenteric lymph node and kidney biopsies was consistent with pyogranulomatous inflammation. Due to deterioration of the condition, the ferret was euthanized five months after the first presentation and post-mortem examination revealed disseminated nodular lesions, mainly localized in kidney, spleen, mesenteric and mediastinal lymph nodes, diaphragm and lung. Histology confirmed a systemic pyogranulomatous disease. Immunohistochemistry was performed using anti-FCoV monoclonal antibody and positive staining for Coronavirus antigen was detected in the cytoplasm of macrophages in the pyogranulomas, providing a definitive diagnosis of ferret systemic coronavirus-associated disease. In conclusion ferret systemic Coronavirus-associated disease should be considered in the differential diagnosis of young ferrets presenting with enlarged lymph nodes, hyperproteinemia and hyperglobulinaemia and histology and immunohistochemistry represents the gold standard for a definitive diagnosis.

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Patologia del furetto

Ferret, Coronavirus, granulomatous diseases

COMPARISON OF DIFFERENT PROCEDURES TO ISOLATE FELINE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) FROM SMALL VOLUMES OF BLOOD

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Studies on leukocytes isolated from feline blood do not provide details on the performances of isolation techniques.¹ From the few numerical data available, however, it can be assumed that the purity of isolated cell populations is high but their recovery rate is low.² Therefore, large volumes of blood (difficult to collect from cats with spontaneous diseases) are required to obtain enough leukocytes for in vitro studies. The aim of this study was to assess the performances of isolation techniques on small volumes of feline blood.

Blood samples (1 to 5 mls) were drawn from clinically healthy cats and placed in EDTA-coated tubes. Fifteen session of tests (10 using Ficoll, 5 using Percoll) were performed. In 9 cases cells were further separated by adherence on Petri dishes (PD) and in 5 cases using iron-labelled monoclonal antibodies against leukocyte antigens followed by magnetic sorting (MS). Cell purity (i.e. the percentage of each population) and recovery (i.e. the percentage of cells of each population recorded after isolation compared with blood) were then calculated.

The purity of lymphocytes was significantly higher ($P=0.015$) with Ficoll (79.6 ± 3.3) than with Percoll (61.0 ± 12.0); the purity of monocytes was low, and significantly higher ($P=0.015$) with Percoll (32.7 ± 13.5) than with Ficoll (9.0 ± 1.6). The recovery rate of lymphocytes was low, and significantly higher ($P<0.001$) with Ficoll (54.7 ± 27.5) than with Percoll (12.0 ± 4.2). The recovery rates of monocytes recorded with Ficoll (59.4 ± 34.7) or Percoll (38.5 ± 9.38) were not significantly different. The purity of cell types in PD was not morphologically determinable. However, assuming that at least 90% of adherent and non adherent cells were monocytes and lymphocytes, respectively, the recovery rate for both the populations was lower than 10% in most cats. The purity after MS was generally high, but the recovery rate was variable and the number of yielded cells very low.

In conclusion, none of the techniques applied in this study provides good performances in terms of number of cells, purity and recovery rate, when applied to small volumes of blood. This suggest that the isolated cells could not be representative of the population in blood, and limits the use of these 3 techniques in cats with spontaneous diseases. Therefore, cell functions in spontaneous feline disease should be investigated in whole blood rather than on isolated cell populations.

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Veterinary clinical pathology
Leukocyte isolation, Feline, in vitro studies

COMPREHENSIVE MANAGEMENT OF A PITUITARY ADENOMA WITH MALIGNANT FEATURES IN A MALTESE DOG

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The aim of the study is to describe the long-term survival of a dog affected by an ACTH producing-pituitary adenoma treated by surgery, medication and radiation therapy.

A 7-yr-old old male Maltese dog was referred to DIMEVET for epileptic seizures, polyuria, polydipsia, polyphagia and symmetrical alopecia. Pituitary-dependent hypercortisolism was diagnosed by endocrine tests. Computed tomography (CT) revealed a pituitary mass with a P/B ratio 0.75, reference <0.311 that was treated with transsphenoidal hypophysectomy; Immunohistochemistry of the surgical specimen confirmed an ACTH-producing adenoma. Hypercortisolism went into remission for three years but then polyuria/polydipsia and alopecia reappeared and CT scan revealed a small sellar abnormal structure (interpreted as pituitary tissue regrowth). Hypercortisolism was confirmed by endocrine tests and medical treatment with trilostane was started, with good control of the clinical signs for one year, until the occurrence of obtundation, seizures and stupor. CT scan showed a sellar mass and radiation therapy was performed with a protocol of 20 fractions of 2.25 Gy. Neurological signs regressed and trilostane treatment was continued with good control of the disease. Six years after the first admission, the dog developed lumbosacral pain and inability to walk and was euthanatized for animal welfare reasons. Histological sections were stained with H&E and PAS and immunostained with antibodies against ACTH, MSH, GH, C-erb-B2 and Ki67.

Macroscopically the formalin fixed brain revealed grey tissue in the pituitary region extending caudally to the mesencephalon. Histologically, unencapsulated neoplastic tissue in contiguity with the meninges and infiltrating the neuropil was detected; neoplastic cells arranged in islands and cords were embedded in a rich amorphous eosinophilic extracellular matrix; neoplastic cells showed an intensely eosinophilic cytoplasm. The nucleus was vesicular, often two or three nuclei were seen, with chromatin margination and a prominent nucleolus. Anisocytosis and anisokaryosis were moderate. Mitotic figures were three in ten random selected fields at 400x magnification. A diagnosis of a recurring infiltrative ACTH-adenoma was made; Ki67 labelling index was 4,8%.

Pituitary tumors that recur or progress despite resection and radiotherapy are often termed “atypical” adenomas, as they do not appear overtly malignant by histological criteria, but exhibit aggressive biologic behavior, have a Ki67 labelling index >3%, p53 immunoreactivity.² Reports of invasive canine pituitary tumors are rare; this is the first report of a long-term survival of a dog affected by this type of tumor that was treated by surgery and subsequently by radiation therapy. Comprehensive management of pituitary adenomas using the various treatment modalities may significantly prolong the dog’s life.

1Kooistra et al., 1997; 2Mamelak et al., 2011.

neuropathology, endocrine pathology

pituitary adenoma, dog, survival

COORDINATED IMMUNE RESPONSE OF MEMORY AND CYTOTOXIC T CELLS TOGETHER WITH IFN- γ SECRETING CELLS AFTER PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) NATURAL INFECTION IN CONVENTIONAL PIGS.

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection in the field usually dampens the pig immune system both as innate and acquired immunity. The extent of immune dysregulation and/or depression depends on the virulence of the PRRSV isolate, the intrinsic ability to interact with the immune system and on the age of the animals. (1)

The present study aims at evaluating the antibody and cellular immune response in pigs naturally infected by PRRSV in order to highlight the immune modulation.

Twenty conventional pigs were selected from a herd with a history of PRRSV infection and monitored for 22 weeks from weaning (4 weeks of age) through the fattening phase (up to 26 weeks of age). The pigs were divided in two groups: a group was naturally exposed to PRRSV infection (N=10, PRRSV-exposed) by comingling with infected resident animals and the unexposed group was used as control (N=10, C).

Blood samples were collected 2 weeks apart and PRRSV infection was detected by quantitative PCR in serum. The humoral immune response was quantified as total serum PRRSV-specific antibodies by ELISA while the cellular response was characterized by flow cytometry and IFN- γ ELISPOT to enumerate circulating T cell subsets and PRRSV-specific IFN- γ secreting cells (SC) in PBMC. (2, 3) In this study, the distribution of cells involved in cell mediated response and IFN- γ producing cells were investigated through innovative methods (flow Cytometry and ELISpot assay) in pigs naturally infected by the PRRSV through exposure to infected animals. Clinical signs were recorded throughout the study.

The results showed that all exposed pigs became infected at 16 weeks of age and viremia lasted until 20 weeks in almost 50% of the exposed animals, whereas the C group remained negative. The PRRSV-exposed group developed an antibody response since 18 weeks of age.

In infected pigs, total CD4⁺ and CD8 α ⁺ T cells increased from 18 weeks onwards, due to a significant increase of cytotoxic T CD8 β ⁺ and memory T helper CD4⁺CD8 α ⁺low lymphocytes. An early and transient increase was observed for naïve T helper CD4⁺CD8 α ⁻ cells. Also virus-specific IFN- γ SC were significantly recalled from 18 weeks, peaking at 22 weeks. Control animals showed non-significant fluctuations in cell percentages and negligible SC levels. In this study, the IFN- γ SC response was strongly induced in parallel with the positive modulation of cytotoxic and memory T cells suggesting the potential activation of these subsets to secrete the immune cytokine.

This approach demonstrated a strong IFN- γ response but also some peculiar aspect of the immune response, likely depending on the delayed infection time of animals exposed to the virus. Overall, taking into account that PRRSV infection was more delayed compared to what is generally observed in the field, the age of pigs may have favoured a more pronounced immune response.

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Patologia generale

PRRSV, PIG, IMMUNITY

CUTANEOUS NEOSPOROSIS IN A GOLDEN RETRIEVER

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This report describes cutaneous cytological and histological lesions caused by *Neospora caninum*.

A 10 years old, intact female Golden Retriever under treatment with cyclosporine for an autoimmune disorder had sudden development of multifocal dorsal cutaneous nodules. Skin cytological specimens obtained by imprint and fine-needle aspiration were stained with May-Grünwald Giemsa. Skin punch biopsy specimens were obtained. Biopsies were fixed in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin. Unstained smears and deparaffinized sections of skin were immunochemically stained with polyclonal anti-*Toxoplasma gondii* and anti-*Neospora caninum* primary antibodies.

Cytology demonstrated a prevalence of degenerated neutrophils admixed with fewer macrophages, rare neutrophils associated with adipocytes and fibroblasts. On the background and within macrophages numerous crescent shaped, 4-6 µm microorganisms, with a light basophilic cytoplasm and a central nucleus (tachyzoites) were visible. Histology revealed diffuse and severe neutrophilic, histiocytic, eosinophilic dermatitis and panniculitis associated with necrotizing vasculitis. Elevated numbers of free and cytoplasmic tachyzoites within macrophages and keratinocytes of the epidermis and follicular infundibula were present. Immunocytochemistry and immunohistochemistry warranted a diagnosis of cutaneous Neosporosis (CN).

Clindamycin administration (11 mg/kg PO every 12 hours) and withdrawal of immunosuppressive medication resulted in prolonged clinical remission.

Cutaneous nodules are a rare manifestation of Neosporosis.1-4 Age-related immunodeficiency and immunosuppressive therapies seem to predispose to CN.1-4 Information on cause and prognosis are fragmentary in this instance. The current dog was alive 1 month after diagnosis.

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DERMATOPATOLOGIA

Dog, *Neospora caninum*, Skin

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON SOME HAEMATOLOGICAL PARAMETERS IN COWS.

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The aim of this study was to evaluate physiopathological responses to different environmental conditions (ambient temperature, relative humidity and temperature-humidity index) on haematological parameters. Blood samples were collected at 4 time points under different environmental conditions (T1, T2, T3 and T4) by 43 Piemontese cows aged 2–12 years and analysed for haematological parameters. For each period, ambient temperature and relative humidity were recorded by means of a data logger and the temperature-humidity index (THI) was calculated as indicator of thermal comfort for cattle. Data were then measured by one way analysis of variance (ANOVA).

The obtained results showed a statistical significant effect of time on the following parameters: RBC ($P<0.0001$), Hb ($P<0.0001$), Hct ($P<0.0001$), MCV ($P<0.0001$), MCH ($P<0.0001$), MCHC ($P<0.0001$), Plt ($P<0.0001$), WBC ($P<0.0001$), neutrophils ($P<0.0001$), lymphocytes ($P<0.0001$), monocytes ($P<0.0001$) and eosinophils ($P<0.0001$).

The majority of haematological values obtained in the present study, even though within the physiological range for cattle, showed that variations in haematological parameters are related to changes in ambient temperature, relative humidity and temperature-humidity index. These results provide insight into the physiological responses of Piemontese cow to different environmental conditions, allowing to better evaluate its ability to adapt and cope with environmental stress (1-3).

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Patologia Clinica

cows, haematology, environment

EFFECTIVENESS OF ENDOMETRIAL CYTOLOGY OBTAINED BY LOW-VOLUME UTERINE FLUSH TECHNIQUE IN POSTPARTUM ENDOMETRITIS OF DAIRY COWS

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Aim of this study was to evaluate the effectiveness of endometrial cytology obtained by low-volume uterine flush technique to identify endometritis in postpartum period of dairy cows, correlating data with bacteriological examination and clinical findings.

Endometritis, responsible of great economical losses in dairy industry, is difficult to diagnose, due to the lack of gold standard criteria accepted by practitioners (1). Subclinical endometritis (SEND), defined as uterine inflammation in the absence of clinical signs, is frequently associated with significant reduction of reproductive performance and often diagnosed through endometrial cytology (2).

The study was performed in 40 multiparous Holsteins. From October to December 2013, cows underwent clinical evaluations from the day of parturition until 40 days postpartum. Intrauterine samples were collected between 30 and 40 d postpartum by infusing 100-150 ml of sodium chloride 0.9% sterile solution and then recovering the fluid; samples were delivered to the laboratory within 12 hours. After centrifugation of the fluid, an aliquot was used for bacteriological examinations and another for cytology. According to the published literature, a cut off of 10% of polymorphonuclear leucocytes (PMNs), was used to identify cows with endometritis (3).

Between 30 and 40 d postpartum, all cows were flushed and examined: 17 cows (42%) showed a positive cytology; 6 of these cows were diagnosed with a clinical endometritis, whereas the remaining 11 were clinically healthy (SEND cows). Of the 23 remaining cows with a negative cytology (58%), 20 were healthy, whereas 3 cows showed clinical endometritis (false negative). Bacteriology allowed the isolation of several bovine uterine pathogens both in clinical endometritis cases and in clinically healthy cows. Six different groups of bacteria were isolated such as *Trueperella pyogenes* (n=6), *Escherichia coli* (n=5), *Enterococcus faecalis* (n=7), coagulase-negative staphylococci (n=8), *Bacillus* spp. (n=1), *Pseudomonas* spp. (n=3) and *Enterobacter agglomerans* (n=1). Bacteriology was negative in 9 cows.

Low-volume uterine flush cytology between 30 and 40 days postpartum has proved to be a useful technique to reveal SEND especially in cases associated with bacteria considered only potentially pathogenic for the uterus, such as *E. faecalis* or *B. licheniformis*. However, we underline the limits of the technique that can lead to obtain false negative. The results of our study emphasize the need for integration of the two diagnostic tools, namely cytology and bacteriology.

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Patologia della riproduzione

endometrial cytology, cow, uterine bacteriology

ERYSIPELOTRIX RHUSIOPATHIAE ENDOCARDITIS IN A SHEEP

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Erysipelotrix rhusiopathiae is a pathogen of a wide variety of animals. Chronic polyarthritis are reported in sheep and lambs. The authors describe one systemic erysipelas infection in an adult sheep in Sicily.

A female, cross-breed, 18 months old sheep showing clinical signs of phosphate esters suspected poisoning, was sent for diagnostic investigations to the Department of Veterinary Sciences of Messina University. A complete necropsy was made and samples of affected organs were partially fixed in 10% neutral buffered formalin to perform histological investigations and partially stored at -80°C for biomolecular researches. Total DNA was extracted from heart tissue and employed in PCR test targeted to the 23S ribosomal region. The DNA sequences were amplified by primers annealing at 23S of ribosomal genes as reported in the literature (Takeshi et al., 1999). PCR products were sequenced and the obtained data were analyzed by Wu Blast 2 sequence alignment software for strain identification.

At necropsy abundant foamy exudates in the trachea and bronchi and pleural hemorrhages were observed. Moreover, hypertrophy of the left heart showing multiple grayish white foci were present. The mitral valve showed a severe acute endocarditis characterized by irregular friable vegetations. In the kidney a voluminous necrotic area with hemorrhagic limits between cortical and medullary was detected. Histology confirmed the presence of multiple myocardial and renal septic infarcts. The flaps of the mitral valve were fibrotic, infiltrated by polymorphonuclear cells, with adherent multiple thrombi. DNA extracted and the sequence analysis showed a complete overlapping with the 23S rDNA of Erysipelothrix rhusiopathiae sequence.

Erysipelothrix rhusiopathiae is a bacterium commonly isolated in swine. In author's opinion this report describes an unusual cardiac localization in sheep previously only rarely described (Chineme et al., 1973; Maclachlan, 1978). The occurrence of this unusual localization imposes the necessity do not neglect common lesions using always regularly laboratory investigations.

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Systemic erysipelas infection

Sheep, E. rhusiopathiae, endocarditis

GLIOBLASTOMA IN AN EWE

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Tumors of neuroepithelial origin (gliomas) include astrocytomas, oligodendrogliomas, and ependymomas. In veterinary medicine, primary neoplasms of the central nervous system (CNS) are most frequently reported in dogs and less commonly in cats, whereas the same tumors are considered distinctly uncommon within the other domestic species [1].

The present report aims at describing the pathological and immunohistochemical findings observed in a Sarda breed sheep with glioblastoma.

A 6 years old Sarda breed sheep was euthanized and necropsied after showing severe and worsening neurological signs, which started 8 months before and mainly consisted of depression and head pressing. At necropsy a wide range of tissues were sampled, promptly fixed in 10% neutral buffered formalin and routinely processed for histopathological investigations (hematoxylin and eosin stain). Furthermore, selected tissue sections of the cerebral mass were subject to immunohistochemistry (IHC) using the following primary antibodies: anti-glial fibrillary acidic protein, anti-vimentin, anti-synaptophysin and olig2.

At necropsy, a large (about 5 cm in diameter) whitish to grayish mass was seen partially replacing the left cerebral hemisphere.

Microscopically, the tumor consisted of polymorphic cells, with a high number of mitotic figures and large areas of necrosis and dystrophic calcification surrounded by pseudo-palisading of cells. The neoplasm was highly vascular with glomeruloid vascular proliferation.

Foci of "oligodendroglioma-like" differentiation and few multinucleated giant cells were also occasionally seen. Mitotic figures were very frequent, up to 5 mitoses per high magnification power field (Ob. x40), and often atypical. At IHC, neoplastic cells proved to be immunoreactive (IR) for vimentin and olig2 but constantly negative for synaptophysin. Only few scattered neoplastic cells were GFAP-IR. However, IHC for GFAP demonstrated the presence of few reactive astrocytes, mainly surrounding the foci of necrosis.

Few cases of primary brain tumors have been described in sheep: medulloblastoma in lambs [2], a case of ependymoma in a Suffolk sheep aged less than 1 year [3], and more recently a case of oligodendroglioma in a 1 year old male Iranian fat-tailed sheep [4]. To the best of our knowledge, the present is the first report of glioblastoma in sheep. Even in sheep, glioblastoma is similar to anaplastic astrocytoma with the additional features of necrosis and vascular proliferation and share many of the histopathological and immunohistochemical features observed in dogs [5].

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PATHOLOGY

Glioblastoma, Sheep, Immunohistochemistry

GLYCOGEN RICH CARCINOMA OF CANINE MALE AND FEMALE MAMMARY GLAND

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Glycogen rich clear cell carcinoma (GRCC) is a rare subtype of human invasive mammary gland (MG) carcinoma, in which at least 90% of the neoplastic cells have clear cytoplasm containing glycogen(1). The aim of this study was to describe the histological, histochemical and immunohistochemical (IHC) features of GRCC of canine MG.

Serial formalin fixed paraffin embedded tissue sections of two GRCC canine mammary carcinoma, one female (case I) and one male (case II) were stained with H&E, PAS, PAS diastase (dPAS), and Alcian Blue (AB). IHC was performed with anti-ER, -PR, -cerbB2, -CK19, -CK14, -CK5/6, -p63, -vimentin, -SMA, -calponin, -S100, -EGFR, -c-KIT, -E-cad and -ki67 antibodies. Sudan III was carried out on formalin-fixed frozen tissue in case II.

Histologically, 90% of neoplastic cells showed sharply distinct borders, clear or finely granular cytoplasm and low N:C ratio. In case II, residual 10% of the neoplastic cells had lipid-like vacuolated cytoplasm. In both tumors, the cytoplasm resulted strongly positive with PAS. Treatment with diastase abolished PAS reactivity. Case I showed PAS+ and dPAS- staining also in lymph node metastasis. In case II, 10% of neoplastic cells were positive to Sudan III. No stain with AB was obtained. Case I showed positivity for CK19 and CK5/6, negativity for ER, PR and c-erbB2, resulting a basal-like phenotype in primary tumor and lymph node metastasis. They were both positive for EGFR, E-cad, c-KIT, and weakly for calponin. Case II was a basal-like phenotype, presenting CK 19, E-cad, c-KIT, weak CK14 and strong vimentin positivity. The proliferative ki67 index was 26.75% in case I and 8.2% in case II.

Based on the morphology, typical features of human MG GRCC are the “fried eggs appearance”, clear cytoplasm and small dark punctate nuclei (2). On the best of our knowledge, this is the first report regarding GRCC in canine MG. The diagnosis was confirmed by PAS+ of intracytoplasmic glycogen granules and lack of stain with dPAS. Case II was considered a GRCC with lipid rich differentiation. Both cases had a basal-like phenotype and the expression of EGFR and c-KIT was suggestive of an association of cell proliferation with signal transduction of surface molecules (3). GRCC can be considered a new rare histological subtype of canine mammary tumors, with clear cytoplasm, PAS+ and dPAS-, expressing the triple negative phenotype, a tumor with clinical aggressive behavior that should be differentiated from lipid rich carcinoma.

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ANATOMIA PATOLOGICA

Canine, Mammary carcinoma, Glycogen rich

GRANULOMATOUS DERMATITIS OF THE AURICULAR PINNA IN A HEIFER

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a unique case of granulomatous dermatitis.

A 2 y.o. Italian Friesian heifer showed a papillomatous-like lesion at the edge of the right auricular pinna. The lesion sized 9 cm x 11.5 cm, was exophytic, globular in shape, firm and with the outer surface uneven, depigmented and ulcerated. Cytological examination revealed the presence of an inflammatory population mainly composed by neutrophils and macrophages. On the basis of the cytological observation, the mass was excised and subjected both to histological staining (H&E, PAS, Gram) and molecular biology examination (PCR).

The histological examination allowed the diagnosis of a pyogranulomatous inflammation characterized by a epithelioid and giant cells, neutrophilic and macrophagic infiltrate organized around focal areas of eosinophilic homogeneous material consistent with the Splendore-Hoeppli phenomenon. PAS staining highlighted numerous coccoid formations within the piogranulomatous reaction or freely standing in the tissue. Gram stain revealed Gram-positive bacterial colonies confirmed by PCR as *Corynebacterium mucifaciens*.

Granulomatous dermatites are caused by agents against whom the body is sensitized and react through an immunomediated response(1). Sometimes the above reactions are histologically characterized by the Splendor-Hoeppli phenomenon that may represent the deposition of antigen-antibody complexes (immunoglobulins and major basic proteins) and debris from the host inflammatory cells(2). The lesion described in this work seemed worth of description because of the etiologic agent. In fact, *Corynebacterium mucifaciens* is a newly-described species belonging to the the largest genus in the group of coryneform bacteria(3). Moreover, the seat of development was extremely atypical for the bovine species. *Corynebacterium mucifaciens* is better differentiated from closely-related species by molecular biology techniques, such as sequencing of the 16S rRNA gene and is isolated from human blood or other normally-sterile body fluids, often considered as part of the normal skin flora or contaminants. To date, literature data report the occurrence of disease due to *Corynebacterium mucifaciens* only in humans where it has been related to severe infections, lethal bacteremia (4), cavitary pneumonia (5), corneal ulcer (6), otitis and nasal polyps (7). To the author's knowledge, this is the first report of a lesion induced by *C. mucifaciens* in veterinary medicine.

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Patologia Veterinaria

C. mucifaciens, Skin granulomas, Splendore-Hoeppli

GRANULOMATOUS MIOSITIS DUE TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN A HORSE

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This study is aimed to describe the morpho-pathological and etiopathogenic aspects of a case of granulomatous myositis in a horse.

A 12 years old Quarter horse mare was evaluated because of the presence of different subcutaneous nodules and masses. The lesions were cytologically diagnosed as pyogranulomas and were resistant to the given pharmacological treatment. The persistence of the lesions along with the deterioration of the clinical status suggested to surgically remove three of them. Macroscopically they were fixed to the muscles, painless and firm in consistency. On cut section, they showed a purulent exudate. Tissue samples from the lesions were fixed in 10% buffered formalin and paraffin wax embedded. Histological sections were stained with H&E, PAS, Masson trichrome, Grocott and Gram. Molecular biology assay (PCR) was performed too. Histological examination revealed, in all samples, a diffuse mixed inflammatory infiltrate characterised by polymorphonuclear granulocytes (neutrophils and mainly eosinophils), macrophages, lymphocytes, plasma cells, epithelioid and multinucleated giant cells invading the endomysium. Different sized round foci of caseous necrosis with calcification and areas of collagenolytic degeneration were present in all sections. PAS and Grocott stains didn't show fungi whereas Gram stain revealed the presence of blue pleomorphic Gram + microorganisms free or inside macrophages.

The described results indicated a severe diffuse granulomatous myositis. PCR analysis revealed the etiologic agent as *Corynebacterium pseudotuberculosis*.

Corynebacterium pseudotuberculosis infection occurs world wide as caseous lymphadenitis in small ruminants and granulomatous infection in horse and cattle.

The bacterium can survive for extended periods in the environment and in the soil. Disease transmission is thought to occur thorough a contaminated environment by direct contact between animals and insects such as house flies (*Musca domestica*), stable flies (*Stomoxys calcitrans*), and other arthropods serving as mechanical vectors (1). Clinically, the infection in horses most commonly causes external abscesses or "pigeon fever" (90% of cases), however, internal abscesses (8%) or ulcerative lymphangitis (1%) may also occur (2).

In our case, none of the known pathogenetic pathways reported in the literature seems to be completely comparable being some aspects common to the so called pigeon fever and others to the chronic form of ulcerative lymphangitis. The presented case is, therefore, very rare and interesting for the pathological findings and, overall, from the epidemiological point of view.

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Patologia Veterinaria

horse, muscle granulomas, pigeon fever

HISTOLOGICAL ASPECTS OF AN UNUSUAL COLONIC DUPLICATION CYST IN A CONSTIPATED CAT

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In animals as in humans the main anomalies of canalization of enterocolic tract are represented by the persistence of Meckel's diverticulum, intestinal duplication cyst, vitelline or omphalic duct cyst or finally, diverticula losing a direct connection with the colonic lumen^{1,3}. In these condition histology may be characterized by the indistinguishable mucosal type between normal and accessories tract, or by the presence of metaplastic epithelial types. We describe a case of 5-year-old domestic short-haired spayed female cat presenting abdominal pain and suspended defecation, in which abdominal ultrasonography revealed the presence of an oval formation connected to the colon, whose wall was similar to the colonic one. Ultrasonographically the content appeared inhomogeneous. Surgery and related histopathology allowed a most likely diagnosis of a colonic duplication cyst.

For histology analysis, samples were routinely processed and stained with hematoxylin-eosin and Alcian-PAS using different types of mucins, then characterized also immunohistochemically.

Mucosal histology and histochemistry revealed particular heterotopic tissues represented by some spotted areas of gastric metaplasia, antral/pyloric in type, and a very particular area of mucosa that resemble respiratory mucosa in type. No heterotopic pancreatic tissue, Brunner glands or biliary epithelium, frequently reported in other cases¹, were observed in this cat. Two nonreactive lymphoid aggregates were observed in the lamina propria of the cyst, in absence of elements evidencing inflammation and/or subsequent bacterial superinfection of the cystic mucosa. After surgery the cat returned to defecate normally.

Duplication cyst is an uncommon congenital abnormality of the alimentary tract. Most often the patients are asymptomatic and colonic duplication cysts remain undiagnosed for years. In this case report we present a fourth description of intestinal duplication cyst case in a cat^{1, 2, 3, 4} with a descending colon location and a mucosal characterization by histology/immunohistochemistry. The importance of description of this rare congenital malformation is finalized to the inclusion in the differential diagnosis of cystic masses of the gastrointestinal tract.

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General and Special Pathology

disontogenic cyst , histochemistry, immunohistochemistry

HSP32 AND HSP90 IMMUNOEXPRESSION, IN RELATION TO GRADING AND OTHER HISTOPATHOLOGICAL FEATURES, IN CANINE CUTANEOUS MAST CELL TUMOURS

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Literature data indicate Hsp32 and Hsp90 as interesting molecular targets in canine neoplastic mast cells. However, their immunoexpression patterns in canine mast cell tumours (MCT) have not been investigated so far. Thus, the aim of the present study was to evaluate the immunohistochemical expression of Hsp32 and Hsp90 in 22 samples of canine cutaneous MCT, in relation to histological grade and other pathological variables, such as absence/presence of epidermal ulceration or tumour necrosis, growth pattern and mitotic index

Grading was established on the basis of the systems proposed by Patnaik et al. (1984) and Kiupel et al. (2011) as follows: 13 grade II (10 low-grade and 3 high-grade) MCT and 9 grade III (all high-grade) MCT. A semi-quantitative method was used to analyse immunoreactivity. Fisher's exact test and Cramer's V were used to evaluate the associations between the examined parameters.

Hsp32 showed a variably intense and distributed cytoplasmic immunostaining, not associated with histological grade. However, the low Hsp32 immunoexpression (<50% of positive cells) detected in the majority of grade III/high-grade MCT samples suggests the need for further investigating the efficacy of the pharmacologic Hsp32-inhibitors on aggressive canine MCT. Reduced Hsp32 immunoreactivity was associated with presence of tumour necrosis ($p=0.035$; Cramer's $V=0.542$), a finding in agreement with the pro-survival functions of Hsp32 in neoplastic canine MC.

Hsp90 cytoplasmic immunosignal was variably associated with nuclear and/or membranous staining. Proportion of Hsp90 immunoexpression was not associated with histopathological features and grade.

Noteworthy was the detection of a membranous expression of Hsp90, in light of its molecular chaperone functions towards the receptor tyrosine kinase Kit. Further studies are currently underway in order to investigate the possible relationship between Hsp90 and Kit immunoexpressions in canine MCT.

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disciplinare

HSPs, Mast cell tumor, Dog

IDENTIFICATION OF REGULATORY T CELLS IN CANINE MAMMARY GLAND TUMORS

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In the present study we evaluated foxp3 expression in benign and malignant canine mammary gland tumors by immunohistochemistry and we investigated its prognostic significance. Foxp3 is a member of the forkhead /widge-helix family of transcription regulators involved in regulating immune system development and function (2). This gene plays a crucial role in the generation of CD4+/CD25+ regulatory T cells. Tregs seem to enable tumors to elude host antitumoral immune response (1, 3).

Animals: a total of 33 samples of canine mammary gland tumors were selected from the archives of Veterinary Pathology of the Department of Veterinary Medical Sciences over a period between 2006 and 2013. 15 neoplasia were diagnosed as benign and 18 as malignant, based on the WHO (1999) classification. Among the benign tumors 10 were complex adenomas, 3 papillar adenomas and 1 mixed benign tumors. Among the malignant tumors 5 were solid carcinomas, 3 complex carcinomas, 9 tubulo-papillar carcinomas and 1 in situ carcinoma.

All the samples were immunohistochemically tested with an anti Foxp3 primary antibody (purified Anti-Mouse/Rat Foxp3 clone FJK - 16s of the firm eBioscience), at a 1:200 dilution in PBS overnight 4°C.

Each slide was evaluated microscopically on 5, 20X fields and all the positive cells were counted in peritumoral and intratumoral infiltrates. Benign tumors showed fewer infiltrates than malignant neoplasias. Foxp3 expression was observed in 9 benign tumors out of 15. In 6 complex adenomas the number of tregs observed was between 1 and 12, only one showed more than 13 tregs, 2 papillar adenomas showed less than 12 tregs.

Foxp3 expression was observed in 14 malignant tumors out of 18. In solid carcinomas foxp3 was highly expressed (more than 40 tregs) while in complex carcinomas, expression was less (between 1 and 12) and in 3 tubulo-papillar carcinoma between 1 and 12 cells were positive and in 4 between 13 and 40. In the in situ carcinoma foxp3 was very highly expressed (more than 40)

In the present study the number of tregs was counted and compared with the histological grade of canine mammary tumors and we observed that foxp3 (tregs transcription factor) was mainly expressed in intratumoral and peritumoral infiltrating areas of neoplasias with poor prognosis.

These results suggest that the increase number of tregs lymphocytes in mammary neoplasias may play a role in tumor progression as they suppress the immune response against it, and allow the tumor progression.

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IMMUNOREGULATION

T LYMPHOCYTES, MAMMARY GLAND TUMORS, FOXP3

MOLECULAR DETECTION OF OVIS ARIES PAPILLOMAVIRUS TYPE 3 IN FORMALIN FIXED, PARAFFIN EMBEDDED (FFPE) SHEEP SQUAMOUS CELL CARCINOMA SAMPLES.

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Recently (Alberti et al., 2010) the prototype of a novel papillomavirus genus (OaPV3) was detected in normal skin and squamous cell carcinoma (SCC) lesions of Sardinian sheep. OaPV3 belongs to the Dyokappa genus and its genome significantly differs from the 2 species of ovine papillomaviruses previously reported in Australia that instead group with the artiodactyl Deltapapillomavirus species, and have been isolated from benign cutaneous lesions. Here we investigate the relevance of OaPV3 in SCC by screening a panel of FFPE sheep SCC samples

Based on the sequence of OaPV3 L1 and E6 two DNA probes and a set of 4 primers were designed and respectively used to develop an In situ Hybridisation test (ISH) and two RT-PCR assays. These assays were applied to a collection of 41 FFPE sheep SCCs samples obtained from cutaneous tumours (5 nasal, 6 ear, 10 periorcular, 3 dorsal, and 17 mammary tumours).

Diagnosis of SCC was confirmed by histopathological examination of the 41 samples. Molecular tests, summarised in the table, revealed that 26 out of 41 (63%) samples were positive to at least one test. Different tests showed different sensitivities. Also, tumours localised in different parts of the sheep body seemed to show variable degrees of positivity to OaPV3.

Results demonstrate a high prevalence (63%) of OaPV3 in sheep SCCs. This level of prevalence is particularly important and comparable to the prevalence of HPVs in human SCC (50 to 69%, Meyer et al., 2000), and suggests that OaPV3 represent an important risk factor for the development of sheep SCC. The level of positivity of nasal and periorcular lesions was greater respect to other tumour locations. This can be explained by the greater level of solar exposition and/or to traumas of these area respect to other locations. As expected, RT-PCR has a greater sensitivity than ISH. However, only combining these two tests the total number of positives can be detected, and both the presence/expression and localisation in the tumour can be investigated. Concluding, we cannot rule out the presence of unknown viral types in negative tissues. Further investigation is needed to investigate the presence of viral variants associated to different tumour locations and the presence of uncovered papillomaviruses in negative samples.

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2. Meyer et al., 2000. Dermatology 201:204-11

Anatomia Patologica e Malattie Infettive
OaPV3, squamous carcinoma, RT-PCR and ISH

Tumour location	N° samples	N° of positive	positive to all tests	ISH positive	RT-PCR positive	ISHE6 positive	ISHL1 positive	RT-PCR positive E6	RT-PCR positive L1
Nose	5	5	2	4	5	4	4	4	3
Ear	6	6	1	4	5	4	3	4	4
Eye	10	4	0	4	4	3	2	2	2
Flank	3	2	0	2	2	1	2	1	1
Udder	18	9	1	5	8	3	2	4	6
Tot.	42	26	4	19	24	15	13	15	16

MYOGENIC REGULATORY FACTORS EXPRESSION IN MURINE C2C12 CELLS TREATED WITH 17BETA ESTRADIOL: PRELIMINARY DATA

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Myogenesis is controlled by a family of transcription factors known as Myogenic Regulatory Factors (MRFs) including Myogenic Differentiation 1 (MyoD1), Myogenic Factor 5 (Myf 5), Myogenin (Myog) and Myogenic Regulatory Factor 4 (MRF4), which are differently expressed throughout the myogenic process. MyoD1 and Myf5 are mainly involved in the satellite cells commitment into proliferating myoblasts, whereas Myog and MRF4 are expressed during middle and late differentiation stages. MRF4 is also involved in fibers maturation (Dedieu 2002, Ferri 2009). These factors are regulated by extrinsic signals such as extracellular matrix substances, growth factors and hormones (Rhoads, 2009).

It is known that estrogens control myogenesis and skeletal muscle fibers changes. However the hormones mechanism of action are not yet completely understood (Boland 2008, De Jager 2011). In this preliminary study we evaluated the 17 β estradiol (E2) influence on MRFs expression in C2C12 cells.

C2C12 cells were seeded and, at about 80% confluence, cells were switched into a 10% horse serum medium to induce differentiation.

Cells were treated 5 times with 10⁻⁵ M or 10⁻⁸ M E2 every 48 hours and were harvested every 48 hours to evaluate expression of MyoD, Myf5, Myog, MRF4 by Real-time PCR and Western Blot. The evaluation of Myosin heavy chain I (MHCI) gene expression was carried out to confirm myotubes formation. Data were properly analyzed using GraphPad InStat (vers. 3.05).

No difference in myoblasts MRFs gene expression was revealed, while a difference in MRF4 gene expression was detected between treated myotubes and controls culture. In particular, 10⁻⁸ M E2 administration induced a greater MRF4 expression in myotubes than in untreated culture and 10⁻⁵ M E2 administration. Western Blot analysis confirmed these data.

The study confirmed that MRFs expression varie throughout the myogenic process, as previously described by Dedieu, 2002 and Ferri, 2009. In particular MRF4 expression, which is physiologically up-regulated during the differentiation phase, is further increased on myotubes by E2 administration.

Results confirm the over expression of MRF4, already described in adult bovine skeletal muscle cultures treated with E2 (Divari, 2013), and could help to understand the estrogen pathway regulation in muscle hypertrophy

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Pathology

myogenic factors, estrogen, mouse cell culture

NECROSCOPIC FINDINGS ASSOCIATED WITH EARLY REPLACEMENT IN BREEDING DOES

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In commercial rabbit farms, mortality and culling are of great relevance from the financial viewpoint.¹ Lesions in breeding rabbits are recorded mainly on animals at the end of their reproductive life and the replacement is usually performed based on reproductive parameters without further diagnostic investigations. The aim of this study was to assess the main necroscopic lesions in does replaced from reproduction and not slaughtered.

Sixty-seven dead does were collected from a commercial rabbit farm (Centro genetico Martini s.p.a., S. Maria Codifiume, FE) housing 5000 does, and submitted for postmortem examination. During necropsy, samples for histological and microbiological examinations were also collected. Information about age, number of deliveries and number of mating were recorded. To evaluate the effect of age, does were arbitrarily divided into two categories based on the median of number of deliveries (if available).

The age of does ranged from 115 to 587 days (median 286), the number of deliveries ranged from 0 to 13 (median 2). On postmortem examination, respiratory and genital tracts were the most frequently affected (n = 40; 59.7% each), the digestive system was involved in 30 does (44.77%) and other concomitant lesions, such as traumatic fractures, abdominal effusion, splenomegaly or cardiac injuries were seen in 36 cases (53.73%). Presence of pododermatitis was also observed in 42 animals (62.69%). Genital lesions included 16 uterine torsion (40%), 12 endometritis (30%), 12 uterine retention (30%) and others, such as dystocia or rupture (n= 6; 15%). Uterine torsion was significantly more frequent in does with more than 2 deliveries (P< 0.05). No other significant differences were observed in the 2 sets of animals grouped according to the number of deliveries. Bacteriological examinations performed on randomly selected pleural effusions were positive for *Pasteurella* spp. (n = 2); *Staphylococcus* spp. were isolated from endometritis (n = 2), macerated fetus (n = 1) and pododermatitis (n = 1).

Mortality in does was highest during the first two deliveries, similar results of highest mortality risks in the first three deliveries are reported also in literature.² Respiratory and genital lesions were the most relevant, in contrast with other authors,² who reported a higher prevalence of respiratory and digestive pathologies. Moreover, in our study, uterine torsion resulted the most important lesion associated with increased number of deliveries, although the related pathogenesis is still unclear.

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2 Rossel JM & de la Fuente LF (2009): Culling and mortality in breeding rabbits. Preventive Veterinary Medicine, 88:120-127.

Pathology

Necropsy, Rabbit reproduction, Rabbit Pathology

PARASITIC DISEASES IN MEDITERRANEAN JOHN DORY (ZEUS FABER)

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The aim of the present paper was to identify, describe and compare tissue changes due to parasites occasionally found in different subjects belonging to a wild teleost species, *Zeus faber*.

A total of 28 John dory (*Z. faber*) were examined. 4 fish had been collected during an experimental trawl survey carried out along the southern Tyrrhenian coasts in May 2012. 24 fish were collected from the fish market of Porto Empedocle and were coming from southern Mediterranean sea (Strait of Sicily). Scientific investigations were performed following routine histopathology methods. For parasitology investigation, parasite specimens were fixed in 70% ethanol, clarified by lactophenol and finally observed under the stereoscope. The molecular investigation was performed for anisakid larvae identifications by RFLP-PCR, amplifying ITS1, ITS2 and the 5.8S subunit.

Two fish, out of a total of four coming from Tyrrhenian sea, showed macroscopic parasites on the skin. Particularly, on the external surface of one fish a marine leech (*Hirudinea*) belonging to the genus *Calliobdella* was found. In the second specimen an adult taenia, 24 cm long, was found. The parasite was identified at genus level as *Alloptychobothrium* sp. At macroscopical evaluation of internal organs, within coelomic cavity of the same fish few anisakid larvae encysted in the stomach serosa were seen.

Only a single fish, out of a total of 24 subjects coming from the Strait of Sicily, at macroscopical external examination, showed grossly evident tissue changes. This fish showed a nodular bulge, 1 cm in size, in the left jaw. Histologically, such newborn tissue, was characterized by a granulomatous reaction containing pieces of parasitic bodies surrounded by an external chitinous cuticle; these parasites were identified as copepods, likely belonging to the genus *Chondrachanthus*. At the level of the caudal and the left pectoral fin, several whitish nodules (more than 50), about 1-2 mm sized, were seen as seriated or grouped growths; histologically, such nodules resemble granulomas found in the jaw. Within the coelomic cavity of all the fish a large number of anisakid larvae, identified by PCR as *Anisakis pegreffii*, were detected. Only two fish were negative for parasites. Moreover, in two fish, adult cestoda, belonging to the same genus described above, were detected within the intestinal lumen. Finally, slight fatty liver degeneration was demonstrated in all the examined fish.

By the results here obtained and according to the literature on teleost parasitoses, it can be assumed that parasites are fully integrated in all the different aquatic ecosystem and can be found in both wild and farmed teleost fish, although generally in wild conditions parasites have a low impact and only rarely can cause mortality. Although copepods and leeches are a common finding in the John dory, no data are available on intestinal cestoda. Finally, according to Angelucci et al. (2011), the presence of anisakid larvae in a so high percentage of specimens is in contrast with data recently reported on *Z. faber* fished in Mediterranean sea (Costa et al., 2010).

1) Angelucci G., Meloni M., Merella P., Sardù F., Madeddu S., Marrosu R., Petza F., Salati F. Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. *J Food Prot.* 2011 Oct;74(10):1769-75. doi: 10.4315/0362-028X.JFP-10-482.

2) Costa A., Sciortino S., Reale S., Alio V., Cusimano M., Caracappa S. 2008. Indagine sulla presenza di Nematodi Anisakidae in specie ittiche marine. *Atti X Congresso Nazionale S.I.Di.L.V.* Available at: http://www.sidilv.org/index.php?option=com_docman&task=cat_view&gid=4&Itemid=6. Accessed 10 September 2010.

fish pathology

ZEUS FABER, MEDITERRANEAN SEA, PARASITE

PATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATIONS OF EQUINE INFECTIOUS ANEMIA INFECTION IN MULES

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Following the implementation of a National surveillance program for equine infectious anaemia (EIA), a high seroprevalence was detected among Italian mules population. EIA infection in mules has been studied limitedly. To increase the knowledge about the pathogenesis EIA virus (EIAV) in mules and to better understand their role in the epidemiology of the infection, a study was conducted.

Naturally EIA-infected mules were immunosuppressed. At the end of the observation period, the mules were euthanized, and their organs were examined for gross and microscopic lesions. Furthermore, immunohistochemistry (IHC) was conducted to investigate the distribution of the virus.

At necropsy, no relevant gross lesions were observed. Microscopic examination of the different animals revealed mild multifocal haemorrhages in several tissues, a moderate to severe hemosiderosis in spleen and lymph nodes and mild to moderate lymphoid infiltrates, mainly in kidney, and lesser in liver and in the adrenal glands. Membranous glomerulonephritis was detectable in about 40% of the kidney. In the lungs, a mild interstitial pneumonia characterized by slight hypercellularity and thickening of the alveolar septa was evident. Interestingly, mild lymphocytic myocarditis with fibres degeneration, together with a multifocal, mild to moderate, lymphocytic meningoencephalitis, with perivascular cuffings thicker in the brain stem, were observed.

IHC, performed with a monoclonal antibody anti-p26 protein, detected EIAV infected cells in different tissues, both in sites as well as in the absence of lesions; this is particularly true in the adrenal glands, where a high number of positive reacting macrophages were detected in the medulla, and in the heart, with a lower positivity in the interstitial space. In contrast to what described in horses, the amount of positive cells in spleen seem to be lower. A positive signal was revealed mainly in the periportal areas of the liver, the alveolar septa of lungs and interstitium of the kidney cortex, and generally limited to cells morphologically resembling macrophages.

Microscopic lesions and EIAV localization in mules, highly resembles the infection described in horses. However, differently from what is reported for certain strains of EIAV in horses, no virus was detected in the endothelia. The present description is a preliminary contribution for the study of pathological alterations and EIAV localization in non-horse species.

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Sanità animale, virologia, anatomoistopatologia
EIA virus, Mules, Immunohistochemistry

PRIMARY “AMYLOID TUMOR” OF THE MAMMARY GLAND IN A DOG**Giudice C.*^[1], Baldassarre V.^[2], Turati L.^[3], Rondena M.^[2], Grieco V.^[1]**^[1]*DIVET, Università degli Studi di Milano ~ Milano*, ^[2]*Laboratorio Privato d'Analisi Veterinarie San Marco ~ Padova*,^[3]*Ambulatorio De Amicis ~ Milano*

The present report describes an unusual mammary lesion in a dog, with gross and histological features similar to the lesion known as “amyloid tumor of the breast” in the woman.

A fourteen year-old female spayed Dalmatian dog was presented to the practitioner with a large, bilobated lump involving left and right IV mammary gland. The mass was surgically excised and submitted for histopathological examination.

Grossly, two large subcutaneous nodular, firm masses, merging on the median line, expanded left and right IV mammary glands. Histologically, masses were encapsulated and mainly composed of large, coalescing lakes of pale eosinophilic, homogenous hyaline amorphous material, that stained positive with Congo Red (amyloid). Numerous plasma cells with moderate anisocytosis and anisokariosis and rare mitoses were multifocally recognizable. Multinucleated giant cells (MNGCs) with nuclear features similar to plasma cells were occasionally present. Moreover, MNGCs with scant eosinophilic cytoplasm and up to 25 dense nuclei were visible closely associated with amyloid deposits. Moderately atrophic mammary parenchyma was present at periphery. Immunohistochemistry (ABC method) was performed to characterize MNGCs and amyloid, applying anti-Lysozyme; Lambda-light-immunoglobulin-chains and HLA antibodies. Plasma cells and MNGCs were Lysozyme and HLA negative. Lambda-chains stained plasma cells, the majority of MNGCs and amyloid (AL amyloid).

Bilateral mammary extramedullary plasmacytoma with abundant AL amyloid deposition was diagnosed. Clinical workup did not reveal systemic amyloidosis or multiple myeloma. Sixteen months later the dog was humanely killed because of cardiac failure. No signs of multiple myeloma or recurrence of the tumor were reported.

Primary amyloidosis of the breast not associated with mammary carcinoma (amyloidoma), is a rare entity that has been documented in women since 1973, affecting mostly elderly patients, with bilateral involvement and related neither to systemic amyloidosis nor to multiple myeloma. Despite similar gross and histological appearance, some cases have been described as primary amyloidosis, while others as plasmacytoma with massive amyloid deposition

In the canine species, mammary gland amyloid deposition was described in association with mammary carcinoma whereas mammary primary amyloidosis or plasmacytoma with massive amyloid deposition have never been reported so far. To the best of author's knowledge this is the first report in the dog of a bilateral mammary extramedullary plasmacytoma with features consistent with primary amyloidoma of the breast in the woman.

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Veterinary and comparative pathology
amyloid, dog, mammary gland

SKELETAL MUSCLE EXPRESSION OF MYOSTATIN, IGF-1 AND GATA-2 IN CATTLE TREATED WITH GROWTH PROMOTERS

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Hormones are key regulators of mammalian muscle metabolism both in health and disease. Myostatin (MSTN), a member of transforming growth factor-beta family is a negative regulator of skeletal muscle mass. Several authors reported a direct relationship between myostatin expression and hormones treatment (Santos et al., 2012). Moreover, insulin-like growth- factor 1 (IGF-1) as well as GATA-2 have been broadly implicated in skeletal muscle growth, hypertrophy and regeneration (Musrò et al., 1999). This study aimed to determine the effects of different growth promoters on myostatin, IGF-1 and GATA-2 expression in skeletal muscle of veal calves and beef cattle.

Trial 1: 24 Charolaise beef cattle, randomly divided into 3 groups received: group A (n=6) 5 doses of 17beta-estradiol (20 mg/week/animal, IM), group B (n=6) DEX per os (0.7 mg/day/animal) for 40 days, group C (n=6) prednisolone per os (15 mg/day/animal) for 30 days; and group K1 (n=6) was untreated. Trial 2: 24 Friesian beef cattle, divided in 4 groups were treated as follows: group D (n=8) Revalor-200 subcutaneous implant for 89 days; group E (n=8) Revalor-200 subcutaneous implant for 89 days and dexamethasone per os (0.7 mg/day/ animal) for 40 days; group F (n=8) Finaplix-H subcutaneous implant for 89 days; group K2 (n=8) was the control.

Trial 3: 18 Friesian veal calves, randomly allotted in 3 groups received: group G (n=6) 35 mg/week of 17beta-estradiol, for 6 times; group H (n=6) 175 mg/week of testosterone, for 6 times; and group K3 (n=6) was untreated.

Trial 4: 30 Friesian veal calves, divided in 4 groups received: group L (n=8) was 17beta-estradiol (5 mg/week/animal) for 6 times and brotizolam (0.25 mg/day/animal) for 31 days; group M (n=8) 17beta-estradiol (5 mg/week/animal) for 6 times and dexamethasone (0.4 mg/day/animal) for 31 days; group N (n=8) nandrosol (150 mg/biweekly/animal) for 4 times and ractopamine (80 mg/day/animal) for 31 days; and group K4 (n=6) was untreated.

Samples of the sternocleidomastoideus muscle were collected from each animal and subjected to quantitative PCR for MSTN, IGF-1 and GATA-2 genes.

No statistical difference was observed in skeletal muscle for MSTN and GATA-2 genes examined in all experimental groups compared to controls.

The IGF-1 expression was significantly up-regulated in the group A ($P<0.01$) as well as in the group F beef cattle ($P<0.01$). Also in veal calves IGF-1 gene expression was significantly up-regulated. In particular, group L and group N gene expression were respectively 2.18-fold increase ($P<0.05$) and 2.48-fold increase ($P<0.01$) compared to controls.

The growth-promoting effects of sex steroids administration in livestock are well known.

IGF-1 is a potent anabolic factor that can activate myocyte proliferation and differentiation, leading to muscle hypertrophy. This study demonstrate the up-regulation of IGF-1 in skeletal muscle of cattle treated with estrogenic and androgenic molecules, as reported in humans and rats (Gentile et al., 2010; Pöllänen et al., 2010). However, in our study the increase of IGF-1 did not induce the up-regulation of mRNA GATA-2, as previously reported by Musrò and colleagues (1999). Further studies are needed to understand the role of sex steroids in the regulation of skeletal muscle hypertrophy.

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pathology

skeletal muscle, growth promoters, cattle

SPONGY POLIOENCEPHALOPATHY IN TWO BELGIAN MALINOIS PUPPIESSalvadori C.^[1], **Mandrioli L.*^[2]**, Gandini G.^[2], Cantile C.^[1]^[1]*Dipartimento di Scienze Veterinarie ~ Pisa*, ^[2]*DIMEVET ~ Bologna*

Spongy degeneration is seen in prion diseases and in a variety of progressive, invariably lethal neurodegenerative disorders. In various dog breeds it can involve the white or the grey matter. Amongst those involving the gray matter, cases are described in Bull Mastiff, Rottweiler, Saluki, Cocker Spaniel and Australian Cattle dog. A hereditary spongy degeneration has been reported in Malinois puppies, which suffer from congenital tremors with ataxia. In this report two cases of spongy degeneration in Malinois puppies are described, emphasizing the morphological and immunohistochemical features of the CNS lesions.

Two unkindred Belgian Malinois puppies were presented for mild depression, tremors of the head, cerebellar ataxia, and progressive ascending paraparesis. Complete blood count and serum biochemistry were normal. Serology for canine distemper virus, Toxoplasma spp. and Neospora spp. was negative. Cerebrospinal fluid showed no abnormalities. Because of the progressive ataxia and development of proprioceptive deficits on all limbs, and poor prognosis, the owners elected for humane euthanasia.

Brain, spinal cord, and samples of major organ were fixed in 4% buffered formaldehyde. Transverse sections of brain and spinal cord were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin, Luxol fast blue. Immunohistochemistry for 2',3'-cyclic nucleotide-3'-phosphohydrolase (CNPase), Olig2, glial fibrillary acidic protein (GFAP), phosphorylated neurofilaments, heat shock protein (HSP)70 and ubiquitin were performed on selected sections.

No lesions were detected at gross examination.

Histological lesions were restricted to the brain and were characterized by the presence of bilateral and symmetric large interneuronal vacuoles in the area of cerebellar nuclei, granular cell layer and foliate white matter of the cerebellum. Phosphorylated neurofilaments revealed numerous axonal spheroids; no ubiquitin-positive intracellular deposits were detected and a normal myelin pattern was evident with Luxol Fast Blue and CNPase immunohistochemistry. Oligodendrocytes showed a normal nuclear reaction for Olig2.

Lesions detected in these puppies are similar to previously described cases (1,2); the predominant lesions were found in cerebellar nuclei and flocculonodular cerebellar cortex, whereas spongy degeneration was not detected in the cerebral cortex. These are the first cases of spongy polioencephalopathy of Malinois dogs described in Italy, and the first time that immunohistochemical features are reported.

1.Cachin et al., 1991, 2.Kleiter et al., 2011

neuropathology

neuropathology, malinois, immunohistochemistry

STEM CELL MARKERS IN BENIGN AND MALIGNANT CANINE PROSTATE TISSUES: AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Canine prostatic carcinoma (PCa) is considered a relevant model for studying advanced, hormone refractory PCa in men. It has been proposed that cancer contains a minor population of cells that can self-renew while simultaneously giving rise to tumour cells (cancer stem cells). Survivin is an acknowledged cancer therapy-resistance factor overexpressed in several tumour types, proposed as a valid cancer biomarker for human prostatic cancer for an early screening for malignancy. Sox9 is a stem cell marker expressed in several adult tissues, required for prostate development. Accumulating evidence indicates that it contributes to the development of human PCa. No studies have been published concerning the immunolocalization of survivin and Sox9 in canine prostatic hyperplasia (BPH) and neoplasia. AIM: to evaluate the patterns and levels of expression of survivin and Sox9 in canine BPH and PCa, in order to correlate their expression with malignant histological features.

immunohistochemistry with specific antibodies in a set of canine BPH and PCa.

Survivin nuclear and rare cytoplasmic immunostaining were present among the basal/reserve cell layer of normal and hyperplastic prostatic lobules. An increase of survivin expression was observed in PCas compared with BPHs. 6/11 PCas showed rare positive nuclei, mainly among the "basaloid" neoplastic cells in the areas with a tubular-papillary arrangement. In the areas with a solid pattern the cytoplasmic immunostaining was more diffuse. Sox9 expression was absent in normal prostatic glands and in all BPHs. 6/9 cases of PCa were highly positive.

Based on the role of survivin as a stem cell marker and the main role in proliferation of nuclear survivin, the positive cells among basal cell layer in normal and HBP cases could represent transit amplifying cells maintaining some stem cell proprieties. The increased survivin expression in Pcas would indicates the molecule as a valid prognostic marker. The absence of expression of Sox9 in the normal gland and all the BPHs suggests that Sox9 is not a stem cell marker of canine adult prostatic stem cells. The high expression observed in PCas clearly suggests an important role of the molecule in canine prostatic carcinogenesis and malignant progression. Further studies should be done in order to confirm this hypothesis.

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Disciplinare

dog, stem cell, prostate cancer

STUDY OF THE HIPPO-TRANSDUCER TAZ IN CANINE AND FELINE MAMMARY TUMORS: PRELIMINARY DATA

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Among others, HIPPO signaling was recently indicated as a key regulator in both physiological and neoplastic cell growth, differentiation, and survival. One of the major output of HIPPO pathway is the inhibition of the coactivator transducer TAZ which controls cell growth, proliferation and survival but also cell differentiation, stem cell renewal, reprogramming, and patterning. TAZ activity correlates with high grade, metastasis, and Cancer Stem Cells (CSCs) content in human breast cancer. Particularly, TAZ is required to sustain self-renewal and tumor-initiation capacities in breast CSCs, moreover its biological activity is associated with epithelial-mesenchymal transition which disrupts TAZ inhibition, increasing its activity. The aim of our study was to evaluate TAZ expression in canine (CMCs) and feline mammary carcinomas (FMCs) particularly investigating correlation to grading and histopathological morphology.

We investigated TAZ expression in 60 CMCs and FMCs and surrounding non-neoplastic mammary gland by immunohistochemistry. The selected mammary tumors equally belonged to the following histopathological groups: simple tubular carcinomas grade I, simple carcinomas grade III, ductal-associated tumors grade I (cats), complex carcinoma grade I (dog), simple-carcinoma-and-myoepithelioma (SCMM) grade I (dog). Three different scoring systems were applied and compared.

TAZ was found to be diffusely overexpressed in grade III simple CMCs and FMCs. In addition, basal cells were TAZ positive both in neoplastic and hyperplastic canine glandular tissue. Interestingly, a moderate positivity was detected also in basal undifferentiated cells of ductal-associated FMCs. Canine SCMMs showed higher TAZ expression compared to complex CMCs.

These preliminary results indicate that also in CMCs and FMCs TAZ may be an important pathway transducer for aggressiveness and might regulate basal cells proliferation and fate. Further samples collection and analyses are required to give insights into its role and regulation in mammary cancers and eventually its additional prognostic information.

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Anatomia patologica veterinaria - Tumori mammari

Hippo, TAZ, Mammary tumor

THE ASSESSMENT OF FEED EFFECTS ON LIVER AND INTESTINE OF REARED FLATFISH: WHAT IS THE BEST STANDARD ANATOMOPATHOLOGIC PROTOCOL?

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This presentation highlights the importance of using standardized methods by the fish pathologists to assess the effects of feed on reared flatfish. The liver and intestine are the most important organs for the digestion and absorption of nutrients from feed, and monitoring their histological structure is of fundamental importance in assessing the effects of nutrient mixtures. A critical analysis of the histological methods on feed effects will be presented, aiming to suggest a standardized protocol that should be adopted, on the basis of the advantages and disadvantages that have been encountered in the course of our recent research trials.

Six feeding trials have been carried out on juvenile common sole (*Solea solea*), Senegalese sole (*Solea senegalensis*) and turbot (*Psetta maxima*) (fed diets with increasing dietary protein levels; 2, 3 increasing dietary plant protein in replacement of fishmeal; 4 increasing lipid levels and increasing mussel meal, respectively).

Routine histology (haematoxylin-eosin stain on formalin-fixed and paraffin-embedded sections) was carried out to check degenerative and/or inflammatory changes. In the liver, as well as the graduation of the lipid content, further histochemical techniques have been applied in order to characterize the type of intracellular accumulation (OilRedO, Toluidine blue, PAS) and transmission electron microscopy (TEM), while in intestine in situ techniques to assess the cellular turnover and trophism of epithelial mucosa (anti-PCNA immunohistochemistry and TUNEL method for apoptosis) evaluated by image analysis and TEM were employed.

The routine histological evaluation is helpful only to demonstrate degenerative-inflammatory changes and its usefulness increases when combined with a grading system for the lipidic content of the liver that revealed the most frequent degeneration assessed. To confirm the lipidic content, Toluidine Blue on semithin sections revealed better than OilRedO which run on frozen sections that require a more difficult management of the samples.

IHC on intestinal sections and the evaluation of cellular kinetics and mucosal turnover are necessary tools to assess the influence of a diet on mucosal trophism.

For both tissues TEM is the elective method to further characterize the type of intracellular accumulation and the early signs of cell damage.

On the basis of our experience it is necessary to adopt a standardized approach to evaluate the effects of experimental diets on liver and intestine; in particular, investigations in liver require Toluidine Blue on semithin sections and TEM, while for intestine IHC together with digital image analysis and morphometry are suggested in addition to routine histology.

1Raskovic et al., 2011; 2Gatta et al., 2011; 3Bonaldo et al., 2011; 4Mandrioli et al., 2012; 5Bonvini et al., 2014 (Proceedings of ISFNF); 6Bonaldo et al., 2014 (Proceedings of ISFNF).

fish pathology

flatfish, liver and intestine, histopathology

Unusual mass stranding of *Caretta caretta* in the North Adriatic Sea: relevant gross and histological lesions.

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Caretta caretta is the most abundant species of marine turtles in the Mediterranean Sea. The greatest threats for the sea turtles in many parts of the world include, marine pollution, loss of nesting habitats, accidental capture by surface fishing with longline hooks or to lesser extent other types of fishing. Worldwide, the loggerhead sea turtle is currently classified as an "endangered" species (IUCN), and its situation is probably critical in the Mediterranean Sea. Between October and December 2013 an unusual mass stranding involving 300 dead *Caretta caretta* occurred on the coasts of the North Adriatic Sea. Depending on the tidal currents and on the wind directions, the sea turtles stranded in 4 main waves on the coasts of Emilia Romagna (ER), Friuli Venezia Giulia (FVG) and Marche (Ma). 54 necropsies were performed at the Department of Comparative Biomedicine and Food Science (BCA) of the University of Padua, 10 subjects from FVG and 44 from ER. Regarding the biometrical data the sex distribution was females 63%, males 28%, and 9% undetermined. On the basis of Curved Carapace Length (CCL) they were 11% juveniles, 72% subadults and 17% adults. The conservation status of the carcasses was very uniform with 46% of the subjects in moderate decomposition and 45% in advanced decomposition; only 7% of the carcasses were mildly decomposed and 2% mummified. Generally, all the subjects were in good status of nutrition (70%); only 24% had scarce fat deposits (6%, not detectable). About the main lesions, 63% of the subjects had haemorrhagic oedema between the pectoral ventral muscles, 60% haemorrhagic effusions within the body cavities (30% in both coelomic and pericardic cavities), and 56% severe and diffuse often haemorrhagic enteritis, lack of ingesta and moderate quantity of haemorrhagic catarrhal contents. Considering all the data together, the dead stranded *Caretta caretta* seem to belong to a singular group that died suddenly and later drifted to the Italian coasts. Parasitological, bacteriological and histological analyses were done; toxicological and biological analyses are in progress. Unfortunately, the scarce status of conservation of the turtle tissues prevent any interpretation of possible lesions (except very rare granulomas or parasite eggs), but strange small round formations appear in different organs of most of the histologically examined turtles: in 18 out of 21 turtles (86%), unidentified roundish eosinophilic cell-like structures are visible, mostly with dark brown crowns, often with very small basophilic structures inside, from 5 to 50 µm large. These corpuscles are mainly in the intestine (48%), spleen (48%) and thymus (29%) suggesting that from the gut they have a tropism for the lymphoid system. Electron microscopy analyses are in progress. Until now, the data does not support any valid theory for the death of so many turtles.

UTERINE LEIOMYOMA ASSOCIATED WITH CYSTIC ENDOMETRIAL HYPERPLASIA-PYOMETRA COMPLEX IN ASIAN ELEPHANT (ELEPHAS MAXIMUS).

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Leiomyoma is the most common uterine tumor in women, occurring also in many domestic animals³. Most captive female elephants are nulliparous and aged and many have endometrial diseases, factors that may hinder fertility^{1,4}. We here describe a case of uterine leiomyoma associated with cystic endometrial

hyperplasia—pyometra complex occurred in a 42-year-old female Asian Elephant (*Elephas maximus*), maintained at the Rome Zoological Garden.

The Elephant, nulliparous, occasionally expelled necrotic material from uro-genital opening for nine years before death. Histologically, this material was always identified as an exudate in the process of organization, resulting positive to *Escherichia coli*. Repeated cycles of antibiotic (moxifloxacin) were made. Two days before death, the animal showed systemic malaise, weight loss and ataxia.

At necropsy, the uterus was significantly increased in volume and abundant purulent material was present in the lumen; the wall was considerably thickened, with the presence of numerous cysts mixed with nodular lesions of variable size, whitish in color and with increased compactness. Masses of necrotic material could also be seen adhering to the wall or free in the lumen. Histologically, numerous nodular lesions that showed neoplastic proliferation of smooth muscle cells in diffusion-type arrangement were observed. The mitotic index was low and there was absence of nuclear atypia. In addition, diffuse hyperplasia of the endometrium with formation of numerous cysts containing liquid amorphous eosinophilic were seen. In some areas there were extensive purulent-necrotic foci. Histological sections stained with Masson's method emphasized the presence of smooth muscle cells and, immunohistochemically, neoplastic cells were constantly positive for vimentin and for α -smooth muscle actin, while they were negative for cytokeratin and S-100. In this case report, the features of the neoplastic cells and the positivity for Masson's method, vimentin and for α -smooth muscle actin clearly express the origin of the tumor from smooth muscle². In addition, *Candida* sp., *Aeromonas* sp. and *E. coli* were isolated from the uterus.

To our knowledge, this is the first case of uterine leiomyoma associated with cystic endometrial hyperplasia-pyometra complex described in an Asian Elephant.

1)Aupperle et al., Chronic endometritis in an Asian elephant (*Elephas maximus*). *J Zoo Wildl Med.* 2008;39(1):107-10. 2)Ilha et al., Uterine lesions in 32 female miniature pet pigs. *Vet Pathol.* 2010;47(6):1071-5. 3)Meuten DJ, *Tumors in Domestic Animals*, 4th Edition Iowa State Press, 2002 Ames, Iowa, USA, 4)Sapundzhiev et al., Fibroleiomyoma in elephant uterus. *J Vet Med A Physiol Pathol Clin Med.* 2007;54(9):499-500. 5)Schaudien et al., Omental leiomyoma in a male adult horse. *Vet Pathol.* 2007;44(5):722-6.

ONCOLOGIA ANIMALI ESOTICI

UTERINE LEIOMYOMA, CYSTIC ENDOMETRIAL H, *ELEPHAS MAXIMUS*

WINTER DISEASE IN ASSOCIATION WITH INTESTINAL NON-FORMING XENOMA MICROSPORIDIA IN GILTHEAD SEABREAM (SPARUS AURATA)

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Winter disease (WD) is a multifactorial disease found primarily in sea caged gilthead sea bream (*Sparus aurata*) along the Mediterranean coast.^{1,2} An emaciative syndrome has been recently observed in Spain and *Enterospira nucleophila*, a new microsporidian species of the family Enterocytozoonidae has been described; these intracellular, non-forming xenoma microsporidia, opportunistic in nature exploit a weakened host immune status, as it could happen in WD.⁶ Teleost intestine contains Mast Cells (MCs), whose functional properties are similar to those of mammalian mast cells;⁵ recruitment of MCs to sites of persistent inflammation is a general response in parasites-affected fish. An increased number of the MCs is also reported in WD-affected fish.^{3,4} In December 2013 a disease outbreak in sea caged gilthead seabream in Italy, affecting 0+ year fish occurred. The aim of this study was to evaluate the histopathological changes related to these two conditions, to characterize the MCs by immunohistochemistry and the microsporidia by PCR.

From twenty gilthead sea bream intestinal tracts were fixed in buffered formalin at the sample site and sent to DIMEVET. Routine histological sections were obtained; Luna stain was also performed. Immunohistochemistry with CD117 antibody (1:100, Dako) was also carried out. Intestinal tissue was also subjected to molecular analysis; a fragment of the 18S rDNA was amplified and then sequenced.

Hindgut showed a moderate dilatation of the lumen in association with whitish casts, similar to the milk-like mucous casts reported in WD outbreaks.^{1,2} A severe mucosal atrophy with total folds flattening was present; within lamina propria and submucosa a mild to moderate MCs hyperplasia and mild mucous cells hyperplasia were observed. Multifocally, within enterocytes and rodlet cells, the nucleus and/or cytoplasm contained microsporidian spores, more evident with Luna stain. The sequences obtained from intestines showed 99.9% identity with *E. nucleophila*. Within perivisceral exocrine pancreatic acini, focal necrosis and MCs infiltration have been observed, as reported by other authors during WD outbreaks.

MCs are interpreted as “standing force” in particular tissues consistently exposed to pathogens, in contrast to a “mobilization force” that has been an advantage in those being exposed to noxious agents only occasionally.⁷

The severe mucosal flattening could be interpreted as an effect of a chronic insult, not only related to the microsporidia infection; other concurrent predisposing factors as those reported in WD could be then considered into the development of an overt pathology.

¹Birincioglu et al, 2013; ²Colorni, 2011; ³Contessi et al, 2006; ⁴Ibarz et al, 2010; ⁵Lauriano et al., 2012; ⁶Palenzuela et al., 2014; ⁷Reite and Evensen, 2006.

fish pathology

winter disease, *sparus aurata*, microsporidia

DILATION OF THE VERTEBRAL CANAL IN A PARETIC BOA CONSTRICTOR (BOA CONSTRICTOR IMPERATOR) AFFECTED BY PROLIFERATIVE OSTEOARTHRITIS

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Proliferative vertebral lesions are a common finding in snakes and have been classified into three distinct categories: bacterial osteoarthritis, including active septic osteomyelitis or osteoarthritis with multiple inflammatory foci, and non-bacterial degenerative ankylosing osteoarthritis. Computed tomography (CT)-guided biopsy could be especially useful to rule out active bacterial infections.

AIM. To describe a case of proliferative osteoarthritis and dilation of the vertebral canal in a paretic boa constrictor (*Boa constrictor imperator*).

Radiography and CT-guided biopsy, haematoxylin-eosin staining and histopathological examination.

A 1-year-old, male boa constrictor presented with paresis of the trunk originating cranial to the cloaca. Radiographies were consistent with proliferative bone lesions involving several vertebrae. CT demonstrated the presence of lytic lesions, characterized by widening of the spinal canal. CT-guided biopsies of the lesions were performed. Histology was consistent with bacterial osteomyelitis and osteoarthritis. Gram-negative bacteria (*Salmonella* sp.) were isolated from cultures of the biopsies. A medical treatment was attempted for several weeks without clinical or radiographical improvements. The animal was euthanized and necropsy confirmed the findings observed upon CT. The main lesion comprised 3 vertebral bodies and the vertebral foramen appeared dilated and filled with friable gray/green to brown (purulent/necrotic) material, surrounded by abnormal vertebral bone. Another lesion, which was clinically undetected, consisted in accumulation of purulent material on the ventro-lateral aspect of the vertebra, involving the left rib. Histologically, the vertebral foramen was filled with degenerated heterophils and necrotic material, with bacterial colonies, completely replacing the spinal cord, surrounded by a rim of reactive macrophages and fewer multinucleated giant cells.

Dilation of the vertebral canal is an uncommon finding in snakes presenting proliferative osteoarthritis and possibly carries poor prognosis. Considering that proliferative vertebral lesions may be secondary to bacterial or other causes, and blood culture is not one hundred per cent sensitive, CT-guided bone biopsies may be useful to reach a definitive diagnosis.

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generale

snake, *Salmonella*, osteoarthritis

Comunicazioni orali Sira

DYNAMIC CHANGES OF LH RECEPTOR CONTENT IN CORPORA LUTEA DURING THE BOVINE ESTRUS CYCLE

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Luteinizing hormone (LH) plays a crucial role in the development and maintenance of the corpus luteum (CL) in cattle. Upon binding to its receptor (LHR), LH stimulates adenylyl cyclase activity. The consequent production of cyclic adenosine 3',5'-monophosphate causes an increase of steroidogenesis, and, in particular, progesterone production (Davis et al., 1996). Based on this paradigm, it is possible to assume the amount of LHR in CL as an indicator of the progesterone secretory capacity and, hence, CL competence to support an adequate luteal phase.

CL life span in relation to the estrus cycle may represent the most important physiological source of variation of LHR content. Such variability has been previously evaluated in bovine LHR transcriptional expression (Kawate et al., 1998); however, to our knowledge, there are no information related to LHR protein.

For this study, bovine ovaries were collected from slaughterhouse and stored at 4°C. After a gross morphological in situ evaluation (Ireland et al., 1980), CLs were classified in relation to the estrus cycle stage (estrus= day 0); in particular, Stage I, II, III and IV included intervals between days 3-4, 8-11, 14-16 and 17-19, respectively.

Frozen/thawed CL samples were kept in lysis buffer on ice for 1 h, homogenized and centrifuged at 13000 rpm for 10 minutes. Proteins (100 µg) were resolved on SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. After blocking with non fat dry milk in phosphate buffered saline with 0.05% Tween 20 (PBS-T), membranes were incubated overnight at 4°C with 1:200 goat anti-LHR antibody (Santa Cruz Biotechnology) and with 1:500 mouse anti-α-tubulin antibody (Sigma-Aldrich) followed by incubation with 1:5000 donkey anti-goat IgG-HRP antibody (Santa Cruz Biotechnology) and with 1:3000 goat anti-mouse IgG-HRP antibody (Sigma-Aldrich), respectively. Proteins were detected with Pierce ECL Plus WB Substrate (Thermo Scientific) and CL-XPosure Film (Thermo Scientific). The films were scanned with GelDoc-It (UVP) using Vision Works LS software and proteins were quantified by ImageJ software. LHR/α-tubulin band ratios were analyzed by ANOVA (Systat 11.0).

A significant ($P < 0.001$) decrease of LHR protein content was found through the CL life span. In particular, LHR protein pattern showed the highest content at Stage I (93.7 ± 19.3), a rapid decline at Stage II (28.2 ± 6.6) and a smaller decline in the later stages (Stage III= 26.5 ± 9.0 and Stage IV= 16.8 ± 1.1).

This pattern may reflect a dependence of the CL to LH stimulus during its preliminary growth and a progressive refractoriness to such influence in later stages of CL life span.

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Reproductive Biology

Bovine, corpus luteum, LH receptor

SUBCLINICAL ENDOMETRITIS IN PIEDMONTESE COW: CYTOLOGY, BACTERIOLOGY, INFLAMMATION MARKERS AND TEST STRIP

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In dairy cows subclinical endometritis (SEM) affect the fertility, increasing the Days open (DO), decreasing the Conception Rate (CR), although very little information are stated about this pathology on beef cows (Santos N. et al 2009). The objective of this study is to evaluate the impact of SEM, on day 30 and 60 from parturition and to estimate the correct cut-off for neutrophils concentration (PMN) on same intervals.

A selected group of 45 cows were enrolled and were submitted to uterine flushing and blood sample at 30 and 60 days postpartum (pp): uterine fluid bacteriology, test strip and cytology, to evaluate neutrophils percentage (PMN), were executed furthermore. Haptoglobin (Hap) and Pregesterone (P4) was analyzed from blood serum. Uterine flushing, pH, Pr and LE were evaluated using test stripes. To define Infertility, 120 days postpartum open day was chosen. The results were correlated with presence or absence of Bacteria, housing conditions, Hap, P4. and test strips results.

A 64% cows were positive to SEM at day 30 with at cut off point for PMN of 6.5% and 44.5% at day 60, with 2.5% of PMN on day 60. Only 30% of cows were positive on day 30 but not on day 60 (DO 149 d) and 17.5 % were negative on day 30 but positive on day 60 (89 days) ($P<0.05$). Number of service/IA and the presence of a calving pen, are correlated with positivity to SEM, cows with SEM increase the DO compared with negative to SEM (104 vs 141; $P<0.05$). Suckling condition and parity not affect SEM ($P<0.05$). SEM cows at day 30 increase DO of 46 days than negative, but only 10 days on day 60 ($P<0.05$). 80% of cows had bacteria on day 30 and 67% on day 60. Only cows positive for SEM and Bacteria in uterus show a DO of 145 days (OR=6.3) on day 30 compared with the negative ones. Only Pr was correlated on day 30 with SEM, Pr cut-off score at 30 days was 2, cows with score 2 or more have a 127 days DO than <2 cows with a DO 67 ($P<0.05$). Hap and P4 in blood serum didn't show any association with SEM neither at 30 (Hapt. 16.60 vs 12.1 mg/dl, P4 1,6 vs 1,7 ng/ml) or 60 (Hapt. 9.6 vs 3.56 mg/dl) days nor between the two period. In conclusion cows positive to SEM at 30 and not at 60 have and increased DO (149 days) than cows positive for 30 and 60 (123 days) and cows negative for day 30 but positive on day 60 (89 days).

In conclusion positivity to SEM on day 30 seems to impact more on fertility ($P<0.05$) than on day 60 ($P>0.05$), probably due to a decreased capacity to resolve inflammatory conditions in earlier postpartum. Protein seems to be an interesting marker for infertility at day 30.

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uterine pathology in postpartum of bovine
endometritis, haptoglobin, test strips

CONTRAST-ENHANCED ULTRASOUND AND ITS UTILITY IN BUFFALOES CORPUS LUTEUM

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Ultrasonography is the first-line and frequently the only imaging modality employed in the assessment of ovarian and uterine abnormalities. Technical advances in transducer design and image processing has further improved the quality of diagnosis impending ovulation and luteogenesis, with colour Doppler adding important information and newer techniques (contrast-enhanced ultrasound and elastography) awaiting further evaluation.

The aim of the present study is to evaluate the corpus luteum perfusion kinetics in buffaloes without ovarian abnormalities using CEUS

Ultrasound examination was performed with a 5–7.5 MHz linear transducer with coded harmonic capability (Mylab 30, Esaote-CnTI System; Esaote, Genova, Italy). A second-generation contrast agent SonoVue (Sulphur hexafluoride microbubbles; Bracco Imaging S.p.A., Milan, Italy) and a dedicated contrast-enhanced ultrasound analytical software (Contrast Tuned Imaging – CnTITM-Contrast Tuned Imaging Technology, Esaote, Genova, Italy) were used. The mechanical index was always lower than 0.1 (range 0.05–0.1) to reduce the acoustic impact of the ultrasound waves on the micro bubble contrast agent, and to increase the persistence of the contrast medium in the blood. The contrast injection consisted of an intravenous bolus of contrast agent (SonoVue, Bracco S.P.A., Milan, Italy) at a dose of 0.03 ml / kg of prepared solution (5 mg/ ml injected into the jugular vein catheter followed by a saline flush of 10 mL, which ensured that all the contrast in the line was administered to the animal. Small adjustments were made to find the optimal plane of the CL to image. A commercial software application (QONTRAST, Milan, Italy) was then used to construct time-intensity curves.

Results The flow of microbubbles was visible in the ovarian parenchyma after about 40-45 seconds. The enhancement pattern was different between the two groups of buffaloes. During the wash-in phase, subcapsular arteries, followed by intraparenchymal arteries could be visualised. After few seconds, an homogeneous and a strong enhancement of the parenchyma could be seen, with parenchymal vessels still distinguishable.

The assessment of microvascular blood flow of the buffaloes CL are possible to measure with CEUS. In particular in the time intensity curves the HVCL showed higher TTP and RBF values compared with the LVCL. Quaia, E., 2005b. Physical basis and principles of action of microbubble- based contrast agents. In: Quaia, E. (Ed.), Contrast Media in Ultrasonography. Basic Principles and Clinical Applications. Springer, Germany, pp. 15–30.

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Reproductive ultrasonography in Buffaloes
ovarian ultrasound, buffaloes , contrast ultrasound

FOLLICULAR GROWTH AND EMBRYO PRODUCTION IN MARCHIGIANA COWS SUPEROVULATED WITH TWO ADMINISTRATION OF PORCINE FSH DILUTED IN HYALURONAN

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The most widely used superovulation (SO) treatment consists of 6-8 FSH injections at 12-h intervals over a 3 or 4 day period. A series of studies were conducted in order to simplify the SO treatment schedule in cattle. Recent researches have demonstrated that SO can be successfully induced using 2 injections of FSH diluted in hyaluronan [1-2]. The aim of this work was to compare ovarian follicle growth profile and SO response of Marchigiana beef cattle treated with two administrations of Pluset® diluted in hyaluronan with the traditional 8 injections protocol.

Marchigiana cows (n=36) were randomly allocated in two groups referred as Control (C) and Slow release treatments (SR). Control animals received FSH diluted in saline administered in twice-daily decreasing doses over 4 days whereas SR protocol consisted in 2 administration of FSH diluted in a 5 mg/ml hyaluronan solution given at day 0 (66% of the total dose) and the second after 48 hours (33% of the total dose). Superstimulation protocol started between day 8 and 11 of the cow's estrous cycle for both the groups (Day 0). All cows received 2 PGF2α injections on day 3, 12 hours apart and starting from day 5 all animals were artificial inseminated. Ova-Embryos were collected on day 12, and evaluated according to IETS recommendations. Cows were submitted to transrectal ultrasonography at the time of the first FSH injection (T0), 48 hours later (T1), at time of estrus, and immediately after embryo recovery. CLs and follicles larger than 3 mm in diameter were counted, measured, and allocated to three size classes: small (3–5 mm), medium (5–7 mm), and large (7 mm).

The number of small and medium follicles at T0 and that of medium follicles at T1 were significantly higher in SR than in the C group. Despite these differences the pattern of follicular growth was highly similar between the groups. Furthermore, no difference were found in the number of pre-ovulatory follicles, CLs and transferable embryos.

These data support the literature regarding the usefulness of a 2 injections protocol of FSH diluted in hyaluronan to induce a satisfactory ovarian response in donor cows. Moreover the reduction of injections, decreases the stress of donors, improves animal welfare and allows to reduce efforts and possibility of failure due to mishandling or incompliance, without compromising the final results of SO.

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Bovine Theriogenology

Superovulation, Hyaluronan, Cattle

	3-5mm		5-7mm		>7mm		Pre-ovulatory Follicle	CLs	Transferable Embryos
	T0	T1	T0	T1	T0	T1			
C (n=18)	10.1±2.0*	12.7±1.1	1.3±0.3*	7.4±0.9*	1.4±0.2	2.9±0.4	14.6±1.3	12.3±1.2	7.4 ±1.4
SR (N=18)	14.4±1.4*	18.1±2.4	1.7±0.5*	10.4±1.2*	1.1±0.1	2.9±0.3	15.8±1.8	13.5±1.8	8.8±1.5
OVERALL (N=36)	12.2±1.3	15.4±1.4	1.5±0.3	8.9±0.8	1.3±0.1	2.9±0.2	15.2±1.1	12.9±1.0	8.1±1.0

PRELIMINARY RESULTS FROM A FIELD STUDY ON THE USE OF ET TO IMPROVE REPRODUCTIVE PERFORMANCE IN REPEAT BREEDER COWS

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Repeat breeders are defined as “Cows that failed to conceive after 3 or 4 inseminations without significant detectable disorders associated with the reproductive tract”; despite these cows exhibit normal signs of estrus every 18 to 24 days they require more than 3-4 services to become pregnant. In recent years, there has been a decline in the fertility of lactating dairy cows and in addition, the number of services per conception has increased; which would mean that the proportion of repeat-breeder cows has also increased. Nowadays repeat breeding is an important factor affecting economic success in dairy management and the first step in correcting this problem is to diagnose its cause or causes. Unfortunately, this can be a difficult task since repeat breeding is a multifactorial syndrome. Several approaches have been tested to improve fertility performances in repeat breeder cows and recent studies suggest that ET may be an effective alternative to achieve this result.

Forty-four referred as clinically normal repeat breeder cows (having $\geq$ three artificial inseminations) of 6 different herds were examined using trans-rectal ultrasonography to assess ovarian structures and uterine conditions. Only cows confirmed without any uterine or ovarian pathological conditions were selected as potential recipients (n=39) and submitted to oestrus synchronization program. Based on estrus detection, 38 recipients were presented to be evaluated for ET. At the scheduled time of ET transrectal ultrasound examinations of the ovarian structures were performed. Only cows that presented a CL with a normal morphology and echotexture and that measured at least 10 mm (n=35) received a single fresh or frozen-thawed embryo using a standard ET technique in accord with the IETS. Pregnancy diagnosis was performed by ultrasonography 30 to 40 days after ET.

Pregnancy rates after ET was 37.14%. Results achieved were slightly lower than those reported in other studies (41.7% [1] 47% [2]) but is higher respect to those commonly achieved by the use of AI in repeat breeder animals. In fact, the pregnancy rate after AI ranged in the six selected farms from 20 to 35 %.

This preliminary results suggest that ET could represent a valid method to improve fertility in repeat breeder cows, but further studies are required to carefully investigate its efficacy.

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[2] Buniotto P. Utilizzo della Embryo Therapy nelle Repeat Breeder . 22nd SIET conference, October 2013, Modena, Italy. http://www.sietitalia.it/Buniotto_SIET_2013a.pdf

Bovine Theriogenology

Embryo Transfer, Repeat breeder, Cow

SERUM IGG CONCENTRATION AFTER INTRAVENOUS PLASMA TRANSFUSION IN COLOSTRUM-DEPRIVED DAIRY CALVES

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Plasma transfusion has been empirically employed in the management of newborn calves with failure of passive transfer (FPT i.e. serum IgG concentration <10 g/L) [1]. Nevertheless, the effect of an IV transfusion of plasma on serum IgG concentration in FPT calves needs to be evaluated systematically. The aim of this study is to evaluate the effect of the administration of an adequate dose of IgG in colostrum-deprived calves on their serum IgG concentration

Twenty-eight Italian Friesian newborn calves, belonging to a single dairy herd, were randomly assigned to Colostrum Group (CG, n=14) or Plasma Group (PG, n=14). CG calves received 4 L of high quality bovine colostrum (> 21 of the 0:32 Brix refractometry scale) within 12 hours after birth [2]. PG calves received a dose of IgG from pooled plasma within 6 hours after birth. The IgG dose needed to obtain the minimum IgG serum level of 10 g/L in our calves has been determined by the following formula [3]:

$$[(\text{body weight} \times \% \text{ blood volume}) \times (\text{pretreatment serum IgG}) + (\text{Total IgG transfused})] / (\text{body weight} \times \% \text{ blood volume})$$

Plasma was collected from 20 healthy IBR free cows vaccinated against rotavirus, coronavirus and E. coli, selected from animals of the same herd with a high γ -globulin rate. To obtain plasma, 4L of blood have been collected from each cow into sodium citrate transfusion packs and subsequently centrifuged. Plasma obtained was tested for IgG concentration by ELISA and then stored at -20 °C until transfused. Serum IgG concentration before (T0) and after plasma transfusion or colostrum administration (1 day - T1, 3 days - T2, 7 days - T3) were determined by ELISA. Both groups were monitored by a daily clinical examination for a period of 3 week

PG calves received a quantity of $52 \pm 4,40$ g of IgG in a volume of $2,23 \pm 0,38$ L of plasma. Four PG calves died for neonatal diarrhea complicated by E. coli septicemia at respectively 5, 11, 12, 16 days of age. On the other hand, none of the CG calves showed signs of disease or died during the study. Serum IgG concentration increased in both CG and PG calves. Mean concentration of IgG in all observation times, was higher in CG calves than PG calves (Fig.1). Although all CG and PG calves showed an IgG value > 10g/L, the means between groups were statistically different at each observation ($P < 0.01$)

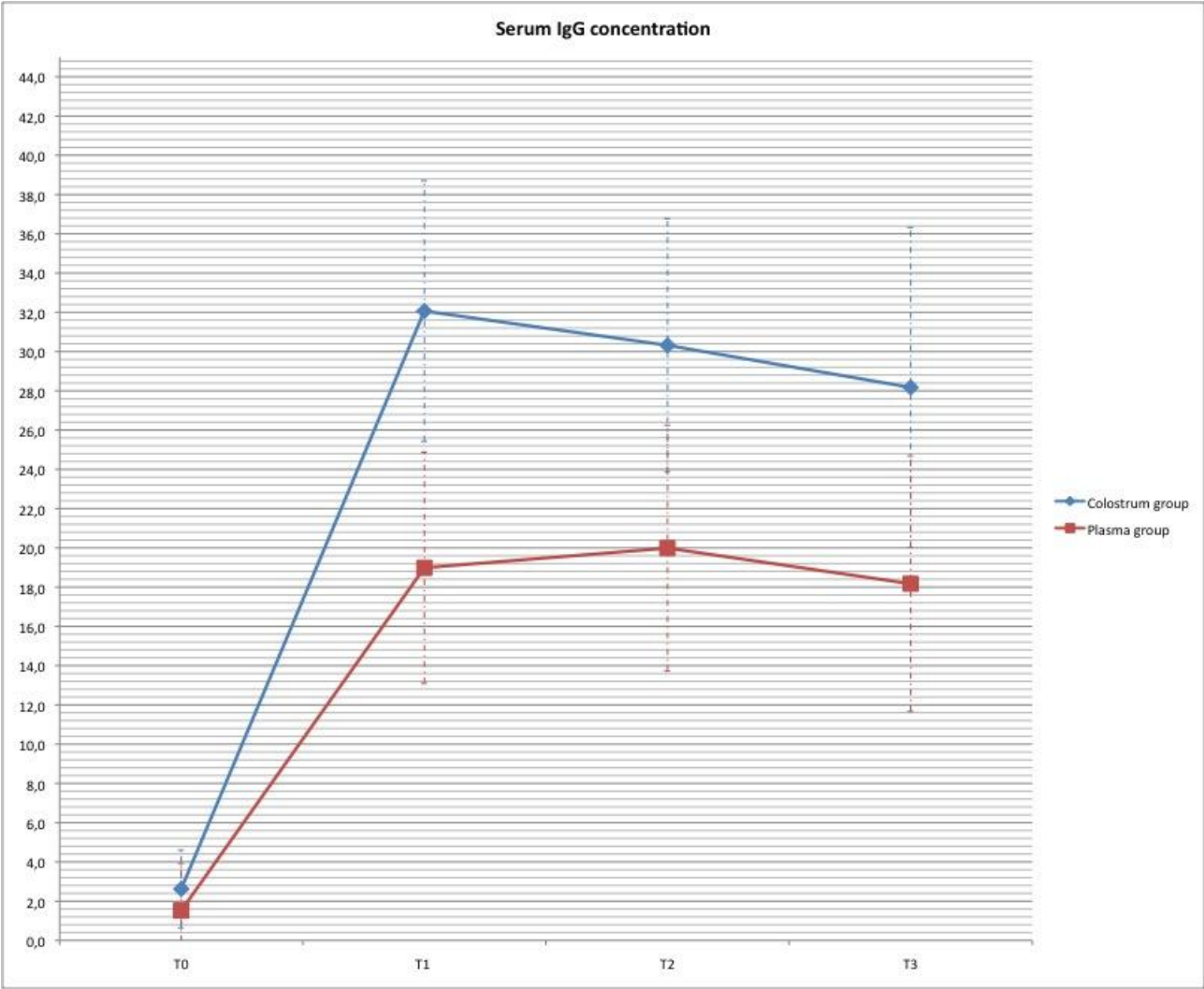
These results suggest that IV infusion of IgG is not a valid alternative to the administration of a good-quality colostrum, although both groups of animals reached an adequate quantity of serum IgG. Nevertheless, plasma trasfusion could represent an effective supportive therapy for FPT

[1] Weaver DM et al. (2000). Passive transfer of colostral immunoglobulins in calves. J Vet Intern Med 14:569-77.

[2] Biemann V et al. (2010). An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. J Dairy Sci 93:3713-21.

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bovine neonatology
FPT, IgG, calves



IGG AND LYSOZYME CONCENTRATIONS IN FETAL FLUIDS OF TERM PREGNANCY DOGS

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In humans, the timing and the factors influencing the trans-placental transfer of immunoglobulins of class G (IgG) were widely studied, but in the dog, in which IgG are the primary antibodies class transferred through the placenta, these knowledge are very scarce. Among the immune system non-specific factors, lysozyme has been demonstrated in amniotic fluid of humans, but still not investigated in dogs fetal fluids. The present study was aimed to evaluate IgG and lysozyme levels in amniotic (AM) and allantoic (AL) fluids of healthy bitch at term pregnancy and to assess possible differences according to, maternal breed size and parity and to fetal gender.

The study enrolled 41 bitches, belonging to several breeds, submitted to elective cesarean section (CS) at term. The AM and AL were collected aseptically from each puppy and stored at -20°C until analysis for IgG by ELISA and lysozyme by lysoplate. At birth, the newborn puppies were evaluated for maturity, viability, gender, absence of gross malformations, and weighted. IgG and lysozyme levels in AM and AL, in relation to maternal breed size and parity, and to newborn gender were evaluated by one way ANOVA.

A total of 142 mature, viable, without gross malformation and normally weighted newborns puppies, 74 males and 68 females, were enrolled. On the base of maternal bodyweight, the 41 bitches were divided into 2 groups: small (n=30) and medium-large (n=11) size. According to parity, 11 bitches were primiparous and 30 pluriparous. IgG mean levels were higher ($p<0.01$) in AM vs AL (0.14 ± 0.17 vs 0.06 ± 0.06 mg/ml), while lysozyme was not significantly different in the two fluids (1.92 ± 1.76 vs 3.01 ± 1.58 µg/ml). Mean ($\pm$ SD) AM and AL IgG and lysozyme in the fluids collected from the 41 litters, in relation to maternal breed size and parity, and to newborn gender are reported in Table 1.

Table 1- IgG and lysozyme levels (mean $\pm$ SD) in AM and AL samples, in relation to maternal breed size and parity and to newborn gender. In parenthesis the number of fluids measured for each parameter.

a,bDifferent superscript within column refers significant differences ($p<0.05$)

In dogs at term, IgG and lysozyme are detectable in both AM and AL, and IgG levels are higher in AM vs AL; in AM IgG levels were about 1/100 of the adult dog serum level, and the amount was similar to what reported for the AM in humans, despite the different type of placenta in the two species. IgG levels in AM were higher in pluriparous as compared to primiparous bitches, suggesting a more efficient immune passive transfer in multiparous compared to primiparous bitches, as reported for bovine colostrum [1]. Unfortunately, maternal serum IgG and lysozyme levels were not investigated, because not all owners allowed the use of maternal serum for research purposes.

1. Kehoe SI et al Prof Anim Sci 2011; 27:176-180.

neonatologia veterinaria

dog, fetal fluids, immunity

	IgG (mg/ml)		Lysozyme (μg/ml)	
	Amniotic fluid (129)	Allantoic fluid (84)	Amniotic fluid (129)	Allantoic fluid (84)
Small breed (30)	0.15±0.19 (88)	0.06±0.05 (60)	1.91±2.07 (88)	3.12±1.75 (60)
Medium-large breed (11)	0.10±0.10 (41)	0.06±0.07 (24)	1.94±0.77 (41)	2.95±1.03 (24)
Primiparous (11)	0.07±0.05 ^a (24)	0.06±0.07 (23)	1.59±0.75 (24)	2.82±0.87 (23)
Pluriparous (30)	0.15±0.18 ^b (105)	0.06±0.05 (61)	1.99±1.91 (105)	3.17±1.77 (61)
Newborn males (74)	0.14±0.16 (66)	0.06±0.06 (47)	1.82±0.73 (66)	3.01±1.34 (47)
Newborn females (68)	0.13±0.17 (63)	0.05±0.05 (37)	2.03±2.41 (63)	3.14±1.85 (37)

OPERATOR'S EFFECT DURING ULTRASONOGRAPHIC CANINE PREGNANCY MONITORING ON MEASUREMENT OF FETAL PARAMETERS

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The aim of the study was to verify any effect of the operator on the ultrasonographic (US) measurement of canine fetal parameters.

Nineteen US exams performed in physiological early and late pregnancy of nine 2.5-8.5 year old, 22-40 BW kg bitches of different breeds (5 German Shepherd dogs, 2 Collies, 1 Flat Coated Retriever, 1 Australian Shepherd dog) were included in the study. US examinations were performed using a General Electric LOGIQ P5/A5 (Soligen, Germany) US machine, with a 6-10 MHz microconvex probe, clipping hair of the ventral abdominal region and positioning bitches in lateral recumbency. In early pregnancy (Day 21-37 from breeding), measurement of ICC (Inner Chorionic Cavity) and CRL (Crown-Rump Length) were done after visualization of gestational chambers with a viable fetal structure. Gestational chambers were visualized as uterine anechoic spherical structures surrounded by a hyperechoic wall (1); fetal structures were an oblong structure apposed to the gestational chamber wall (2). In late pregnancy (Day 37-50 from breeding), BP (Biparietal Diameter) and BD (Body Diameter) were measured. Each measurement was taken at least in 2 fetuses. The date of mating, of whelping and the number of puppies were recorded. Each US exam was performed twice, by 2 different operators, who independently made their measurements.

The operator effect on fetal measures was investigated through the application of the Analysis of Variance using the software SIGMASTAT 2.03.

Any statistically significant difference due to the effect of operator was observed for all the parameters taken into account, in both periods of observation (BD: P=0.612; BP: P=0.396; CRL: P=0.254; ICC: P=0.654).

Taking into account the interaction between operator and fetus, any significant difference was revealed by the statistical analysis for all parameters (BD: P=0.950; BP: P=0.995; CRL: P=0.764; ICC: P=0.495).

It is well known that US is an operator dependent exam. Fetal measurements are useful for monitoring normal fetal growth and to estimate parturition date (3). US studies in this field often involve > one operator, and data collection may be much faster when more operators are involved. Based on our results we can conclude that, provided operators are sufficiently skilled and the research protocol is strictly adhered to, there should be no significant difference among operators as well as among fetuses. Data collection for studies concerning the estimation of canine gestational age may be performed by 2 operators without any consequences on the reliability of results.

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3. England GCW, Edward Allen W, Porter DJ– J Small Anim Pract 31: 324-329, 1990

Reproduction of small animal
Ultrasound exam, Dog, Pregnancy

OVARIOHYSTERECTOMY IN DOGS: OPEN TECHNIQUE VS LAPAROSCOPY

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Ovariohysterectomy (OHE) is an operation which is made to remove ovaries and uterus. The main indications of OHE are: prevention of heats and unwanted pregnancies, prophylaxis of udder tumours and transmission of congenital pathologies, prevention and therapy of some uterine pathologies such as cystic endometrial hyperplasia-pyometra complex, subinvolution of placental sites, uterine rupture and prolapse. A study made in Virginia in 2003 by Austin et al. showed that in human medicine the laparoscopic ovariohysterectomy was made to reduce pain, infection incidences, hospital admitting and the same results could be getting in veterinary medicine (Austin et al., 2003).

Another study made by Davidson, Moll and Payton demonstrated that laparoscopic surgery in 16 dogs was longer and produced more complications than open surgery (69 minutes in open technique versus 120 minutes in laparoscopic technique) but this study showed also that laparoscopic technique produced less pain than open OHE made on 18 dogs and it was proved thanks to pain scores recorded at 0, 2, 8 and 24 hours after surgery (Davison et al., 2004).

Other studies proved that laparoscopic technique was longer but less painful than open technique (Hancock et al., 2005) (Devitt et al., 2005).

Another study made on dogs with pyometra proved the success of laparoscopic-assisted ovariohysterectomy (Minami et al., 1997).

Laparoscopic surgery did many progresses in these years because of its evident advantages compared with traditional technique. Its main advantages are: less handling of tissues during surgical procedures, less incidence of postoperative complications (injury infection, suture opening, training of hernias), less risk to cause adhesions, less postoperative pain and so less days of hospital admitting.

In laparoscopic surgery we have to consider a learning curve: the quality of results increases with the experience and it's in relation with the type of operation. Indeed some surgical procedures are easy to do instead the formative training is long for other ones. It can be improved and reduced with experience and using well-advanced instruments to simplify surgical procedures and to reduce surgical times.

The laparoscopic surgery is becoming a practice very diffused in veterinary medicine but it can't substitute in every case the traditional technique.

So the aim of this study is to confront open and laparoscopic ovariohysterectomy to explain some aspects that we have to consider to choose one of the two surgical methods. On this subject we considered some aspects: patient selection, surgical methods with their advantages and disadvantages, duration of surgery, postoperative measures and possible complications.

In this study 27 dogs, from 4 to 16 years old, 2,5 to 56 kg body weight, had an operation of OHE, 15 with open method (2,5-39 kg b.w.) and 12 with laparoscopic method (3-56 kg b.w.). We utilized three laparoscopic accesses in six dogs of the second group, two accesses in two dogs and in four patients we utilized laparoscopic-assisted method.

The 15 dogs treated with open technique e 4 of the dogs treated with laparoscopic method were operated at the Veterinary Teaching Hospital Mario Modenato of the University of Pisa instead 8 dogs treated with mini-invasive method were operated at the Veterinary Clinic "Galilei" of Prato.

Examining the 15 open cases, for 13 of them the reason of surgery was pyometra and we diagnosed it with clinic visit and thanks to ultrasonography exam. One of these patients were operated in urgency in reason of the serious clinic conditions, instead the other 12 cases did a therapy with Alizin® to open the cervix and then Ceftriaxone (20 mg/kg bid) and Enrofloxacin (5 mg/kg sid). In 1 dog of the same open group we made the operation in urgency in reason of metrorrhagia 10 days post-partum for lacking involution of placental

sites. Finally we operated one dog of OHE open in reason of unwanted pregnancy at 32 days and we saw with ultrasonography exam that four of six fetuses were dead.

Examining the 12 laparoscopic cases, we operated 6 dogs with pyometra in progress upon ultrasonography exam of uterus to verify that its dimensions were smaller of around 3 cm and we utilized the three accesses technique. In two cases of this group we made laparoscopic-assisted OHE because uterus dimensions passed 3 cm and the uterine horns were too long.

In 1 dog we utilized the two accesses method after therapy with Alizin®; indeed when we made the scan exam after the therapy, we saw that the uterus was empty but there were many endometrial cysts and so we made the operation to prevent pyometra relapses.

We treated 3 dogs of the same laparoscopic group with Alizin® to achieve appropriate uterine dimensions and then we operated them. For two of them we utilized laparoscopic-assisted technique because uterine dimensions passed 3 cm whereas in the other dog we started with two accesses method but then we had to change it in a traditional surgery because uterine dimension was too large.

We prepared the patients of the two groups in the same way. They didn't eat 12 hours before operation, except the patients operated in urgency. We shaved the fur of abdomen from xiphoid apophysis of sternum to inguinal region, enlarging laterally until the inferior limit of dorsal region. We inserted bladder catheter to every patient and they were anesthetized. Then we positioned the patients on the operating table and we disinfected the operating field.

In 8 dogs of laparoscopic group we used harmonic scalpel Ultracision (Ethicon Endosurgery) for coagulation/resection and electro coagulation with bipolar forceps and laparoscopic scissor in the others. All dogs were admitted in hospital for one (laparoscopy) to three days (open) post surgery for analgesia.

The results showed that an appropriate patient selection and a careful knowledge of surgical methods and possible complications enable us to choose the most suitable surgical method for every patient, to get better surgical results.

All surgery cases was completed without complications.

Time of surgery were: 49' (30' to 65') open method, 68' (40' to 95') laparoscopic method; this difference is statistically significant ($P < 0,05$).

Time of surgery of two accesses technique was 75', three accesses technique was 67' (40' to 95') and laparoscopic-assisted method was 69' (50' to 90').

In 8 operations we used Ultracision and the average duration of surgery was 63' (40' to 95'); in 4 operations we used electro coagulation and the average duration of surgery was 82' (75' to 90').

Examining postoperative management we admitted the 15 dogs of the open group for one day, except for three days the two dogs operated in urgency. They received fluid therapy, antibiotics and analgesic therapy with methadone IM at 0,2-0,3 mg/kg every 4 hours. We admitted the 12 patients of laparoscopy group for one night; they received fluid therapy, antibiotics and analgesic therapy with fans.

The open method is significantly shorter than laparoscopic one (49' to 68') but advantages are in the postoperative period: less pain, less handling of tissues, less drop of body heat, reduction of adhesions and postoperative complications are features of endoscopy that reduce days of hospital admitting.

The time of surgery is strictly correlated with experience of surgical team and high technology surgical instrumentation like harmonic scalpel. The Ultracision reduces postoperative pain because it doesn't produce heat; it speeds the surgery, and so reduces the consequences of pneumoperitoneum on vital patient parameters seeing that postoperative pain generally is caused by residual pneumoperitoneum.

From this study we also saw that laparoscopic OHE can be generally made, upon suitable patient selection, without run the risk of have particular postoperative complications.

High costs that have laparoscopy may be reduced in consideration of low time of admitting and analgesic drugs administration and smaller probability of postoperative complications than traditional surgery.

So we can conclude that laparoscopic method for OHE can be really considered when uterine dimensions are right, and before surgery it is necessary to do, if possible, medical treatment to avoid intraperitoneal complications. An extension of surgical cases and further studies are desirable to confirm the results we got.

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riproduzione piccoli animali

Ovariohysterectomy, laparoscopic surgery, dog

EFFECTS OF ANTIOXIDANT SUPPLEMENTATION TO IN VITRO MATURATION MEDIUM ON OOCYTE DEVELOPMENT OF DOMESTIC CAT (FELIS CATUS).**Cocchia N.*^[1], Abbondante L.^[1], Ciani F.^[1], Tafuri S.^[1], Tortora G.^[1]**^[1]~ *Napoli*

Mammalian oocytes require a variable period of in vitro maturation (IVM) to develop to metaphase II stage. Oocytes recovered from preovulatory follicles show a reduction in cleavage frequency and blastocyst development of cat embryos produced. It was demonstrated that high oxygen concentration compromises nuclear maturation rates and worsens the oxidative stress during IVM. Incubated oocytes show severely high quantities of superoxide dismutase (SOD), glutathione reductase (GSR), glutathione peroxidase (GPX1) and catalase (CAT) mRNA and this effect results in a protective mechanism against oxidative stress. Examining the follicular fluid oxidants and antioxidants it is possible to understand the composition changes in vivo and optimize the conditions of IVM. To improve the development of cat oocyte, we studied the effects of the addition of SOD and CAT to IVM medium on developmental competence and embryo production.

Cat oocytes were collected from fresh excised ovaries and cultured in a maturation medium supplemented without (untreated group) or with (treated group) SOD (25UI/ml) and CAT (50UI/ml) for 24h. Both groups were subjected to fertilization in vitro (IVF) with fresh epididymal sperm extracts for 12h. After IVF, the zygotes were cultured in a synthetic oviduct fluid medium for 8 days.

There were no significant difference in the maturation rates (86.4% vs 87.6%) between the groups, nevertheless antioxidant supplementation. The frequency of blastocyst development from oocytes of treated group (36.0%) was higher ($p < 0.05$) than untreated group (28.4%).

Our data indicate that the supplementation of SOD and CAT into IVM medium may improve blastocyst development of cat oocytes after IVF.

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Biotecnologie riproduzione

Oocyte, Antioxidant, Cat

HORMONAL CHANGES DURING PROESTRUS AND ESTRUS IN THE BITCH. FOCUS ON FT3, FT4 AND CORTISOL

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The aim of this study is to evaluate serum levels of fT3, fT4 and cortisol during bitch estrous period in order to understand whether a crosstalk between the thyroid and the adrenal glands may modulate the breeding activity of this species in physiological conditions.

Seven healthy female dogs (age: 3.6 ± 1.05 years; body weight: 20.8 ± 3 kg), which had reached the 3rd heat, were included in the study. All of them, at the end of their anestrus phase, were examined twice a week until the appearance of vaginal discharge (T0). During each clinical visit a vaginal cytological smear was evaluated by Diff quik staining and starting from T0 a blood sample, was taken. Afterwards new blood samples and vaginal smears were obtained at T1: 3.7 ± 0.7 days after the onset of proestrus ($P4 < 0.5$ ng/ml); T2: 8.14 ± 0.6 days after the beginning of proestrus ($0.5 < P4 < 1$ ng/ml); thereafter they were got at intervals of about 48 hours. T3: at estrus; T4: at the alleged LH peak; T5 and at the survey of the first day of cytological diestrus (T6) after 5.14 ± 0.6 days from T5. Each plasma sample was subjected to quantitative assay of fT3, fT4, P4 and cortisol by enzyme immunoassay kits (EIA WELL®, Radim, Italy). Data were analyzed by ANOVA test.

For both free thyroid hormones an increase of plasma concentration, was observed, starting from the beginning of proestrus, with a peak at T1 more pronounced for fT3 ($P < 0.001$), at each time considered. A new increase at the beginning of the estrous phase was observed for fT3 (T3vsT0, T1, T4 $P < 0.001$; and T3vsT2, $P < 0.01$). Also fT4 showed a pick at T3 (T3vsT6 $P < 0.05$). Subsequently, both hormones had reached baseline values, to increase again at T5 (fT3: T5vsT1 $P < 0.001$); fT4 T5vsT4 $P < 0.05$) during the periovulatory period. Another pick was recorded at T6 (fT3: T6vsT0, T1, T4 $P < 0.001$; vs T2 $P < 0.01$; fT4: T6vs T0, T1, T3, $P < 0.005$; T6vsT4 $P < 0.01$). Cortisol values did not show significant differences.

Under physiological conditions circulating fT3 and fT4 are in continuous equilibrium, due to the homeostatic mechanisms ensuring gradual changes in the concentrations of fT3, which is the most active thyroidal hormone (Kelly and Chopra, 2000). In our study in fact curves describing serum concentrations of the two hormones maintained an almost similar trend. Peaks evidenced in proestrus and in estrus underline their involvement in the mechanism regulating follicular development and ovulation (Cecconi et al., 2007; Kobayashi et al., 2009). The increased metabolic request during the estrus cycle responsible of the thyroid hormones rise probably does not involve the adrenal glands since cortisol concentrations do not show statistical significant variations.

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REPRODUCTION

fT3, fT4, CORTISOL

LACTATE, URIC ACID, CREATININE AND GLUCOSE CONCENTRATIONS IN AMNIOTIC FLUID OF TERM PREGNANCY DOGS

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In humans the analysis of amniotic fluid (AF) composition provides useful clinical information for the correct management of both the process of parturition and of birth. To date, canine AF composition from a clinical perspective has not been completely investigated. The present study was aimed to evaluate lactate (L), uric acid (UA), creatinine (C) and glucose (G) AF concentrations in term pregnancy dogs, in relation to some clinical maternal and neonatal parameters, to provide information for a better management of both mothers and puppies at the time of parturition.

The study enrolled 8 primiparous and 22 pluriparous bitches, submitted to elective or emergency cesarean section (CS) at term. The AFs were aseptically collected from each puppy and stored until analysis for L, UA, C and G by commercial kits. At birth, the newborn puppies were evaluated by Apgar score [1], and for maturity, gender, absence of gross malformations, and weight. Concentrations of L, UA, C, G and the L/C ratio in AF, in relation to newborn viability and gender, the type of birth, the maternal breed size and parity, were statistically evaluated by Anova.

RESULTS

Forty-nine males and 54 females normal newborns were enrolled. On the basis of Apgar score, 82 puppies had Apgar ≥ 7 and 21 Apgar < 7 . Based on type birth, 29 AF samples were collected from puppies born by emergency CS, while 74 from puppies born by elective CS. According to maternal bodyweight, 87 AF samples belonged to small size breed (SB) and 16 to medium/large size (LB). Mean ($\pm$ SD) amniotic L, UA, C, G and L/C measured on a total of 103 AF samples belonging to the 30 litters, are reported in Table 1.

Table 1- Amniotic L, UA, C, G levels (mean $\pm$ SD) and L/C in the 103 AF samples, grouped in relation to newborn viability and gender, to the type of birth, and to maternal breed size and parity.

Data obtained in this study showed strong inter-individual variations for most of the studied parameters, as evidenced by the wide SDs. However, amniotic UA was significantly higher in SB compared to LB, differently to the absence of significant differences among races in amniotic UA in humans [2] and could be supposed to be indicative of the major oxidative stress occurring at birth in small sized animals. Amniotic G was surprisingly higher in males compared to females puppies, difference not reported for humans, although the mean levels were similar to data reported for AF glucose in human normal pregnancies (22 ± 12 mg/dl) [3]. No significant associations were found among the studied parameters and newborn viability or type of birth.

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small animals reproduction
dog, fetal fluids, lactate

Amniotic fluid					
	Lactate (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Lactate/creatinine
Apgar ≥ 7 (n=82)	73 $\pm$ 24.91	1.4 $\pm$ 1.58	2.3 $\pm$ 1.82	22 $\pm$ 15.8	45 $\pm$ 32.06
Apgar < 7 (n=21)	69 $\pm$ 30.73	1.1 $\pm$ 0.96	3.2 $\pm$ 4.05	19 $\pm$ 14.65	43 $\pm$ 27.43
Males(n=49)	68 $\pm$ 22.23	1.5 $\pm$ 1.84	3 $\pm$ 3.62	26 $\pm$ 13.31 ^b	41 $\pm$ 28.94
Females(n=54)	76 $\pm$ 29.14	1.3 $\pm$ 1.04	3.1 $\pm$ 3.83	16 $\pm$ 15.88*	47 $\pm$ 32.78
Elective CS (n=74)	72 $\pm$ 24.93	1.4 $\pm$ 1.65	3.1 $\pm$ 3.97	23 $\pm$ 15.86	46 $\pm$ 31.65
Emergency CS (n=29)	72 $\pm$ 29.46	1.2 $\pm$ 0.92	3 $\pm$ 2.98	15 $\pm$ 12.87	40 $\pm$ 29.51
Small breed (n=87)	72 $\pm$ 27.77	1.5 $\pm$ 1.55 ^a	3.2 $\pm$ 3.96	21 $\pm$ 15.43	43 $\pm$ 29.14
Medium/large breed (n=16)	72 $\pm$ 18.69	0.9 $\pm$ 0.99 ^b	2.3 $\pm$ 1.85	21 $\pm$ 16.59	50 $\pm$ 40.35
Primiparous(n=23)	67 $\pm$ 26.88	1.3 $\pm$ 0.89	2.1 $\pm$ 1.40	23 $\pm$ 21.43	45 $\pm$ 37.03
Pluriparous(n=80)	73 $\pm$ 24.83	1.3 $\pm$ 1.41	3.3 $\pm$ 4.17	20 $\pm$ 13.45	44 $\pm$ 29.34

CORTISOL FETAL FLUIDS CONCENTRATIONS AND NEWBORN OUTCOME IN TERM PREGNANCY CHIHUAHUA DOGS: PRELIMINARY RESULTS

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In humans the amniotic fluid cortisol (C) concentration towards term pregnancy was associated to fetal lung maturation [1] and well correlated with gestational age [2]. To date, canine fetal fluids composition has not been completely investigated from a clinical perspective. The present study was aimed to evaluate C amniotic and allantoic concentrations in term pregnancy Chihuahua dogs, in relation to some clinical maternal and neonatal parameters, to provide information for a better management of both mothers and puppies at the time of parturition.

The study enrolled 10 Chihuahua bitches, 2-5 years old, healthy at a general examination, both regularly vaccinated and dewormed. All these bitches underwent an elective Caesarean Section (CS) at term. The parturition day was scheduled on the basis of the ovulation date estimation, on the fetal ultrasonographic measurements of both the inner chorionic cavity and biparietal diameter, and on progesteronemia. The amniotic and allantoic fluids were aseptically collected from each puppy and stored until analysis for C by RIA. At birth, the newborn puppies were evaluated for viability by Apgar score [3], maturity, gender, absence of gross malformations, and weight. Cortisol concentrations in the two types of fluids were statistically evaluated by t-test, in relation to newborn viability and gender as well as to maternal parity.

Sixteen males and 10 females normal newborns were enrolled. On the basis of Apgar score, 23 puppies had Apgar ≥ 7 and 3 Apgar < 7 . According to parity, 3 bitches were primiparous and 7 multiparous. Mean ($\pm$ SD) amniotic and allantoic C concentrations in total samples, in relation to newborn viability and gender as well as to maternal parity, are reported in Table 1.

Table 1- Amniotic and allantoic C concentrations (mean $\pm$ SD) in total samples, in relation to newborn viability and gender, and to maternal parity.

In this study, the preliminary results obtained in a small number of animals, did not show significant differences in C levels between the two type of fluids. Additionally, significant differences in C concentrations were not found neither on the basis of newborn viability and gender nor on the basis of maternal parity. The absence of significant differences, most likely due to the small number of samples and high inter-individual variations as demonstrated by the wide SDs, did not allow to draw conclusions, but, on the opposite, require further investigations.

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small animals reproduction

dog, fetal fluids, cortisol

	Amnios	Allantois
	Cortisol (ng/ml)	Cortisol (ng/ml)
Totalsamples	9.7±5.50 (n=19)	11.3±7.74 (n=18)
Apgar ≥ 7	9.8±5.74 (n=17)	11.58±8.16 (n=16)
Apgar < 7	8.5±3.61 (n=2)	9.5±3.58 (n=2)
Males	9.5±6.00 (n=9)	8.5±3.69 (n=8)
Females	9.7±5.61 (n=9)	14.1±10.66 (n=8)
Primiparous	12.4±4.86 (n=6)	19.7±9.54 (n=5)
Multiparous	8.4±5.48 (n=13)	7.9±3.84 (n=12)

EFFECT OF SOD (SUPEROXIDE DISMUTASE) AND CATALASE ADDITION ON LIQUID STORAGE (4°C) OF CHILLED EPIDIDYMAL CAT SPERMATOZOA

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The aim of this work was to estimate the quality of cat epididymal sperm stored for short time at +4°C in extender with or without antioxidant (SOD and Catalase) supplementation and to assess the percentage of motility, viability, acrosomal integrity, DNA integrity and phosphorylation of ERK protein (Cocchia N et al.2011, Siemieniuch M et al.2007, Thiangtum K et al. 2009, Thuwanut P et al.2010).

Epididymides were collected from 20 domestic cats during routine neutering procedure. Each set testicular-epididymis collected, it was immediately dipped in Dulbecco's PBS into a test tube Deware (20-24 ° C) and they were carried rapidly to the laboratory. The epididymis were separated by the testicles and blood vessels were removed to isolate the proximal epididymis and vas deferens (Zambelli D. et al.2006). The caudal portion of each epididymis was placed in 1 ml of Tris extender in a Petri dish was dissected with a scalpel blade to release the spermatozoa. After 10 min of incubation (38 °C), the epididymal tissue was removed with forceps, and the medium was collected in a Falcon tube and centrifuged at 700 g for 6 min. The supernatant was removed, and the pellet concentration was evaluated by phase-contrast microscopy (400×) using a Bürker chamber. The pellet was resuspended in a fresh Tris extender to obtain a final concentration of 10×10⁶ spermatozoa/ml. A total of 20 samples was divided into 2 aliquots: spermatozoa diluted after centrifugation in extender without (control) or with (experimental) SOD and Catalase (50UI/ml and 100UI/ml respectively). Each sample was analyzed for motility, viability, acrosome status and DNA integrity, immediately after semen preparation and again after storage at 4 °C for 24h, 48h and 72h.

Acrosome integrity was evaluated by Fluorescent-labeled peanut lectin agglutinin (PNA-FITC conjugated staining). In order to assess the DNA fragmentation, the APO-BrdU™ TUNEL Assay Kit was used. A classic proteomic approach of quantifying extracellular signal regulated kinase (ERK) was also evaluated by Western Blot as an indirect indicator of oxidative stress. At 24h, 48h and 72h, incubation with commercial SOD and Catalase (Exp1 and Exp2) was associated with a significant increase both in motility (P <0.001) and viability (P < 0.001) compared with the control by a one-way analysis of variance (ANOVA). Quality parameters of sperm were significantly higher (Progressive Motility P 0.01; Viability P 0.001) in aliquots supplemented with SOD and Catalase. ERK phosphorylation, analyzed by Western Blot, was statistically higher (P 0.01) in aliquots without SOD and Catalase. Apoptosis increased significantly and progressively from 3h to 72h (P<0.0001). However the number of apoptotic cells was significantly lower in semen added with SOD or with Catalase compared with the control (P<0.0001), at each time.

The Authors concluded that addition of SOD and Catalase to semen extenders improves the quality of chilled epididymal cat spermatozoa semen.

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biotechnology, reproduction

Sod and Catalase, Feline Sperm, Frozen Semen

EFFECTS OF VERBASCOSIDE, A BIOACTIVE COMPOUND FROM OLIVE OIL MILL WASTEWATER, ON IN VITRO DEVELOPMENTAL POTENTIAL AND BIOENERGETIC/OXIDATIVE PARAMETERS OF PREPUBERTAL LAMB OOCYTES

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Juvenile in vitro embryo production from oocytes of prepubertal subjects is a promising assisted reproductive technology (ART). In farm animals, it reduces the generation gap and in human reproductive medicine, it helps to overcome premature ovarian failure. Oxidative stress in germ cell in vitro culture and cryopreservation procedures is an emerging problem in ART and studies aimed to evaluate the potentially antioxidant activity of natural bioactive compounds, such as phenolic compounds and polyphenols, present in plant-derived by-products would be beneficial to improve cryopreservation and in vitro culture protocols of prepubertal oocytes. Verbascoside (VB) or acteoside is a phenolic bioactive compound with known antioxidant activity, which is present in a good amount in olive oil mill wastewater (1). The aim of this study was to test the effects of VB on the developmental competence of ovine prepubertal oocytes and the bioenergetic/oxidative stress status of fresh and vitrified oocytes.

Previously published methods were used for: VB extraction, purification, HPLC analysis and uptake by the cumulus-oocyte complex (1); in vitro oocyte maturation (IVM) and assessment of bioenergy redox biomarkers, such as mitochondrial distribution pattern and activity; intracellular reactive oxygen species (ROS) levels; mitochondria/ROS colocalization; catalase and total superoxide dismutase activities (2); oocyte vitrification (3); in vitro fertilization and embryo development (4). VB effects were tested at microM concentrations (1.03, 2.06 and 4.11).

In fresh oocytes, 4.11 microM VB exerted pro-oxidant short-term effects, i.e. catalase activity increase and uncoupled increases of ooplasmic mitochondria and reactive oxygen species (ROS) specific fluorescence signals. It also induced pro-oxidant long-term effects, i.e. reduced blastocyst formation rate, thus indicating an affected developmental competence of exposed oocytes. In vitrified oocytes, 1.03 microM VB increased ROS levels. Pro-oxidant VB effects in ovine prepubertal oocytes could be related to higher VB accumulation, which was found as being almost one thousand times higher than that reported in other cell systems in previous studies. Also, long exposure times of oocytes to VB, throughout the duration of IVM culture, may have contributed to significantly increase oocyte oxidation.

In conclusion, VB, added at microM concentrations in a continuative 24 hours IVM exposure protocol, acts as a pro-oxidant molecule by impairing bioenergetic potential, oxidative status and embryo developmental competence of prepubertal lamb oocytes. Further studies are ongoing aimed to identify suitable conditions, lower concentrations and/or shorter exposure times, to figure out VB antioxidant effects in juvenile ARTs.

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Reproductive biotechnologies in domestic animals

Prepubertal oocytes, Verbascoside, Pro-oxidant effect

IN VITRO STUDY ON THE DIRECT ROLE OF DOPAMINE IN THE MODULATION OF RABBIT CORPORA LUTEA LIFE-SPAN

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Dopamine (DA) is a catecholamine neurotransmitter that is extensively distributed in the brain and also in different peripheral organs of numerous species. In mammals, DA receptors (DR) are expressed in several organs and tissues; in particular, in the ovary D1R was identified also in luteal cells, suggesting that DA may exert a novel physiological regulatory pathway directly involved in the function of the corpora lutea (CL). The main objectives of the present study were to examine the gene expression of DRs and DA and their immunolocalization as well as the in vitro effects of DA on the production of progesterone, prostaglandin E2 (PGE2), and PGF2 α during early, mid, and late luteal stages of pseudopregnancy in rabbit CL.

Sexually mature New Zealand white female rabbits were used. Pseudopregnancy was induced by buserelin. At day 4 (early stage), 9 (mid), and 13 (late) of pseudopregnancy, animals were sacrificed by cervical dislocation. Immunohistochemical investigation was performed using goat-polyclonal anti-D1R and D3R and mouse monoclonal anti-DA primary antibodies. The intensity of immunostaining was assessed microdensitometrically. RNA extraction and real-time PCR analysis were performed to assay DA, D1R and D3R gene expressions. Western blotting was performed using the same immunohistochemistry primary antibodies. In in vitro experiments, early, mid, or late CL were incubated with DA, D1R agonist (dihydroxydine hydrochloride), D1R antagonist (SCH 23390 hydrochloride), D3R antagonist (GR 103691), or D3R agonist (7-hydroxy-PIPAT maleate).

Immunoreactivity and gene expression for D1R decreased while that for D3R increased in luteal and blood vessel cells from early to late pseudopregnant stages. DA immunopositivity was evidenced only in luteal cells. DA and D1R agonist increased in vitro release of progesterone and PGE2 by early CL, whereas DA and D3R agonist decreased progesterone and increased PGF2 α in vitro release by mid and late CL.

These results provide evidence that the DA/DR system exerts a dual modulatory function in controlling the lifespan of CL: the DA/D1R is luteotrophic, while the DA/D3R is luteolytic. The present data shed new light on the physiological mechanisms regulating luteal activity that might improve our ability to optimize reproductive efficiency in mammal species, including humans.

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Reproductive biology

Corpora lutea, Dopamine, Dopamine receptors

EFFECTS OF BLUETONGUE VIRUS SEROTYPE 1 ON RAM FERTILITY IN SARDINIA

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In Sardinia, during the 2013 Bluetongue virus serotype 1 (BTV 1) epidemic, the disease in rams was characterized by the involvement of the genital tract with severe hyperthermia and edema of the scrotum. Herein we describe the pathological and virological findings as well as the effects on fertility caused by BTV 1 infection.

The study was carried out on 12 rams collected from 8 sheep flocks, in which clinical Bluetongue (BT) infection was observed during the summer and the autumn in 2013. Rams were serially euthanized 15 (n. 1), 30 (n. 2), 60 (n. 2), 100 (n. 6) and 140 (n. 1) days after the onset of the disease. At the necropsy blood, spleen, testis, epididymis, male accessory glands and regional lymph nodes were adequately sampled for serological, virological and pathological examinations.

All the rams were positive by ELISA for antibodies against BTV.

BTV RNA was also detected by RT PCR in blood, spleen and regional lymph nodes of all rams whereas in male accessory glands and testis up to 30 and 60 days from onset of symptoms, respectively.

Histopathological examination of the testis revealed oligospermia to azospermia, diffuse degeneration of spermatogenic epithelial cells with reduced number of the cell layers and presence of multinucleate giant cells. In the testis, examined at 15, 30 and 60 days after the clinical onset of the disease, testicular degeneration was characterized by intertubular edema as well as by the lack of mature spermatids and germ cells. In the animals euthanized at 100 and 140 days intertubular fibrosis was observed together with a less severe grade of degeneration of the spermatogenic epithelial cells.

Hypofertility has been reported in rams [1] vaccinated with BTV 2 live modified vaccine, while BTV 1 and BTV 8 field strains did not cause any lesion in the reproductive tracts of experimentally infected rams [2]. In this study we observed that rams naturally infected with BTV 1 displayed severe degeneration of spermatogenic epithelial cells which a partial recovery being observed only after 100 days. Whether BTV directly affects germ cells or exerts detrimental effects through other pathways remains to be determined.

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REPRODUCTION

BlueTongue, Hypofertility, Rams

EFFECT OF SEMINAL PLASMA INFUSED AT THE TIME OF FROZEN SEMEN ARTIFICIAL INSEMINATION ON JENNIES (EQUUS ASINUS) UTERINE CYTOLOGY EVALUATED 24 HOURS LATER

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Fertility rates obtained by frozen semen artificial insemination (AI) in the donkey species are often disappointing (0-36%) (1). In the horse, seminal plasma (SP) modulates polymorphonuclear cells (PMN) activity in vitro and post mating induced endometritis in vivo (2, 3). In jennies SP did not decrease uterine PMN 6-10 hours post frozen semen AI (1).

The aim of this study was to evaluate uterine cytology in donkeys 24 hours after frozen semen AI with or without post-thaw addition of seminal plasma.

Two cycles of 5 Amiata jennies were assigned in random order to either frozen semen AI (AI) or frozen semen AI with post-thaw addition of 70% v/v seminal plasma (AI+SP). Ovulation was induced (h0) by a GnRH analogue (Suprefact®, Sanofi Aventis, IT; 0.1mg SC) and a single AI was performed 30 hours later with 500x10⁶ spermatozoa (pooled semen of two jacks). Low volume uterine lavage (LVL) (4) was performed at h0, to exclude jennies with PMN, and 24 hours after AI. Amount of recovered fluid, adsorbance (Accucel, IMV, FR) and cell concentration by hemocytometer were evaluated. The proportion of PMN was assessed on a smear stained with Diff Quik® (Microptic, ES) on 200 cells. Concentration and total number of PMN were determined as well. Eight days after ovulation the uterus was flushed to evaluate embryo recovery rate. Statistics: Paired T-test (P<0.05 significant).

Amount of recovered fluid (42.4±7.1% and 42.0±8.9%), adsorbance (0.222±0.12 and 0.171±0.13), cell concentration (0.284±0.01 and 0.233±0.16), %PMN (53.7±39.3 and 47.7±24.3), PMN concentration (0.221±0.2 and 0.266±0.2), total PMN number (8.5±8.4 and 10.4±8.3) and embryo recovery rate (1/5 and 1/5, 20%) did not differ between AI and AI + SP.

Post-thaw addition of SP did not reduce the number of PMN into the uterus 24 hours after AI, thus suggesting that uterine clearance was not improved.

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Riproduzione

donkey, seminal plasma, endometritis

IN VITRO CHARACTERIZATION OF GRANULOSA PROGENITOR CELLS ISOLATED FROM BOVINE OVARIAN FOLLICLES

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Ovarian granulosa is a highly available cell cluster that could be used as an alternative source of mesenchymal stem cells (MSCs) for veterinary medicine applications.

To this, presumptive granulosa MSCs (gMSCs) were isolated by aspirating the follicular fluid from 2-3mm growing follicles from bovine ovaries collected at slaughterhouse. gMSCs were divided in three culture groups in basic medium supplemented with a different factor: group 1 was added with 2% fetal calf serum (FCS) only, group 2 with 10 ng/ml epidermal growth factor (EGF) and group 3 with 0.02% leukemia inhibitory factor (LIF). These cells were evaluated until passage (P) 5 for their proliferative potential and the expression of pluripotent (c-Myc and Oct-4), mesenchymal (CD29, CD44, CD73 and CD166), functional (FSHR and FST) and hematopoietic (CD34) markers by RT-PCR. Oct-4, CD73 and FSHR expression was also investigated by quantitative PCR (qPCR). Adipogenic and osteogenic differentiation potential was assessed at P3.

Each culture condition supported the growth of cells that retained the capability to adhere to the plastic dish and expand exponentially with a low doubling time (1.21, 1.33, 2.13 days for FCS, EGF and LIF, respectively). All cells expressed pluripotent and mesenchymal markers but not hematopoietic markers until P5. A significant decrease in the FSHR expression and an increase in the CD73 and Oct4 expression were observed by qPCR at P3 compared to P0. A 100-fold decrease in the expression of FSHR, and a 10-fold and a 8 fold increase in the expression of CD73 and Oct-4 respectively, were detected in gMSC cultured in presence of LIF. The FST marker was expressed in all the tested conditions. Although the expression of mesenchymal and pluripotent markers was found in gMSCs, these cells did not differentiate into the adipogenic and osteogenic lineages. Our results differ from those reported for humans and gilts. We hypothesize that these differences might be attributable to the collection method and the follicular development stage.

The present study was performed on cells collected by needle aspiration from growing follicles, whereas in human and gilts a scraping protocol from luteinizing ones was applied (Kossowska et al., 2009; Mattioli et al., 2012). Our findings demonstrate that the conditions we used allowed for the isolation of progenitor cells from the ovarian granulosa, although these cells did not show the differentiation capability probably due to epigenetic memory. This hypothesis was further confirmed by the expression of FST in each passage studied. In conclusion, the cells obtained in this study cannot be considered gMSCs and the question about the presence of isolable presumptive gMSCs from growing follicles remains open.

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reproduction and biotechnologies
granulosa cells, stem cells, regenerative medicine

LAPAROTOMIC INTRAUTERINE INSEMINATION WITH FROZEN SEMEN IN THE EWE: PRELIMINARY RESULTSSylla L.^[1], [Pistolesi A.*^{\[1\]}](#), Biancucci A.^[2], Palombi C.^[1]^[1]*Dipartimento di Medicina Veterinaria ~ Perugia*, ^[2]*Dipartimento di Scienze Agrarie e Ambientali ~ Udine*

In all domestic species, acceptable pregnancy rates with frozen-thawed semen are only achievable with intrauterine insemination. Unlike the cow and the goat, the cervix of the ewe is a formidable barrier to penetration for transcervical intrauterine insemination not only due to the physical size of the canal and a narrow luminal diameter (even at estrus), but also due to its caudally facing, eccentric series of four to eight funnel-like rings.

The objective of the present study was to evaluate the pregnancy rate upon an intrauterine insemination through the laparotomic approach by using a frozen/thawed semen in the ewe.

The present study was carried out during the period of May - October 2013 and consisted in the intrauterine artificial insemination in a group of 15 Sopravvissana breed ewes with a Merino's frozen semen imported from Australia. Ewes were subjected to hormonal synchronization of estrus with endovaginal sponges of Chronogest® and ovulation induction with Folligon®. The intrauterine insemination was performed through a median laparotomy; the exteriorized uterine horns were catheterized with a 18 G intravenous catheter and an inseminating dose of frozen/thawed semen of 100 x 10⁶ spermatozoa were inoculated in the lumen. The abdominal incision was then routinely closed and after 55 days, pregnancy diagnosis was carried out by transabdominal ultrasound examination.

All animals recovered promptly from the surgery and there were any complication. We performed a pregnancy rate of 47%; the ewes lambed at the term of gestation except for one who delayed for 3 days as she was carrying an hydroanencephalic lamb and required an elective cesarean section. Our results are similar to those obtained by other authors laparoscopic intrauterine insemination with 44% of pregnancy (McKusick et al., 2000; Anel et al., 2005). The main advantage of the technique reported herein consists in its simplicity requiring any expensive equipment such as the laparoscope and air insufflating device in order to perform pneumoperitoneum.

In conclusion, the present study showed that intrauterine insemination with frozen semen by a laparotomic approach is very advantageous, in order to overcome with the difficulties imposed by the complex anatomy of the ovine cervix. This technique can be widely used in sheep breeding, whenever lacking a laparoscopic equipment, mainly for genetic improvement, as it offers advantages on both the economic incomings and the pregnancy rate.

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Riproduzione animale

intrauterine AI, ewe, frozen semen

POSITIVE EFFECT OF NANOCERIA ON MOTILITY PARAMETERS OF RAM SEMEN DURING LONG TERM STORAGE AT 4°C

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Oxidative stress is one of the major limitation in the preservation of ram semen, reducing its vitality and thus its ability to fertilize. It has been demonstrated that nanoceria (nanoparticles of Cerium Oxide, CeO₂) have a ROS scavenger activity in several tissues and cell types (1). The aim of this preliminary study was to test the effect of nanoceria on ram semen parameters preserved at 4°C.

Semen samples from 2 rams of proved fertility were collected by artificial vagina once a week from October 2013 until March 2014. Motility parameters of the undiluted samples were analyzed using CASA (Ivos, Hamilton Thorne, Biosciences). After analysis, samples were diluted 1:5 in OVIXcell (IMV Technologies) and divided into 4 groups, a control group, and 3 groups supplemented with increasing doses of nanoceria (22, 44 and 220µg/mL). Samples were then allowed to gradually cool to 4°C for 2 h and then analyzed by CASA at different time steps, 0h, 24h, 48h, 72h, and 96h for total motility (TM), progressive motility (PM) and specific kinematic parameters (VAP, VSL, VCL, ALH, BCF, STR, LIN). After 2h and 24h of incubation with nanoparticles an aliquot (50µL) from each group was fixed and processed for TEM to assess the uptake of CeO₂ by the cells. The data collected were analyzed by the General Linear Model considering time and treatment as fixed factors (PSPP, Gnu Free Software Foundation).

Our results showed a positive effect of the highest concentration of nanoceria (220µg/mL) on both TM and PM of ram spermatozoa. In particular, the group incubated with 220µg/mL nanoceria showed significantly higher TM scores compared to the control group at 24h, 48h, 72h and 96 h (P<0.05). No significant differences were observed among the control group and the 22 and 44µg/mL and among the treated groups at 0h, 24h and 48h of incubation (P>0.05). The PM followed a similar trend although the effect of 220µg/mL nanoceria was significantly visible at 24h and 48h of incubation (P<0.05) compared to the control group but not to the 22µg/mL and the 44µg/mL groups. No significant differences were found among groups for the other kinematic parameters. The analysis carried on with TEM showed no uptake of the nanoparticles by spermatozoa and no evident interaction or contact between the cells and the CeO₂.

The present preliminary study showed that 220µg/mL nanoceria have a beneficial effect on motility parameters of ram semen during long term storage at 4°C. Although no visible uptake from the spermatozoa have been observed, we can speculate that nanoparticles can exert their antioxidant activity improving the environment in which the cells are stored. Further studies are needed to test the effects of nanoceria on sperm membrane integrity and fertilization capability in vitro and in vivo and to understand the mechanisms of their scavenger activity.

(1) Cerium Oxide Nanoparticles protect Endothelial Cells from apoptosis induced by oxidative stress; Chen S., Hou Y., Cheng G., Zhang C., Wang S., Zhang J.; Biol Trace Elem Res (2013) 154:156-166

Reproduction

Semen, Nanoceria, Ram

EVALUATION OF EQUINE ZONA PELLUCIDA IN POLARIZED LIGHT MICROSCOPY

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ARTs (Assisted Reproduction Technologies) are essential means for the preservation of valuable genetic resources; furthermore, they are powerful tools which often provide clinical answers for hypofertile patients recovering.

The observation of female gametes immediately after collection or cryopreservation, with polarized light microscopy, could be a useful technique for the investigation of effective gamete quality without causing cell damage.

Purpose of this study was to evaluate the correlation between parameters obtained from the measurement of oocyte birefringent structures (area, retardance and thickness of the inner layer of zona pellucida (IL-ZP), and total thickness of ZP) and the parthenogenetic activation response of the cells, in order to identify markers of good gamete quality in horses.

199 COCs (Cumulus-Oocyte Complex) were collected from 40 ovaries obtained from a local slaughterhouse. After collection, the gametes were incubated in maturation medium and then, denuded of cumulus cells, 51 of them were analyzed with PLM (Polarized Light Microscopy) after 36 h of IVM (In Vitro Maturation). Later, 28 mature oocytes were submitted to parthenogenetic activation, transferred in SOF for 3-6 days and daily inspected in order to evaluate the progression of activation.

It was obtained an average of cleaved oocytes per work session equal to $80,24 \pm 5,36\%$.

The ZP birefringent proprieties were estimated and then correlated to the activation outcome.

The data obtained showed that the thickness of ZP was significantly increased in immature oocytes compared to mature oocytes ($20,78 \pm 3,12 \mu\text{m}$ vs $18,85 \pm 2,38 \mu\text{m}$, $p < 0,05$).

The comparison between parthenogenetically activated and non-activated oocytes showed a significantly increase of IL-ZP thickness in parthenogenetically activated oocytes compared to the parthenogenetically non-activated ($4,79 \pm 1,00 \mu\text{m}$ vs $4,18 \pm 0,30 \mu\text{m}$, $p < 0,05$).

Furthermore, it has been observed an increasing trend for IL-ZP area values in the parthenogenetically activated oocytes compared to the parthenogenetically non-activated ($2456,00 \pm 448,76 \mu\text{m}^2$ vs $2220,00 \pm 113,70 \mu\text{m}^2$).

As for women, the PLM in equine oocytes allows the non-invasive observation of zona pellucida, a birefringent oocyte structure, considered as marker of gamete quality. The application of this method could permit to direct towards subsequent ARTs just the cells with a high quality level, having an increase of fertilization percentage. In order to pursue this aim it could be necessary to increase the number of analyzed oocytes to confirm and enhance the results of this preliminary work.

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Riproduzione equina
horse, oocyte, polscope

Sira Poster

THE EFFECTS OF ECG ON OVULATION PARAMETERS IN BOS INDICUS BEEF COWS SUBJECTED TO TIMED ARTIFICIAL INSEMINATION PROTOCOL.

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The aim of this study was to evaluate the effect of equine chorionic gonadotrophin (eCG) on estrus occurrence, mean of interval from PGF2 α to estrus occurrence and estrus dispersion, in suckled Bos indicus beef cows subjected to timed AI (TAI) protocols.

A total of 98 cows in random stage of the estrus (D0) received at beginning of synchronization of ovulation an intravaginal progesterone device (P4, CIDR® 1.9 gr progesterone, Zoetis) plus 2 mg of estradiol benzoate i.m. (EB; Gonadiol®, MDS Animal Health) and the BCS was taken. Eight days after, device was removed and 500 μ g of cloprostenol sodium i.m. (PGF; Sincrocio®, Ouro Fino Animal Health) was administered. At this moment, cows were randomly assigned to one of three treatments: 300 IU of eCG (Group eCG; n=33); 300 IU of eCG plus 1 mg of ECP im (Group eCG+ECP; n=32) or no additional treatment (Control; n=31). Cows were timed inseminated 48 after P4 device removal (D10) and were administrated simultaneously, 100 μ g of gonadorelin im (Profertil® - Tortuga Companhia Zootécnica Agrária). The ovulation was confirmed by ultrasonic exam on days 10, 11 and 12 and determined by the presence of a CL and disappearance of the ovulatory follicle. The data were analyzed using the GLIMMIX procedure of SAS and differences with $P < 0.05$ were considered statistically significant.

Cows that received ECP treatment (eCG+ECP=90.6%; 29/32) presented a higher ovulation rate than the control group (64.5%; 20/31), but no difference was found between this group and the eCG group (84.9%; 28/33). Furthermore, no differences were founded between the mean interval from PGF2 α to ovulation ($P=0.21$) and ovulation dispersion ($P=0.43$).

In conclusion, Bos indicus cows receiving eCG treatment (with or without estradiol supplementation) have greater ovulatory responses after estradiol/P4-based synchronization protocol, but were unable to concentrate the ovulation or alter the ovulation moment.

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Estrous synchronization beef cows
Synchronization, eCG, Timed AI

CYTOLOGY AND BACTERIOLOGY OF CANINE COLOSTRUM

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Subclinical mastitis is suspected when pups fail to gain weight or neonatal mortality rate is too high, although milk generally appears normal and the bitch is not systemically ill. Milk culture and somatic cell counts, with the determination of differential cell patterns, are important tools for the evaluation of udder health in cows. Studies are limited in dogs: the normal parameters in the numbers and types of cells present in canine milk have been described in a single investigation (1); data on colostrum are lacking.

The objective of this work was to describe the cytology and bacteriology of normal canine colostrum, for a future assessment of parameters indicative or predictive of subclinical mastitis.

27 postpartum bitches of different breeds were included in the study. Twenty bitches whelped spontaneously while 7 underwent Cesarean section. On postpartum day 1 some drops of colostrum were collected from each inguinal mammary gland and directly smeared on a microscopic plate for May-Grunwald Giemsa staining. Other drops were collected on a bacteriological swab for microbiologic exams. A single bitch had clinical signs of acute mastitis.

Colostrum cytology was similar to that reported in other mammals, like cow, sheep and woman. At microscopic observation (ten fields, 40x), among inflammatory cells, lymphocytes were the least numerous population (maximum 6 cells/ten fields), followed by macrophages (N=21) and neutrophils (N=59). In the single case of acute mastitis, neutrophils were the only visible cells (N>400). Culture results showed that only 4/49 samples were negative and 6/49 yielded nonspecific microbial contamination; in the other cases different bacteria were isolated, with higher frequencies for *Staphylococcus pseudintermedius* and *Escherichia coli*. In contrast with a previous work (2), but in agreement with our previous observations (3), *Staphylococcus aureus* was never isolated: the strain isolated from a single swab was confirmed to origin from cross-contamination (i.e. the operator). When comparing cytology and culture results, we did not find a correlation between inflammatory cell numbers and isolation of bacteria, either if in pure culture, or if potentially pathogen species (*S.pseudintermedius*, *S.aureus*, *Streptococcus canis*, *E.coli*, *Klebsiella pneumoniae*, *Pasteurella multocida*). The inflammatory response might become apparent later on, after the initial bacterial colonization and proliferation that we can have detected at day 1; alternatively, bacteria may colonize the mammary glands without causing infection and without affecting pup survival.

Past literature does not offer data for comparison in the bitch and it will be interesting to analyse milk samples collected later on in the postpartum period.

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Riproduzione animale

dog, colostrum, post-partum

HEMATOCOLPUS IN TWO BITCHES WITH A HISTORY OF GNRH TREATMENT TO POSTPONE PUBERTY

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To report a rare case of hematomocolpus in two bitches following a contraceptive treatment.

Two mixed breed bitches (18 months), 1 year before treated with deslorelin acetate to postpone puberty, were admitted for the suspicion of a heat, despite the absence of vulvar bloody discharge. The animals were hospitalized and undergone to various diagnostic procedures, including physical genital tract examination, vaginoscopy, vaginal cytology, endocrine assay, ultrasound and X ray using vaginal infusion of iodine and pneumatic bladder as positive and negative contrasts. Both animals were submitted to exploratory laparotomy followed by removal of the genital tract. Specimens were bacteriologically, cytologically and pathologically processed.

In bitch 1, vaginal cytology and progesterone levels confirmed the presence of an ovulatory “dry” oestrus. Ultrasound showed preovulatory follicles and, in the following days, transition to corpora lutea. The caudal abdomen presented a large ovoid cystic structure filled with echogenic fluid, next to the bladder. Radiographic scans demonstrated a normal bladder profile, while the iodine failed to enter into the cranial vagina. At laparotomy, a vaginal sac (10x5 cm), from which brown fluid was aspirated, was found and resected together with uterus and ovaries. There was no communication between the sac and the remaining vaginal tract. Bitch 2 had the same diagnostic route and findings, but was laparotomized 3 months after the heat. During this period no spontaneous regression of the lesion was observed. At laparotomy, the vaginal sac (8x4 cm) was only aspirated and the bitch was regularly neutered. In both cases, cytology of the fluid, aspirated from the vaginal sac, revealed mucosal superficial epithelial cells and abundant degenerate red blood cells. Bacteriology was negative. Histology (bitch 1) confirmed the vaginal origin of the sac despite its immature appearance, and revealed a Gärtner’s duct cyst.

Bitches were cycling but showed an atypical “dry heat”. The impressive vaginal distension (hematomocolpus) probably originated by an inadequate drainage of proestrous bloody discharge for a vaginal abnormality [2-3]. It is unlikely a congenital origin of the lesion, since vaginal abnormalities are uncommon and bitches were not related. It is likely that the GnRH agonist treatment of the prepubertal bitches interfered, by an irreversible way, with the normal development of the vaginal tract, which in some traits maintained the prepubertal characteristics and an incompetent lumen. On the other hand, Gärtner’s duct cysts are rare lesions in the bitch associated with disorders of development of the vaginal tract [1].

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Reproduction

BITCH, HEMATOCOLPUS, GNRH

HAEMATOLOGICAL PARAMETERS DURING THE DIFFERENT PHASES OF THE ESTROUS CYCLE IN RAGUSANA JENNIES (EQUUS ASINUS).

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Changes in haematological values during the estrous or menstrual cycle have been reported in some species but there are no data for the donkey [1, 2, 3, 4, 5]. The aim of this study was to evaluate the influence of the various phases of the estrous cycle on haematological parameters in jennies.

The work was carried out from January to February 2014 on 7 clinically healthy and cyclic Ragusana jennies, aging 2-16 years, living in Sicily. The animals were monitored by ultrasound examination every 3 days from the time of ovulation during one regular oestrus cycle (21-24 successive days). Blood samples were collected with each ultrasound examination (luteal phase: day 1, 4, 7, 10, 13, 16 and follicular phase: day 1, 4, 7) by jugular venipuncture into EDTA tubes. A complete blood count (CBC) was performed using the hematology analyzer ProCyte Dx® (IDEXX). The parameters measured included: red blood cell count (RBC), hematocrit (HCT), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count (WBC), neutrophils (NEU), lymphocyte (LIM), monocyte (MONO), eosinophil (EOS) and basophil (BASO) count, platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT). Test results were analyzed by analysis of variance (ANOVA).

No significant changes were found for haematological parameters during the study period.

The results of this work indicate that the oestrus cycle is not significant determinant of variation in haematological values in jennies.

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Reproduction

DONKEY, ESTROUS CYCLE, HEMATOLOGY

SUBINVOLUTION OF PLACENTAL SITES IN A LEISHMANIOTIC BITCH

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To report a case of subinvolution of placental sites (SIPS) in a bitch, discussing the probable relationship with the concomitant leishmaniosis.

A 12 month Jack Russel bitch was admitted for a persistent vulvar bloody discharge 9 weeks post partum. The bitch underwent complete physical examination, haematology and serum biochemistry screen, vaginal cytology and ultrasound.

Physical examination of the bitch revealed pale mucosae, normal lymph nodes, weakness, a mild ulcerative facial dermatitis and abundant bloody discharge from the vulva. Haematological parameters showed a mild anaemia with a marked thrombocytopenia. Vaginal cytology revealed parabasal cells, many red blood cells, few neutrophils, and some scattered trophoblastic cells. Abdominal palpation revealed multiple ovoid structures. Ultrasound examination of the caudal abdomen confirmed the presence of at least 4 solid uterine swellings (up to 1.2 cm) with a variably echoic appearance. The subinvolved areas were interspersed with involuted segments (0.5 cm) with an evident echoic central line. A diagnosis of SIPS was done. An antibiotic coverage was given (metronidazole and spiramycin) and the bitch was re-evaluated after 3 days. The clinical picture did not change, platelet number were slightly increased. Tests for haemoparasites were started. A low dose progestin therapy was administered in addition: megestrol acetate 0.1 mg/kg os SID for a week and half dose for another week [1]. After 4 days, the vaginal discharge disappeared; after 15 days the subinvolved placental sites were reduced to 0.8 cm. A 1:20480 IFAT titre for leishmaniosis was found and an appropriate therapeutical approach was applied.

Post partum uterine involution requires about 14 weeks in the bitch, but bloody discharge should not exceed 6 weeks post partum. SIPS is an enigmatic and uncommon gestational trophoblastic disease; it affects young bitches after the first whelping and it is characterized by persistent bloody discharge and collagenous/necrotic masses at placental site level [2]. Rarely, SIPS is an emergency, necessitating blood transfusion and surgery. Ecboic or progestin therapies have been proposed, but spontaneous recovery may occur [3]. Pathogenesis is unknown, but it is likely that residual trophoblastic cells invading the uterine wall are firstly responsible of the lesion. Clotting disorders have been proposed as complicating factors of SIPS. To our knowledge, this is the first reported case of SIPS in a leishmaniotic dog. Although a coincidence cannot be ruled out, authors' hypothesis is that the parasitic disease may have played a role in the worsening of SIPS interfering with the clotting mechanism.

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Reproduction

BITCH, PLACENTAL SITES, LEISHMANIOSIS

PREGNANCY IN A FEMALE FERRET FOLLOWING TREATMENT WITH A DESLORELIN IMPLANT: A CASE REPORT

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Description of a clinical case

Reproduction control in pets can be achieved using slow release GnRH-agonist implants (1; 2; 3). This report describes the case of a 4-year old jill that was treated with a 4.7 mg deslorelin implant (SuprelorinTM; Virbac: France).

The jill gave birth to viable kits in 2010 and to deformed kits in 2011. The following breeding season, despite several matings, she did not conceive. In March 2013 the jill started to show signs of heat and was mated 3 times, but even if she went off of heat following breeding and showed a progressive abdominal volume increase during next 40 days, she did not give birth.

In early May 2013, the jill showed signs of heat again and following multiple matings over a period of 2 weeks, she went off heat again, but this time started losing weight. She was then taken to the veterinarian who, based on her history, thought that she was facing another period of prolonged cycling and potential worsening of her body conditions and therefore decided to treat her with a deslorelin implant (Suprelorin; Virbac) to stop her from cycling.

The deslorelin treatment was administered 4 days following the last mating (22 May) and with the ferret still showing some degree of vulvar edema. The implant was positioned under the skin, on the midline a few cm cranial to the umbilicus.

Subsequently the owner noticed the disappearance of vulvar edema a few days later, and a swollen abdomen and swollen mammary glands 30 days later. Therefore, the jill was taken to our Small Animal Reproduction Service where she was found to be pregnant. Five days later she gave birth to 6 viable kits.

Even if the ferret had a normal mammary development and milk production, she did not show adequate maternal instinct, and 5 kits died during subsequent weeks of life presumably due to lack of maternal care. Necropsy was not performed. The last kit survived and is currently growing normally. Following delivery the ferret has been in good health and has not shown any sign of heat until March 2014. Currently the implant is still in place and the jill is in good condition and has finished heat manifestations.

In this case, the deslorelin implant did not have any detrimental effects on pregnancy with regard to fetal health and pregnancy duration (44 days from the last mating). After treatment the jill improved her general body condition and gained some weight. In addition no other physical side effects were observed, similarly to what observed by other authors in dogs and cats (3;4).

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Reproduction of small animal

Deslorelin, female ferret, pregnancy

VAGINA FOLD PROLAPSE IN A BITCH WITH PYOMETRA AND OVARIAN PAPILLARY CYSTOADENOCARCINOMA

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Vaginal prolapse is a rather uncommon condition in the bitch. The vaginal mucosal hyperplasia occurs during the proestrus or oestrus phases of the ovarian cycle under estrogen influence more rarely may also occur in the late pregnancy period due to decreased progesterone level and increased oestrogen level. Vaginal prolapse has also been described in a bitch treated with oestrogens and in an ovariohysterectomised bitch. An edematous swelling of the vaginal mucosa immediately develop cranial to the urethral orifice and expanding caudally over the urethral orifice and can involve the entire circumference of the vaginal mucosa. Hyperplasia of the vaginal mucosa may regress spontaneously during diestrus but it recurrence can be observed during the following oestrous cycle, from 66% up to 100% of cases. Hyperplasia of the vaginal mucosa must be differentiated from the uncommon true prolapse. In these cases other organs, such as the bladder, and the uterine body and/or distal part of the colon, may be present in the prolapse.

To the best of our knowledge, we present here the first report on vaginal prolapse concomitant with pyometra and ovarian papillary cystadenocarcinoma

An 7-year old nulliparous bitch, Corso breed weighing 42 kg, with ovarian papillary cystadenocarcinoma and pyometra. The oestrus lasted 16 days and ended after about 25 days. The prolapsed mass increased gradually over the 3 days before bringing the bitch to our clinic showed “trilobed shape”: the median portion corresponded to the vaginal floor and the lateral portions to the walls of the vagina. According to abdominal palpation, trans-abdominal ultrasonography, and laboratory analysis the pyometra was diagnosed. Under general anesthesia Ovariohysterectomy was performed. The protruding tissue was cleaned with a 0.2 % chlorexidrin solution and relocated in the vaginal cavity. The vulvar lips were temporarily locked by using a Bünner suture.

The ovaries were inspected and number of luteal bodies estimated (seven to the right and five on the left). The right ovary was twice the size of the left and had a cystic structure. Histologically the cystic structure was a papillary cystadenocarcinoma. Serum Estradiol (E2) and progesterone (P4) concentration were evaluated by commercial solid-phase radioimmunoassay. Progesterone and oestrogen in the serum reached respectively 9.36 ng/ml e 30.42 pg/ml, while in the cystic fluid were 72.72 ng/ml e 792 pg/ml.

Since the history showed a predisposition of the bitch to the vaginal prolapse, we can conclude that oestrogens produced by the ovarian adenocarcinoma, besides being a predisposing factor for the cystic hyperplasia-pyometra complex, played a key role in determining the hyperplasia of the vaginal mucosa and the prolapse.

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riproduzione

BITCH, VAGINA FOLD PROLAPSE, PYOMETRA

DIETARY SUPPLEMENTATION IN THE DOG BREEDING: INFLUENCE ON THE SEMEN QUALITY, ENDOCRINE FUNCTIONALITY AND BEHAVIOUR

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The influence of a diet supplemented with antioxidants on semen quality and reproductive function has been documented in numerous animal species, especially those of economic interest (cattle, pigs, horses, and dogs), as well as in humans, in recent scientific works (1,2,3,4) and increase the metabolic activity. Given the growing interest in using supplemented diets to improve semen quality, the aim of this study was to compare the changes in endocrine function over time, and the main parameters of semen quality and quantity in response to the administration of a diet enriched with specific phytochemicals, vitamin E acetate, carotenoids, folic acid, and zinc chelate from amino acids hydrate.

The study involved 14 male dogs of various races, divided into four age groups, selected after a general and clinical examination to rule out any anatomical or functional disorders, or behavioral issues affecting the reproductive sphere.

The animals were from 1 to 10 years old. Research lasted nine months, divided into two phases of 90 and 135 days respectively (PRE SUPPLEMENTATION – use of previous diet- WITH SUPPLEMENTATION - supplemented diet). During each phase, biological material was collected 3 times, every 45 days (T-3, T-2, T-1 - T1, T2, T3): (I) quantitative evaluation (blood sample) for endocrine activity Tst (ng/ml), fT4 (pg/ml); (II) evaluation of semen; (III) general examination; (IV) follow-up with owners (potential changes in behaviour) and Body Condition Score evaluation. Pearson correlation and Student T test ($p < 0,05$) were performed.

Data show a rapid response to the new diet: (T1) constant increase in metabolic activity (fT4, Tst) within 45 days, which has a positive influence on thyroid activity and reproduction; the data on behaviour showed an increase in territoriality (80%) and dominance (60%), and the BCS underlines increased muscle tone could be due to the testosterone increase. All subjects (T basal vs - T1, T2, T3) showed significant difference in body weight, Tst level, semen concentration, volume and motility. Instead in the 2-7 year group (PRE SUPPL/WITH SUPPL)

significant differences were in Tst level, semen volume, motility and vitality.

The diet was created especially for dog between 2-7 years. Supplementation does not seem to positively influence the semen values in mature (senior) subjects.

The diet was created especially for 2-7 years aged group because this group reaches a peak in performance. Supplementation does not seem to positively influence the semen values in mature subjects: probably due to the ageing in tissues or metabolism. Considering this encouraging outcome, the diet should be used for dog breeding, to maximize reproduction management.

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riproduzione, miglioramento riproduttivo

reproductive function, dietary supplementat, dog breeding

INDUCTION OF HYPERACTIVATION IN BULL SPERM IN DIFFERENT INCUBATION CONDITIONS

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The sperm cells must undergo two processes which are essential to ensure their ability to penetrate the zona pellucida and fertilize the oocyte: capacitation and hyperactivation that in vivo physiologically occur within the female genital tract.

With capacitation the spermiatic movement pattern is subjected to changes which can be evaluated through computer analysis: they become more rapid and linear; furthermore during this phase the acrosome reaction happens. The hyper-activated sperm shows an increase in curvilinear velocity (VCL), a widening of the lateral movements of the head (ALH) and the flagellum and a gradual decrease in speed.

Several techniques were identified and proven to achieve in vitro capacitation of bovine semen, whereas the different proposed procedures for the induction of hyperactivation are still under investigation.

The aim of the study was to induce in vitro sperm capacitation and hyperactivation of four Piedmontese breed bulls whose fertility was proven. Capacitation was induced with the standard methods for in vitro fertilization (IVF) in cattle (1), whereas to achieve hyperactivation the sperm cells were incubated in a medium added with Procaine, a molecule commonly used as a local anesthetic, but able to induce sperm hyperactivation by increasing membrane permeability to calcium ions(2).

We analyzed the sperm of four Piedmontese breed bulls, for each animal three paillettes were thawed out by placing them in a water bath, set to 37°C, for 1 minute. The sample thus obtained has been divided into 2 parts. One of them was processed using swim up, with an incubation of 45' at 38,5°C in an atmosphere of 5% CO₂. The other share has been used as it was.

The latter was shared and incubated at 38,5°C in an atmosphere of 5% of CO₂ after dilution with FERT TALP (C1, suspension), FERT TALP-PHE (C2, capacitation), FERT TALP-PROCAINE (C3, hyperactivation), FERT TALP-PHE-PROCAINE (C4, capacitation and hyperactivation). After swim-up, both sample with separated semen and that with sperm which didn't separate (C7 capacitation, C8 hyperactivation), were resuspended in FERT TALP-PHE (C5 capacitation), FERT TALP-PROCAINE (C6 hyperactivation).

All the obtained shares were evaluated through computer-assisted semen analysis (CASA), immediately after dilution (T0) and after 15', 30', 60' of incubation (T1, T2, T3).

The statistic analysis, performed with SPSS 19, proved that semen capacitation (VSL and BCF increased for samples C5 and C7. VSL C1= 93,96 µm/s ± 6,48 C5= 96,52 µm/s ± 3,38 C7= 95,02 µm/s ± 4,25 – P< 0.05 - BCF: C1= 31,27 Hz ± 7,09 C5= 38,13 Hz ± 3,42 C7: 34,69 Hz ± 5,4 – P< 0.05 and progressive) and hyper activation (VCL increased C1= 211,10 µm/s ± 22,78 C3= 235,39 µm/s ± 39,09 C4= 245,32 µm/s ± 41,17 C6= 248,03 µm/s ± 44,51 C8= 272,52 µm/s ± 35,29 – P< 0.05 - and percentage of progressive cells decreased C1= 47,5% ± 4,38 C3= 21,5% ± 13,32 C4= 20,30% ± 11,25 C6= 8,38% ± 11,18 C8= 3,63% ± 3,68 – P< 0.05) occurred with values statistically greater in samples that undergoing swim up (C5,C6,C7,C8), compared with those used as they were.

Time evolution of analyzed parameters showed a faster decay in samples treated with swim-up, probably because of stress-induced damages correlated with this procedure.

Finally, performing an individual analysis of the bulls, it was possible to observe a wide variability between subjects. Confirmation of this fact based on a larger number of samples and correlation between field fertility and in vitro capacitation and hyper activation response could allow the use of these tests as fertility indicators for bulls.

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Ripro

Hyperactivation, Sperm Bull, Procaine

GENETIC CHARACTERIZATION OF ALGERIAN CAMELS (CAMELUS DROMEDARIUS) USING STR LOCI

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The camel (*Camelus dromedarius*) represents an important economic resource in many arid areas across several countries, as a pack or racing animal or since providing milk, meat and hair. Notwithstanding, knowledge on the genetic diversity and structure of the camel population is still very poor. Here we contributed to fill this gap by thoroughly characterizing Algerian camels using multi-allelic STR markers.

A total of 198 animals from 7 sampling areas (representatives of 17 municipalities and 79 herds) were considered. Individual genomic DNAs isolated from whole blood were typed at 20 STR loci, out of which 18 belonged to the recommended ISAG/FAO panel (FAO, 2011). Three previously developed multiplex PCR reactions (Almathen F., unpublished data) were adapted to capillary electrophoresis (CE) analysis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and the GeneScan™500 ROX™ as internal size standard. Raw CE data were analyzed using the GeneMapper software (Applied Biosystems). Classical population genetics parameters were calculated using the Arlequin computer package (Excoffier and Lischer, 2010). Model-based Bayesian clustering was carried out using STRUCTURE (Pritchard et al., 2000).

Nineteen loci were polymorphic (excluding the locus CMS17), with an average of 8.7 ± 5.4 alleles, ranging 2 (YWLL59, CVRL8, VOLP32) to 23 (CVRL1D). The average observed heterozygosity was 0.60 ± 0.17 and the average expected heterozygosity was 0.64 ± 0.19 . Four loci (CVRL4, CMS121, CMS9 and VOLP10) deviated significantly from Hardy-Weinberg proportions ($P \leq 0.01$) due to excess of homozygous genotypes. Out of 190 possible pair-wise comparisons, only four displayed a significant ($P \leq 0.01$) "linkage" disequilibrium. There was no clear genetic structure according to the Log probability of data in the STRUCTURE analysis.

Overall, relatively high levels of genetic variability were observed in the Algerian camel population. Together with the lack of genetic structure among different geographic isolates, this may likely reflect traditional camel management practices still in use today.

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Genetica di popolazione

Camelus dromedarius, Genetic diversity, Microsatellites

QUANTITATIVE CHARACTERIZATION OF ANTIOXIDANTS THIOL-DERIVATIVES IN FOLLICULAR FLUID OF MARES

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The chemical composition of follicular fluid (FF) is an indicator of the secretory activities and of the metabolism of follicular cells and thus could be related to the follicular quality. It could also provide a useful indication of the oocyte growth and maturation [1]. Additionally, there are many antioxidants found in FF which promote healthy oocyte maturation and oocyte viability. The aim of the present preliminary study was to evaluate the changes in the concentrations of different antioxidants, such as glutathione(GSH), taurine, glutamylcysteine(Glu-Cys) in the FF collected from different follicular size categories in mare. As well as malondialdehyde (MDA), as an indicator for lipid peroxidation, was estimated.

Mare ovaries were collected at a slaughterhouse during reproductive season. The follicles were classified into three groups according to their diameter as small (2-3 cm), medium (3.5-4.5 cm), and large (5-6 cm). The follicular fluids were centrifuged in order to quickly separate the fluid from the cell fractions and supernatants were kept at -80°C until analysis. The determination of antioxidants was carried out according to the method described by Zinellu et al. [2].

GSH concentration significantly decreased ($P<0.05$) in the FF obtained from small sized follicles compared with the medium and the large ones (6.913 ± 1.1 , 2.83 ± 1.3 and $2.05\pm0.8\ \mu\text{M}$), respectively. Furthermore, taurine level was significantly ($P<0.05$) less in FF of medium ($71.4\pm0.4\ \mu\text{M}$) compared with large ($121.3\pm0.3\ \mu\text{M}$) follicles. In the other hand, the concentration of MDA and Glu-Cys did not appear significant different between the experimental groups. It is worth noting that the MDA activity appeared higher in large follicles ($7.939\pm1.6\ \mu\text{M}$) and in contrast, the concentration of Glu-Cys was higher in the small ones ($1.146\pm0.4\ \mu\text{M}$).

The present study presents for the first time the quantitative characterization of low molecules-weight thiols derivatives in mares' FFs. However, further experimental support is necessary in order to understand the biochemical modifications of antioxidants and their possible role in process of follicular development.

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Reproduction

antioxidants, follicular fluid, mare

SCREENING FOR SEQUENCE VARIABILITY AT THE CAMEL (CAMELUS DROMEDARIUS) MYOSTATIN GENE

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Dromedary camels play a major economic role as suppliers of meat for human consumption across several countries. Notwithstanding, a comprehensive characterization of the sequence variability at the MSTN locus coding for myostatin, a negative regulator of skeletal muscle responsible in several livestock species for increased skeletal-muscle mass, is still lacking. The aim of this study was therefore to perform the first extensive sequence and polymorphism analysis of the MSTN gene in the Camelus dromedarius species.

A total of 22 Camelus dromedarius samples from Tunisia (5) Egypt (7) and Algeria (10) were considered. The MSTN locus was amplified using heterologous oligonucleotide primers designed using Primer3 (www.primer3.ut.ee/, version 4.0.0) on the available Camelus ferus contig (GenBank Accession No AGVR01040332.1) which includes the whole-gene MSTN sequence. Purified PCR products were Sanger sequenced. Sequence alignments were carried out using ClustalW (www.genome.jp/tools/clustalw/).

Shah et al. (2006) previously sequenced a small (256 bp) region in the first exon of the C. dromedarius MSTN locus. Here we sequenced more than 3.6 kb, including the three exons, part of intron 1 and intron 2 and part of the 3' and 5' ends of the C. dromedarius myostatin gene. The NJ tree obtained from the pairwise sequence identity among C. dromedarius and other species belonging to the Cetartiodactyla clade was consistent with previous reports (Kacskovics et al., 2006). A close similarity between C. ferus and C. dromedarius was observed (sequence identity, 99.73%). The polymorphism analysis carried out on 22 animals from three different geographic regions (Algeria, Tunisia and Egypt) highlighted three variant nucleotide sites (486_G/C, 798_G/A and 799_C/T). All the polymorphic sites were located in the first intron. We did not observed any polymorphism in the second exon of the myostatin, which is known from other species to harbor functional mutations (Baron et al., 2012).

Our results confirmed previous evidences of the myostatin gene as a highly conserved gene across mammals. Notably, the low diversity observed at the MSTN locus in C. dromedarius may reflect the evolutionary history of this species, which likely developed as a domesticates from a low variable and geographically isolated wild ancestor population in the Arabian Peninsula.

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Genetica di popolazione

Myostatin, Polymorphism, Camelus dromedarius

SEMEN CHARACTERISTICS DURING BREEDING SEASON IN MELOPSITTACUS UNDULATUS

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Semen characteristics of *Melopsittacus undulatus* have been studied in depth for decades^{1,2}, but the increase in availability of CASA analysis in avians has only recently allowed its use for the selection of individuals that are suited for testing semen cryoconservation and assisted breeding in this species. The aim of this work is the identification of semen characteristics during breeding season while comparing the results with those present in literature³ so that a standardized protocol for collection and evaluation can be established.

19 males in breeding, age range 1-4 years, part of a mixed colony with females of breeding age. The subjects underwent two weekly collections using a modified sacro-abdominal-cloacal massage technique⁴ between February and March, yielding 82 samples. Each bird was physically restrained by an assistant, using a knotted towel in the form of a doughnut, so that the assistant only had to hold the wings together and legs well spread apart. Ejaculation was produced by massaging the dorsal aspect of the abdomen towards the cloaca with thumb and index or middle finger and then by gentle rhythmic squeezing at the base of the cloaca with the same finger of the other hand. The ejaculate was collected in graduated microcapillary tubes (Microcaps® - Drummond Science Company) for the evaluation of color and volume. After dilution in TALP (pH 8.4), concentration, motility, VAP, VSL and VCL were evaluated using a Computer Aided Sperm Analyzer (CEROS, Hamilton Thorne Research Inc.) on 10 µl of extended semen placed in a pre-heated Makler chamber.

The values observed in this are shown in Table 1. Only VAP, VSL and VCL could be compared to those found by Gloria et al.³. The mean values are slightly higher than those recorded in other work for the same type of animal.

The results obtained in this work are compatible with those from previous works³, despite the different pH, collection dose and CASA system. These results need further studies to determine the minimum fertilizing amount and the best protocol for cryoconservation, necessary to set up a donor bank and improve the breeding performance of this species.

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Avian seminology and reproduction

Budgerigar, Semen evaluation, CASA

	Unit	Mean	SD	Median	IQR
Volume	ul	2,93	1,85	2,63	2,35
Concentration	N sperm/ml x10 ⁶	9730	486,65	6450	7775
Motile spermatozoa/ejaculate	(x 10 ⁶)	22,740	46,316	6,783	23,377
Total motility	%	69,9	21,77	73	31,75
VAP	μm/s	75,97	21,51	77,3	31,55
VSL	μm/s	65,9	19,36	65,9	25,9
VCL	μm/s	99,96	24,02	101,5	39,35
N= 82	Sampling interval:			February – March 2014	
NB: mean and SD are outlined in bold in the case of a normal distribution, median and IQR otherwise. The limit for statistical significance was set at p<0.05.					

Table 1. Semen characteristics of analysed *Melopsittacus undulatus*.

BUDGERIGAR SEMEN VARIATIONS BOTH BETWEEN COLLECTION PERIODS AND WITH DIFFERENT DILUTION PROTOCOLS

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The aim of this study is to assess 1) the effect of collection training on semen quality of budgerigars and 2) to evaluate the effect of different semen extender pH values.

For the first part of the study, 19 males (age 1-4 years) were divided in two groups according to initial semen quality: groups S (9 individuals; low motility parameters), group D (10 individuals; high motility parameters). The birds underwent two weekly collections using a modified sacro-abdominal-cloacal massage technique between February and March. Group S originated 40 samples, Group D 48. Volume, concentration, motility, VAP, VSL and VCL were evaluated using a Computer Aided Sperm Analyzer (CEROS, Hamilton Thorne Research Inc.) on 10 µl of extended semen placed in a pre-heated Makler chamber.

For the second part of the study, 54 ejaculates were diluted with TALP either at pH 8.2 (N=12) or at pH 8.4 (N=42). The evaluated parameters were motility, VAP, VSL and VCL. The statistical tests employed were Shapiro-Wilk's normality test, Kolmogorov-Smirnov's two-sample test and Student's T-Test. All tests were conducted using the R statistical software (version 3.1.0) with statistical significance set at $p < 0.05$.

1) Both groups experienced a statistically significant increase in volume, VAP and VSL as the collection period continued, while motility, concentration and VSL failed to achieve statistical significance in following this trend (Table 1).

2) No statistically significant difference was found for motility between TALP pH 8.2 and pH 8.4. pH 8.2 exhibited higher values for VAP, VSL and VCL compared to pH 8.4 (Table 2).

The average increase of the seminal characteristics, particularly in volume, underlines the importance of donor training at the start of the breeding season, both increasing the performance of a suboptimal group and granting the ability to select the best donors in a breeder collection. An increase in volume with the same or higher concentration is of particular importance to the original aims of the study, working towards obtaining multiple fertilizing doses per single ejaculate.

TALP 8.2 appears to perform better, both when compared to the 8.4 pH group and the results in literature, where a lower pH was used (pH 8.0)².

TALP 8.2 appears to perform better, both when compared to the 8.4 pH group and the results in literature² (using pH 8.0).

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2. Gloria A, Contri A, Carluccio A, Parrillo S, Cicconi M, Robbe D. The breeding management affects fresh and cryopreserved semen characteristics in *Melopsittacus undulatus*. *Anim Reprod Sci* 2014, 144: 48-53.

Avian seminology and reproduction

Budgerigars, Donor training, Dilution protocols

	Group S		Group D	
	Beginning	End	Beginning	End
Volume (μL)	1.32 $\pm$ 0.39	1.99 $\pm$ 0.58	1.23 $\pm$ 0,76	3,29 $\pm$ 1,37
Concentration ($\times 10^6$ sperm/ml)	4012 $\pm$ 2771.45	3220 $\pm$ 1952	2249 $\pm$ 1228	4117 $\pm$ 2599
Motility (%)	50.4 $\pm$ 25.31	64.8 $\pm$ 23.35	68.33 $\pm$ 21.25	68.17 $\pm$ 23.39
VAP ($\mu\text{m/s}$)	41.7 $\pm$ 6.15	85.66 $\pm$ 16.35	85.65 $\pm$ 21.97	99.18 $\pm$ 20.57
VSL ($\mu\text{m/s}$)	36.02 $\pm$ 5.65	76.94 $\pm$ 15.77	75.52 $\pm$ 20.21	87.80 $\pm$ 16.53
VCL ($\mu\text{m/s}$)	65.34 $\pm$ 8.97	108 $\pm$ 16.45	112.5 $\pm$ 23.36	125.4 $\pm$ 23.87
<i>Observations</i>	40		48	

Table 1. Motility, VAP, VSL and VCL values (means $\pm$ SD) observed during the collection period.

	TALP 8.2	TALP 8.4
	Mean $\pm$ SD	Mean $\pm$ SD
Motility (%)	68.83 $\pm$ 22.53	73.62 $\pm$ 19.68
VAP ($\mu\text{m/s}$)	92.96 $\pm$ 18.30	75.50 $\pm$ 18.49
VSL ($\mu\text{m/s}$)	82.32 $\pm$ 15.73	65.35 $\pm$ 19.92
VCL ($\mu\text{m/s}$)	117.7 $\pm$ 20.81	99.65 $\pm$ 21.01
<i>Observations</i>	12	42

Table 2. Motility, VAP, VSL and VCL values (means $\pm$ SD) observed using different dilution compounds.