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Università degli Studi di Perugia

Dipartimento di Medicina Veterinaria

Via S. Costanzo, 4 - 06126 Perugia

SOCIETÀ ITALIANA DELLE SCIENZE VETERINARIE
Joint meeting

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ATTI 2015

LESIONI MUSCOLARI IN CORSO DI LEISHMANIOSI, DALLA PATOGENESI ALLA LESIONE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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LESIONI RENALI IN CORSO DI LEISHMANIOSI, DALLA PATOGENESI ALLA LESIONE

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

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

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

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

LESIONI OCULARI IN CORSO DI LEISHMANIOSI, DALLA PATOGENESI ALLA LESIONE

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

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

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

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

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

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

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

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EMATOBIOCHIMICO: MODIFICAZIONI E MARKERS DI RECENTE INTERESSE

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

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

1) La sede delle lesioni renali

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

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

2) L'entità delle risposte infiammatorie/ossidative

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

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

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

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

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

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

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

Part VII

XV CONVEGNO A.I.P.Vet

THE PRESENCE OF SHORT FORM OF RON/STK TRANSCRIPT COULD BE A PREDICTOR OF POOR OUTCOME IN FELINE MAMMARY CARCINOMA

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Since 1980, feline mammary carcinoma (FMC) has been recognized as a suitable animal model for studying human breast cancer because it shares epidemiological, morphologic and prognostic features with human breast carcinoma(1). RON/stk tyrosinase receptor, identified in cat as feline-stk (2), is activated by Macrophage Stimulating Protein (MSP) and over-expressed in human breast cancer (3). Human RON gene is able to generate both the full length (fl) and the short forms (sf) of the transcripts. Sf-RON is generated from an alternative transcriptional start from a second promoter, within the intron 10 (4). The sf-RON lacks the N-terminus of the protein, including most of the extracellular domain, but conserves the kinase activity of the COOH terminus after heterodimerization. In human the short form of RON plays an important role in breast cancer and its expression is correlated to invasive capability in vitro (5,6,7).

The aim of this research was to investigate the expression of both RON and MSP and to identify the presence of the sf-RON transcript in feline mammary carcinomas (FMCs) in relation to clinico-pathological findings.

Tissue samples of spontaneous mammary tumours were collected from 50 queens. All the animals underwent a complete clinical staging and were surgically treated with surgery and then they were followed until the recurrence of the neoplasm or death. All the samples were histologically evaluated and immunohistochemically tested for RON and MSP. Histological and immunohistochemical results were evaluated in relation to clinico-pathological data. RNA was extracted from each formalin fixed paraffin embedded case and RT-PCR was performed to detect sf-RON, with primers annealing on exon 10 and exon 11.

IHC expression of RON and MSP was observed in the 68% and 58% of FMCs respectively while the 52% of the cases co-expressed both proteins. IHC expression of RON, MSP or both in FMC was not correlated with clinical outcome.

RON protein is associated to MSP by IHC suggesting a similar interaction in feline as seen in human as well as a possible involvement of RON in tumor progression For the first time, RT-PCR performed on FMC tissues, revealed the presence of the short-form in the 51% of feline mammary carcinomas. This form originates, as in humans, from the alternative promoter (P2) and codifies the proper feline short form (sf-RON). sf-RON resulted statistically associated with the poorly differentiated histological grade, with a shorter disease free (DFI) period and a shorter survival (OS). These results confirm FMC as suitable model in comparative oncology and identify sf-RON expression as new predictor of outcome for this disease.

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EXPRESSION OF PDGFRS RECEPTORS IN CANINE MAMMARY CARCINOMAS: PATHOLOGICAL AND CLINICAL IMPLICATIONS

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Canine mammary tumors (CMT) are the most frequent tumors in bitches (1) and several similarity between CMT and human breast cancer have been found (2). Platelet growth factor receptors (PDGFR α and β) are tyrosine kinases receptors over-expressed in several human and canine cancers (5) and play an important role in the tumoral transformation as well as in tumoral-stromal interaction.

The aim of this study was to evaluate the immunohistochemical expression (IHC) of α and β PDGFRs in tumoral and stromal compartments, and to correlate their expression with grade, histological type and clinical follow-up.

IHC against α and β isoforms of PDGFRs was performed on 83 CMT (42 simple, 25 complex and 16 mixed carcinomas) samples. Immunohistochemical expression of PDGFR α and β was evaluated both in tumoral and stromal compartment and relation with histological type, grade and clinical follow-up was investigated. cDNA from 11 fresh CMT surgical samples was subjected to q-PCR and quantitative expression (2 - $\Delta\Delta$Ct) was determined.

IHC and q-PCR revealed that PDGFR α and β are expressed in 88% and 78% of tumors respectively, and that their expression are significantly more frequent in mixed and complex tumors compared to simple carcinomas (p=0.0262 and p=0.0447). PDGFR α and β loss of expression is significantly correlated to high grade in mixed CMT and simple CMT, respectively. Over-expression of α isoform confers a better prognosis in all CMT, while β isoform only in mixed CMT. Moreover, PDGFR β loss of expression in the stromal compartment was significantly correlate with high grade of malignancy in CMT (p=0,05) and in simple carcinoma compared with mixed and complex carcinoma (p=0,019). PDGFR α and β transcripts are expressed respectively in 8/11 and 5/11 fresh tissues. PDGFRs have a different role in the pathogenesis and histological differentiation of CMTs. As in humans also in CMTs, the loss of beta isoform had shown to be correlate with a high grade phenotype (3,4). Collectively, these data suggest that continued characterization of PDGFR expression in CMTs should present opportunities for improved accuracy in prognosis and also to assess or not the efficacy of PDGFR-directed tumor therapy.

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SOMATOSTATIN RECEPTOR (SSTR2A) IN CANINE MENINGIOMA: PRELIMINARY RESULTS OF IMMUNOHISTOCHEMICAL AND QRT-PCR INVESTIGATIONS

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The neuropeptide somatostatin (SST) plays an important regulatory role in the proliferation of both normal and neoplastic cells. Five subtypes of somatostatin receptors (SSTRs) have been identified in several human tumors. Among human brain tumors, meningiomas show the highest incidence of somatostatin receptor expression by either immunohistochemical (IHC) or RT-PCR analyses. The receptor most commonly identified is the SSTR2a subtype. Although the exact functional role remains unclear, in vitro studies indicate that the activation of SSTRs may result in cytostatic effects on neoplastic cells. Long half-life somatostatin analogues (i.e. octreotide) are today included in chemiotherapy schedules for unresectable or radiation-refractory recurrent human meningiomas.

The aim of this study is to test the expression of SSTR2a in canine meningioma by immunohistochemical and qRT-PCR analyses. The presence of SSTRs may be predictive of a positive response of canine meningioma to somatostatin analogue therapy, especially for cases that cannot receive conventional treatments.

Twenty one FFPE meningiomas were used for IHC investigations performed with rabbit anti-human Somatostatin Receptor Type 2a antibody (1:500, Alomone Labs, Jerusalem, Israel) and avidin-biotin-peroxidase complex method. FFPE canine pancreas and gastric wall were used as positive controls. For each tumour, area of labeling was assessed in five grades, ranging from (-) = absent to (++++) = > 75% of tumor. Twenty four cases including the main histotypes were also submitted to qRT-PCR investigations performed with Taqman probe (Life Technologies). Total RNA was extracted from 5 μm sections of FFPE tissue with FFPE-RNA Purification Kit (Norgen), and mRNA was reverse-transcribed with iScript cDNA synthesis (Bio-rad).

At IHC, SSTR2a was expressed in 18/21 cases (86%) showing a diffuse cytoplasmic immunoreaction pattern. The most common histotypes, including meningothelial, fibroblastic, transizional, and psmammomatous meningiomas as well as papillary meningioma were positive, ranging from (+) to (++++). Anaplastic type (grade III) did not show any immunoreaction. In all positively stained tumors, SSTR2a immunoreactivity was uniformly present on nearly all tumor cells. The PCR-amplification tests of the extracted and reverse transcribed RNA gave positive results confirming the expression of SSTR2a in the majority of the canine meningiomas. Therefore, these preliminary results encourage continuing this study aimed to find new chemiotherapeutic protocols for dogs, usable as additional or as an alternative to the most traditional. We expect these preliminary results to be supported by further biomolecular and functional studies. The absence of somatostatin receptor (SSTR2a) in the anaplastic meningiomas remains to be confirmed.

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GLOMERULOID MICROVASCULAR PROLIFERATION IN CANINE CHOROID PLEXUS TUMORS

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Choroid plexus tumors (CPT) are intraventricular neoplasms, accounting for 7% of all canine primary brain tumors.1 Canine CPTs are classified according to the human WHO classification of central nervous system tumors (2007)2 into choroid plexus papilloma (CPP, grade I), atypical choroid plexus papilloma (aCPP, grade II) and choroid plexus carcinoma (CPC, grade III).3 Tumor-related microvascular proliferation (MVP) is morphologically defined as vessels lined by multilayered and mitotically active endothelial cells, pericytes and smooth muscle cells and occurs classically in glial tumors.2 In these tumors, MVP appears as glomeruloid bodies and is associated with higher grades and poor prognostic outcome in human patients. We have observed the occurrence of glomeruloid MVP in a subset of canine CPT. The aim of this study was to characterize MVP in canine CPT and to investigate whether the appearance of glomeruloid MVP is associated with high histological grades. Fifty-five cases of canine CPT were included in this study. Tumors were graded according to the human WHO 2007 classification on HE stained sections and the presence/absence of MVP were recorded. Immunohistochemistry with antibodies against aSMA, vWF, and Ki67 was performed on 25 canine CPT. Labelling was recorded as either positive or negative. The Ki67 labeling index was obtained from the evaluation of 10 HPF, using an image analysis program (ImageJ). Statistical analysis was performed using t test (P value < 0. 05). According to the human WHO-classification2, 32/55 tumors were CPC and 23/55 CPP. The Ki 67 proliferation index was 1.065 % in the CPPs (range of 0.593-1.644) and 9.44 % in the CPCs (range of 1.86-24.75). MVP was observed in 33/55 tumors. Two types of tumor-associated MVP occurred: simple hypervascularity and glomeruloid bodies. Simple hypervascularity was characterized by increased density of vessels with a single vascular lumen lined by vWF+ endothelial cells and bordered by plump aSMA+ pericytes. This type of MVP occurred in 8/23 CPPs and 11/32 CPCs. Glomeruloid bodies resembled aberrant glomerular structures and were characterized by exuberant proliferation of plump and disorganized aSMA+ pericytes. These inconsistently surrounded multiple poorly defined vascular lumina, lined by weakly vWF+ endothelial cells. Glomeruloid bodies occurred in 13/32 CPCs and only one of 23 CPPs. In conclusion, our data provide evidence that histological grade of canine CPTs statistically correlates with Ki67 proliferation index. Even though glomeruloid MVP occurs only in a proportion (40%) of CPC, its occurrence clearly correlates with high grade tumors (CPC). No relationship was found between the simple hypervascularity and tumor grade. These data suggest that glomeruloid MVP may serve as histological marker of malignancy in canine CPT. Obviously, this hypothesis needs to be confirmed by prospective studies as it is currently not clear whether the human classification system 2 reflects the biological behavior of canine CPTs. Pathogenetic mechanisms of MVP in canine CPTs have to be identified.

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PDGF RECEPTORS AS PREDICTOR OF OUTCOME IN CANINE ORAL MELANOMAS

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Malignant melanoma represents one of the most frequently diagnosed oral neoplasm of dogs(1-2). Canine Malignant Melanoma (CMM) has an aggressive behaviour and poor surviving rate. The Platelet-derived growth factors (PDGFs) are involved in some physiologic processes and to several diseases, including cancer, in which these factors promote angiogenesis and autocrine stimulation of tumour cells (1). PDGFRα and PDGFRβ are tyrosine kinases receptors (TKR) whose dysfunction are identified in some human cancers and a lot of studies have observed similar expression in canine and feline cancers(3-4). Tyrosine kinase inhibitors (TKI) specific for PDGFRs receptors and others are currently studied in veterinary oncology and used in the treatment of canine mast cell tumours(5). The aim of this research is to evaluate the expression of PDGFR-α and -β in CMM, in order to identify their role in the tumour pathogenesis and their correlation with prognosis.

Tissue samples were collected from 36 dogs with stage II-III surgically resected CMM followed for at least 6, and up to 24 months. All the samples were histologically evaluated and immunohistochemically tested for PDGFRα and β and Ki-67 (as prognostic factor) and PNL-2 (to confirm the diagnosis). Histological and immunohistochemical results were evaluated in relation to clinic-pathological data.

All samples analyzed showed positivity to PNL-2 antigen. PDGFR-α and -β positivity were observed in 50% and 44.4% of cases respectively, while they were co-expressed in 38.9% of cases. The positivity to each receptor as well as their co-expression were associated to shorter disease free period (Log-rank test, P<0.001) and a shorter survival (Log-rank test, P<0.001), suggesting that they can be considered good prognostic indicator and good target for specific therapies. The majority of cases (75%) had an high Ki67 index (associated to a poor prognosis according to literature). These results emphasize the importance of PDGFRs in improving the accuracy of the prognostic evaluation of CMM and pose the basis for the potential use of PDGFR-directed tumour therapy.

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EFFECTS OF HSP90 INHIBITOR 17-AAG ON VASCULOGENIC MIMICRY IN D22 AND D17 CANINE OSTEOSARCOMA CELL LINES

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Vasculogenic mimicry (VM) is a neovascularization pattern of aggressive malignancies and an unfavorable prognostic factor in human osteosarcoma (OSA) (1). VM differs from traditional tumor angiogenesis, since neoformed microcirculatory channels are lined by nonendothelial pluripotent embryonic-like and highly invasive tumor cells (2). VM was observed in three-dimensional (3D) collagen cell cultures of several cancer models (3,4). Key factors involved in neovascularisation, such as VEGF, HIF-1α, FGF, PDGFα, PDGFβ, TGFβ-1, are all overexpressed in OSA tissue and cell cultures (5-8) and VEGF and HIF-1α involvement in VM has been confirmed (3,9). The functional folding of these factors and their receptors is guaranteed by Hsp90. Anti-cancer treatments based on Hsp90 inhibition could negatively influence the action of these factors and the consequent VM process in metastatic OSA.

Aim of the study was to evaluate the capability of two canine OSA cell lines, D22, derived from a primary bone tumour and D17, isolated from a metastatic site, to form tubular networks (VM marker), when grown in 3D cultures, as well as the efficacy of the Hsp90 inhibitor 17-AAG in preventing the formation of VM structures.

The ability of OSA cell lines to form vascular channels was assessed in 3D cultures on Collagen Rat Tail Type 1. D22 and D17 cells were seeded on solidified gel and maintained in culture hood for 3 week. VM marker was morphologically evaluated after 24, 36, 48 h and 5-8 days of cultures. After 3 weeks, cultures were fixed, paraffin embedded, sliced in 5 μm serial sections and haematoxylin and eosin (H&E) stained. Once established the ability to form endothelial-like structures, cells were treated with 0,5 μM 17-AAG for 24 and 48 h and principal VM features (number of junctions, meshes, segment and branches) were quantified by an ImageJ macro Angiogenesis Analyzer. Results were analyzed through Univariate Analysis of Variance.

D22 cells formed isolated clusters with round shape, but they did not show VM pattern, probably being unable to grow on collagen. On the contrary, D17 cell line exhibited tubular structure formation after 24 h of culture and tubular network surrounded by clusters of tumor cells, losing their typical epithelioid shape and extending cytoplasm, after 36 h. H&E stained serial sections showed the presence of tubular cavities, supposing a longitudinal stretch longer than 25 μm, surrounded by endothelial-like flat cells. 17-AAG-treated D17 cells also showed a significant decrease ($p<0.05$) of junctions, meshes, segment and branches number after 48 h. The results of this study give further confirmation to the involvement of VM on cancer cell malignancy and the possibility to impair VM process by Hsp90 inhibition. The latter result confirms the ability of 17-AAG to intervene on the common molecular pathways that induce both VM and classical neoangiogenesis.

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CHLAMYDIA SPP. INFECTION AND MALE REPRODUCTIVE PATHOLOGY IN KOALAS (PHASCOLARCTOS CINEREUS)

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Koala chlamydiosis presents with a range of clinical signs resulting in hundreds of animals being admitted to koala hospitals in South-east Queensland each year. The most common impact of this disease is infertility in the female, associated with ovarian cysts, salpingitis and/or metritis [1]. Unlike the female, male koala infertility from chlamydiosis is not well defined and its impact on semen quality has yet to be documented. Therefore, the aim of this study is to assess the incidence and aetiology of male reproductive pathology associated with Chlamydia infection in koalas submitted to koala hospitals in South-east Queensland. Thirty koalas referred for suspected clinical chalmydiosis and presented for necropsy at the Moggill Koala Hospital were examined. Chlamydia spp. was detected in the semen and/or urogenital swabs by real-time PCR. The effects of Chlamydia infection on the reproductive tract was evaluated through the histopathological examination of selected tissues. The sperm DNA fragmentation (SDF) was analysed by means of the sperm chromatin dispersion test (SCDt), recently developed and validated for koala spermatozoa [2]. Twenty-six koalas showed evidence of histological lesions suggestive of Chlamydia infection. Chronic lymphoplasmacytic inflammation has been observed in the kidney 12 times, the urinary bladder 16 times, the prostate and prostatic urethra 24 times, the membranous urethra on 13 occasions, the bulbourethral glands 8 times, and the testis and epididymis on 1 and 2 occasions, respectively. Twenty-nine out of 30 samples were positive for Chlamydia spp. (14 semen, 7 swabs, 8 semen and swabs). The mean SDF of the semen samples was approximately 30% and compared to only 5% SDF from a captive koala population that showed no clinical signs of the disease [3]. Chlamydia spp. may be therefore able to induce inflammatory and/or degenerative lesions in the reproductive tract of the male koala and our results underline the potential adverse impact of Chlamydia spp. infection on male koala reproduction. The increased SDF may represent how Chlamydia can potentially interact with and affect sperm morphology and function and ultimately fertility.

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HEALTH STATUS OF GREY SQUIRREL POPULATIONS (SCIURUS CAROLINENSIS) IN PIEDMONT: ANATOMO-PATHOLOGICAL AND MICROBIOLOGICAL INVESTIGATIONS

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The grey squirrel (Sciurus carolinensis), an American species introduced in Europe, is considered a pest with high potential for widespread, and represents a serious threat to the red squirrel (Sciurus vulgaris) because of their interspecific competition (MARTINOLI et al., 2010). Since 2010 the north regions of Italy (Liguria, Lombardy and Piedmont) have joined the LIFE + EC-SQUARE European Project, acting for the protection of the red squirrel through the control of the grey squirrel (BERTOLINO et al., 2012).

The aims of this study were to increase the knowledge of the viral, bacterial, parasitic and fungal diseases affecting a grey squirrel population captured following a containment programme, and to assess possible risks to public health and for the transmission of pathogens to the red squirrel.

Forty grey squirrels (21 males and 19 females) were captured and euthanized in 2013 and 2014 in the province of Cuneo (Piedmont Region, north-west Italy). Each squirrel was recorded, the main biometric measures were registered, samples of the body surface were collected to detect dermatophytic fungi, and age was determined by the dry weight of the crystalline. At necropsy, samples were collected and fixed in 10% buffered formalin (pH7) for histological investigations, and frozen at -20°C for microbiological and biomolecular investigations. Fixed tissues were routinely processed and stained with haematoxylin-eosin and additional stains were performed. Investigations for Hepatitis E virus (qPCR), Francisella spp.(PCR), Salmonella spp.(bacteriological cultures), Squirrel Poxvirus (nested PCR), Toxoplasma gondii (nested PCR) and dermatophytic fungi (fungal cultures and PCR) were also carried out. All data were statistically analysed with the software GraphPad InStat (vers. 3:05; GraphPad Software, California, USA).

Lesions were found in the lungs (n=28), heart (n=28) and skin (n=10). All the squirrels resulted negative for viral, bacteriological and parasitological analyses, except for the presence of bacteraemia in three squirrels showing positive for Staphylococcus spp. and Streptococcus spp. Mycological and biomolecular investigations revealed 14 squirrels positive to keratinophilic fungi belonging to 9 different genera. In Europe the main cause of extinction for the red squirrel is the competition for food with the grey squirrel; the competition is also mediated in UK and Ireland by a squirrel poxvirus, which so far is not found in Italy. However, other bacterial or viral pneumonia, fungal infections or parasites might be involved in the competition and should be investigated in the two species. Moreover, the role of the grey squirrel as a zoonotic carrier for mycotic diseases should not be underestimated.

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TEHV3 OUTBREAK CHRACTERIZATION IN CAPTIVE TESTUDO SP

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Tortoises represent a popular non-conventional pet in Italy. Of these, several species are either considered endangered (Testudo hermanni) or near threatened (T. marginata and T. graeca) according to the Italian commission of the International Union for Conservation of Nature. When pet tortoises are abandoned or found injured or seized following illegal detention, they are sent to wildlife rehabilitation centers. Starting from 2008, the Testudo spp. population housed in the WWF Vanzago's oasis exhibited clinical signs of variably severe lethargy, nasal discharge, conjunctivitis and diphtheronecrotic glossitis. During that period of time 50 tortoises died with reported mortality peaks during March and October. In Spring 2012, the Vanzago center population was composed of 9 T. marginata, 7 T. hermanni and 2 T. graeca, still variably exhibiting the same abovementioned clinical signs. By the end of 2012 all Testudo species had died. Based on these findings, Testudinid herpesvirus 3 (TeHV3) infection was suspected. The presence of TeHV3 was investigated by molecular biology and anatomical pathology. All the tortoises housed in Vanzago were tested for the presence of anti-TeHV3 antibodies by ELISA and they all resulted positive but one T. hermanni. Of these, 3 T. marginata, 2 T. graeca and 7 T. hermanni died and were all necropsied. Lesion frequency distribution was lingual and oral diphtheric plaques (15.4%), serous atrophy of the fat (23.1%), hepatic lipidosis (15.4%) ulcerative stomatitis and/or glossitis (7.7%), pneumonia with emphysema (43%), focal intralesional bacterial aggregates (17%), intravascular bacterial thrombi (25%), hepatic granulomas (28.6%), necrotizing tracheobronchitis (25%) and intranuclear amphophilic/eosinophilic inclusion bodies (8.33%). PCR confirmed the presence of the virus in 8/12 tortoises. To better complement the epidemiological evaluation of TeHV3 distribution in northern Italy tortoises, 20 retrospective cases were selected from the archive of the University of Milan. Selection criteria were the presence of inclusion bodies or necrotizing lesions of the respiratory or gastrointestinal tract. Of the 20 cases, 5 were TeHV3 PCR positive tortoises. Lesions closely resembled those of the Vanzago's population but there were more cases with diphtero-necrotic glossitis and stomatitis and inclusions bodies. These results are consistent with a high prevalence of TeHV3 in northern Italy tortoises household population. The finding of intranuclear inclusion bodies was specific but did not represent a sensitive diagnostic tool. TeHV3 diagnostic gross and microscopic lesions have been reported to vary according with the host immune response and by the viral replicative status, and can be obscured by autolytic changes, thus gross and microscopic findings are not always diagnostic and the support of additional techniques is often necessary to confirm viral infection. In the current caseload, TeHV3 infection was often associated with secondary lesions suggestive of immunodepression that can be attributed to virus itself associated with abnormal hibernation. According to the literature and to our findings, T. hermanni spp. seems the species with higher mortality and lower antibody concentrations when infected with TeHV3

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DIETARY INSECT MEAL INCLUSION IN CHICKENS: PRELIMINARY RESULTS ABOUT ANATOMOPATHOLOGICAL INVESTIGATIONS

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Insects are being considered as a novel protein source for poultry feed, because they have high quality and quantity of protein, show low competitiveness with human feed and reduce the environmental contamination (1, 2). Previous studies found that chickens fed diets containing insect meal can improve growth performances in terms of feed intake, body weight gain and feed conversion efficiency (2), but limited anatomopathological data are available. The present study aims to investigate the anatomopathological findings in different chickens strains fed with standard or experimental diets including insect meal.

160 male broiler (group 1) and 100 female medium-growing hybrid chickens (group 2) were divided in 4 (basic feed, 5%, 10% and 15% Tenebrio molitor inclusion) and 2 (basic feed and 7.5% Tenebrio molitor inclusion) dietary treatments, respectively. For each experiment birds were distributed over 5 replicates for each dietary treatment. Diets were isoenergetic and isonitrogenous. At the age of 53 (male) and 100 (female) days the animals were slaughtered and 2 birds for each replicate were taken for analysis. 5 animals for each dietary treatment in the group 1 and 10 animals for each dietary treatment in the group 2 were submitted to anatomopathological investigations. Samples of liver, spleen, thymus, bursa of Fabricius, kidney, heart, glandular stomach and intestine (4 standardized segments of duodenum, jejunum, ileum and caecum) were collected, fixed in 10% buffered formalin solution and paraffin embedded to obtain 5μm histological sections stained with Haematoxylin & Eosin. Histopathological lesions were scored using a semiquantitative scoring system as follows: absent or minimal (score 0), mild (score 1) and severe (score 2). Data were compared by Kruskal-Wallis and Mann-Whitney U tests (GraphPad Prism® software, P value < 0.05).

Histopathological findings were similar in both groups and were not significantly different (P > 0.05) between broilers fed with standard diet and with dietary insect meal inclusion. Spleen, thymus, bursa of Fabricius, liver and glandular stomach were the most frequently affected organs, while heart and kidney showed no significant alterations. Spleen showed white pulp hyperplasia or depletion. In thymus there was cortical depletion. Bursa of Fabricius showed follicular depletion with or without intrafollicular cysts. In liver there was lymphoid tissue activation. The etiopathogenesis of the lymphoid tissue activation and/or depletion remains to be elucidated. Glandular stomach showed lymphoplasmacytic flogosis with lymphoid tissue activation and epithelial squamous metaplasia. Interestingly, glandular stomach of medium-growing hybrids was affected by more severe alterations (P = 0.0008) than broilers. This finding could be related to the free range farming of this group. These preliminary results suggest how insect meal could be included in chickens diet without inducing histopathological changes. Studies are in progress to evaluate the effects of dietary insect meal inclusion on intestinal mucin composition and morphometry.

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HISTOLOGICAL FINDINGS IN LUNGWORM INFECTION IN ROE DEER (CAPREOLUS CAPREOLUS)

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Introduction: Roe deer (Capreolus capreolus) is the most common wild ungulate living in Italy; despite this abundance, little is known about the sanitary status of this species. As in many wild animals, parasitism is one of the most common and dangerous sanitary problem and, among parasites, lungworms are relatively common in roe deer. The most commonly reported lungworms in roe deer are Dictyocaulus eckerti and Varestrongylus capreoli1,2.

Aim: to investigate distribution presence of parasites and associated lesions from roe deer

Materials and methods: organs from roe deers culled in three areas of the province of Arezzo, Tuscany, during the hunting seasons 2009-2011.

Results: The lesions found with greater frequency were those of parasitic origin due to Sarcosporydium spp in myocardium and skeletal muscle, gastrointestinal nematodes and lungworms. In particular 94 samples of lung were examined, 42 of which were histologically normal, while 42 showed histological lesions consisting in diffuse interstitial pneumonia (24 cases), bronchitis-peribronchitis of medium-sized bronchi (22 cases), hyperplasia of BALT (25 cases), smooth muscle hypertrophy (20 cases) and lymphomonocytic to granulomatous pneumonia (15 cases), seldom associated with intralesional nematodes (12 cases). Parasites were seen in 16 cases; in 2 cases a single section of an adult nematode was present in medium-sized bronchi, while in 14 cases rare adults and variably numerous ova and larvae were present in alveolar spaces. Intrabronchial parasites, morphologically consistent with Dictyocaulus spp, were associated with severe catarrhal bronchitis, characterized by infiltration of lymphocytes and eosinophils. Bronchioloalveolar parasites were associated to a wide range of histological changes: in 2 cases there were scattered ova and larvae associated with minimal inflammatory infiltrate; in 12 cases the parasites were associated with lymphomonocytic infiltrates with multinucleated giant cells. The number of parasites ranged from scarce to very abundant: the parasites were less abundant in samples with marked granulomatous and eosinophilic infiltrates, while more numerous where the inflammation was mainly lymphoplasmacytic. A very suggestive change seen in 20 cases was mild to severe hyperplasia of smooth muscle from small and terminal bronchioles, as well as medial hypertrophy of arterioles; in 10 cases this lesion was associated with the presence of parasites, while they were not evident in the remainder of the cases. In 50 cases parasitological examination of lung tissue or faeces was performed; Dictyocaulus eckerti was found in 33/50 cases (66%), Varestrongylus capreoli in 22/50 (44%) cases, and a dual infection with both species in 6/50 cases (12%). The results of this observational study showed a great degree of lesions in the pathological picture of verminous pneumonia in roe deer, similarly to the great variability also observed in domestic small ruminants, probably reflecting different status of immune system and host natural resistance3; furthermore they can suggest a possible interspecies ecological influence in areas shared between roe deer and other wild animals (especially wildboar) or domestic ruminants.

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WHAT CAN WE LEARN FROM DEAD ANIMALS IN ZOOS?

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During the years 2004-2014, 287 cases of necropsies performed on zoo animals referred to the Department of Veterinary Science of the University of Turin (Italy) from four different Italian zoos were reviewed. Aim of the study was to evaluate the mortality rate due to inadequate veterinary care or zoo management. Macroscopical and/or microscopical necropsy reports have been classified according to the cause of death, including spontaneous pathology, veterinary and management errors. Every year in each zoo under investigation from 0% to 100% of mammals died for inadequate veterinary care and management for a total of 78 cases during the eleven-year period. Causes of death included: trauma (38 cases; 48.7%), inbreeding (13 cases; 16.7%), diagnostic (11 cases; 14.1%) and management mistakes (16 cases; 20.5%).

The investigation reveals how poor management and lack of knowledge about wildlife behavior and medicine are crucial factors responsible for zoo animal mortality. Future studies will include other zoo animal classes and will enquire by questionnaire other Italian and European zoos to estimate how much the human influence can affect the mortality rate of wild animals under human control.

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A COMPARATIVE APPROACH TO FORENSIC NECROPSY PROCEDURES: INTRIGUING SELECTED CASES

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Forensic veterinary science concerns the use of technical and scientific methods to answer questions posed by courts of law in order to establish crimes against animals1. Necropsy is a cornerstone of forensic practice and it has the task to answer questions on cause, mechanism and manner of death. Furthermore, other objectives of a forensic necropsy are: the accurate and complete inspection of crime scene, establishment of time elapsed since death, the proper sampling and handling of evidences, the collection of photographs at any stage of necropsy and the edition of a clear report useful to support judicial investigations. Thus, forensic necropsy should be performed by highly skilled pathologists, avoiding to ignore any details considering that many procedures are unrepeatable.

The aim of the study is to compare the forensic necropsy procedures to support judicial investigations in cases of suspected crime against animals.

In this study we present five cases of crimes against wild and companion animals. Necropsy were requested by magistrates or owners and were performed at the Department of Veterinary Medicine of University of Naples "Federico II" and Experimental Zooprophylactic Institute of Lazio and Tuscany. Results of investigation showed that first dog died for air gun multiple shoots: in this case radiographs were useful to identify and localize bullets. The second dog died for starvation: the investigation on crime scene helped the authorities to confirm the crime of neglect. Last dog died for severe infected wounds due to illegal dogfight: in this case the accurate external examination of cadaver allowed to highlight bite wounds. One bear was shot and the ballistic study was crucial to identify the culprit. Lastly, a wolf was found victim of poaching.

Forensic necropsy requires special and most complex technical procedures, special organization, and, for its legal relevance, should be performed following accurate standard procedures. For this purpose, the definition of "guide lines" must be considered mandatory to ensure the accuracy and efficacy of necropsy and definition of "good quality standards in forensic necropsy".

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PATHOLOGICAL FINDINGS IN WOLF PREY

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The wolf is a skilled and extremely efficient predator. Its predatory behaviour consists of a number of complex and integrated sequences, which are instinctive (so called "fixed action patterns", such as aggression, killing and consumption of the prey) or learned during the "hunting school" (e.g. location of the prey and ambush). Such behaviors deeply influence the pathological findings observed in wolf prey (1,2).

Aims are to report the main lesions observed in domestic and wild animal species preyed on by wolves. Such findings are of functional relevance and can be useful for differential diagnosis.

The present study has been carried out in Gran Sasso & Monti della Laga national park, a large protected area in central Italy. Wolf predation was confirmed in 716 small ruminants (sheep and goats), 83 cattle, 73 equids, 6 roe deers and 1 red deer.

Small ruminants and roe deers always showed characteristic, single (smaller animals, <30 Kgs) or multiple (rams, large meat breed), bite injuries at the parotidean region. In cattle and red deer, bilateral and symmetrical bite injuries severely affected thighs and shoulders. After skinning, wide hemorrhages and lacerations of the following muscles were commonly observed: semitendinosus, semimembranosus, quadriceps femoris, latissimus dorsi, triceps brachialis. Furthermore, bite injuries usually involved the parotidean region, the muzzle and the nose. Due to the skin thickness, bite injuries affecting the head and the neck were mild and superficial. In equids, bite injuries were always seen on thighs and on the parotidean region, their pathological features largely overlapping those observed in cattle and small ruminants, respectively.

Our data confirm that predation result from a number of features of the prey (e.g. size, neck diameter, skin thickness), as well as of the predator (size, bite efficacy, predatory behaviour, experience, "risk analysis"). In small ruminants, bites at the upper neck play a key role for a successful predation, by stimulating the vagus nerve and the carotid baroreceptors, while the laceration of specific muscles (crucial for standing) is a strategic point to prey on larger animals. In equids, bite injuries of the parotidean region have functional significance closely resembling those reported in small ruminants. On the contrary, biting on the head and the upper neck seem useful to immobilize and beat down cattle and calves only by virtue of leverage effects. Finally, pathological findings reported herein are of diagnostic relevance to confirm/rule out predation, and to correctly identify the predator.

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ASSOCIATION BETWEEN KIT EXPRESSION PATTERNS AND EFFICACY OF TREATMENT WITH TYROSINE KINASE INHIBITORS IN CANINE MAST CELL TUMORS

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Canine mast cell tumor (MCT) is a common neoplastic disease in dogs that shows a variable biologic behavior [1]. Several studies [2,3] have shown that canine MCTs express a mutated form of KIT, a receptor tyrosine kinase involved in the control of mast cell growth and differentiation. Three KIT immunohistochemical expression patterns have been identified in MCTs: Pattern I (membrane-associated), Pattern II (cytoplasmic focal) and Pattern III (cytoplasmic diffuse). Patterns II and III have been found to correlate with higher histological grade and with a worsened post-surgical prognosis. However, to our knowledge, there is no study investigating the correlation of KIT staining pattern and efficacy of the treatment with tyrosine kinase inhibitors.

The aim of our study is to address the role of KIT in canine mast cell tumours by studying the correlation between KIT expression patterns and the overall survival in dogs postoperatively treated with tyrosine kinase inhibitors toceranib phosphate (Palladia) and masitinib mesylate (Masivet).

We selected thirty cases of canine cutaneous MCTs submitted to the Pathology Service of the Veterinary Medicine University of Naples between 2011 and 2014. Case selection criteria included 1) original diagnosis of a MCT, 2) immunohistochemical analysis of KIT expression 3) post-surgical chemoterapeutic treatment with a tyrosine kinase inhibitor and 4) complete history and follow-up data. Statistical analysis was performed in order to compare the overall survival times (OS) of dogs postoperatively treated with chemotherapy and the KIT pattern staining.

All dogs with KIT pattern 1 MCT were still alive at the end of the study period, without evidence of tumor recurrence or metastasis. On the contrary, eight out of twelve dogs (66%) with KIT pattern 3 MCT died for recurrence and metastasis, with a mean survival time of 6 months. Two out of twelve dogs (16%) with KIT pattern 2 MCT died for recurrence and metastasis. Our data confirm the key role of KIT in the biopathology of canine MCTs and suggest that the aberrant cytoplasmatic distribution of KIT is negatively related to the efficacy of tyrosine kinase inhibitors giving also a significant prognostic information about the treatment outcome. Further studies are necessary to unravel the cellular mechanisms underlying focal and diffuse cytoplasmic KIT staining patterns and their respective pathologic relevance.

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IMMUNOHISTOCHEMICAL EXPRESSION OF MACROPHAGE MARKER, MAST CELL TRYPTASE, CD 79, IGA, IGG AND IGM IN CANINE HEPATOID GLAND TUMORS

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Introduction: According to the 'field of the organization of tissues' (Toft) theory, cancer arises from the deregulation of the interactions between cells and their stromal microenvironment6. Immune cells that infiltrate tumors play decisive roles at different stages of tumor development2. Among canine neoplasms, tumors of perianal glands are common and their causes and biological behaviour are still not well known.

Aim of the paper: Contribute to the knowledge of the canine hepatoid gland tumors through the assessment of stromal cell population such as macrophages, plasm cells and mast cells.

Material and methods: An immunohistochemical study for Mast cell Tryptase, Macrophage Marker, CD79, IgA, IgM, IgG on 25 lesions classified, according to the WHO classification, in adenoma (10), epithelioma (5) and carcinoma (10) was performed.

Results: Cell markers revealed the number and localization of plasm cells (PC), mast cells (MC) and macrophages (MA). These cells increased in number in benign lesions and progressively decreased in carcinomas. In addition, in normal glands and hyperplasia/adenoma CD79 epithelial positive cells were found inside the glandular lobules. Positive epithelial cells were scattered in carcinomas and epitheliomas. Anticanine IgA, IgM and IgG were localized in PC and hepatoid cells in normal, hyperplastic/adenomatous and cancerous cells.

Discussion and conclusion: Stromal immune components support cancer initiation, progression and metastasis1. We found an increased number of MC, PC and MA in neoplastic lesions of hepatoid gland. Moreover, we found Igs and CD79 both in normal and neoplastic hepatoid glands. In the normal epithelium of human skin, IgA and IgG have a potential antimicrobial activity4. In dog, the expression of antimicrobial substances and the potential involvement of hepatoid glands in local defensive mechanism of the skin, has been suggested5. CD79 and Igs have been found in human cancerous cells and their role in cancerogenesis has raising interest3. On the basis of our preliminary results and literature data, we suggest that such cells and molecules could have a role in local immune responses and could be directly involved in the biology of hepatoid gland tumor.

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CORONARY ARTERIOSCLEROSIS IN VEAL CALVES AND BEEF CATTLE: POTENTIAL RELATION TO HOUSING CONDITIONS

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Arteriosclerosis, defined as chronic arterial change consisting of hardening, loss of elasticity and luminal narrowing, is greater in older animals. However, adverse socio-environmental factors have been reported to be a major stimulus to the development of arteriosclerosis of the intramural coronary arteries in young chicken (1), swine (2), mice (3) and monkeys (4). The present study aims to investigate the prevalence of coronary arteriosclerosis in regularly slaughtered veal calves and beef cattle.

From January 2013 to March 2015 a systematic macroscopic and histological study of 42 bovine hearts was performed. Animals were 25, 6/9-months-old veal calves (60%) and 17, 10/24 months-old beef cattle (40%) housed in intensive livestock farming. Samples of interventricular septum, left and right papillary muscle, left and right ventricular free wall and left and right atrium were collected, fixed in 10% buffered formalin solution and paraffin embedded to obtain 5μm histological sections stained with Haematoxylin & Eosin, Weigert Van Gieson and Alcian Blue. Pathological intramural coronary arteries were manually counted in every localization. Data were compared by Kruskal-Wallis and Mann-Whitney U tests (GraphPad Prism ® software, P value < 0.05). Selected paraffin embedded samples were also submitted to ultrastructural investigations.

Arteriosclerosis of the intramural coronary arteries was observed in all calves and cattle (100%). Intimal hyperplasia, degenerative changes of the media tunica and medial hypertrophy/hyperplasia were the most important observed lesions. In calves there was a greater percentage of intimal hyperplasia (92% vs 88%) and degenerative changes of the media tunica (76% vs 71%). The medial hypertrophy/hyperplasia increased in cattle (59% vs 44%). This finding could reflect a temporal evolution of the arteriosclerotic disease. In calves the interventricular septum and the papillary muscles were significantly more affected ($P < 0.0001$), while in cattle the interventricular septum and the left papillary muscle only showed greater coronary arteriosclerosis ($P < 0.0001$). In cattle there was a greater percentage of stenotic intramural coronary arteries (45%) than in calves (23%), even if there was no significant difference between them ($P > 0.05$). Anitschkow cells, confirmed by ultrastructural investigations, were detected in both calves (60%) and cattle (76%). They were localized almost exclusively in coronary walls, suggesting a potential role in arteriosclerosis development. The preliminary results here described suggest a potential relationship between the development of coronary arteriosclerosis and intensive livestock farming of veal calves and beef cattle. A comparative study on free range cattle is in progress.

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DEVELOPMENT OF A DIAGNOSTIC PROTOCOL FOR MASTITIS IN GOATS

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Mastitis is an inflammation of the mammary gland that can lead to alterations in milk produced and composition. Therefore mastitis represents an important problem for animal welfare and public health (1). A wide range of infectious agents is known to cause mastitis. Contagious pathogens such as Staphylococcus aureus and Streptococcus agalactiae are transmitted among animals. Environmental pathogens such as Streptococcus uberis and Streptococcus dysgalactiae are opportunistic invaders of the gland (2). Among viral agents Small ruminant lentiviruses (SRLV) can cause subclinical mastitis (3). Aim of this research was to develop a diagnostic protocol for goat's mastitis correlating the pathologic findings with the isolated microorganisms.

25 udders with macroscopically suspected mastitis were collected in a small slaughterhouse in northern Italy from regularly slaughtered dairy goats and sent to the Department of Veterinary Science, Torino University. Anamnestic data about the animals (age, breed, characteristics of the farm) were also reported. After gross examination, 2 samples were removed: one, ïň?xed in 10 neutral buffered formalin for histological investigations and the other one, frozen at -20Â°C as tissue bank. The remaining tissue was sent to the Istituto Zooprofilattico Sperimentale of Torino to perform bacteriological, virological (PCR for ecthyma and SRLV) and mycological investigations. Antibiogram and research of inhibitory substances were also made.

According to the literature, the pathological lesions and the bacteria isolated revealed a high prevalence of suppurative-chronic infections. In fact histologically most of the udders (80%) showed chronic mastitis characterized by mixed or suppurative infiltrates. This result was supported by bacterial isolates reporting in particular pyogenic agents. Nevertheless the histological findings were difficult to correlate with the microbiological data because often co-infection of several microbial species were isolated in the same udder(84%). Staphylococcus spp.(37%) - especially S. caprae, S. xylosus and S. aureus- and Streptococcus spp.(9%) -especially S. agalactiae and S. uberis- were the most frequently isolated bacteria. Mycological survey was positive only in one udder (Aspergillus spp.) probably as a contaminant. 20 udders were positive for SRLV (genotype A), only one sample was PCR positive for the contagious ecthyma virus. 22 samples were negative for the research of inhibitors. No particular antimicrobial resistances were observed to routine texted antibiotics. This diagnostic protocol was easy to perform and relatively quickly. It provides a large number of data and it is also applicable to the ovine species whose agents of mastitis are similar. In the absence of an histological classification of small ruminant mastitis, this protocol can be useful to define a specific classification in these species. Moreover it can represents a useful epidemiological tool. In particular antibiotic resistance data can be used to select a correct therapy in the farm. To correlate the pathologic findings with the isolated microorganisms a greater number of samples have to be carefully investigated.

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ELLIS-VAN CREVELD SYNDROME IN GREY ALPINE CATTLE: IMMUNOPHENOTYPIC AND MOLECULAR CHARACTERIZATION.

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Ellis-van Creveld (EvC) syndrome is an autosomal recessive disorder due to a mutation in one of two genes, EVC or EVC2, described in human medicine. A whole genome re-sequencing study revealed a single candidate causal mutation in EVC2 and Sanger sequencing confirmed the deletion of 2bp in exon 19 as the cause of dwarfism in Grey Alpine cattle. Chondrodysplasia is characterized by histological changes affecting the growth plate. Physeal chondrocytes are subjected to a plethora of extracellular factors. When chondrocytes hypertrophy occurs the synthesis of collagen II, one of the major components of the cartilage extracellular matrix, is down regulated, and the synthesis of Collagen X is initiated. Collagen X is found in the hypertrophic zone and its role is to facilitate the deposition of calcium in the matrix. Sonic Hedgehog (SHH) and fibroblast growth factor (FGF) are molecules involved in skeletal development. Transgenic mice overexpressing SHH are affected by disarray of the physis in the tibia and absence of the femur and humerus. FGF signaling is essential for endochondral bone formation, and mutations in FGFR3 cause achondroplasia in humans. The aims of this study were to: 1) evaluate by immunohistochemistry (IHC) the degree of differentiation and proliferation index of the physes in order to better elucidate the pathogenesis of this EvC syndrome in Grey alpine cattle; 2) determine the level of expression of EVC and EVC2 mRNA in affected bones. Five Grey Alpine calves, with a known mutation in the EVC2 gene, were autopsied. Two calves, Grey Alpine breed, not affected by EVC2 gene deletion, were used as controls. IHC was performed on bone sections using anti-Collagen II, -Collagen X, -SHH, -FGF2, and -Ki67 antibodies. RT-PCR was performed using the primers for EVC1 and EVC2 on tissue samples of bone, heart, trachea, testicle and tooth of one affected calf and one control calf. Collagen II labelled diffusely the resting, proliferative, hypertrophic zones, primary and secondary spongiosa in controls, with a loss of labeling in the resting zone of two dwarfs. In the controls Collagen X was expressed in hypertrophic zone in the matrix around hypertrophic chondrocytes, but it was absent in all five chondrodysplastic cases. SHH labeled hypertrophic chondrocytes, and the primary and secondary spongiosa similarly in controls and affected animals. In both controls FGF2 was expressed in the chondrocytes of all growth plate zones, but it was completely lost in 3 of 5 cases, and had scattered expression in 2 of 5 dwarfs. The Ki67 index was lower in dwarf calves compared with controls. Unexpectedly, Both EVC and EVC2 transcripts were detected in affected and healthy calves, in contrast to what inferred in previous works. The premature collagen II degradation, abnormal collagen X expression, and the low proliferation index together with loss of expression of FGF2 are all findings that

suggest the pathogenesis of EvC syndrome in Grey Alpine cattle may involve reduced proliferation, and early hypertrophy of physeal chondrocytes with accelerated differentiation leading to early ossification.

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BLUETONGUE VIRUS SEROTYPE 1 IN THE GENITAL APPARATUS OF AFFECTED SARDA RAMS

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During the 2013 Bluetongue virus serotype 1 (BTV 1)Sardinian epidemic, severe hyperthermia and edema of the scrotum characterized the clinical signs of the infected rams, while histologically, evidences of testicular degeneration were observed.

This study aims to investigate the pathogenesis of the histological changes in the testis of naturally BTV affected rams.

Thirteen rams were collected from different flocks, in which clinical Bluetongue (BT) infection was observed. Rams were serially euthanized from 5 to 140 days after the onset of the disease. At the necropsy blood, spleen, testis, epididymis, accessory glands and regional lymph nodes, tongue and scrotal skin were adequately sampled for viral RNA quantification by Real-Time qRT PCR as well as for viral VP7 and NS2 proteins detection by immunohistochemestry (IHC).

BTV RNA was detected in blood, spleen and regional lymph nodes up to 140 days after the onset of the disease, whereas in accessory glands and testis it was detected up to 30 and 60 days, respectively. By IHC, BTV was found in the endothelial cells of the testicular, epididymal and scrotal skin capillaries only in the early stage of the disease. On the contrary, severe testicular degeneration with oligospermia or azoospermia was observed in the testis by histology up to 60 days, with a partial recovery being evident only after 100 days. Hypofertility has been reported in rams vaccinated with BTV 2 live modified vaccine[1] or naturally affected by BTV 8[2], whereas BTV 1 and BTV 8 field strains did not cause any lesion in the reproductive tracts of experimentally infected rams [3]. In this study, we observed that rams naturally infected with BTV 1 displayed severe degeneration of spermatic epithelial cells. Results obtained by IHC associated to histopatological findings indicate that the degeneration of the germinative epithelium during BTV infection might be ascribed to endothelial damage of the intertubular capillaries of the testis.

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ORAL CONGENITAL FIBROPAPILLOMATOSIS IN LAMBS

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Papillomavirus (PV) are oncogenic, double stranded viruses responsible for tumors in humans as well as in domestic and wild animals1.Congenital papillomatosis or fibropapillomatosis have been reported in piglets2, horses3 and cattle4. In these cases, a viral etiology was suspected but not confirmed. In humans there are controversial data about the vertical transmission of PV; on the contrary, in cattle such an infection has been documented5.

In sheep, the association of PVs with tumors and other disorders has been poorly investigated. Two ovine papillomavirus genotypes OaPV1 e OaPV2 are known to occur in sheep and are responsible for fibropapillomas; more recently, OaPV3 has been found in squamous cell carcinoma and in skin of healthy sheep6. To our knowledge, no previous reports about congenital fibropapillomatosis in lambs are known to occur. The aim of the present study is to report preliminary findings observed in lambs of Sarda breed sheep affected by congenital fibropapillomatosis in the gengiva, palate and muzzle skin.

Lesions were macroscopically observed just few days after the birth. In gengival and palate mucosa lesions were characterized by the presence of a proliferative tissue, white-reddish in color which makes lambs unable to suckle. Muzzle proliferative lesions appeared to be rather greyish in color. The animals died at about one month of age as they were not able to feed.

Tissues from two lambs were collected for histopathological, molecular and electron microscopic investigations.

Histologically, a mixture of epithelial (keratinocytes) and mesenchymal cells was seen. Numerous mitoses were observed in both cell types. Many mitoses appeared to be atypical. Ultrastructurally, the lesions appeared to be composed of heterogeneous epithelial and mesenchymal cells showing severe alterations of nuclei such as deep meandering invaginations which give nuclei a bizarre and lobulated appearance often containing nucleoli located peripherically. Electron dense particles, 40 nm in diameter, consistent with virus particles were scattered in some nuclei. In sheep, lesions induced by PV were only described in adult animals7,8. No lesions caused by vertical PV infections have been described so far in sheep. Here, we report mucosal and skin lesions in lambs. Our preliminary findings (molecular investigations are in progress) seem to indicate, for the first time, the presence of virus particles responsible for congenital fibropapillomatosis of lambs. It has been suggested that Bovine Deltapapillomavirus infect trophoblastic cells and are responsible for reproductive disorders in cattle and buffalo5. OaPV1 and OaPV2 are classified as ovine Deltapapillomavirus9,10. They are characterized, like bovine Deltapapillomavirus, by a tropism for epithelial and mesenchymal cells. It is conceivable to think that, as already shown for bovine Deltapapillomavirus11, ovine Deltapapillomavirus can join the genital apparatus via bloodstream. The role, if any, of ovine Deltapapillomavirus in reproductive disorders in sheep warrants further studies in an attempt to improve our knowledge in molecular pathways responsible for virus infection leading to neoplastic and no-neoplastic events.

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11 Roperto et al., 2011

LYMPHOPLASMACYTIC MYOSITIS AND SARCOLEMMAL EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I AND II ASSOCIATED WITH MUSCULAR SARCOCYSTOSIS IN SHEEP

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Muscular sarcocystosis in sheep is an extraordinarily worldwide common affection caused by Sarcocystis spp., protozoan parasites with an obligatory two-host predator-prey (definitive-intermediate) lifecycle. Sheep is the intermediate host of four species of Sarcocystis: S. gigantea, S. medusiformis, S. tenella and S. arieticanis, whose definitive hosts are felids or canids (1). The aim of this study was to investigate if parasitized muscle fibers could play a role in immune-stimulation, as sporadically described in accidental muscular sarcocystosis in definitive hosts (2).

Skeletal muscle samples from 80 sheep of mixed breed 4 - 5 years old were collected at the slaughterhouse and snap frozen in liquid nitrogen. Cryosection were processed with a standard panel of histological and histoenzymatic stains; immunohistochemical (HRP method) detection of MHC I, MHC II, CD3, CD4, CD8, CD79Îś, CD45RA. Part of the samples was fixed in 2,5% glutaraldehyde for ultrastructural examination. Frozen samples were collected for species identification by PCR.

A moderate to high number of intra-sarcoplasmic cysts with thin wall and internal compartments were detected in 97,2 % of cases examined. 69% of cases were characterized by inflammatory changes scored as mild (58,1%), moderate (36,3%) or severe (5,4%). Inflammation consisted of a mixed mononuclear infiltrate of small lymphocytes and plasma cells mostly located in the perivascular connective tissue or in the endomysium in a scattered fashion, with attendant myofiber degeneration and necrosis. The predominant populations were CD3+, CD8+ with lesser numbers of CD4+ and CD79Îś+ cells. Eosinophils were constantly absent. Notably, moderate to strong sarcolemmal and cytoplasmic labeling to MHC I and II was found both in biopsies with evident inflammatory infiltrate and in cases without inflammation. The wall of the cysts resulted strongly positive to MHC II and occasionally positive to MHC I. Our data suggest that muscle fibers respond to the presence of cysts by expression of MHC I and II that can play a role in stimulating and maintaining the lymphoplasmacellular inflammation. This findings underline the importance of sarcocystosis in the differential diagnosis of idiopathic inflammatory myopathy in all species (humans included) and raises interesting questions about meat consumption safety.

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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NORMAL SERTOLI AND GERM CELLS IN POST-NATAL RABBIT TESTES, FROM NEONATAL TO ADULT AGE

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In the last decades, human male reproductive pathologies and testicular cancer have increased. During the physiological maturation of the testis, from foetal to adult age, both Sertoli (SCs) and germ cells (GCs) switch from an immature to a mature immunophenotype. The re-expression of markers of immaturity in adult has been reported in numerous pathological conditions affecting the testis, in man as in animal species. Rabbits have been commonly employed for scientific research of human male reproductive system, but reports on rabbit testicular cells markers are few and data about the immunophenotype of normal postnatal SCs and GCs are lacking.

The aim of this study was to evaluate the immunohistochemical expression of anti Müllerian Hormone (AMH), Vimentin (VIM), CKAE1/AE3 (CKs), Desmin (DES), Inhibin-α (INH-α) and Placental Alkaline Phosphatase (PLAP) on normal rabbit testes, from the neonatal to adult age.

Twelve neonatal, 17 prepubertal and 7 adult testes were considered in the study. VIM was constantly expressed by SCs independently from the age of the rabbits, AMH and CKs expression in SCs was limited to the neonatal and prepubertal age, and DES and INH-α were never expressed by SCs as well as PLAP in GCs. This latter finding indicates the absence of early GCs (gonocytes) in postnatal rabbit testes.

The immunolabelling of normal GSc and SCs from rabbit testes revealed analogies with the human testicular phenotype in the different stages of testicular postnatal development. In fact, during maturation, in SCs some markers are maintained, as VIM, while other markers are lost as CKs and AMH. Considering GCs, the absence of early, undeveloped, GCs also parallels with findings in human species. The data obtained from this pivotal study suggest that rabbit could a potential good animal model for human testicular pathologies and encourage further investigations focusing on the immunohistochemical phenotype on rabbit testicular neoplasms.

IMMUNOHISTOCHEMICAL ASSESSMENT OF FOLLICULAR DENDRITIC CELLS IN HEALTHY AND PMWS-AFFECTED PIGS

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Post-weaning multisystemic wasting syndrome (PMWS) is caused by porcine circovirus type 2 (PCV2). The pathogenesis of PMWS is largely unknown. Macrophages are considered the main target for PCV2, since viral antigens and genome can be easily detected in those cells. Notwithstanding this, whether PCV2 replicated in monocytes/macrophages is still controversial (5). Some data suggest that follicular dendritic cells (FDCs) could be also infected by PCV2, thus likely playing a role in the pathogenesis of PMWS (1,2,4).

The aims are to quali-quantitatively assess FDCs in tonsils of healthy (n = 8) and PMWS-affected (n = 10) pigs.

Tissue samples were routinely processed for histopathology. Consecutive tissue sections were tested by immunohistochemistry to detect PCV2, FDCs (S-100 and CNA.42) and macrophages (lysozyme). FDCs and PCV2 antigens were quantified by means of the Image J software, and data submitted to statistical analysis.

In healthy pigs, lymphoid follicles appeared normal with well-developed FDC networks. The immunohistochemical pattern markedly differed between S-100 (mainly nuclear) and CNA.42 (mainly cytoplasmic and dendritic). Lymphocytic depletion, infiltration of histiocytes and/or syncytia were confirmed in PMWS cases; large amounts of PCV2 antigens were seen within macrophages, syncytia, and/or showing a "FDC-like" pattern. Both S-100 and CNA.42 immunoreactivity were significantly reduced in PMWS-affected pigs. A positive correlation was seen between S-100 and CNA.42 , while a negative correlation was observed between S-100 and PCV2, as well as between CNA.42 and PCV2.

Our results demonstrate a significant reduction of FDCs in PMWS-affected pigs, which goes âĂIJhand in handâĂİ with the severity of lymphocytic depletion and with the infiltration of macrophages and syncytia. The reduction of FDCs likely compromises the immune response and enhances the occurrence and the severity of secondary infections, which are relevant for the expression of PMWS (3).

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PERFORMANCES COMPARISON OF DIFFERENT ELECTRODES FOR RADIOFREQUENCY THERMAL ABLATION (RTA) ON EX-VIVO BOVINE AND PORCINE LIVER

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Radiofrequency thermal ablation (RTA) is a consolidated, safe and minimally invasive approach for the treatment of hepatic nodular lesions and neoplasm of different organs (kidney, spleen, prostate, breast, lung, bone, and recently thyroid nodules) (Deandrea et al., 2008). Tissue necrosis is achieved around the needle tip, through the heating induced by rapid ion movement, in a controlled fashion.

Aim of the work was to compare the ablation characteristics of the moving-shot technique (MST) and the fixed electrode technique (FET) for radiofrequency (RF) ablation in an ex-vivo bovine and porcine liver tissue model.

In the first pilot experiment different conditions (technique, ablation time, electrode type) were investigated on bovine livers, under ultrasound guidance. Subsequently, MST (both perfused or not) with single or double ablation, and FET were applied on excised porcine livers. The efficacy of induced necrosis was evaluated by monitoring tissue impedance. Following ablation the liver was macroscopically and histologically examined, and morphometrical techniques were applied in order to measure the parameters of each type of ablation (diameters, perimeter and area of surfaces, and morphology of the necrotized tissue). The liver was cut along the longitudinal plane through the longitudinal axis of the electrode and then cut transversely and perpendicular at the center of the ablation area.

Differently from similar studies (Ha et al., 2014), the FET achieved a significantly larger ablation zone than the MST (confirmed by each of the measured parameters). Moreover a double passage with both normal and perfused moving shot electrodes reached values similar to the ones obtained with FET. The non perfused electrode for MST, with a single passage of ablation, induced a smaller ablation zone.

The evaluation of the performances of different electrodes and conditions of application in an ex-vivo model is very useful in order to manage with nodules of different shapes and location in vivo. As the application of ex-vivo data to in-vivo situations could result in different outcomes, further investigations in an in vivo model is fundamental in order to better evaluate the effects of the best combinations identified in the ex-vivo model, before the application on patients. The mechanism underlying the differences detected by our study and other investigations is an important issue to be further investigated.

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MYOPATHY OF THE PIPPING MUSCLES, HEPATOSIS DIETETICA, AND CATARACTS IN EMU CHICKS (DROMAIUS NOVAEHOLLANDIAE)

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Vitamin E is considered one of the key factors in ratites nutrition and management, since its absorption is limited in newly hatched birds and thus the embryonic stores may be depleted before the onset of an efficient dietary assimilation (Tully et al., 1996). Few cases of encephalomalacia and nutritional myopathy caused by vitamin E deficiency have been described in adult emus (Aye et al., 1991; Rae et al., 1992), while reports on similar lesions in embryos or newly hatched chicks are lacking. Seven emu chicks from a farm with poor hatchability (16-18%) and increased neonatal mortality were presented for necropsy with a history of death at or within few days after hatching. Macroscopic examination revealed subcutaneous oedema and haemorrhages and swelling of the pipping muscles in the proximal neck (71.4%), pale liver with haemorrhages (57.1%), non-internalised residual yolk sac (85.7%) and anasarca (14.3%). Histologically, the most remarkable findings were degeneration and loss of cross striations of the musculus complexus (pipping muscle) (100%), as well as myocardial degeneration and mineralisation (14.3%). Liver contained multifocal severe hepatocellular necrosis and haemorrhages (57.1%) and both eyes exhibited swollen and vacuolated lenticular fibres in 5 c hicks in which the eyes were examined. The lesions observed here are suggestive of a nutritional deficiency. The deficiency was confirmed by finding low levels of vitamins E and A in the livers and feed. Although vitamin E deficiency is considered the primary aetiological factor of the lesions observed in our cases, management factors (such as a high relative humidity during incubation) may be potentially considered additional contributing factors. The involvement of the muscles in the neck region, and specifically the musculus complexus - whose contraction at the end of the incubation elevates the head of the chick and therewith the beak (Fisher, 1958) - could have compromised the hatching process.

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EPHRIN A3 RECEPTOR AS TARGET FOR CANCER THERAPY: PRELIMINARY IMMUNOHISTOCHEMICAL RESULTS IN THREE CANINE TUMOURS

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With 16 members, the Ephrin (EPH) receptor family is the largest family of the receptor tyrosine kinases and it is of increasing interest in developmental therapeutics. Recent findings on the elevated expression of EPH in human malignancies as well as in stem cells are of particular interest [1]. The most promising, EPHA3 is highly expressed at various stages of embryonic development [2] while its expression declines, usually being low, if detectable at all, in adults. However, EPHA3 re-expresses in cancers and plays important roles in a variety of biological functions, such as tumour cellular proliferation, angiogenesis and tumour progression [3]. The aim of this study is to evaluate the expression of EPHA3 in three significant cancers of dogs (cutaneous haemangiosarcoma, osteosarcoma and prostate carcinoma) as an important prelude for evaluating an EPHA3-targeting antibody as a potential therapy. Twenty-four cutaneous haemangiosarcoma (HSA), 29 osteosarcoma (OSA) (18/29 osteoblastic, 6 mixed type fibroblastic and osteoblastic, 3 telangiectatic, 2 mixed chondroblastic and osteoblastic), and 22 prostate carcinoma (PC) (13/22 cribriform with comedonecrosis, 5/22 solid, 3/22 small acinar/ductal and 1/22 papillary) cases were studied. For immunohistochemistry, tissue sections were labelled by the avidin-biotin-peroxidase complex (ABC) procedure with a commercial immunoperoxidase kit. The sections were incubated with a primary rabbit polyclonal anti-EPHA3 antibody (Santa Cruz Biotech., dilution 1:800 for HSA and PC, 1:1500 for OSA). In 13 out of 24 haemangiosarcoma samples, a strong cytoplasmic expression was detected in more than 80% of neoplastic cells, while the remaining 11 samples contained 50-60% positive cells. In all osteosarcoma samples, the antibody showed diffuse and strong cytoplasmic labelling with a mean of 86% of cells staining positively. Moderate to strong diffuse cytoplasmic expression was observed in 90-100% of prostate carcinoma cells. Labelling pattern was similar in all histological types of all three tumours. Most normal prostate tissues display weak cytoplasmic positivity. In normal prostate cells, moderate granular cytoplasmic expression was observed in 20-30% of cells, mainly in the basal layer. The present study demonstrated strong immunohistochemical labelling for EPHA3 in neoplastic haemangiosarcoma, osteosarcoma and prostate carcinoma cells suggesting that EPHA3 may play a role in the carcinogenesis of the three entities under consideration and putatively in other canine tumours. Further studies are required to clarify whether EPHA3 overexpression is correlated with survival and could be used as a predictor of disease-free survival time or as a new therapeutic target for these neoplasms.

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FIRST REPORT OF MYCOBACTERIUM BOVIS INFECTION IN A FREE-RANGING MARSICAN BROWN BEAR (URSUS ARCTOS MARSICANUS)

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Mycobacterium bovis (MB),causative agent of bovine tuberculosis (boTB),has a wide host range and is often maintained in complex transmission cycles[1]. Diagnosis of tuberculosis in wildlife relies on necropsy,histopathology and microbiology. MB was cultured from a black bear in absence of lesions[2]. M. avium paratuberculosis was reported as cause of disease in 2 brown bears[3]. To the authors' knowledge this is the first report of MB in a brown bear. Aim This study describes a case of MB tuberculosis in a free-ranging brown bear.

An adult female brown bear (U. arctos marsicanus) died in Abruzzo in 2014.The carcass was submitted for postmortem examination to ascertain the cause of death. Tissue samples for histopathology were formalin-fixed,embedded in paraffin wax,sectioned at 4 μm and stained with HE and Ziehl-Neelsen. Samples were also submitted to cultures for bacterial pathogens including Mycobacteria,viruses isolation (cell cultures) and toxicological analysis(GC-MS).Bacterial identification was obtained by molecular techniques (PCR,sequencing) of selected target genes.

The bear was in fair body condition. Gross findings included peritonitis, enlarged necrotic mesenteric lymphnodes, thickening of intestinal wall, hepatosplenomegaly, rhinopharyngeal exudate, pulmonary edema, subpleural petechiae and meningeal hyperemia. A presumptive diagnosis of mycobacterial infection was made. Histology showed large necrotic foci in the peritoneum and intestinal mucosa, massive necrosis of mesenteric lymphnodes, granulomatous hepatitis, perisplenitis, membranoproliferative glomerulonephritis and granulomatous meningitis. In all the examined organs acid-fast bacilli were observed in macrophages and extracellularly. Slow-growing Mycobacterium sp,identified as M.bovis by molecular methods,was isolated from multiple organs. Staphylococcus schleiferi subsp coagulans was isolated from intracardial clot and peritoneal fluid. Virological tests and toxicology were negative.

The bear was diagnosed with a chronic severe systemic MB infection,with both pathological and microbiological aspects suggesting ongoing generalization. The cause of death is likely to be attributed to MB and a concurrent opportunistic infection with S. schleiferi coagulans. Due to the main gastrointestinal localization of lesions MB infection was thought to be acquired by ingestion. MB infected cattle have been known to graze in the home range of the bear in 2012:some bovines died on pastures and were consumed by scavengers. Gross lesions in cattle are typically caseous and mineralised with histology showing central necrosis surrounded by granulomatous reaction and fibrosis, but lesions in wildlife may differ[4].The case herein described presented with poorly organised lesions,similarly to what have been observed in other carnivores. Bears are thought to be spillover hosts and are likely to play no significant role in the maintenance of boTB.Nonetheless,spillover from cattle to bears may have serious implications for the conservation of this species. Stricter application of health regulations in force is warranted along with wildlife monitoring to assess presence of infection in other scavengers. We underline the importance of personal protection measures when dealing with wildlife forensic cases as zoonotic infections cannot be ruled out based on external findings.

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GLEASON-LIKE GROWTH PATTERNS OF CANINE PROSTATIC CARCINOMA AND THE POTENTIAL APPLICATION OF A MODIFIED GLEASON GRADING IN THE PRACTICE SETTING

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Human prostatic carcinomas (PCs) are graded by pathologists using the Gleason system [1], which remains one of the most powerful prognostic indicators in PC [2], assigning numerical grades (1-5) based upon the architectural patterns of the tumour. Gleason grading was updated at the 2005 consensus conference by the International Society of Urological Pathology in response to evolving clinical practice and understanding of prostate cancer pathology [3]. Since we have recently recognised Gleason-like growth patterns in dogs [4], this study aimed to apply the modified Gleason grading to score the aggressiveness of 45 canine prostate carcinomas. Specimens were represented by tissue samples collected during necropsy (n=20), prostatectomy (n=4) or biopsy (by ultrasound or exploratory laparotomy; n =20). Gleason score (GS) was obtained by adding the primary and secondary grades together. A tertiary pattern higher than the primary and secondary grades has been included in the final GS as the secondary grade. Any amount of Gleason pattern 5 - predicting a worse outcome in men [5] - was considered significant and included for analysis. A single primary growth pattern was observed in 28 cases, a secondary pattern in 11 cases and a tertiary pattern in 6 cases. Cribriform, solid and small acinar/ductal were the most common primary, secondary and tertiary morphological patterns, respectively. Seven (15.6%) dogs were classified as Gleason score 3+3 = 6; 2 (4.4%), 4+3 = 7; 7 (15.6%), 4+4 = 8; 2 (4.4%), 5 +3 =8; 4 (8.9%), 5 +4 = 9; 2 (4.4%), 4 + 5 = 9. The highest Gleason score (GS10) was obtained in 46.7% of cases (n = 21). Nine of 14 metastasising cases were classified as GS10. The most common score observed in tissues collected during necropsy and prostatectomy was GS 10, while GS8 in biopsy samples. Gleason pattern 5 was present in 35 of cases. This study suggests that canine PC may show variable morphological features and Gleason-like growth patterns that would aid in the acceptance of the modified GS as a grading system for histopathology. As expected due to the aggressive biological behaviour of canine PC, the most common GS is 10 and the highest GS was observed in metastasing PCs. We suggest that once carcinoma is detected and the different morphological patterns recognised, the Gleason grading system may be potentially applied in the practice settings in order to complete the clinical assessment for the best management of the patient.

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PATHOLOGICAL FINDINGS IN A FATAL OUTBREAK OF ORTHOPOXVIRUS INFECTION IN TONKEAN MACAQUES (MACACA TONKEANA)

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Orthopoxviruses are known to naturally infect a broad range of host species such as ruminants, cats, rodents, various zoo and exotic animals and humans. Human cowpoxvirus usually causes self-limiting skin lesions but severe complications and fatal disease can occur in immunocompromised people. The cowpox infection in nonhuman primates in Europe is reported with fatal course in an outbreak. As asymptomatic carriers small rodents are considered the most responsible for the spread of infection.

Aim: Describe the pathological findings observed following an Orthopoxvirus infection in a group of macaques (Macaca tonkeana).

In January 2015, twelve macaques of a group of 18 animals, housed in a sanctuary in a wooded area of Central Italy, died between 48 hours and 7-8 days from the beginning of symptoms. Severe respiratory distress, depression and in most of cases skin lesions were observed. Animals were submitted for post-mortem examination. Samples from the major organs and from the skin lesions, were routinely processed for histology and virological investigations. Data relative to animal introduction and movements were recorded to identify a probable source of the infection.

All monkeys presented a good body condition. Animals dead within 48 hours (N=2) showed severe lung congestion and hepatosplenomegaly; erythematous papular and pustular lesions on the oral and tongue mucosa and at the inguinal region, in some cases diffuse, were evident in subjects dead in 7-8 days (N=10). In the latter animals, several lymph nodes were enlarged and haemorrhagic and hepatosplenomegaly and liver degeneration were observed. Histologically, cutaneous lesions were characterized by focal epidermal necrosis, acanthosis and acantholysis and early vesiculation with eosinophilic intracytoplasmic inclusion bodies in enlarged degenerate cells. The liver showed moderate steatosis and scattered foci of necrosis. Splenitis occurred as foci of necrosis of the lymphoid follicles and histiocytosis. Affected lymph nodes showed a severe necrotising lymphadenitis associated with haemorrhages and histiocytosis. In some cases a mild interstitial pneumonia was associated with focal necrosis of bronchial epithelium. Transmission electron microscopy detected Orthopoxvirus particles in all tested animals. The preliminary characterization of virus isolates ruled out the presence of Monkeypoxvirus and lead to suspect a Cowpox infection. Deeper molecular investigation are still ongoing. The introduction of susceptible species in the last year was excluded, but free ranging cats and rodents are present in the area.

Even if Orthopoxvirus infections in Macaca tonkeana had not been previously described, pathological findings observed were similar to those described in New World monkeys in a fatal outbreak occurring in Germany. Epidemiological investigations to define the source of the infection are in progress.

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'BRAIN-ONLY' FORM OF DOLPHIN MORBILLIVIRUS INFECTION IN STRIPED DOLPHINS (STENELLA COERULEOALBA): PATHOGENETIC INSIGHTS

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Dolphin Morbillivirus (DMV), a highly pathogenic agent, can give rise to peculiar, 'brain-only' forms of infection (BOFDI), in which evidence of viral antigen and/or genome can be found exclusively in the brain tissue from striped dolphins (Stenella coeruleoalba)1-4 and, far less commonly, from bottlenose dolphins (Tursiops truncatus)4. These BOFDIs show morphopathological and neuropathogenetic similarities with subacute sclerosing panencephalitis and old dog encephalitis, which are known to occur in Measles Virus (MeV)-infected patients and in Canine Distemper Virus (CDV)-infected dogs, respectively5. We investigated in the brain tissue of 3 BOFDI-affected, male striped dolphins, 2 adults and 1 newborn, the expression levels of 5-lipoxygenase (5-LOX), along with the ultrastructural damage and the neuronal and non-neuronal cell populations colonized by the viral pathogen. The expression levels of 5-LOX, which were evaluated by means of Western Blot (WB) analysis, were significantly ($P \leq 0.05$) higher in the brain parenchyma of the 3 aforementioned cetaceans, when compared with those of 3 additional striped dolphins showing no direct nor indirect evidence of DMV infection. Furthermore, alongside with a number of nuclear (chromatin) and cytoplasmic (mitochondrial) ultrastructural changes, double labeling-indirect immunofluorescence (DL-IIF) microscopy revealed different degrees of viral colonization of calbindin (CALB)-immunoreactive (IR) and nitric oxide synthase (NOS)-IR neurons, but not of (GFAP-IR) astrocytes, within the brain tissue from the two DMV-affected adults as compared to the DMV-affected newborn. Albeit preliminary, this is the first study addressing the ultrastructural pathology and the neuropathogenesis of BOFDI, with special emphasis

on the neuronal and non-neuronal cell populations colonized by DMV in the striped dolphin's brain. Further studies aimed at characterizing the virus- and the host-related factors involved in BOFDI pathogenesis are warranted.

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ELODONTOMA AND CEMENTIFYING FIBROMA IN A DEGU (OCTODON DEGUS)

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Degus are diurnal, caviomorph, small rodents belonging to the family of Octodontidae, characterized by complete elodont (continuously growing) dentition. They are used in research studies and have been increasingly popular as pet animals. Reports of dental diseases are common in hystricomorphs, while in degus there are few descriptions (1; 2). This report aim at describe a case of multiple dental disease in an adult degu. A 4.5 years old intact male degu, kept as pet, was evaluated clinically because of a history of respiratory disease, dysorexia, progressive weight loss, and deformation of the left mandible ventral profile. Necropsy was performed after the death of the subject. The degu was in poor body condition and its bowel was severely dilated by gas content. The examination of the left mandibular branch revealed two ventral lumps at the level of the first and the third molar, respectively of 0.4 and 0.3 mm in diameter. The entire jaw was collected, fixed in 10% neutral buffered formalin, decalcified for 24 hours in a commercial solution, cut transversally, processed routinely and stained with hematoxylin and eosin. Histologic examination revealed two space-occupying lesions. The first molar was affected by a neoplasia composed of conglomerates of haphazardly arranged dental tissue, including cementum directly associated with columnar odontogenic epithelium and fibrous connective tissue. The second lump was a neoplasia surrounding the inferior incisor root, composed by fibroblast-like spindle cells within a collagen matrix, admixed with foci of cementum. An heterophilic gingivitis centered around a plant fragment and bacterial aggregates was also evident controlaterally. The histologic diagnoses were elodontoma of the first molar tooth, cementifying fibroma of the incisor root, and focal heterophilic gingivitis with foreign body. Elodontomas are space-occupying lesions of continuously developing odontogenic tissue. They are considered by many authors hamartomas rather than true neoplasm (3). In 2006 Boy and Steenkamp (3) proposed this term to replace the term "odontoma", aiming at distinguishing hamartomas of continuously growing elodont teeth, from those of anelodonts, detectable only in developing teeth of younger animals. Cementifying fibroma is a rare tumour, already seen in horses and dogs. Its matrix component has features of cemental differentiation including complex basophilic lines growing in a mosaic or lamellar pattern typical of cementum (4). Both these neoformations are locally destructive, disturb normal odontogenesis and require complete excision but have no metastatic potential (4). Molar elodontoma and inflammation of the mouth soft tissue have been bound to molar malocclusion-related laceration and food impaction (1). Maxillary elodontoma can be highly disruptive to the sinuses and nasal cavity, causing severe respiratory deficiency, whereas mandibular elodontoma is generally less symptomatic, frequently causing lumps along the ventral mandible and making eating difficult (1; 3). As dental diseases are frequent in elodont captive animals, it is important to develop a good understanding of these conditions for effective prevention and treatment.

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LIPOMATOSIS OF A MANDIBULAR SALIVARY GLAND IN A DOG

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Salivary gland enlargements in dogs are rare and they include inflammation (sialoadenitis), salivary mucoceles, infarction and neoplasia. Tumors are predominantly carcinomas or adenocarcinomas (3). Another rare condition causing salivary gland enlargement is lipomatous infiltration (lipomatosis of the salivary gland) (1,2,3).

This report describes a case of lipomatosis of the salivary gland in a dog. Material and methods: Cytological smears were obtained by fine needle aspirate, air dried and stained with May-Grünwald Giemsa. Histological sample were formalin fixed-paraffin embedded and stained with Hematoxylin and Eosin.

An 8 year-old, male viszla dog was presented with a 2 years history of a slowly growing mass in the right submandibular region. The dog was asymptomatic. The physical examination revealed a soft, not movable and not painful retromandibular mass. The regional lymph node was not palpable. The laboratory tests, including complete blood count, chemistry profile and coagulation profile were unremarkable. CT examination revealed a soft tissue density mass in the right submandibular space, measuring 10 x 7 cm. The mass had an irregular shape and extended from the temporomandibular joint to the 3rd vertebral body. Based on the CT images, the mass apparently arose from the mandibular/parotid gland. Cytological smears revealed lipid droplets and two types of cells: rare mature adipocytes alone or in small groups and clusters of epithelial cells with acinar structures. The epithelial cells were round to cuboidal, with dark blue to clear foamy cytoplasm and single round nucleus with a small nucleolus. Occasional spindle cells were also present. The cytological features were suggestive of an epithelial glandular neoplasm. The mass was excised and the dog completely recovered after the surgical procedure.

Histologically the lesion was composed by a well differentiated salivary gland, preserving its lobular architecture, and severely expanded by the presence of abundant well differentiated adipose tissue infiltrating the interlobular and intralobular septa. Adipose tissue separated the salivary acini and excretory ducts. The histological features were consistent with lipomatosis of the salivary gland.

Lipomatosis of salivary gland is a rare condition characterized by fatty infiltration of the salivary gland (1,2,3). It can be differentiated from true neoplasm of adipose tissue based on the presence of salivary gland cells scattered throughout the adipose tissue (3). As previously reported in the literature, in this case the lesion was slowly growing and monolateral (1,2,3). Lipomatosis should be considered in the differential diagnoses of monolateral enlargement of the salivary gland in the dog.

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IMMUNOHISTOCHEMICAL EVALUATION OF P62 IN CANINE MAMMARY TUMORS

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In the last years in human and veterinary oncology most of the pathogenetic studies regarding mammary tumors has been paid to apoptosis and autophagy mechanisms. P62 can be considered the crossroad molecule of autophagy and apoptosis (2).

The p62 protein, also called sequestosome 1, is a ubiquitin-binding scaffold protein that polymerizes via an N-terminal PB1 domain and can interact with ubiquitinated proteins via the C-terminal UBA domain. P62 is found in cellular inclusion bodies and in cytosolic protein aggregates that accumulate in various chronic, toxic, and degenerative diseases(1).

In veterinary medicine, the role of p62 in tumors is poorly understood. A recent study has been performed in canine cutaneous mast cell tumors (3). The aim of this study is to evaluate the immunohistochemical expression of p62 in normal mammary tissue, in adenomas and carcinomas of the dog.

The immunohistochemical analysis were performed on thirty-six mammary tumors and eight normal mammary tissues present in archive of Laboratory of Animal Pathology - University of Camerino. The samples were histologically classified according to criteria of WHO. When present, the regional lymph nodes were analyzed too.

Immunohistochemistry was carried out by the Streptavidin-Biotin-Peroxidase method using as primary antibody an anti-p62 antibody (Sigma- Aldrich). Immunohistochemically, we have found specific reaction to p62 in epithelial cells of normal and neoplastic tissues. All normal mammary tissues, normal, and hyperplastic lobules exhibited a strong, homogeneous positiveness towards p62. Almost all epithelial cells showed a brown granular stain in the cytoplasm while the nucleus was negative. Only 5% of myoepithelial cells were immunostained while the stroma was always negative. In all adenomas immunostain to p62 was enough intense but the percentage of epithelial positive cells was lower (65%).

In malignant tumors, the immunoreaction appeared heterogeneous both between samples and within the same sample. In fact, 19 tumors (68%) showed little areas strongly positive close to others hardly negative while 9 tumors (32%) exhibited a diffuse weak stain. Only two of 7 high-grade carcinomas appeared positive to p62. Metastatic cells in lymph nodes were p62 positive in 50% of cases. These data could suggest a correlation between p62 expression and neoplastic progression because in carcinomas p62 overexpression is not observed. To date, as the paucity of samples examined and the complex role of p62 in autophagy and apoptosis, we believe that is not possible to consider p62 a progression marker in canine mammary tumors.

In the future will be interesting to compare these results with data obtained from breast cancer studies where a few authors hypothesize a negative correlation between p62 expression and neoplastic progression while most authors believe that p62 play a role in the interactions between epithelial neoplastic cells and stroma.

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CHRONIC INTESTINAL PSEUDO-OBSTRUCTION WITH SEVERE MYOPATHY AND FIBROSIS IN A YOUNG MINIATURE BULL TERRIER

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Chronic intestinal pseudo-obstruction (CIPO) is a rare clinical syndrome in veterinary medicine defined by severe intestinal dysmotility without evidence of mechanical occlusion of the intestinal lumen. A few canine cases of CIPO have been reported and most have been related to an idiopathic sclerosing enteropathy or fibrosing gastrointestinal leiomyositis, less frequently to dysautonomia (3). In human medicine CIPO can be caused by different gastrointestinal neuromuscular diseases (GINMDs) including primary visceral neuropathies, interstitial cell diseases and myopathies (3). A one-year-old male miniature bull terrier dog was presented with chronic weight loss, regurgitation, vomiting and diarrhoea. On exploratory laparotomy the small intestine was not obstructed but appeared markedly distended with fluid and gas and the wall was thinned. Full thickness intestinal biopsies of small intestine were obtained. Due to the persistent clinical signs of dysmotility the dog's clinical condition severely deteriorated thus euthanasia was elected. Necropsy confirmed that small intestine was severely dilated and filled by a moderate amount of greenish fluid content. The wall was diffusely thinned and atonic. A complete set of tissue was taken for histopathology, including various portions of intestinal tract. Sections of intestinal tract were also stained with periodic acid-Schiff (PAS) and Masson trichrome and were submitted to immunohistochemistry using antibodies to alpha-smooth muscle actin (α-sma), neurofilament, synaptophysin, neuron specific enolase (NSE), CD117, glial fibrillary acid protein (GFAP), CD3 and CD79. Histological findings of the small and large intestines consisted of severe diffuse atrophy of the tunica muscularis and severe locally-extensive to diffuse fibrosis of submucosa as demonstrated by Masson trichrome stain. Additionally intestinal mucosa appear multifocally eroded. The myenteric and submucosal nerve plexuses had intact neurons confirmed by immunohistochemistry for NSE, neurofilament and synaptophysin without inflammatory infiltrates. Also interstitial cells of Cajal were preserved and were strongly stained for CD117. However α-sma immunoreactivity was markedly reduced in the muscular layers of all the different intestinal sections examined with foci of complete loss. Loss of α-sma expression is recognized as a marker for intestinal dysmotility and myopathy causing CIPO in human medicine. Recently a case of CIPO associated with deficient expression of α-sma in the muscular layer and loss of myofibrils has been described in a Bengal cat and a leiomyopathy has been hypothesized (1). The clinical, histopathological and immunohistochemical findings of this rare case is consistent with enteric myopathy and fibrosis and could be referred to a GINMDs as in human medicine.

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CASE REPORT: DIAGNOSTIC APPROACH FOR IDENTIFICATION OF A ZINC PHOSPHIDE POISONING IN A BADGER (MELES MELES)

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Zinc phosphide is a dark grey, crystalline compound used as a rodenticide but in Italy it is frequently used for malicious poisoning of domestic and wild animals. Identification of the cause of death represents a key element of the investigation process aimed at identifying criminals as well as preventing further risks for poisoning in both animals and humans. In the present work we report a case of poisoning in a badger (Meles meles) found in the countryside area of Grosseto, Tuscany, Italy. The animal was discovered by Provincial Police while showing convulsions and foam at the mouth and soon after it died. Based on suspicious of animal poisoning, the carcass was delivered to the National Reference Center for Veterinary Forensic Medicine, located in Grosseto.

The aim of the study is to describe the diagnostic approach adopted in a case of suspected poisoning and to describe the post-mortem and laboratory findings observed in a badger dead for zinc phosphide poisoning.

Post-mortem analyses were carried out using the forensic approach thus, all steps of necropsy were documented by photos taken using a metric reference system (ABFO). Based on anamnestic information and post-mortem findings, brain samples were collected for virological investigation; samples of oesophagus, stomach, intestine, brain, liver, spleen, pancreas, myocardium, lung and kidney were submitted for histological examination. The contents of the stomach and samples of luminal contents of small and large intestine were collected for toxicology.

Soon after death the carcass showed a rectal temperature of 43,4°C. At necropsy, carried out twenty hours after death, the rigor mortis was still present. A generalised subcutaneous congestion was observed. Blood of dark appearance was present in both pleural and peritoneal cavities. Lungs were congested and moderately oedematous. Hydropericardium and congestion of pericardium were recorded at cardiac level. Myocardium was atonic and interested by focal areas of tissue degeneration. Hazel (Prunus spp.) and some dark grey granules with characteristic garlic smell were found in the contents of the stomach; the gastric and intestinal mucosa and the pancreas were congested. The liver was moderately increased of volume, congested and friable. Congestion of meninges was also observed. Histology mainly revealed: multifocal necrosis of myocardium; mild multifocal fatty changes associated with central venous congestion and sinusoidal dilatation in liver; multifocal tubular cloudy swelling and congestion in kidney. All virological tests carried out as differential diagnosis turned out negatives, whereas gastric-enteric contents resulted positive for zinc phosphide.

Following ingestion of zinc phosphide, in presence of gastric acids, it is hydrolysed in phosphine gas that is rapidly adsorbed by gastric mucosa. Once phosphine enters the circulatory system, it causes major metabolic acidosis with systemic consequences. Both post-mortem lesions and hystopathological findings reported in our case reflect those described in the literature and mainly in humans.

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TORSION OF THE URINARY BLADDER IN A DOG

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Diseases of the lower urinary tract frequently occur in dogs. In particular, cystitis, urinary incontinence and urolithiasis are the most commonly reported disorders affecting the lower urinary tract in that animal species (1). On the contrary, urinary bladder torsion represents a very rare condition in dogs.

The aim is to describe a case of torsion of the urinary bladder in a dog.

An adult, female, neutered German Shepherd showed depression and was very painful on abdominal palpation. According to what reported by the owner, the dog lived outdoor in a large garden, clinical signs suddenly appeared and rapidly worsened. The dog was referred to a veterinary clinic, where it died few minutes later. Therefore, the carcass was submitted to diagnostic investigations at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" (Teramo, Italy).

At necropsy, the urinary bladder was extremely expanded and occupied a major part of the abdominal cavity. Both ureters were also distended, while blood vessels of the urinary bladder wall, as well as of the renal capsule, appeared markedly congested. A bladder torsion of approximately 360° was evident at the level of the trigone. On cut section, the bladder wall was thickened and congested, while the mucosal surface was wrinkled, congested and hemorrhagic. No relevant lesion was observed elsewhere. On the basis of the gross findings, the torsion of the urinary bladder was diagnosed.

The torsion of the urinary bladder is occasionally observed in sows and in cattle affected by the torsion of the uterus (2). To the best of our knowledge, only two cases of urinary bladder torsion have been previously described in dogs, as a complication after ovariohysterectomy (3) or of presumable traumatic origin (4). In the present case report, the presence of any reasonable predisposing factor remained unknown.

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WOLF PREDATION ON LIVESTOCK: TRUTHS, SIMULATIONS AND COSTS IN A PROTECTED AREA IN CENTRAL ITALY

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The peaceful cohabitation between large carnivores and livestock is almost impossible. However, various and complementary measures can be carried out to manage such conflict, including compensation for damage to livestock. Compensation should integrate other preventive strategies, without becoming an additional tool for agricultural subsidies. In this respect, veterinary inspections are crucial to evaluate the real impact of predation and to minimize fraudulent behaviors (1).

To report data collected from 2004 to 2014 within a protected area (Gran Sasso Monti della Laga National Park, GSLNP) in Central Italy, in order to confirm/rule out wolf predation.

In total, 1,774 small ruminants, 376 cattle and 230 equids were reported, and therefore investigated as presumed cases of wolf predation. A special emphasis was placed on pathological features useful to differentiate wolf predation from other non-compensable events (e.g. predation by free-ranging dogs, fraudulent simulations, accidental wounds). Data about the costs for compensation were also collected and provided herein.

Wolf predation was confirmed in 1,326 (74.7%) small ruminants, 172 (45.7%) cattle and 125 (54.3%) equids, while fraudulent simulations of wolf attack were detected in 60 small ruminants (3.3%), 19 cattle (5.0%) and 3 horses (1.3%). The ratio wolf predations/total reports progressively increased during the period under study. A drastic drop of costs was observed between 2003 and 2004, with the beginning of veterinary inspections.

Our data indicate that the wolf is the major liable for livestock damage caused by predators in GSLNP. Wolf predation might be overestimated due to the presence of free-ranging dogs. However, wolves and dogs show different predatory behaviors, which deeply influence the pathological findings and could be extremely useful to correctly identify the predator (2).

Fraudulent simulations of wolf predation are relatively few and decreasing within the GSLNP; at the same time, illegal killing of wolves has been never reported during the last 15 years. Taken together, our data suggest that the conflict between wolves and human activities is efficiently managed. The progressive increase of the ratio between wolf predations and total reports - i.e. the reduction of reports due to other causes - further supports such belief. In conclusion, the correct identification of wolf predation, along with the implementation of complementary strategies, seem useful to manage the cohabitation between predators and livestock and, as a consequence, for the conservation of endangered predators.

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ETHYLENE GLYCOL TOXICITY: A RETROSPECTIVE PATHOLOGICAL STUDY IN CATS

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Ethylene glycol (EG) is an organic compound responsible for intoxication by ingestion in humans and animals. EG has in itself a low toxicity, but is rapidly metabolised in toxic compounds that determine severe acidosis, deposition of calcium oxalate crystals with acute, severe and often fatal renal, cardio-respiratory or nervous clinicopathological alteration (1, 2).

Few reports are available on gross pathological signs in animals and are limited to renal changes (3, 4). Aim of this study is to describe anatomohistopathological changes in cats with ethylene glycol intoxication (EGI).

From 2011 to 2014, 637 cats were submitted to necropsy to confirm suspected poisoning, in the framework of a national surveillance program on poisoning (Ministerial Decree "Norme sul divieto di utilizzo e di detenzione di esche o di bocconi avvelenati" 08.12.2008).

If necropsy was sufficient to diagnose the cause of death, no further analysis were performed. Otherwise samples of organs were processed to assess anticoagulant or pesticides poisoning, or other causes of death (i.e infectious agents or degenerative diseases). When renal gross findings were compatible with EGI, histopathology with hemathoxylin-eosin and Pizzolato stain were applied to highlight calcium oxalate deposits

In 452 (71%) cases necropsy alone defined the cause of death. Six cases got a direct suspect of EGI on the basis of medical history and renal changes (bilateral nephromegaly). On the remaining cases (29%) histopathology and/or ancillary exams on lesioned organs were necessary to confirm the presumptive diagnosis. Seventeen of this cases (3%), submitted to histopathology either with vacant diagnosis or with suspect of feline infectious peritonitis (FIP), had diffuse, severe tubulonephrosis with dilation of proximal tubules, flattening, vacuolization and necrosis of the epithelium and intratubular or interstitial deposition of moderate to high amount of lightly yellow, refringent, round-shaped, radially disposed crystals, consistent with calcium oxalate deposits; no other relevant changes were noted in the other organs examined. In all the 23 aforementioned cases the presence of calcium oxalate crystals was confirmed by Pizzolato stain.

Gross findings in the 6 EGI-suspected and in the newly 17 detected cases were respectively: thoracic and/or peritoneal sero haemorrhagic effusion (5/6 and 12/17), hyperemia of lungs (5/6 and 9/17), hyperemia of liver (3/6 and 8/17), enlarged (6/6) or pale/degenerated (7/17) kidneys, and hepatic degeneration (1/6 and 5/17). This retrospective study on cats points out that in EGI, gross findings are not limited to renal changes (nephromegaly and/or pale kidney): serohaemorrhagic cavitary effusions, lungs hyperemia, liver degeneration or hyperemia and degenerated kidney are frequent gross findings, as already described in humans (with the exception of effusions) (1).

EGI should not be ruled out in case of these macroscopic picture, even in the absence of typical renal changes, and especially when medical history is absent or with animals found dead. Microscopic examination, which is the unique postmortem method available, would allow to diagnose otherwise missed EGI cases.

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SOX9 EXPRESSION IN FELINE FIBROADENOMATOUS CHANGE

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Fibroadenomatous change (FAC) is a progesterone-responsive non-neoplastic proliferation of the mammary gland of the cat characterized by sudden, rapid onset. High Ki-67 proliferative index has been reported in FAC in several studies (Millanta et al., 2002) and an important role of autocrine and/or paracrine production of growth hormone and insulin-like growth factor has been suggested in its pathogenesis (Ordás et al. 2004) however, many pathogenetic aspects have not been clearly elucidated yet. Recent studies indicated that SOX9 transcription factor, in cooperation with Slug, controls the mammary stem cell state (Guo et al., 2012) and that increased ductal branching may be observed in transgenic mice overexpressing SOX9 in mammary epithelium (Wang et al., 2013).

To gain insight into the role of SOX9 in FAC development, we analyzed by immunohistochemistry SOX9 expression in FAC, non-FAC hyperplastic/dysplastic mammary lesions and normal mammary tissue of cat.

Materials and methods: Sections from FFPE tissue blocks of surgical biopsy samples of 10 FAC, 6 non-FAC hyperplastic/dysplastic mammary lesions and 3 normal mammary gland from female cats were examined for SOX9 expression by ABC immunostaining method, using a polyclonal rabbit serum produced with a polypeptide with 96% homology with Felis catus Sox9. Intensity was scored as negative, weak, moderate, strong. Percentage of reacting cells was evaluated by counting 1000 cells.

Positive SOX9 immunostaining of variable intensity and percentage was seen in all the samples analyzed and was essentially nuclear or in a few cases nuclear and weakly cytoplasmic. In normal mammary gland SOX9 was detected both in epithelial and in myoepithelial cells. Percentage of positive cells ranged from 33.2% to 55.4% (mean value 40.5%). Sox9 positive staining was seen only in a few stromal cells. Intensity was strong in one case and moderate and in 2 cases. In non-FAC hyperplastic/dysplastic lesions staining was intense and percentage of positive cells varied from 60.3% to 71.9% (mean value 66.4%); Sox9 positive staining was infrequent in stromal fibrocytes. All FAC samples showed moderate to strong SOX9 expression both in glandular and in stromal tissue; positivity ranged from 81.6% and 94.5% (mean value 86.5%) in ductal cells and from 51.4% and 86% (mean value 75.1%) in stromal fibrocytes.

This study provides evidence that in feline mammary FAC both glandular and stromal cells express high levels of SOX9. A possible role of SOX9 in tumor development and progression has been suggested however FAC is a non-neoplastic, benign mammary disease and the high-level expression of SOX9 observed in this condition should not be regarded as indicative of neoplastic transformation. Various studies suggest now that SOX9 plays multiple important roles in branching morphogenesis of several organs (Furuyama et al., 2011; Reginensi et al., 2011; Rockich et al., 2013), we hypothesize therefore that also in FAC SOX9 drives branching morphogenesis by controlling proper balance between proliferation and differentiation.

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ROLE OF NECTIN-4 IN CANINE PROSTATE CANCER

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Aggressive cancer cells are characterized by the ability to self-aggregate in order to survive and proliferate when an appropriate matrix anchorage is lacking. PVRL4 (poliovirus-receptor-like 4), also called Nectin-4, is a component of the E-cadherin-based adherens junctions in epithelial cells and potent mediator of the anchorage-independent colony formation in normal epithelial as well as cancer cells. Clusters of circulating tumour cells (CTCs) have been identified in blood samples of several tumours including prostate cancer (1,2). Targeted therapies aiming to block such cell-cell contacts may represent a novel anticancer treatment approach.

In dog, Nectin-4 expression has been only evaluated in relation to the Morbillivirus infection (3). Since Nectin-4 is a well-known tumour-associated histological and serological marker for several types of adenocarcinoma (lung, breast and ovary) (3) and it is expressed in a human prostate carcinoma cell line derived from a lymph node metastasis (4), we evaluated its expression pattern in canine prostate tissue to understand Nectin-4 role in prostate cancer pathogenesis.

The study was carried on formalin-fixed, paraffin-embedded samples from 42 canine prostate tissues including 2 normal prostates, 10 benign prostatic hyperplasia (BPH), 28 prostatic carcinomas (PCa), 1 pulmonary and 1 lymph node metastatic lesions. Immunohistochemistry was performed using a primary antibody specific for Nectin-4 (1:70). Nectin-4 expression was classified as membranous or cytoplasmic; samples were grouped in four categories based on the number of positive epithelial cells : absent (0%), low (0-30%); moderate (30-80%), high (>80%). The labelling intensity was recorded as weak, moderate,or strong.

Nectin-4 expression pattern showed a progressive loss during malignant progression and a switch in distribution from membranous to cytoplasmic. No immunostaining was observed in solid undifferentiated tumours. In particular, normal and BPH prostates showed high membranous distribution associated with low cytoplasmic positivity in BPH. In PCa samples, a low to high membranous distribution and low to high cytoplasmic positivity was observed, whereas metastatic cells, both in the lymph node and in the lung, exhibited a moderate/high membranous distribution and moderate cytoplasmic positivity. These results suggest the involvement of Nectin-4 in the CTC migration and maintenance during prostate cancer metastatization; furthermore, its loss of function in primary PCa may support the presence of the clivated form of the protein in canine serum (5) and thus its possible application as a serological marker in dog.

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ANGIOSTRONGYLUS VASORUM IN DOGS FROM CENTRAL ITALY, WITH THE DESCRIPTION OF THREE CASES OF DISSEMINATED INFECTION

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Angiostrongylus vasorum is a worldwide distributed nematode living in the pulmonary arteries and right heart of dogs and wild carnivores (1), with red fox considered its reservoir (2). Infection in dogs can be totally asymptomatic or cause respiratory and circulatory disorders (3). Migration of first stage larvae with dissemination to different organs can occur (2, 4).

In the last few years many clinical and pathological cases have been described in central Italy. Due to this increasing number of reports, a retrospective study on dogs from central Italy was performed, focusing on its prevalence and on the occourrence of disseminated infection.

Between January 2009 and February 2015, among dogs coming from Lazio region and submitted to IZSLT for necropsy, 433 cases were selected according to the following features: gross examination of heart and lungs and subsequent histopathology at least on lungs.

At necropsy a chronic, moderate to severe, locally extensive or diffuse pneumonia was noted in 8 cases (1.85%); histopathology revealed adults and larvae of A. vasorum in all lungs. In 4 cases (50%), pneumonia had a clear granulomatous appearance suggesting parasitic etiology, while in the remaining cases it was described as hardening of locally extensive areas of tissue with reddish discoloration, or with aspects of suppurative infection. Three dogs (30%) had adult parasites in the right ventricle .

In 3 cases histology confirmed the disseminated nature of infection, revealing larvae in multiple tissues (3/3 brain; 2/3 kidney and liver; 2/2 heart; 1/2 spleen). In 2 out of 3 cases (66%), adults in the right ventricle were associated with disseminated infection and in the third case, a focal hemorrhage with trombosis in the cerebral ventricles, with no evident intralesional larvae, was observed. In 6 cases (75%) gross pulmonary findings were severe and considered the cause of death; in remaining cases a severe hemoperitoneum due to traumatic liver rupture or cerebral hemorrhage occurred. Histopathology detected no A. vasorum parasites in other lungs of dogs with pneumonia.

Anatomohistopathological examinations revealed 8 cases of A. vasorum infection, with a prevalence of 1.85%, comparable to those described in other works on asymptomatic animals (5, 6) and higher compared to what described in northern Europe (7, 8). Gross pulmonary lesions observed on dogs were severe and frequently considered as cause of death. A. vasorum has not be observed without gross pulmonary findings, conversely to what described in wild foxes (9) Interestingly, disseminated infection was found in a high prevalence of cases (37.5%); this finding can be related to a different host/parasite relation, either for the increasing presence of the latter in specific areas, for changes in its virulence (10) or in case of host immune depression.

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HSP90 IMMUNOEXPRESSION IN CANINE CUTANEOUS EPITHELIAL AND MELANOCYTIC TUMOURS

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Heat shock protein 90 (Hsp90) is involved in the regulation of several biological processes such as cell signaling, proliferation and survival and guarantees the correct folding, functions and localization of numerous key proteins. Thus, Hsp90 inhibition has the potential to affect multiple signaling pathways that frequently contribute to the tumour development and progression2, explaining the recent and increasing interest in this molecule as a potential target for cancer therapy.1 Aim of the present study was to investigate the immunohistochemical patterns and levels of expression of Hsp90 in normal canine skin and cutaneous neoplasms, in order to understand the potential therapeutic application of a Hsp90 inhibitor in these types of tumour. Formalin-fixed, paraffin-embedded samples of 11 squamous cell carcinomas (SCCs), 30 follicular tumours, 8 melanocytomas and 10 melanomas were analysed using a streptavidin-biotin-peroxidase method. A semi-quantitative analysis of the immunoreactivity and Fisher's exact test were used to evaluate the associations between the examined parameters.

SCCs showed an increased cytoplasmic staining of neoplastic cells compared with surrounding normal epidermis, more intense in the outermost layers, with rare nuclear staining. Most of the follicular tumours showed an intense cytoplasmic staining, that was drastically reduced in the infiltrating cords and small clusters of neoplastic cells in the malignant cases analysed (pilomatricoma, trichoepithelioma), where it was associated with an increased nuclear staining. Half of the cases of melanocytoma showed a complete absence of immunostaining, while in most of the melanomas cytoplasmic Hsp90 was highly expressed, with a low to moderate nuclear expression. High levels of cytoplasmic Hsp90 immunostaining (>50% of positive neoplastic cells) were significantly related with malignancy in canine melanocytic tumours.

The present work demonstrates the expression of Hsp90 in the majority of the cases evaluated, indicating a role of the molecule in the development of canine cutaneous SCCs, hair follicle and melanocytic tumours. The diffuse and intense immunostaining observed in SCCs and the significant correlation of Hsp90 expression with malignancy in melanocytic neoplasms, would indicate Hsp90 as a possible molecular targets in the anti-cancer therapy, as suggested by recent experimental studies on non-melanoma skin cancer in murine models3,4, as well as in human melanoma cell lines.5 Interestingly, a partial response to the therapy, with marked decrease in size of the mass in an aggressive oral malignant melanoma of a dog was observed following treatment with the Hsp90 inhibitor STA-1474.6 These data and our results suggest Hsp90 as a potential effective target in canine anti-cancer therapy.

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UNUSUAL MULTIFOCAL PULMONARY NEOPLASTIC LESIONS IN A CAT

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An 8 years old castrated male stray cat, daily fed and looked after by feral cat caretakers was found death near the feline colony where it lived. The body was referred to the University of Milan for the necropsy. The cat was severely dehydrated, had lost incisives with severe gingivitis and abundant tartar accumulation and presented a shrunken, reduced in volume, left eye (phthisis bulbi). The most relevant alterations, affecting the abdominal and thoracic organs, were renal papillary erosion and necrosis associated to irregular renal profile with severe scarring and yellowish, pale cortex with numerous cortico-medullary strikes, and severe pulmonary atelectasis of the caudal lobes. Two pearly white, rounded, flat lesions, less than 1 cm in diameter, were detected in the left and right caudal lobes. Histologically, pulmonary lesions consisted of numerous, well circumscribed, not encapsulated nodules composed of irregularly arranged, tubular and/or dilated acinar structures, lined by a single layer of tall, columnar epithelial cells with abundant clear cytoplasm and basally located nuclei. Mitosis were less than 1 for HPF and anisokaryosis and anisocytosis were mild. Renal lesions were bilateral and diffuse. Histologically, they consisted of ulceration and necrosis of the papilla, numerous perivascular to interstitial aggregates of lymphocytes and plasmacells associated to severe interstitial fibrosis, glomerular synechiae/sclerosis and tubular degeneration, necrosis and mineralization. A diagnosis of end stage kidney, the most likely cause of death, was posed.

Immunohistochemical investigation for thyroid transcription factor-1 (TTF-1), AE1/AE3 cytokeratins (CKs), CK5, smooth muscle actin (α-SMA) and histochemical staining with PAS and alcian blue (AB) (pH 2.5) was performed. Neoplastic glands were diffusely positive for CKAE1/AE3, CK5 and PAS. AB staining was faint and multifocal, while TTF-1 and α-SMA were negative. Based on histological and immunohistochemical findings, a diagnosis of mucus gland adenoma was formulated. Lung tumors, namely bronchial gland carcinoma, bronchiolo-alveolar tumors and squamous cell carcinoma, have been extensively reported in cats, even though they are overall considered rare tumors. Conversely, to the authors' best knowledge, pulmonary mucus gland adenoma has not been reported to date in the feline species. In human beings, mucus gland adenomas are extremely rare tumors, arising mostly within the main, lobar or segmental bronchi and more rarely in the lung periphery. They are often endobronchial and multicystic, causing signs and symptoms of obstruction.

The present report described the first case of peripheral lung nodules arising from the submucosal mucinous gland in a peripheral small airway in a cat, an unusual and rare benign lesion that shares many similarities with the human counterpart.

NASAL CARCINOSARCOMA IN TWO DOGS

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Carcinosarcoma (CS) is a rare tumor composed by two cell types, epithelial and mesenchymal, both showing features of malignancy. It is rarely reported in animals, mostly in canine mammary gland1and more rarely in the head2, thyroid gland3and other organs. Aim of the work is to describe two cases of nasal carcinosarcoma, never previously reported in the dog. Meterials and methods: CASE 1: a 7 years-old, male, crossbred dog was examined clinically for persistent bloody nasal discharge. X-ray examination and computerized tomography scans showed a mass lesion in the right nasal cavity. The cytological exam revealed two types of cells: huge clusters of epithelial cells and single spindle cells admixed with inflammatory cells and scattered osteoclasts. Histological examination revealed a biphasic neoplasm composed by solid trabeculae of epithelial cells with moderately abundant eosinophilic cytoplasm, oval nuclei and multiple prominent nucleoli; the second tumor type was composed by polygonal cells occasionally embedded in a eosinophilic amorphous extracellular osteoid matrix with multifocal mineralization and bone remodelling. Anisocytosis and anisokaryosis were marked in both the populations and the mitotic activity was high. A final diagnosis of carcinosarcoma was done (transitional cell carcinoma and osteosarcoma). The dog was euthanized and submitted to complete necropsy, that failed to show any secondary neoplastic lesion. CASE 2: a 6 years-old, male neutered, crossbred dog was referred for a catarrhal-hemorrhagic nasal discharge with sneezing and noisy breathing. Rhinoscopy revealed an exophytic tumour filling the left nasal cavity and extending till choanae. Histological examination of endoscopic biopsies revealed a biphasic tumor composed by solid lobules of epithelial cells admixed with a more undifferentiated tumor composed by polygonal to spindle cells with multiple areas of osteoid deposition and mineralization. Anisocytosis and anisokaryosis were moderate in the epithelial component, marked in the mesenchymal part of the tumor. A final diagnosis of carcinosarcoma (undifferentiated carcinoma and osteosarcoma) was made. The owner refused any therapy other than palliative treatment with FANS and prednisone, prolonged until euthanasia 3 month after the diagnosis. No secondary lesions was suspected based on clinical examination, but necropsy was not done. In both cases the biphasic nature of the tumor was confirmed by immunohistochemical examination with cytokeratin and vimentin, that stained respectively the epithelial (carcinomatous) and mesenchymal (osteosarcomatous) portion of the tumor. Carcinosarcoma in dogs is rarely reported; mammary carcinosarcomas are highly malignant with reported metastatic rates up to 100%1; other sites are thyroid gland3 and head (frontal skull and maxilla): in this latter the tumour appears to be less aggressive, with no metastases in 4 cases reported2. None of these two cases of nasal carcinosarcoma were associated with evident metastatic disease, even if complete necropsy was done only in one case. Further studies are needed to assess the biological behaviour of nasal carcinosarcoma in the dog.

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Part of this work was performed during the activity of "Registro Tumori Animali" of Umbria region

CANINE ORBITAL PSEUDOTUMORS: A REVIEW OF 9 CASES

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The term orbital pseudotumor was introduced by Birch-Hirschefeld (1905) to describe a space-occupying orbital mass grossly consistent with a neoplasia but histologically composed of a mixed inflammatory infiltration. More recently the definition idiopathic orbital inflammation -(IOI) hasbeen proposed for this entity. Aim of this study is to review and immunohistochemically characterize 9 cases of canine orbital lesions previously diagnosed as orbital pseudotumors.

Six cases were from Veterinary Pathology Archive (University of Milan), 4 cases were provided by Dr Dubielzig (COPLOW, US). Microtomic section were Hematoxylin and eosin stained and immunolabelled with antibodies anti vimentin, alpha smooth muscle actin (α-SMA), MHCII, lysozyme, CD3 and CD20.

Affected dog were aged 4.5 to 13 years (mean 7.7); 5 males/4 females. Different breeds were represented. Three pseudotumors affected the dorso-lateral portion of the orbita with lacrimal gland infiltration in 2/3. Six pseudotumors were located deep in the orbita without connection with any specific structures. A nodular mass, with focal infiltration of surrounding tissues, was surgically removed preserving the eye in 6/9 cases. In 3/9 cases the mass required orbital exenteration. Histologically, all lesions were composed of a mixed inflammatory cell population: in 6/9 macrophages/histiocytes predominate, with a variable number of lymphocytes, scattered plasmacells, neutrophils, large fibroblasts and occasional eosinophils (granulomatous pattern). In 3/9 cases, macrophages/ histiocytes, lymphocytes and plasmacells were almost equally represented, with fewer granulocytes. In 2/3 cases, affecting the lacrimal gland, large bundles of dense, collagen rich fibrous tissue, were evident. These cases were histologically consistent with the sclerosing form of human IOI. Immunohistochemically, histiocytes were consistently MHCII stained, scattered macrophages were lysozyme positive . Lymphocytes T (CD3) and B (CD20) were always present, CD3+ T cells predominating in 5/9 cases. In 2 cases sclerosing IOI-like pseudotumors, fibroblasts were α-SMA and vimentin stained (myofibroblast), fibroblast in granulomatous pseudotumors were α-SMA negative/vimentin positive.

In the present review of canine orbital pseudotumors, all lesions were consistent with an idiopathic inflammation of orbital soft tissues. The authors propose that, consistently with human medicine, the definition "idiopathic orbital inflammation (IOI)" is adopted. Granulomatous inflammation was the most common histological-type in dogs. This pattern was histologically and immunohistochemically strikingly similar to another idiopathic orbital condition: canine nodular granulomatous episcleritis (NGE). Further study could elucidate if NGE and granulomatous-IOI are actually the same disease in different locations. In 2 cases pseudotumors affected the lacrimal and were associated with prominent fibroblastc/myofibroblastic proliferation. In men it has been suggested that these cases could represent chronic dacryoadenitis and that the release of lacrimal secretion could induce fibroblast/myofibroblast proliferation.

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INTRANUCLEAR GLYCOGEN IN OXYNTIC CELLS IN CANINE GASTRIC BIOPSIES

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Foreign material within the nuclear contour seen in histological sections is referred as inclusion (I) or pseudoinclusion (PI), depending on the absence or presence, respectively, of an infolding of the nuclear membrane around the material. Intranuclear glycogen is un uncommon finding described for the first time by Ehrlich in 1883 in hepatocytes of diabetic patients; afterwards this finding was reported in many other diseases (1,2), as well as in the liver of healthy individuals (3).

In animals intranuclear glycogen has been observed in liver of cows(4), of animals with chronic pyrrolizidine alkaloid poisoning(5), of western barred bandicoot with papillomatosis and carcinomatosis syndrome(3) and in few other conditions. Pathological significance of this lesion is unclear. To describe, to investigate the nature and to speculate about the pathological meaning of an histological finding observed in stomach of dogs during routine diagnostics, morphologically consistent with nuclear glycogen inclusions/pseudoinclusions (I/PI) in oxyntic (parietal) cells, never reported in literature in our knowledge.

Samples from 107 dogs submitted to endoscopy because of gastrointestinal clinical signs were routinely processed and evaluated at light microscopy for histological lesions. The samples with nuclear lesions in parietal cells, and an equal number of samples without histological lesions, were stained with PAS with and without diastase pre-treatment. Data were used to evaluate the associations between the presence of nuclear I/PI and signalment / histological diagnosis.

We found nuclear lesions in parietal cells consisting in enlargement of the nucleus with chromatin margination and central pale or slightly eosinophilic area with sharp contours; these nuclear I/PI were observed in scattered cells ranging from occasional (0-1/hpf) to numerous (4-5/hpf). The lesion was detected in 24 dogs and in 19 cases this finding was associated with gastritis, mainly lymphoplasmacytic, of mild severity, while in 5 cases there were no lesions. The nuclear I/PI showed PAS positivity (10/13) and diastase sensitivity (5/10), consistently with the typical pattern features of glycogen. In samples with nuclear lesions generally the cytoplasms were slightly PAS-positive, but did not show diastase sensitivity. We did not find a statistically significant association between gastritis and the presence of nuclear I/PI ($P > 0.05$). Possible pathogenetic mechanisms of glycogen accumulation within the nucleus are discussed: some authors stated that glycogen could be translocated from the cytoplasm through nuclear pores, others suggest the possibility of a pseudoinclusion; otherwise glycogen could also be synthesized in interchromatin regions of the nucleus. Further studies are needed (specifically, TEM examination is ongoing) to better determine the nature, pathological and functional significance of this finding.

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STEM CELL MARKERS IMMUNOEXPRESSION IN CANINE CUTANEOUS MELANOCYTIC TUMOURS

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Nestin and SOX9 were identified as specific markers of melanocytic stem cells. Nestin is a class IV intermediate filament, mainly expressed in the cytoplasm of neuroepithelial stem cells and in developing yet not differentiated endothelial cells of blood vessels. Nestin expression was increased in numerous cancers. In many tumors, such as melanoma, nestin has been identified as a prognostic factor. Transcription factors belonging to the SOX family have been shown to have a role in the survival and migration of oligodendrocyte precursors. A SOX family protein, SOX9, has been demonstrated to interact with SOX10 and BRN2 in melanocytic differentiation and to be strongly expressed in pigmented cells of cultured melanomas. Nestin, SOX9, BRN2 and SOX10 were found to be strongly expressed in primary and metastatic melanomas in humans, while the levels of expression of these molecules in melanocytic nevi were much lower. In particular nestin and SOX9, respectively, were associated with the presence of ulcerations in primary tumors and with a more advanced stage of disease progression and therefore considered as negative prognostic markers.

The aim of our study was to investigate whether these two markers could have a similar prognostic significance, through the correlation of their immunohistochemical expression levels and histologic features of malignancy. A total of 31 melanocytic tumors were included in the present study: 8 melanocytomas and 23 melanomas (4 metastatic melanomas; 9 amelanotic melanomas; 10 pigmented melanomas). Tumors were investigated by immunohistochemistry using a specific rabbit polyclonal anti-human SOX9 and a mouse monoclonal anti-human nestin antibody. SOX9 antibody is reactive with the dog, according to manufacturer's instructions and nestin antibody has already been used in two studies in the dog. With few exceptions, almost all melanocytic neoplasms investigated showed an absent or very low reactivity of neoplastic cells for both markers used. However the positivity of both external and internal positive controls used (hair follicles) confirmed the validity of these markers as putative stem cell markers in the dog, similar to what is described in humans. In particular, SOX-9 positivity was present in <5% of neoplastic cells in only three cases, while nestin immunoreactivity was noted in 5 cases. Three of these five cases, all represented by melanomas, were characterized by a percentage of positive neoplastic cells ranging from 40 to 60%. In general, the nestin-positive cells were located at the periphery (the invasive front) of the tumor. In one of these cases (metastatic melanoma), the reactivity was present both in the primary tumor as well as its metastases (lung, pancreas, adrenal). None of melanocytomas was positive for nestin. In conclusion, the results of this study in the dog, different from what is described in human medicine, dampen the use of nestin and SOX9 as valid prognostic negative markers in canine melanocytic tumors. However, the positivity for nestin in rare cases of melanoma, in the face of a total negativity for this marker in melanocytomas, would lead to speculate that this molecule may be involved in the process of malignant transformation of melanocytic cells. Further studies are needed to investigate this hypothesis.

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EFFECT OF GROWTH PROMOTERS ON APOPTOSIS PATHWAY OF VEAL CALVES TESTIS

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During spermatogenesis, apoptosis is an essential physiological event that controls the number of germ cells. Androgens and estrogens play an important role in the regulation of homeostasis and cell death in testicular cells [1-2], even if a long-term exposure to these molecules may alter the balance between cell survival and apoptosis. There are two main pathways involved in the apoptosis: the extrinsic pathway triggered by the activation of death receptors on the cell surface and the intrinsic pathway activated in response to signals originated from the inside of the cell. The intrinsic pathway involves molecules belonging to the BCL2 family. Both these pathways converge at the level of caspases. The aim of this study was to investigate the expression of some genes, involved in the apoptosis pathway, in testis of veal calves experimentally treated with growth promoters (GPs).

Forty Friesian veal calves, 6 months old, were randomly assigned to 5 experimental groups: group A (n=8) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.25 mg/die of brotizolam for 31 days; group B (n=6) treated with 5 mg/week of estradiol benzoate for 6 weeks and 0.4 mg/die of dexamethasone (DEX) for 31 days; group C (n=8) treated with 150 mg/2 weeks of Nandrosol for 4 weeks and 80 mg/die of ractopamine for 31 days; group D (n=8) treated with 15 mg/die of prednisolone (PRD) for 31 days; group K (n=8) was untreated. The animals were slaughtered at 3 days after the last treatment.

Samples of the testis were collected from each animal. Quantitative PCR (qPCR) of APAF1 (apoptotic peptidase activating factor 1), AVEN (apoptosis, caspase activation inhibitor), BAX, BCL2 and CASP3 (caspase 3) mRNA was performed. Statistical differences were determined by ANOVA, followed by Dunnett's post test. AVEN expression was significantly up-regulated in group A ($P<0.01$), whereas CASP3 expression was up-regulated both in group A ($P<0.05$) and in group C ($P<0.05$). No effect on APAF1, BAX and BCL2 expression and BAX/BCL2 ratio has been detected.

Estrogens and androgens are known to play a critical role in preventing apoptosis in a wide range of cell types. It has been reported that estradiol or DHT (5Îś-dihydrotestosterone) treatment increases the anti-apoptotic BCL2 levels and decreases the expression of both BAX and CASP3, two pro-apoptotic proteins [3]. Conversely, the hormone deprivation causes an increase of BAX expression, a decrease of BCL2 expression and the activation of caspases [4]. Our results point out a balance between BAX and BCL2 expression, whose ratio is often used as a biomarker for the apoptosis detection. Thus, the intrinsic pathway seems to be not involved, but the increase of CASP3 expression following estrogen or androgen administration suggests an increment in the apoptotic signalling mediated by extrinsic factors. AVEN is known to inhibit the apoptosis pathway [5]. The AVEN up-regulation observed in this study could be due to the interruption of the hormonal treatment. Therefore, these findings may be explained considering the withdrawal time. Indeed, in this period the estrogen and androgen protective effect probably declines, resulting in the increase of the downstream effectors of apoptosis.

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A CASE OF WARTY DYSKERATOMA IN A DOG

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Warty dyskeratoma is an uncommon human benign tumour described for the first time in two dogs by Hill (1987). It is a dermal mass with a nodular, cup-shaped or cystic architecture1, with an umbilicated center opening on the skin surface; when cystic, it is filled with keratin and cellular debris. Multiple lesions are rare2. Histologically, nodules are lined by squamous mature epithelium with foci of acantholysis and dyskeratosis3, and the lower portion shows dermal papillae resembling intestinal villi2. Some authors proposed a follicular origin because of its positivity to anti-keratin antibodies for cortex and inner root sheath of normal hair follicle4, but the localization of lesions in oral and genital human mucosa makes the pathogenesis confused5. An association with viruses (i.e. Papillomavirus) failed to be demonstrated2. The main differential diagnosis is acantholitic squamous cell carcinoma1,3. As warty dyskeratoma is a rare disease in dogs, with only 5 descriptions in veterinary literature, the aim of our work is to describe a new case. A 4-year-old crossbreed dog was presented to the clinician for a partially exophytic, dermal mass of 16 x 13 x 5 cm at scapula-humeral joint, without bone invasion. The mass was sampled in two different locations and the biopsies were submitted for histopathologic examination. Samples were routinely processed and observed at light microscopy; special stains were used (i.e. PAS, Gomori's trichrome and Giemsa) and IHC techniques (anti-keratin and anti-laminin antibodies) were applied to characterize the lesion. Histologic examinations showed the presence of multiple dermal cystic structures lined by a mature squamous epithelium with foci of acantholysis, often creating suprabasal clefts, as well as keratinocytes apoptosis and dyskeratosis; the lower portion showed dermal papillae lined by a single layer of basal cells resembling intestinal villi. Cystic structures were surrounded by an abundant fibrous stroma. Numerous neutrophils and multiple foci of mineralization were observed in these structures; occasionally, cystic rupture was associated with macrophagic and neutrophilic inflammation around epithelial elements. Anisocytosis and anisokaryosis were mild and mitotic index was low (4-5/10 hpf). PAS staining for fungal elements was negative. Immunohistochemistry with anti-cytokeratin and anti-laminin antibodies confirmed the epithelial nature of the lesion and the integrity of the basement membrane, respectively.

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COMPARATIVE ASSESSMENT OF CYTOLOGICAL VERSUS HISTOPATHOLOGICAL BIOPSIES IN THE DIAGNOSIS OF CANINE OSTEOLYTIC BONE LESIONS

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Primary bone tumors account for 2-5% of canine malignancies. Affected dogs often present with typical radiographic changes including cortical bone lysis, sclerosis and periosteal reaction. Osteosarcoma is the most common primary bone tumor in dogs. Being an extremely aggressive tumor, it should be differentiated from other less common tumor and from tumor-like lesions, such as fibrosarcoma, chondrosarcoma, hemangiosarcoma and osteomyelitis, as therapy and prognosis can vary greatly. Accordingly, a histological diagnosis is generally preferred before surgery, requiring general anaesthesia and collection of bone samples, with possible complications such as pathological fractures. An early and accurate diagnosis obtained by lesser invasive methods should be important to decrease patient discomfort and allow owners to make informed treatment decisions. Nevertheless, there is a paucity of information regarding the utility and accuracy of aspirate cytology of bone lesions in dogs.

Aim of the work/Objectives - The purpose of this study was to compare the diagnostic accuracy of cytological and histopathological biopsies of bone lesions in dogs with the definitive diagnoses performed by the histology on surgical samples.

A computer search of canine medical records at the Department of Veterinary Medical Sciences, University of Bologna, from January 2000 to present identified 41 cases of bone lesions that were sampled for cytology by fine needle aspiration (n = 21) or by incisional biopsy for histology (n = 20). Seven cases were sampled by both methods. The accuracy of both methods was assessed by comparing the former diagnosis with the final histological diagnosis on surgical samples or post mortem samples, when applicable.

The examined case series included 18 primary bone tumors, including osteosarcomas, chondrosarcomas, giant cell tumors, 4 carcinoma metastases and 12 non-neoplastic lesions, including osteomyelitis, osteonecrosis and reactive bone. Accuracy was 85% for cytology (86% for tumor lesions and 83% for non-tumor lesions) and 80% for histology (75% for tumor lesions and 87.5% for non-tumor lesions). Cytology correctly identified the tumor histotype in 6 out of 11 cases (54.5%).

The results of this study indicate that fine needle aspiration cytology is a reliable technique in the diagnostic work up of bone lesions in dogs. Accuracy is higher for neoplastic lesions compared with non-neoplastic lesions. Among bone tumors, cytology is moderately effective in distinguishing osteosarcoma from other tumors. Being an efficient, inexpensive and minimally invasive technique, cytology should be further considered to aid decision making in the preoperative setting of aggressive bone lesions.

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

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INTRODUCTION - Osteoarthritis and articular degeneration are the most important causes leading to a poor life quality both for patients and the owners. Many approaches had been developed in veterinary medicine and in the last decades clinicians are paying attention to infiltrative substances such as non steroid anti-inflammatory or steroid drugs, hyaluronic acid and blood derivates. One of the latter is platelet rich plasma (PRP), whose use is increasing but lacking of evidences and standardized procedures of preparation and administration. Its regenerative potential had already been established in human medicine, both in vitro and in vivo, and numerous experimental animal in vitro studies had been performed suggesting a promising outcome (1). Therefore in vivo studies are required in order to assess a real efficacy of PRP, a safety and relatively easy clinical application (2). AIM-The aim of this study is to point out the effect of a single PRP injection in osteoarthritic joints of dogs in a period of three months examining clinical and radiographical scores, other than owners satisfactory grade. MATHERIALS AND METODS-Autologous PRP preparation - Autologous anticoagulated venous blood samples were centrifuged twice, and platelet pellet was than resuspended in platelet poor plasma (PPP) at a final concentration ranging from 7 to 10 folds above whole blood platelet count under aseptic conditions .Patients - Six dogs (3.6 Âś 2.56 years old, body weight 35.05Âś16 Kg) with osteoarthritis involving a single joint were enrolled. All patients underwent a general visit and serum blood analysis. After sedation and intravenous anaesthesia, the autologous PRP was injected intra-articularly, after a clinical evaluation of synovial fluid aspect. Clinical and radiographical evaluations - Patients underwent a clinical evaluation (lameness, clinical objective assessement, response to manipulation) using standard tables with three grades at 0, 15, 30, 60 and 90 days from the infiltration. Radiographs of both the affected joint and the normal one were made at day 0, 30, 60 and 90 from injection. Projections for elbows were lateral standard at 130Â°,at 90Â°, at 45Â°, at maximum extension, and standard antero-posterior and antero-posterior with 15Â° of pronation; for other joints, standard lateral antero-posterior and postero-anterior were made. Same radiographical parameters were maintained during the study. Radiographs were evaluated following the International Elbow Working Group graduation by two radiologist blinded to the study. Satisfactory grade of the owners were assessed using the Liverpool Osteoarthritis in dogs questionnaire at the first visit and at day 90 of the follow up. CONCLUSIONS -An overall positive effect of the autologous treatment was observed in all dogs. Radiographical scores showed no statistical difference between all time points of the study, suggesting a stability of both affected and normal joints. Clinical lameness scores at 90 days were significantly different from those observed at 0 and 15 days ($p<0,036$); while clinical objective assessements and manipulation response were significant between day 0 and 90 days ($p<0,05$). All owners' questionnaires indicate a reduction in lameness and pain. BIBLIOGRAPHY - 1) Everts PA,et al. J Extra Corpor Technol. 2006 Jun;38(2):174-87. 2) Fahie MA et al. JAVMA, 2013;,243: 1291-97.

A PATHOLOGICAL SURVEY ON SICILIAN RAPTORS

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This study was carried out on 20 raptors obtained from some regional recovery centres. Birds have been collected during the years 2013 and 2014 and were stored frozen at -20 C. Later specimens were subdivided per species as follows: 8 buzzards, 3 kestrels, 3 honey buzzards, 1 marsh harrier, 1 lesser kestrel, 1 red-footed falcon, 1 owl, 1 barn owl and 1 horned owl. Necropsies were performed and tissue samples were obtained from all organs and tissues for histology and molecular biology. At necropsy undigested food with abnormal dilation of oesophagus and stomach was found in 8 subjects. In a buzzard, several granulomatous changes with a cavernous core were detected at the abdominal and thoracic air sacs, as well as in different coelomic organs. Grocott's staining showed several dichotomous and septated black hyphae; molecular exam confirmed perfect homology with Aspergillus niger. A metallic foreign body (steel nail, 20mm x 3mm) was found driven in an abdominal air sac. A lung mycetoma due to Candida sp. was found in a lesser kestrel. In another buzzard, 3 parasites identified as acanthocephala belonging to the species Centrorhynchus globocaudatus. Several trematodes belonging to the species Physaloptera alata were found in gizzard of a kestrel. 4 nematodes belonging to the species Dispharynx nasuta were found in a horned owl, fixed to the gizzard cuticle which showed erosion and inflammation. Finally, in another buzzard a carpus-metacarpus join luxation with ulnar epiphyseal fracture was found; histological exam performed on lungs and liver showed several cartilaginous emboli in the blood vessels and some free in the parenchyma. Pathological findings here reported provide useful information considering the lack of data available in literature. The application of molecular exam to identify specific pathogens confirms the meaning of this diagnostic tool in routine pathological examination. The unusual localization of D. nasuta in the gizzard must be underlined. Finally, the presence of huge amount of undigested food within the entire gastro-intestinal tract of 8/20 raptors probably suggests the need of an improvement of animal care and animal welfare, as well as of health management in recovery centres, considering also the difficulties related to the various interspecific differences among the wild species present in the Sicilian area.