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Immunohistochemical study of a nonepitheliotropic cutaneous lymphoma involving mammary gland

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Introduction

An 8-years-old, female, german shepherd dog was referred to our Institution due to a recidive of a mammary gland tumor that was rapidly growing. The initial tumor was a non ulcerated nodule, excised 2 months ago by the referrer. Histopathological study had not been performed. Therefore, the owner had noted some days ago multiple skin nodules in the trunk and head. During examination, both mammary chains (3-4 right and 3-5 left mammary glands) were affected with firmness, erythema and thickening, without ulceration or pain. The skin nodules were also firm, erythematous, non ulcerated, and had a depressed centre. A fine needle aspiration was performed from skin nodules and from mammary gland lesions. The cells were large lymphocytes, with round nuclei and indistinct nucleoli. Cytoplasm was scant and slightly basophilic. No inflammation or plasma cell infiltration were seen. No neoplastic lymphocytes were present in peripheral blood.

Aim of the study

Cutaneous lymphoma is a rare neoplastic condition in the dog. The aim of this study was to determine the immunophenotype of these neoplastic cells.

Material and methods

Biopsy samples were taken from the skin nodules for histopathological and immunohistochemical study with the antibodies CD3, CD79 and HLAII, using the avidin-biotin-peroxidase complex method.

Results and conclusions

Histopathological and immunohistochemical study identified the lesion as a cutaneous nonepitheliotropic T-cell lymphoma. The initial presentation of this lymphoma as a mammary tumor is extremely rare. The tumor was biologically very aggressive and exhibited a rapid progression. Due to the poor prognosis the owner rejected any treatment and the dog died 2 weeks after diagnosis.

References

tion with normal saline was performed. Clinical and histopathological evaluations were performed in each group at 7, 14, 21 and 28 days after operation. According to results of this study, wound closure was accelerated in the experiment group in compare to control at days 14, 21 and 28 after honey application. Color and consistency of granulation tissue and epithelization were also more improved in experiment group from day 14. From histopathological aspect, in treatment group especially from the second week on, the compact connective tissues were developing from the base of the wound and a little sepsis was observed in some superficial points. In addition, less neutrophilic chemotaxis was found in treatment group than in the control group. Honey can be used as a natural substance to accelerate the healing process of the skin wounds.

Key words: non-boiled honey, healing of wounds, dog

Results
57 cats were included in the study. Mean age was 79 months (6-194), 30 were neutered females and 27 neutered males. All murmurs were systolic and varied in intensity from 2/6 to 5/6. The PMI was the left or right parasternal region in 24/57 (42%) of murmurs. Murmurs were caused by dynamic left ventricular outflow tract obstruction in 25/57 (44%) of cases, dynamic right ventricular outflow tract obstruction in 9/57 (16%) of cases and combined dynamic left and right outflow tract obstruction in 11/57 (19%) of cases. In 5/57 (9%) of cats no cause of the murmur could be identified. Heart disease was present in 50/57 (88%) of cats, namely left ventricular hypertrophy in 44/57 (77%) and congenital defects in 6/57 (10%) of cats.

Conclusion
Heart murmurs in apparently healthy cats can often be heard parasternally and are frequently caused by dynamic left and right ventricular outflow tract obstruction. A heart murmur is a reasonably reliable indicator of heart disease.

CAUSE OF HEART MURMURS IN 57 APPARENTLY HEALTHY ADULT CATS
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Introduction
Heart murmurs are caused by turbulent blood flow or by vibration of cardiac structures. Turbulent blood flow may originate from structural heart disease or from physiological phenomena. The aim of this study was to establish the cause of heart murmurs in apparently healthy adult cats and to determine whether a heart murmur is a reliable indicator of heart disease.

Materials and Methods
In this retrospective study we reviewed medical records from cats in which a heart murmur was heard during physical examination by one of the observers from January 2008 until December 2009. Cats aged < 6 months and with systemic disease were excluded. Timing, grade and point of maximum intensity (PMI) of the murmur was determined by a single observer (MJD) preceding 2D-, M-mode and Doppler echocardiography.

BACTERIAL FLORA OF THE CONJUNCTIVA IN NORMAL CAPTIVE BIRDS OF PREY
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Purpose: To identify the normal conjunctival flora in a Falconiformes order.

Materials and Methods: 8 birds of the Falconiformes order (7 belonging to the genus Falco and 1 to the genus Parabuteo) underwent ophthalmic routine examination as well.

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based on conventional, morphological and biochemical tests.

**Results:** All samples were negative to fungus and only 6 birds (75%) were positive to bacteria. Fifty percent of the animals contained a single species of bacteria. In total there were isolated and classified 11 species: 9 (82%) were Gram-positive and 2 (18%) were Gram-negative. The most common bacteria were Staphylococcus and Micrococcus, followed by Aerococcus and Pantoea. Staphylococcus spp. was isolated 4 out of 5 birds analysed (80%), while Micrococcus spp. was less widespread and it was found only in 2 (40%) animals.

**Conclusions:** The animals involved in this report are all captive birds. The preliminary results showed the prevalence of Gram-positive bacteria according to other studies on birds orders. Furthermore no records of fungi were detected. A relationship may exist between resident normal flora and the etiology of ocular infections but up to now there have been no studies. It might be interesting to investigate these events on infected birds.

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**Materials and methods**

Faecal samples were collected prospectively from 316 puppies of 32 breeding kennels and screened for canine coronavirus using real-time PCR. Puppies’ age, gender, breed, and the number of dogs in the kennel were recorded. For each puppy the faecal quality was scored using a 5-point numerical scale.

**Results**

A mean number of 10 puppies were sampled per kennel (range: 4–19). 30 % of puppies had gastrointestinal troubles. The prevalence of coronavirus infection was 53 % among the puppies and 85 % among the kennels. The virus was isolated in 54 and 52 % of puppies respectively with and without gastrointestinal problems. No significant association was detected between the level of excretion and incidence of digestive problem.

**Discussion**

Coronavirus is cited as a diarrheic agent in dogs, however no significant association was detected between coronavirus shedding and gastrointestinal problem in our study. This observation is in accordance with some studies [1]. This lack of association can be explained by the circulation of different strains with different levels of pathogenicity or the necessity of an association between the virus and other pathogens (parvovirus, giardia…) to induce clinical signs.

**References**

Introduction: Intervertebral disc degeneration (IVDD), in humans, is defined as “an aberrant, cell-mediated response to progressive structural failure”. In degenerating human intervertebral discs (IVDs) the glycosaminoglycan (GAG) content is negatively correlated (1) and the matrix metalloproteinase 2 (MMP-2) activity is positively correlated (2) to increasing grades of IVDD. There are many similarities in the pathogenesis of human and canine IVDD but the biochemical cascade of events during IVDD has not been investigated in dogs.

Objective: To quantify the GAG content and the MMP-2 activity in the nucleus pulposus of canine IVDs in different stages of degeneration and to correlate these levels with the gross morphologic appearance using the five-category grading scheme according to Thompson (3).

Material and methods: A total of 123 nuclei pulposi, obtained from intervertebral discs of 13 randomly selected dogs older than one year of age, were used. None of the 13 dogs was euthanized for reasons related to IVDD. For the quantification of the GAG content in the samples, the Farndale (dimethylmethylene blue) assay was used and to evaluate the MMP-2 activity, gelatin zymography was performed.

Results and conclusion: A general decrease in GAG content was observed with increasing grades of IVDD. For the MMP-2 activity there was an increase in activity up to Thompson grade III and thereafter the activity level decreased. These findings are in accordance with findings in similar studies on IVDD in humans.

References

MORE THAN 2 YEARS OF SURVIVAL TIME IN A DOG WITH SPLENIC HEMANGIOSARCOMA TREATED WITH METRONOMIC THERAPY.
López I, Guerrero F, Montes A, Diez E, Pineda C, Aguilera-Tejero E.

Introduction
Median survival time for dog with splenic hemangiosarcoma (HS) treated by surgery plus conventional chemotherapy was described no more than 10 months, even with the addition of immunotherapy. Recently one work in dogs with HS treated with continuous low-dose chemotherapy (etoposide + cyclophosphamide + piroxicam) showed both higher median survival time and disease-free interval compare with conventional chemotherapy alone. Here we present a case of dog with stage III splenic HS treated, after surgery, with conventional doxorubicin chemotherapy plus continuous oral low dose chemotherapy (cyclophosphamide + meloxicam) that is still alive and disease-free after 2 years.

Methods
Eight years old male cocker spaniel was diagnosed for stage III splenic HS, with confirmed metastasis in mesenteric lymph nodes. Two weeks after Splenectomy, the dog was treated with VAC chemotherapy protocol. After 5 cycles of VAC protocol, dog started oral low dose chemotherapy based on cyclophosphamide (10 mg/m2/EOD), metotrexate (2.5 mg/m2/twice per week) and meloxicam (0.1 mg/kg/daily). To prevent gastric problems, 20 mg capsules of omeprazole were also administrated daily.

Results
After 9 month of oral metronomic therapy, dog developed a moderated cytopenic episode and then oral therapy was discontinued. One month later hemogram normalized and dog came back to the same protocol of low dose chemotherapy, except metotrexate. Currently, more than 700 days after the surgery, dog is still enrolled in this oral therapy, and is doing well, no signs of disease are shown, and no new drug adverse effects are presented.

Conclusions
Continuous low dose oral chemotherapy (metronomic or antiangiogenic chemotherapy) combined with conventional chemotherapy can be very effective for treated dog with splenic HS.
SERO PREVALENCE OF ANAPLASMA PHAGOCYTOPHILUM IN DOGS IN TEHRAN, IRAN

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Anaplasma phagocytophilum is an obligatory intracellular gram-negative bacterium that causes anaplasmosis in human and numerous animal species, including horses, cats, dogs, ruminants, and wildlife. The organism is transmitted by tick and its infection has a worldwide distribution. Clinically, canine anaplasmosis causes subclinical to acute situation. Despite having high hygiene and health importance there is no data on seroprevalence of Anaplasma phagocytophilum in Iran. The purpose of this study was to determine the seroprevalence of A. phagocytophilum antibodies in dogs in Tehran. We selected 240 stray and owned dogs randomly from different area of Tehran province. After complete clinical examination, blood samples were collected for complete blood cell count, serum biochemical analysis and serological test. Buffy coat smears were made, stained with gimsa and microscopically examined for the presence of intracellular bodies. Serum samples were serologically tested against A. phagocytophilum by immunofluorescent antibody (IFA) test. Fifty dogs (20.83%) were seroreactive with A. phagocytophilum antigen. Ten percent of seropositive dogs were thrombocytopenic. Two seropositive dogs had the morulae within eosinophils and platelets. This is the first serological study for the prevalence of A. phagocytophilum in dogs in Tehran province. Although positive reactions against A. phagocytophilum were observed in this experiment but the possibility of cross reaction with A. platys can not be excluded. Therefore, further studies to determine prevalence of each infection using different approaches are recommended.

HISTOPATHOLOGIC AND HISTOMORPHOMETRIC EVALUATION OF THE PLATELET-RICH PLASMA (PRP) EFFECTS, ON FEMORAL CANCELLOUS BONE DEFECT HEALING IN RABBITS

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Abstract

Introduction: Platelet-rich plasma (PRP) is an autologous product that ability to carry a high concentration of growth factors, which are well known to stimulate different tissues healing. The purpose of this study was to determine the effect of PRP on healing of rabbit femoral cancellous bone defect.

Material and Methods: The experiment was conducted on 16 male adult New Zealand white rabbits which were divided into two groups of control and experiment (I month & II months duration) of 4 rabbits each. After induction of general anesthesia, a hole in size of 4x5 mm in diameter and depth was made using a dental bit in the inner aspect of the medial condyle of left femor. In control group, defect was left untreated and in experiment group, the created hole was filled with autologous PRP. Rabbits were sacrificed at 1 and 2 month after surgery and evaluated histopathologically and histomorphometrically.

Results: In control group, within 1 month, defect seemed to be filled with adipous tissue with sparse hematogenic cells. In 2 month, in spite of a poor osteogenic activity and some osteoblasts could already be seen. In experiment group, within 1 month, many osteoblast groupings, and neoformed vessels occupied the gap, within 2 month, young bone trabeculas increased in number and bone neoformation was more compact, with bone trabeculas more organized. Differences observed in histomorphometrically findings were significant between control group and experiment group.

Conclusions: The results of this study show that PRP provide a more rapid regeneration of bone defects.
COMPARATIVE EFFICACY OF TWO FIPRONIL SPOT-ON FORMULATIONS AGAINST REPEATED FLEA INFESTATIONS IN DOGS
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Introduction
The insecticidal efficacy of 2 fipronil spot-on formulations, Effipro® (Virbac) and Frontline® (Merial), was evaluated against challenge flea infestations in a randomized, blinded laboratory study in dogs.

Materials and Methods
Twenty-four mongrel dogs (7-18kg) free from any insecticidal treatment in the 12 weeks preceding the trial were housed in environmentally controlled rooms. They were infested with 100 fleas (laboratory strain of ) before treatment on Day-6 and Day-1. The dogs were randomly allocated to one of 3 groups: untreated control group (n=8), Effipro® Spot-on treatment (n=8) or Frontline® Spot-on treatment (n=8) performed once on Day0 according to label. Each dog was then repeatedly infested with 100 fleas weekly from Day7 to Day91. Flea counts were performed by combing 48h after treatment (Day2) and each new challenge infestation (from Day9 to Day93) by trained staff unaware of treatment allocation.

Efficacy against flea infestation was calculated from geometric mean numbers of fleas on dogs in the treated (GMT) and control (GMC) groups using Abbott’s formula: %efficacy = 100x(GMC-GMT)/GMC.

Results
Geometric mean flea counts in the control group ranged between 42.1 and 88 from Day2 to Day93, indicating a sufficiently high flea challenge throughout the study period. Lower flea counts were recorded with both fipronil spot-on treatments versus the control group at all times (P<0.05). Geometric mean flea counts ranged from 0 to 0.1 with Effipro® and from 0 to 0.3 with Frontline® from Day2 to Day58, with no significant difference between groups. Higher flea counts were recorded subsequently: up to 2.1 (Effipro®) and 18.3 (Frontline®) fleas per dog at day 93. Treatments achieved >99% efficacy over 10 weeks (Effipro®) and 9 weeks (Frontline®).

Conclusions
Under controlled experimental conditions, both products provided similar useful residual protection against flea infestation regardless of differences in excipient vehicles between the two formulations.

EFFICACY OF TWO FIPRONIL SPOT-ON FORMULATIONS AGAINST REPEATED EXPERIMENTAL INFESTATIONS OF BEAGLE DOGS WITH IXODES RICINUS TICKS
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Introduction
A blinded, randomized study was performed to evaluate the efficacy of two fipronil spot-on formulations, Effipro® (Virbac) and Frontline® (Merial), against artificially induced infestations of dogs with Ixodes ricinus ticks.

Materials and methods
Eighteen adult Beagle dogs (9-15kg) free from any parasitical treatment in the 8 weeks preceding the trial were housed individually in environmentally controlled rooms. They were infested with 50 unfed ticks (30±2 females and 20±2 males, laboratory strain of Ixodes ricinus) before treatment on Day-7 and Day-2. The dogs were randomly allocated to one of 3 groups: untreated controls (n=6), Effipro® Spot-on treatment (n=6) or Frontline® Spot-on treatment (n=6), adjusting for sex and bodyweight. On Day0, test products were applied according to label on skin between the shoulder blades. Repeated tick infestations were performed weekly after treatment from Day7 to Day35. Attached and free live ticks were counted by staff unaware of treatment allocation 48h after each challenge infestation, except on Day-2 (counts performed on Day2). Efficacy was calculated from geometric mean numbers of ticks on dogs in the treated (GMT) and control (GMC) groups using Abbott’s formula: %efficacy = 100x(GMC-GMT)/GMC.

Results
Tick attachment rates in the untreated group were ≥25% throughout the study period, indicating adequacy of infection. Lower attached and free live ticks were counted by staff unaware of treatment allocation 48h after each challenge infestation, except on Day-2 (counts performed on Day2). Efficacy was calculated from geometric mean numbers of ticks on dogs in the treated (GMT) and control (GMC) groups using Abbott’s formula: %efficacy = 100x(GMC-GMT)/GMC.
ments versus the control group at all times (P<0.05), with no significant difference between treatments (table).

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Treatments achieved >90% efficacy over 5 weeks (Effipro®) and 3 weeks (Frontline®).

Conclusions
Under experimental conditions, both products provided similar protection against I. ricinus infestation, regardless of differences in excipient vehicles between the two fipronil formulations.

Aim of the study
The aims of the present study were to identify and characterize a SP in the anterior pituitary gland of normal, clinically healthy dogs and characterise this cell fraction with quantitative PCR (qPCR).

Materials & Methods
FACS was used to identify and sort a SP in six normal pituitary glands, obtained from healthy laboratory dogs. RNA was isolated from SP and MP cells and analysed with qPCR for the expression of markers associated with the stem cell phenotype.

Results
In this study for the first time a SP was identified in the canine anterior pituitary. A high expression of stem/progenitor cell markers (BMI-1, nestin, CD133) was shown in the SP. Furthermore, the SP expressed markers of signalling pathways (Notch, Wnt, SHH), which are essential in embryonic pituitary development. However, also the pituitary cell lineage markers Prop1, T-Pit and the haematopoietic stem cell and endothelial marker CD34 were highly expressed. Further studies are needed to analyse the SP in more detail and to further support the presence of the stem/progenitor cell phenotype in the canine pituitary gland.

References