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Determination of plasma and skin concentrations of orbifloxacin in clinically normal dogs and dogs with pyoderma following oral administration at a dose of 7.5 mg kg^{-1}

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The objective of the study was to characterize orbifloxacin concentrations in the plasma and skin of 14 clinically normal dogs and 14 dogs with pyoderma following single and multiple oral administration of orbifloxacin at a dose of 7.5 mg kg^{-1}. Skin biopsies and whole blood samples were obtained prior to dosing and at the time of the expected peak concentration of orbifloxacin in skin (3 h post dose) on the first (day 0) and fifth (day 5 ± 1) day of dosing. Biopsies and plasma were analysed for orbifloxacin using validated high-performance liquid chromatography methods. Statistical comparisons were performed by *t*-test ($P = 0.05$). Following single and multiple dose administration at 7.5 mg kg^{-1}, dogs with pyoderma had significantly higher mean skin concentrations of orbifloxacin (day 0: 7.80 ± 3.40 mg g^{-1}, day 5 ± 1: 9.47 ± 6.23 mg g^{-1}) than dogs with normal skin (day 0: 3.85 ± 1.08 mg g^{-1}, day 5 ± 1: 5.43 ± 1.02 mg g^{-1}). On day 0, the range of skin orbifloxacin concentrations was 3.17–15.79 mg g^{-1} in dogs with pyoderma, but only 1.45–5.29 mg g^{-1} in dogs with normal skin. By day 5 ± 1, the range of skin orbifloxacin concentrations was 0.82–25.50 mg g^{-1} in dogs with pyoderma, although only 3.66–6.97 mg g^{-1} in dogs with normal skin. On both day 0 and day 5 ± 1, dogs with pyoderma had a significantly higher mean orbifloxacin skin/plasma ratio (1.40 and 1.44, respectively), than normal dogs (0.81 and 0.96, respectively). There were no significant differences between mean plasma orbifloxacin concentrations in clinically normal dogs and dogs with pyoderma following single or multiple daily dosing with orbifloxacin. The accumulation of orbifloxacin in diseased skin following multiple daily oral administration may contribute to the efficacy of this compound for the treatment of bacterial skin infections.

Clinical efficacy of cefalexin administered by oral route at two dosages in the treatment of deep pyoderma in dogs

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This study was performed to evaluate the clinical efficacy of a cefalexin-based oral paste administered once daily at two dosages (30 and 60 mg kg^{-1}) in the treatment of deep pyoderma in dogs. A multicentre, randomised field trial was conducted in 12 veterinary practices in France. In total, 103 dogs aged between 3 months and 14 years with a clinically diagnosed deep pyoderma were included in the trial: 51 dogs were administered the test product (cefalexin oral paste Virbac) as a single daily dose of 30 mg kg^{-1}, and 52 dogs were administered the same paste as a single daily dose of 60 mg kg^{-1}. In both groups, the treatment was administered until complete disappearance of lesions +10 days, without exceeding 3 months. On day 0 (D0), a cutaneous swab for bacteriological analysis was taken from each animal included in the trial. Efficacy of the treatments was assessed through the percentage of clinical cure (total disappearance of lesions) rates and the recovery period (time interval between D0 and the day of clinical cure). On D0, 49.5% of the dogs presented a furunculosis, 27.5% a cellulitis and 16.5% an interdigital pyoderma. Gram-positive bacteria represented 65% of the pathogens isolated. Of the 91 dogs with interpretable results, 75 (82.4%) were considered to be cured after treatment: 40 (85.1%) in the 30 mg kg^{-1} group and 35 (79.5%) in the 60 mg kg^{-1} group ($P = 0.4862$). Recovery was observed on average after 39.1 ± 17.4 days (14–98 days) and 35.2 ± 14.3 days (14–84 days), respectively, in the 30 and 60 mg kg^{-1} groups. ($P = 0.6021$). No significant difference was found on clinical criteria between dosages. Considering these results, cefalexin administered orally once daily is effective in the treatment of deep canine pyoderma at the two dosages tested: 30 and 60 mg kg^{-1} day^{-1}.

Clinical efficacy of cefalexin administered once or twice daily by oral route in the treatment of pyoderma in dogs

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The study was performed to compare the efficacy of cefalexin administered orally at a dosage of 30 mg kg^{-1} once daily or 15 mg kg^{-1} twice daily in the treatment of superficial pyoderma in dogs. A multicentre, randomised field trial was performed in France and Germany. In total, 83 dogs aged between 3 months and 15 years with a clinical diagnosis of pyoderma were included in the trial: 41 dogs were administered a cefalexin-based oral paste (Virbac) in a single dose of 30 mg kg^{-1} day^{-1} and 42 dogs were treated with cefalexin-based tablets (Virbac) at the recommended dosage of 15 mg kg^{-1} twice daily. Both treatments were administered until the complete disappearance of lesions +10 days, but not exceeding 2 months. On day 0 (D0), a sample for bacteriological analysis was taken from each animal included in the trial. Efficacy of the products was assessed through the percentage of clinical cure (total disappearance of lesions) rates and the recovery period (time interval between D0 and the day of clinical cure). On D0, 79.5% of the dogs had folliculitis and *Staphylococci* represented 79% of the pathogens isolated from cutaneous swabs. Of the 83 dogs treated on D0, 77 (92.8%) no longer had cutaneous lesions after treatment: 38 (92.7%) in the oral paste group and 39 (92.9%) in the tablet group ($P = 0.9756$). Recovery was observed after 29.1 ± 15.5 days (7–69 days) and 26.7 ± 12.3 days (7–61 days), respectively, in the oral paste and tablet groups ($P = 0.4503$). No significant difference was found on clinical criteria between dosage regimens. Based on these results, cefalexin administered orally once or twice daily is effective in the treatment of canine pyoderma.

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Usefulness of an ethyl lactate shampoo in conjunction with systemic antibiotics in the treatment of canine generalized superficial pyoderma: a controlled clinical study

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Shampoo therapy has been advocated to help resolve canine superficial pyoderma. The purpose of this study was to evaluate whether using a 10% ethyl lactate shampoo in conjunction with systemic antibiotics would accelerate the resolution of superficial pyoderma and whether owners perceived any benefit in shampooing. Twenty privately owned dogs diagnosed with primary generalized superficial pyoderma were assigned in alternating order to two groups. In group 1, 10 dogs were treated with cephalexin 25–30 mg kg^{-1} orally every 12 h and baths were not allowed. The ten dogs in group 2 were treated with cephalexin 25–30 mg kg^{-1} orally every 12 h and shampooed twice weekly with the ethyl lactate product. An ANOVA of clinical lesional score at day 0 demonstrate no significant difference between the groups. Covariance analysis was used with lesional score at day 0 as covariate. Animals were re-evaluated every 14 days until day 42 or until clinical cure was achieved. Clinical lesional scores were significantly different between groups at day 14 and 28 ($P < 0.01$). Owner-perceived improvement was scored every 14 days for haircoat appearance ($P < 0.01$, day 14), degree of pruritus (no significant difference), body odour ($P < 0.05$, day 14) and overall improvement ($P < 0.06$, day 14). Complete resolution of lesions for animals in group 1 required 37.8 days, compared with 29.4 days in group 2 (ANOVA, $P < 0.02$). These results suggest that utilization of a shampoo twice weekly reduces the length of systemic antibiotic therapy needed in canine superficial pyoderma and increases owner perception of improvement.

Juvenile-onset generalized demodecosis due to a short-tailed demodectic mite and *Demodex canis* infection

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An unidentified species of canine *Demodex* has been reported previously in other countries, but not in Portugal. This report describes a case of mixed infection due to a short-tailed demodectic mite and *Demodex canis* in a dog. Biometrical data from this possible new species are also presented. A referred 4-year-old, neutered, male Shar-pei cross-breed, with juvenile-onset generalized demodecosis and pyoderma, which was refractory to treatment, was examined. Physical examination revealed widespread erythema, alopecia, hyperpigmentation, crusts, scaling and lichenification, accompanied by pruritus. *Staphylococcus intermedius* was cultured from skin lesions, and fungal culture on Sabouraud's dextrose was positive for *Microsporum canis*. Smear cytology revealed *Malassezia pachydermatis* overgrowth and staphylococcal infection. Complete blood cell count, biochemistry and urinalysis were within reference values. Skin histopathology revealed severe hyperkeratosis of telogenic follicles, perifollicular-pattern dermatitis and the presence of various forms of *Demodex* mites within the follicles. Mites were mounted on glass slides in Hoyer's medium and examined using light microscopy and a calibrated eye-piece micrometer. In 15 deep skin scrapings 16% (8/50) short-tailed mites were found. *Demodex canis* were present in larger numbers. The unidentified species total body length ranged from 120 to 145 μm (mean 135.5 μm) for a total of five mites measured. The gnathosoma, podosoma and opisthosoma measured 22.5–25 μm (mean 24.5 μm), 50–65 μm (mean 57 μm) and 40–60 μm (mean 51 μm), respectively. The measurements found are similar to those reported previously. In conclusion, the morphological characteristics of this unidentified *Demodex* mite included a shorter body than *Demodex canis*, in particular the opisthosoma, and an obtuse or blunt terminal end.

Comparison of skin scrapings, trichograms and skin surface biopsy for the diagnosis of demodicosis in dogs

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Diagnosis of demodicosis in dogs is usually performed by the microscopic observation of deep skin scrapings (SS). Anecdotal reports have suggested that trichograms (T) may also be useful. In human medicine, skin surface biopsies (SSB) have been shown to be ideal for collecting *Demodex folliculorum*.[1] The aim of the study was to compare SS, T and SSB to collect *Demodex canis* from dogs. Five dogs with generalized demodicosis were included. For each dog, 10 lesional sites were chosen. Three adjacent areas of exposed skin (4 cm^2) were sampled. SS were made using a surgical blade, until bleeding occurred. The material collected was spread in a drop of mineral oil on a microscope slide. For the trichogram atuft of hair was gently removed and placed in a drop of mineral oil on a microscope slide. SSB's were obtained by placing a drop of cyanoacrylic acid (SuperGlue®) on a microscope slide, applying it to the skin and gently removing it.[2] The sample was then covered with one drop of mineral oil and a cover slip. Each sample was examined microscopically (magnification ×10) and the total number of mites was counted. Fifty samples were obtained by each method. At least one mite was observed on every sample obtained by SS (50/50), compared with 40% of samples for T (20/50) and 38% for SSB (19/50). The mean number of mites was higher with SS (12.1 mites/slide) than with T (2.2 mites/slide) and SSB (2.1 mites/slide). SS was shown to be the best of the three methods to detect *Demodex* mites in dogs. T is less sensitive and detects fewer mites than SS. SSB does not seem to be the ideal technique in dogs; however, further studies are necessary to better evaluate the usefulness of this technique in the canine species.

Serological diagnosis of sarcoptic mange in cats

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In the early 1970s previously naive wild red foxes (*Vulpes vulpes*) in Sweden became infected by the burrowing itch mite, *Sarcoptes scabiei*. The infection spread throughout the country. Other animal species were found to be susceptible to infection, in particular, dogs, wolves (*Canis lupus*) and lynx (*Lynx lynx*). Domestic cats seemed to be refractory to the infection. However, we have confirmed, using skin scrapings, a number of cats with sarcoptic mange and by using a modified enzyme-linked immunosorbent assay (ELISA) to detect specific antibodies to *S. scabiei* the diagnosis of sarcoptic mange in cats has been improved. An indirect ELISA detecting specific antibodies to *S. scabiei* in dogs, red foxes and swine, modified with respect to secondary antibody, conjugate and substrate was used. Sera from cats were diluted 1:200 and tested in duplicate. A monoclonal anticat IgG, diluted 1:8000 and a rabbit antimouse horseradish peroxidase diluted 1:2000 were used. The substrate TMB was allowed to act for 10 min after which time the reaction was stopped by addition of a 10% solution of H_2SO_4. Optical density (OD) was measured at 450 nm. A preliminary cut-off value based on the results of the ELISA on sera from 35 cats visiting one small animal clinic and exhibiting no skin disease and no pruritus, was determined at 0.19. Preliminary evaluation of the test showed promising results. All cats with verified *S. scabiei* (8) had OD values > 0.27 (0.27–2.2) and all 13 specific pathogen-free cats had OD values < 0.05. Using Western blot analysis the same dominant characteristic bands seen in studies of infected dogs and red foxes were also found in infected cats.

Hair growth and hair density in dogs from 8 to 52 weeks of age

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The objective of this study was to determine changes in hair growth in puppies over a 1-year period. Fifteen 8-week-old black Labradors from two litters (A and B) were evaluated every 4 weeks for hair regrowth and hair follicle number. A 7-cm^2 section of hair was shaved on the left dorsal side every 4 weeks. Each subsequent month, hair was shaved just adjacent to the previous site. Hair follicles were counted in a 5 mm^2 area using a phototrichogram technique. Hair regrowth was measured in the previous month's shaved area. The results presented in the Table are for weeks 8, 12, 30 and 52. The two unrelated litters of black Labrador puppies had different hair regrowth rates and hair follicle densities.

Age (weeks)	Measurable hair follicle density		Mean hair follicle density Decrease from age 8 weeks		Mean hair regrowth	
	Litter A	Litter B	Litter A	Litter B	Litter A	Litter B
8	84.9^{γ}_{a}	67.7^{μ}_{a}	N/A	N/A	13.07^{χ}_{e}	13.64^{χ}_{e}
12	66.6^{γ}_{b}	51.9^{μ}_{b}	18.2^{α}_{c}	15.9^{α}_{c}	16.60^{χ}_{f}	11.82^{χ}_{f}
30	47.7^{γ}_{b}	38.1^{μ}_{b}	37.1^{α}_{d}	29.6^{α}_{d}	10.24^{χ}_{g}	13.08^{η}_{ef}
52	45.0^{γ}_{b}	38.9^{μ}_{b}	39.8^{α}_{d}	28.9^{α}_{d}	N/A	N/A

Values with different superscript Greek letters denote differences between litters ($P < 0.05$). Values with different subscript letters within a column denote differences between evaluations ($P < 0.05$). Using phototrichogram technique, the hair tract orientation was visually different between litters and changed as time progressed.

Dietary tyrosine and red hair syndrome in dogs

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There are reports from breeders that the coat colour of dogs changed from black to reddish brown when fed certain commercial pet foods. Until recently, this 'red hair syndrome' has been considered unfounded. Lately, it has been reported that levels of tyrosine (Tyr) and phenylalanine (Phe) required to support maximal melanin synthesis in cats were above current recommendations for growth. The purpose of this study was to evaluate whether a similar observation could be made in dogs. Twelve puppies (six Newfoundlands, six black Labradors) were weaned on a puppy diet. They were then divided in to three groups and fed three similar diets (A, B, C) with levels of Phe + Tyr in diet A = 1.9 and diet B = 2.6 times American Association Food Control Official requirements for growth. Diet C was diet B coated with free Tyr so that it reached 3.2 times requirements for growth. Food intake, body weight and coat colour were monitored for a year. Hair samples were collected monthly and pictures taken bimonthly. Dogs remained healthy and showed similar growth patterns. After 2 months, hair growth of dogs on diet A was red and that on diet C darker than on diet B. After 5 months, the coat of dogs fed diet A appeared reddish brown. It was possible to distinguish blindly between dogs fed diet B and those fed diet C based on the intensity of blackness of the coat. Thus, in dogs, addition of free available Tyr to foods can prevent 'red hair syndrome' and optimize hair pigmentation, even for diets that have a level of Phe + Tyr above current recommendations.

Candida albicans cheilitis in guinea pig may be caused by commensal strains carried in the lower genital tract

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Two unrelated cases of chronic crusted cheilitis in adult male guinea pigs were previously shown to be caused by *Candida albicans* infection. They were successfully treated with oral ketoconazole at 10 mg kg^{-1} twice daily. A concomitant preputial candidiasis was observed in one guinea pig, while *C. albicans* could be isolated from the skin of the lip and from the prepuce in both animals. It is not known whether this opportunistic cutaneous candidiasis could result from auto-inoculation from the penis and/or the prepuce during grooming. This study was undertaken to determine whether *C. albicans* isolates from the penis may be responsible for subsequent cheilitis. In each guinea pig, the two *C. albicans* isolates, one from the lip and the other from the penis, were typed by random amplified polymorphic DNA (RAPD) using primer M13 (5′-GAGGGTGGCGGTTCT-3′) together with 12 epidemiologically unrelated isolates of *C. albicans*. For each guinea pig, RAPD molecular types were identical for the yeasts isolated from the lip and the penis, although the patterns were different between the two animals and different to those of the unrelated *C. albicans* isolates. In these two cases of crusted cheilitis caused by *C. albicans*, the yeast strains isolated from the lip were the same as those isolated from the penis, even in the absence of a symptomatic genital candidiasis. As *C. albicans* can be carried in the lower genital tract, these results suggest that *C. albicans* cheilitis in guinea pig could be due to auto-inoculation from the genital mucosa during grooming.

The failure of lufenuron and enilconazole to control dermatophytosis due to *Microsporum canis* infection in a Persian cattery

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The use of lufenuron, a chitin synthesis inhibitor, has been recently described for the treatment of cats with dermatophytosis. In this study, oral lufenuron in conjunction with topical enilconazole was evaluated for the management of endemic *Microsporum canis* dermatophytosis infection in a Persian cattery. All cats and kittens over two weeks of age were treated with oral lufenuron at 100 mg kg^{-1} and rinsed in 0.2% enilconazole solution on days 0, 14, 28, 42, 56, 70, 84, 98 and 112. In addition, any pregnant queen was treated at day 60 of gestation with oral lufenuron at 100 mg kg^{-1} and bathed in 2% miconazole and 2% chlorhexidine shampoo. Woods lamp examination and fungal culture of all cats was performed on days 0, 28, 56, 84 and 112. Environmental decontamination with vacuuming and washing all nonporous surfaces with 1:10 bleach solution was completed every 7 days. Thirty-one cats and 32 kittens were treated. All cats and kittens were positive for *M. canis* on fungal culture on day 0. At day 112, no lesions were evident but all adult cats were positive on fungal culture for *M. canis*. Clinical lesions ranging from focal areas of alopecia, papules, scaling and crusting developed in 65% (17) of kittens at 6 (10), 10 (5) and 12 (2) weeks, respectively, and 97% (31) of kittens were positive on fungal culture for *M. canis* at day 112. This protocol utilizing lufenuron and enilconazole was not effective for controlling endemic dermatophytosis in a Persian cattery.

Clinical and histopathological aspects of dermatophyte kerion in the dog: a retrospective study of 20 spontaneous cases

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Dermatophyte kerion (DK) is a deep pyogranulomatous to granulomatous dermatitis, often associated with *Microsporum gypseum* or *Trichophyton mentagrophytes* infection and complicated by *Staphylococcus intermedius*. The main aims of this study were the identification of local fungal pathogens, the prevalence of bacterial complications and the diagnostic accuracy of periodic acid Schiff (PAS) and Gomori stains compared to that of haematoxylin and eosin (H&E). A total of 20 dogs, belonging to various breeds and ranging in age from 3 months to 4.5 years, entered the study. Inclusion criteria were positive Dermatophyte test medium (DTM) culture and/or the presence of fungal elements on histopathology. In total, 91 lesions strongly indicating DK were noticed (1–12/dog, median 3.5), on the head (46/91, 50.5%), the trunk (14/91, 15.4%) and the extremities (31/91, 34.1%). DTM cultures were positive in 13 dogs. *Microsporum canis* was isolated in 10 and *M. gypseum* in 1 case. Negative DTM results were more often seen when topical and/or systemic antifungal treatment had been applied ($P < 0.05$). Fungal elements were found in 100% of the cases with H&E, while the corresponding figure in PAS and Gomori stains was 85% each. *S. intermedius* was isolated in 5/17 (29.4%) dogs. *M. canis* is the most common dermatophyte associated with DK, at least in Greece. Bacterial complications occur in approximately one third of cases. The diagnostic accuracy of H&E in DK is equal, if not superior, to that of special stains.

An open clinical trial with heat-treated amphotericin B in canine leishmaniasis

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Heat treatment of amphotericin B deoxycholate (Fungizone®) at 80 °C for 15 min has previously been shown to decrease toxicity of the drug to mammalian cells and increase its activity against *Leishmania donovani* in mice. A total of 15 naturally infected dogs with leishmaniasis were treated using this formulation of amphotericin B. Clinical signs were scored before and 1–2 months after the end of the treatment. Eleven of the 15 dogs showed good or high efficacy of the treatment. Eleven of 12 dogs tested by a polymerase chain reaction technique of bone marrow were negative for leishmania after treatment. In this study it was shown that this heat-induced reformulation of Fungizone® allows *L. infantum*-infected dogs to be treated with higher dosages and few side effects. A mean of six injections gave results statistically equivalent to those obtained with 13 injections of conventional Fungizone®. Therefore, heat treatment offers a new opportunity, which is nonexpensive and easy to develop, for increasing the dosage/duration ratio of the treatment.

Immunohistochemical and PCR detection of *Leishmania* organisms in canine cutaneous sterile pyogranuloma/granuloma syndrome (SPGS)

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Cutaneous sterile pyogranuloma/granuloma syndrome (SPGS) is an uncommon canine skin disorder of unknown aetiopathogenesis. Histopathological findings and failure to demonstrate an aetiologic agent using light microscopy and microbiological examination of lesioned skin are suggestive of this syndrome. Nevertheless, it has been hypothesized that SPGS might be related to an immunological response against persistent endogenous or exogenous antigens. Canine leishmaniasis (CL) can mimic SPGS histopathologically. The aim of this study was to investigate the presence of the *Leishmania* organism using immunohistochemical (IPI) and polymerase chain reaction (PCR) techniques in canine skin specimens histopathologically diagnosed as SPGS. A retrospective study was conducted on 21 skin biopsy samples with histopathological lesions compatible with SPGS and no visible microorganisms on haematoxylin and eosin (H&E) stain or foreign body material under polarized light. PAS, Ziehl-Neelsen and Gram stains were performed on these samples to rule out the presence of fungi, acid-fast and gram-positive bacteria and immunohistochemical and PCR techniques were applied to detect *Leishmania* organisms. Both immunohistochemical and PCR techniques gave positive results in 12 of 21 skin samples. In areas endemic for CL the presence of *Leishmania* organisms should be routinely ruled out in skin specimens histopathologically suggestive of SPGS. Immunohistochemical and PCR techniques are useful to confirm the presence of *Leishmania* organisms in cutaneous biopsies.

Behaviour of two different strains of *Ctenocephalides felis* in a canine flea allergy dermatitis model

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The objectives of this study were to evaluate the difference between membrane fed fleas and naturally raised fleas in a dog model for flea allergy dermatitis (FAD). Sixteen sensitized Beagle dogs, who had previously shown signs of FAD, were exposed to a flea strain raised on cats (strain A) for 6 weeks to provoke FAD. Five months after recovery they were exposed to fleas fed with bovine blood through membranes (strain B). Throughout both experiments 50 fleas were released once weekly on the back of each dog. The dogs were combed weekly and the number of fleas recovered was noted. Signs of FAD were assessed by clinical examination. FAD was induced by both flea strains. Flea recovery rates varied between 21.3 and 67.5% for strain A, and 2 and 8.5% for strain B. A large number of strain B fleas was found dead but engorged on the kennel floor. Both flea strains induced FAD within a 3-week period. Strain A failed in one dog and strain B failed in four dogs. Intensity, type and localization of lesions were the same in both trials. Flea strain B has lost its ability to live on dog blood. We conclude that development of FAD is related to the density of flea infestation and frequency of biting by fleas. We question the dogma that one flea bite is sufficient to trigger FAD in sensitized dogs. Variations between individuals are obvious.

Survival performances of the dog flea, *Ctenocephalides canis* and the cat flea, *Ctenocephalides felis felis*: a comparative study

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Three experiments were conducted to evaluate and compare the survival performances of *Ctenocephalides canis* and *Ctenocephalides felis felis* in the haircoat of dogs and cats and in the environment. In Experiment 1, survival of *C. canis* in the haircoat was evaluated on five dogs and five cats. Each animal was experimentally infested with 50 young adults. A combing count was performed twice a week until negative results were obtained (16 and 2 weeks for dogs and cats, respectively). Twenty-four hours after deposition 78.4% of fleas were still present, 72.4% were recovered 5 days later and 63.6% were recovered 8 days after infestation. Mean survival was estimated to be 8.6 days. Under the same conditions, 24 h after deposition in cat haircoats, only 12.4% of fleas were still present, 5.6% were present after 5 days and 2% 8 days after infestation. Maximal survival was 106 days for one *C. canis*. In Experiment 2, six adult Beagle dogs were infested simultaneously with 25 unfed, young adult *C. canis* and 25 unfed, young adult *C. felis* (≈ 30 females and 20 males). Seventy-eight per cent of the 600 *C. canis* which were deposited were recovered by combing 48 h later. Under the same conditions, only 59% of the *C. felis* were recovered. The difference was significant ($P < 0.001$). In Experiment 3 the survival of 250 unfed fleas of both species was evaluated at 19 and 27 °C (relative humidity 70%). The mean survival of 50% of *C. canis* was estimated to be 15.9 days (19 °C) and 9.0 days (27 °C). Under similar conditions, the mean survival of *C. felis* was estimated to be 11.7 days (19 °C) and 9.6 days (27 °C). After a feeding period (48 h on a dog), mean survival of females was 7.9 days (19 °C) and 4.8 days (27 °C) for *C. canis* and 4.9 days (19 °C) and 3 days (27 °C) for *C. felis*. Male survival was inferior.

Localized exposure of dogs to *Ctenocephalides felis* does not cause generalized signs of flea allergy dermatitis

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The objectives of this study were to investigate whether canine flea allergy dermatitis (FAD) can be induced by localized exposure to a limited number of flea bites. Twenty-nine Beagle dogs were exposed to 30 fleas (*Ctenocephalides felis*) in chambers equipped with a membrane through which the fleas could bite for 30 min per day, 5 days a week, for 5 weeks. Thirteen dogs had shown signs of FAD during full body exposure to fleas in a previous experiment, eight dogs had previously been exposed to fleas but not shown FAD, and eight dogs had never been exposed to fleas. Signs of FAD were assessed by clinical examination. Intradermal skin test (IDST), flea-specific serum immunoglobulin (Ig)E and IgG levels, and skin biopsies were used as additional test parameters. Generalized FAD was not induced. Dogs with a history of FAD showed moderate to severe erythema, exudation and excoriation at the flea feeding sites. Control dogs showed either no or only mild reaction. Results of IDST and IgE/IgG serum levels were inconclusive. Biopsies from flea feeding sites revealed a higher level of inflammation in FAD dogs than in control dogs before and after 21 and 35 days of flea exposure. On day 3 after beginning of flea exposure the average levels of inflammation were similar in all dogs. We conclude that FAD develops at the locations where the fleas feed. A predisposition or sensitization is nevertheless necessary to permit induction of FAD. Generalized FAD can develop only under the burden of multiple flea bites at different locations.

Efficacy of an insect repellent (spot-on formulation) in dogs and cats experimentally and naturally exposed to mosquito infestation

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Two trials were conducted to assess the efficacy of an association of dimethyl phthalate and neem oil in spot-on formulation as an insect repellent. In Trial 1, five dogs were treated and exposed to bites of a laboratory strain of *Aedes aegypti* (20 insects/dog) 15, 30, 60 min, and again 12, 24, 36, 48, 60, 72, 84 and 96 h following treatment. Five untreated dogs served as controls. At each time point, engorged mosquitoes were collected and counted and the efficacy was assessed. In Trial 2, 20 dogs and 10 cats were naturally exposed to mosquito bites in an area with an abundance of mosquitoes (mostly *Culex pipiens* and *Aedes caspius*). Each animal was observed for 3 h for a period of 10 days from the treatment. Efficacy was assessed on the basis of the number of animals bitten. In dogs experimentally exposed, a high repellent activity was observed 24 (98%) and 48 h (92%) after treatment. After 60 and 72 h, the activity was low (68 and 45%, respectively). No more activity was observed from 84 to 96 h. In the first 48 h, the total number of engorged mosquitoes collected from the untreated dogs ranged from 143 to 172. In dogs and cats naturally exposed to mosquito bites, efficacy was complete (100%) during the first 24 h and after 48 h 80% in dogs and 90% in cats. At 72 h activity was 45% in dogs and 50% in cats. The study shows that a spot-on formulation can be helpful in controlling insect bites. The insect repellent activity is long lasting and the spot-on easy to use, safe and well tolerated by animals.

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Clonality analysis of cutaneous lymphocytosis in 20 cats

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Cutaneous lymphocytosis in humans refers to a heterogeneous group of benign proliferation of lymphocytes which can mimic cutaneous lymphoma clinically and histologically. Similar lesions have been observed in dogs and cats. Feline cutaneous lymphocytosis is characterized by a perivascular to diffuse infiltration of T cells. B cells are commonly seen in small aggregates. Histomorphology alone cannot predict clinical outcome. However, cutaneous lymphocytosis may be indicative of a systemic lymphocytic proliferative disorder with variable clinical progression, particularly in cases that exhibit the CD4/CD8 double-negative T-cell immunophenotype. The objective of this study was to investigate the clonality of feline cutaneous lymphocytosis in order to provide additional criteria to differentiate cutaneous lymphocytosis from cutaneous lymphoma. DNA was extracted from 25 formalin-fixed, paraffin-embedded tissues obtained from lesional skin of 20 cats that had been previously diagnosed with cutaneous lymphocytosis. Glyceraldehyde-3-phosphate dehydrogenase was used as a positive control to assess the integrity of the DNA in each sample. Polymerase chain reactions (PCR) were performed with all DNA samples using primers specific for feline variable joining segments of immunoglobulin (Ig)H and TCRγ for B-cell and T-cell clonality, respectively. PCR products were evaluated by heteroduplex analysis in order to differentiate between polyclonal and monoclonal populations. Monoclonal populations of B cells were not seen. PCR confirmed monoclonal T-cell populations in two cats. One of these had a dermal CD4+/CD8+ T-cell infiltrate and became systemically ill. The other cat is still alive, and does not currently show evidence of systemic disease. PCR did not confirm clonality in two cats with double-negative T-cell population and lymphocytic infiltration of internal organs. However, negative PCR results on clonal populations have been reported. Therefore, both immunophenotype and evaluation of clonality represent diagnostic tools adjunctive to morphological characterization to differentiate reactive from neoplastic lymphocytic infiltrates in cats.

A case of seasonal lymphocytic mural folliculitis with spontaneous resolution in a cat

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A 9-year-old spayed Domestic Short Hair cat was presented for recurrent truncal hyperkeratosis and alopecia of 2 years duration. The cat had been lethargic and heat-seeking for some months and had difficulty lifting the head. At first presentation (November) patchy truncal alopecia, hyperkeratosis and squamosis had been present since the previous August, gradually involving the legs and tail. Pruritus was moderate. Both external ear canals were filled with beige cerumen. Skin scrapings, ear swabbing, fungal culture, coproscopy, biochemistry profile, chest X-ray and ultrasonography were normal. Eosinophilia varied between 1385 and 1918 cells mm^{-3} (10–20%). Histopathological examination revealed lymphocytic mural folliculitis and perifolliculitis and the absence of sebaceous glands. Treatments were ineffective or aggravated clinical signs. The condition then recurred each year from roughly August to December. Intradermal skin testing (IDST) was carried out and showed a mild positive reaction to a grass mix. Neither these results nor annual medication/vaccination could be correlated with observed seasonality. At 12 years of age, a 10-day course of marbofloxacin and prednisolone was associated with dermatological cure and no recurrence. Eighteen months later, the cat became blind and a meningioma was diagnosed (scanner, necropsy). Feline lymphocytic mural folliculitis is uncommon and unified by its histological aspect. Underlying causes recognized include allergy, dermatophytosis and early epitheliotropic T-cell lymphoma. In this case, no underlying cause was recognized during the course of the disease. Steroid and antibiotic treatment had little effect. The clinical cure observed for 18 months was probably spontaneous. To our knowledge, this is the first report of a clearly seasonal recurrence of the disease ended by remission of the symptoms.

An unusual case of toxoplasmosis in a cat being treated with cyclosporin for feline atopy

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At the ESVD meeting in Copenhagen cyclosporin was considered to hold great promise for future use in feline dermatology, especially atopic dermatitis. The feline patient was a 4–5 years old, neutered, male, Domestic Short Hair, which had been diagnosed with atopic dermatitis in 1999. Initial dosage of CSA was 25 mg once daily, but the cat became anorexic after a month. Blood chemistry was normal at this stage. Dosage was then reduced to 25 mg on alternate days, with good clinical control of the atopic dermatitis, for 6 months, after which some reappearance of symptoms was noted and dosage was again increased to 25 mg once daily. Three weeks later the cat became severely depressed, developed acute hepatic failure with rapid clinical deterioration and was euthanased. At autopsy organ samples were collected in 10% formalin for histopathology. Throughout the treatment period blood CSA levels had been high. Histopathology was characterized by a severe multifocal hepatic necrosis with nonsuppurative triaditis, acute necrotizing interstitial pancreatitis, necrotizing, granulomatous lymphadenitis and proliferative, nonsuppurative interstitial pneumonia. Immunohistochemical (IHC) staining of tissue sections for *Toxoplasma gondii*, revealed strong positive staining of toxoplasma tachyzoites in the necrotic foci of various organs. On the strength of severe hepatic, mesenteric lymph node and pancreatic pathology associated with numerous intralesional *T. gondii* tachyzoites on IHC staining, it was thought that this cat acquired the first infection with toxoplasma during the cyclosporin therapy. CSA has been reported to be immunosuppressant and downregulatory of the host parasite response, and there is concern that such effects in this cat, may have contributed to the development of acute toxoplasmosis.

Cyclosporin concentration in the skin following oral administration

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Cyclosporin A (CsA) has proven to be an effective therapy for canine atopic dermatitis. The drugs anti-allergic and antipruritic effects are probably explained by the inhibition of cytokine release lymphocyte affecting T, mast cell survival and activation, eosinophil recruitment, Langerhans cell function and keratinocyte cytokine production. As skin is the target tissue for the therapeutic effect of CsA, concentration of the drug in this tissue was measured. Four Beagle dogs were dosed with 3.8 mg kg^{-1} CsA once a day for 14 days. Skin biopsies were taken under local anaesthesia on four sites of the body (shoulder, neck, back and hind quarter) at 4 and 24 h after the last drug administration. Blood samples were taken at the same time. Blood and skin samples were analysed using a high-performance liquid chromatography assay. The mean concentrations were 167 ng mL^{-1} and 660 ng g^{-1} at 4 h and < 25 ng mL^{-1} and 206 ng g^{-1} at 24 h, in the blood and skin, respectively. The individual skin/blood concentration ratio varied from 2.5 to 6.4 at 4 h. This study confirms that, as in man; CsA concentrations are higher in canine skin than in blood, and that tissue depletion is slower in skin than in blood. The activity of CsA in atopic dermatitis is probably related to the immunomodulation effects at the skin level. Once-a-day dosing is possible because of the slow depletion from the skin.

The effects of cyclosporin a and oral prednisolone on flea allergen specific serum IgE and intradermal tests in experimentally sensitized laboratory Beagles

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The objective of this study was to measure the effects of cyclosporin A (CSA) on the two standard methods of evaluating sensitization status, namely intradermal test (IDT) and allergen-specific immunoglobulin (IgE) serology in comparison with oral prednisolone. CSA is an attractive alternative to long-term corticosteroid therapy. However, the effect of CSA on allergen-specific IgE, measured either *in vivo* or *in vitro*, is not known. Laboratory Beagles with experimentally induced flea allergy dermatitis (FAD) are a well-controlled model system which may be used to measure the effects of CSA. Three groups of eight Beagles with reproducible symptoms of FAD upon flea exposure, were untreated, or treated with CSA (5 mg kg^{-1} day^{-1}) or prednisolone (1 mg kg^{-1} day^{-1}) for one week before flea infestation (20 fleas/week). Flea infestation and drug treatment were continued for ≈ 7 weeks. IDT with flea salivary allergens was performed before (week 0) and after 5 weeks of treatment. Serum was obtained at 0, 5 and 7 weeks. Serum IgE specific for the major flea allergen Cte f 1 was measured in a quantitative Fc_R1_-based enzyme-linked immunosorbent assay (ELISA). All dogs had positive IDT to Cte f 1 at weeks 0 and 5. Neither CSA nor prednisolone significantly suppressed the immediate reaction. The level (ng mL^{-1}) of serum IgE specific for the major flea allergen Cte f 1 varied markedly from 17.5 to 48,600 ng mL^{-1} on day 0. There were no differences in levels of serum anti-Cte f 1 IgE between groups at any time point. A 5-week course of a therapeutic dose of CSA has no significant effect on levels of antiflea IgE as measured by intradermal test or ELISA.

Importance of psychogenic factors in canine atopic dermatitis

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Atopic dermatitis (AD) is a genetically determined inflammatory and pruritic skin disease with characteristic clinical features. The mechanisms of AD have not been fully elucidated, but a number of interdependent factors are involved. In humans, it is considered that psychogenic factors play a role in AD. The purpose of this study was to evaluate the roles of psychogenic factors in canine AD. All 34 dogs described in this study were diagnosed with AD using both the revised Willemse's criteria (1997) and a positive reaction to intradermal testing using *Dermatophagoides* spp. antigens. Psychogenic factors were identified by the following features:[1] broken hairs and excoriation due to pruritic behaviour such as grooming, and scratching;[2] onset of pruritic behaviour associated with emotionally unstable situations;[3] existence of inductive life events, daily hassles or concurrent behaviour problems;[4] and need for behavioural treatment. The dogs showing all features were diagnosed as having psychogenic factors. The incidence of psychogenic factors and the efficacy of the treatment in canine AD were evaluated. In 34 dogs with AD, 23 cases (67.6%) were compatible with having psychogenic factors, 14 cases (41.2%) were apparently improved with behavioural treatment. In dogs with psychogenic factors, 17 cases (73.9%) showed broken hairs at the lateral thigh, and excoriation at the trunk. In dogs without psychogenic factors, only two cases (18.2%) showed these lesions. Skin biopsies at the lesions were performed in seven cases with psychogenic factors and one case without psychogenic factors, and histopathological findings were unremarkable in all cases. Psychogenic factors should be considered as an aetiology in canine AD, and as partially contributing to the itch–scratch cycle. Further investigation, especially psychoneuroimmunologic and psychiatric analysis will be needed.

A review of 200 cases of otitis externa in the dog

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This study details 200 cases of canine otitis externa referred to the author over a 3-year period because of a lack of response to antimicrobial therapy, often prescribed on the basis of culture and sensitivity. In all cases, a primary inciting dermatological disease was identified and treated. In every case the infection was identified to be a perpetuating factor and not a primary problem. Otitis externa was the major presenting sign. Where other dermatological lesions were present these were mild not requiring specific therapy. In no case were the animal's ears chronically irreversibly damaged although many cases had had corrective aural surgery without deriving any benefit. A history, physical, dermatological and otoscopic examination was performed in every case, as were skin scraping and tape strippings from the ear pinna and cytology of primary lesions where present. Ear wax was examined in both potassium hydroxide and using Diff Quik staining. Biopsies of ear flap or vertical ear canal were taken where appropriate. General tests included hypoallergenic diets, intradermal allergy tests, routine blood samples and thyroid assessment. More than 75% of all cases had atopy identified as a primary trigger. In many cases the perpetuating infection had been unresponsive to therapy without concurrent therapy for the atopy. Hypothyroidism, hyperadrenocorticism, pemphigus foliaceus, sebaceous adenitis, demodicosis and erythema multiforme were identified as other triggers. Ear mites were not identified in any dogs. Approximately 50% of cases had *Staphylococcus* as a perpetuating infection. *Pseudomonas*, *Streptococcus*, *Bacteroides* and *Pasteurella* were also isolated. *Malassezia* pachydermatis was identified in 45% of all ears. Cases of otitis externa that are poorly responsive to antimicrobial therapy have an underlying primary trigger. Atopy is the most common cause of otitis externa in the dog. Ear mites rarely cause ear problems in this species.

Pemphigus foliaceus in the horse – 13 cases

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The objectives of this study were to evaluate the age of onset and clinical signs of pemphigus foliaceus in the horse and diagnostic tests used in these patients. Records of 13 horses with pemphigus foliaceus diagnosed by history, clinical signs, histopathology and exclusion of differential diagnoses were evaluated retrospectively. Breeds included six Quarterhorses, two American Paints, two Ponies, one Spanish Mustang, one Arab and one Warmblood. Six were mares, five geldings and two stallions. Three of the patients were foals younger than 6 months, 9 were over 9 years and one was 4 years old. Mean age was 9 years with a range from 3 months to 25 years. Crusting (11), scaling (10) and alopecia (9) were the most common lesions and were present most commonly on the face (9), neck (9) and trunk. (9) Extremities were involved in eight horses; pustules were seen in three. Pruritus was present in more than half (7). Acantholytic cells were identified in four of the six horses in which cytology was evaluated. Histopathology was supportive in all horses. Pemphigus foliaceus in the horse typically presents as a scaly or crusty skin disease in very young or older horses. Face, neck and trunk are most commonly affected. Cytology provides diagnostic clues in the majority of patients and is a useful diagnostic tool, histopathology confirms the diagnosis.

A case of acquired alopecia resembling pseudopelade in a dog

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Pseudopelade (PP) has been recently described and immunologically characterized in the dog. We report a case of an acquired, patchy, noninflamed, generalized and progressive alopecia, of 2 months' duration, in a 13-year-old male English Setter, characterized by comedones, follicular casts and brown hair coloration. Histopathological examination showed a lymphocytic infiltrative mural folliculitis and perifolliculitis targeting the follicular isthmus. A diagnosis of pseudopelade-like dermatosis was made. Immunohistochemistry staining showed an infiltrate composed mainly of CD3+ lymphocytes. Indirect immunofluorescence showed an antibody reactivity situated in the most proximal matrix and in the outer root sheath. Immunoblotting detected several antihair follicle antibodies targeting strongly hair keratins (45–52 kDa). With time, new lesions appeared and older lesions improved. Pseudopelade is a rare dermatosis of dogs with distinctive clinical, histopathological and immunological features, that fails to respond to immunosuppressive therapies and is apparently permanent. In our case, the diagnosis of pseudopelade-like dermatosis was based upon the following criteria: (i) an adult-onset patchy, noninflamed and progressive alopecia; (ii) a lymphocytic infiltrative mural folliculitis with atrophy of the hair follicles; (iii) infiltrate of CD3+ lymphocytes and circulating immunoglobulin (Ig)G autoantibodies specific for follicular antigens. Similar autoantibodies are present in alopecia areata (AA) but not detected in demodicosis. Although there is no evidence of their pathogenicity in the dog, they may perhaps be used as markers of this form of mural folliculitis. This case differs from those described previously in that alopecia did not appear permanent and comedones and follicular casts were prominent. However, slightly increased superficial or infundibular keratin was reported in canine PP. The condition reported may be a particular phenotype of PP or may be a distinctive idiopathic dermatosis, as different immunological events may initiate this form of mural folliculitis in dogs, leading to different clinical expressions.

A case of subepidermal bullous dermatosis due to topical corticosteroid in a dog

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Prolonged topical corticotherapy may induce local cutaneous effects or prolonged adrenal suppression. A distinctive syndrome of localized bullous skin lesions, secondary to prolonged use of topical corticosteroids (steroid dermatosis), was recently described. A 7-year-old, male, Dalmatian was presented with a chronic ulcerated dermatosis of axillary and inguinal areas, of 12 months' duration. This dog had received daily application of topical product containing betamethasone dipropionate for 18 months. Histopathological examination showed an acanthotic epidermis, clefting at the dermo-epidermal junction level or just beneath mixed, lichenoid dermal infiltrate, mild proliferation of small superficial blood vessels, homogenization of superficial dermal collagen and a few nodular pyogranulomas, centred on colonies of *Cocci*. A diagnosis of steroid dermatosis was made. Complete clinical resolution of lesions occurred in 2 months, after withdrawal of topical corticotherapy and cephalexin administration for the pyoderma. No relapse was observed during a follow-up period of one year. The features of our case are identical to those of steroid dermatosis, except for the presence of pyogranulomas. These formations are probably secondary to a bacterial infection than the cause of the dermatosis. This condition seems very rare, partly because prolonged use of topical corticosteroid therapy is requisite for inducing bullae. In our case, betamethasone dipropionate was used for 6 months prior to the development of ulcerations. As postulated, subepidermal clefting is likely to be due to local, corticosteroid-induced skin fragility. The clefting seems to occur in the superficial dermis rather than in the basement membrane zone. A striking unexplained feature of this dermatosis is the acanthosis of the epidermis. This rare, distinctive and benign syndrome, is important to consider because the main differential diagnoses are subepidermal bullous autoimmune dermatoses.

Desmoglein-3 is a target autoantigen in spontaneous canine pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering skin disease of humans and companion animals. In human patients, PV is associated with the production of immunoglobulin (Ig)G autoantibodies specific for keratinocyte desmosomal glycoproteins of the cadherin family. The purpose of this study was to determine whether antikeratinocyte IgG autoantibodies were present in the skin and serum of dogs with PV, and also to identify the canine PV autoantigen(s) targeted by circulating autoantibodies. Eleven dogs with PV were selected because of the microscopic demonstration of suprabasal epithelial acantholysis. Direct immunofluorescence revealed the presence of IgG autoantibodies bound to the membrane of keratinocytes in skin biopsy specimens of 8/9 dogs (89%). Using indirect immunofluorescence with normal canine gingiva and cultured oral keratinocyte substrates, IgG autoantibodies were detected in the serum of 10/11 (91%) and 5/11 (45%) dogs, respectively. By immunoblotting using cultured canine oral keratinocyte protein lysates, IgG autoantibodies from 7/9 (78%) tested dogs recognized a 130 kDa antigen that co-migrated with that identified by rabbit polyclonal antibodies raised against desmoglein-3. This 130 kDa antigen was confirmed to represent the canine equivalent of human desmoglein-3 by immunoprecipitation–immunoblotting. Results of these studies provide evidence that the canine desmoglein-3 homologue is a major autoantigen in dogs with PV. These observations further establish spontaneous canine PV as a natural model for research on pathogenesis, aetiology and novel therapeutic approaches for this disease of humans.

Canine epidermolysis bullosa acquisita is an autoimmune blistering skin disease directed against collagen VII (12 cases)

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Epidermolysis bullosa acquisita (EBA) is a rare autoimmune skin disease of humans. It is characterized by blistering and scarring that predominate on areas of friction and trauma, microscopic subepidermal vesicles and autoantibodies that target collagen VII in anchoring fibrils. The purpose of this study was to establish clinical, microscopic and immunological characteristics of canine EBA in a retrospective case study of 12 dogs. Six dogs belonged to the Great Dane breed. The median age of onset was 12 months. Males outnumbered females by two to one. In 11 dogs, a generalized inflammatory phenotype was observed. Turgid vesicles, rapidly evolving into ulcers, arose acutely on erythematous skin in the groin, axillae and oral cavity. Footpads were affected in nine patients. Half of the subjects were euthanased because of disease severity or lack of response to therapy, while the disease of other patients responded to multidrug immunosuppression. In one dog, a mild localized phenotype was characterized by vesicles and ulcers restricted to the ears. Histologically, subepidermal vesicles were seen in all cases. Noninflammatory vesicles were visualized in 10 dogs, whereas neutrophil-rich blisters were observed in 11 subjects. Eosinophils were detected in vesicles of four patients. Direct immunofluorescence (IF) revealed immunoglobulin (Ig)G deposited linearly at the epidermal basement membrane zone in 9/11 dogs. With an indirect IF method using canine salt-split gingival lip sections, basement membrane-specific autoantibodies were detected in all cases and were predominantly of IgG_1 and IgG_4 isotypes. Using an enzyme-linked immunosorbent assay (ELISA), IgG serum autoantibodies were found to target recombinant human NC1 and NC2 segments of collagen VII in 12 (100%) and 3 (25%) patients, respectively. These observations suggest that canine EBA is a relevant model to further the understanding of the human disease.

Antigen specificity of antinuclear autoantibodies in vesicular cutaneous lupus erythematosus (idiopathic ulcerative dermatosis) of Collies and Shetland Sheepdogs

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Vesicular cutaneous lupus erythematosus (VCLE) is an acutely arising erosive and ulcerative photosensitive skin disease associated with lymphocyte-mediated cytotoxicity directed against basal epidermal keratinocytes. This disease exhibits some clinical, histological and immunological resemblance with a variant of photosensitive subacute cutaneous lupus erythematosus of humans. The purpose of this study was to investigate the antigen specificity of circulating antinuclear autoantibodies in 11 dogs with VCLE using immunofluorescence, immunoblotting and enzyme-linked immunosorbent assay (ELISA). The indirect immunofluorescence method, performed with Hep-2 hepatoma cell substrates did not reveal any specific nuclear fluorescence. An immunoblotting assay, using Hep-2 cellular protein extracts, permitted the detection of serum immunoglobulin (Ig)G autoantibodies targeting antigens of 60/52 kDa (SSA/SSB; three dogs), 74 kDa (M2; five dogs) and/or 29 kDa (unidentified; six dogs). Subsequently, ELISA was performed with purified recombinant human proteins and confirmed the presence of IgG autoantibodies directed against SSA/SSB in five dogs, against Sm in three dogs, against JO1 in three dogs and anti-Sm/RNP in two subjects. When all methods were combined, antibodies against nuclear antigens were detected in 9/11 dogs (82%). These observations suggest that antinuclear autoantibodies specific for extractable nuclear antigens are commonly found in Rough Collies and Shetland Sheepdogs with idiopathic ulcerative dermatosis, thus supporting the hypothesis that this disease represents a cutaneous variant of lupus erythematosus.

Successful management of exfoliative cutaneous lupus erythematosus in three German Shorthaired Pointer siblings

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Exfoliative cutaneous lupus erythematosus (ECLE) is a rare, familial exfoliative dermatitis recognized in German short-haired pointers. Three German short-haired pointer siblings presented with scaling, crusting and alopecia involving the face, pinnae, dorsum and limbs. In one dog, the lesions were painful and the dog was pyrexic with a peripheral lymphadenopathy. Haematology, serum biochemistry, serum ANA, urinalysis, urine protein/creatinine ratio analysis and multiple joint aspirates revealed no abnormalities. Histopathological evaluation confirmed a lymphocytic interface dermatitis. Immunoglobulin (Ig)G and IgM, but no IgA or C3, were detected at the basement membrane zone using direct immunoperoxidase techniques. All three dogs were treated with prednisolone 1 mg kg^{-1} every 12 h, sulphur/sodium salicylate shampoo twice a week and propylene glycol humectant spray every 24 h. In one dog, azathioprine 1.5 mg kg^{-1} every 24 h was included in the treatment regime. After 18 months of therapy, all three dogs are in remission. These findings are consistent with the clinical, histological and immunohistochemical findings reported for ECLE. The sustained response to therapy is a feature not previously reported. The occurrence in sibling German short-haired pointers further supports the hereditary basis of this syndrome.

Hereditary junctional epidermolysis bullosa in the German Shorthaired Pointer: an epidemiological and clinical prospective study of 21 cases

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Recently an hereditary junctional epidermolysis bullosa (HJEB) was characterized in the German Short-haired Pointer. This HJEB is caused by the homozygous missense mutation 1514C → T in the laminin-5 α3 cDNA (5.1 kb). The purpose of this study is to report the epidemiological and clinical features of this genodermatosis. A prospective study relating to the epidemiological and clinical aspects of HJEB was performed between 1997 and 2001 in the German Short-haired Pointer. Diagnosis of HJEB was based on histopathological lesions, immunohistochemical features and in some cases on ultrastructural aspects. We observed 21 cases of HJEB in the German Short-haired Pointer; 18 cases belonged to 6 litters from different kennels. No sexual predisposition was observed (11 males/10 females). Cutaneous lesions developed in 9 cases (42.8%) within 3 weeks, in 5 cases (23.8%) within 6 weeks, in 5 cases (23.8%) within 2 months, in 1 case (4.8%) within 4 months and in 1 case (4.8%) at the age of 6 months. Age of diagnosis was: 23.8% (5) within 6 weeks, 28.5% (6) within 3 months, 28.5% (6) within 4 months, 4.8% (1) within 7 months, 9.6% (2) within 11 months and 9.6% at the age of 4 years. At 2 months cutaneous signs were characterized by paronychia and onychomadesis affecting several digits in 5 cases (100%). Circumscribed ulcers were observed on the medial aspect of the pinnae, on the lateral aspect of the elbows, carpi and tarsi in 3 cases (60%). Ulcers and bulles were noticed on the gingivae, lips and tongue in 2 cases (40%). Between 3 and 4 months, lesions were similar but the frequency of lesion distribution was different: paronychia and onychomadesis were observed in 9 cases (75%), ulcers were noticed on diverse pressure points (elbows, tarsi, carpi, footpads). Ulcers and bullae were reported in the oral cavity (tongue, lips, hard palate, gingivae). One case diagnosed at 7 months was characterized by a predominantly mucous form with multiple small ulcers and bulles in the oral cavity, pharynx and oesophagus and on the medial aspect of the pinnae. At 11 months, circumscribed ulcers were observed on the majority of pressure points, notably the footpads. In both cases onychodystrophy and onychogryphosis, enamel hypoplasia and failure to grow were reported. Two cases were diagnosed in adult German Short-haired Pointers aged 14 years. Clinical signs were characterized by chronic circumscribed ulcers on the footpads, scrotum and teats and premature wear of the teeth with scarring in the oral cavity. Clinical signs of HJEB in the German Short-haired Pointers seem to be characterized by different phenotypes: a classical form observed in pups and young adults, rapidly severe and fatal, a chronic form, less severe and compatible with a almost normal life and a form characterized by predominantly mucous lesions which are extremely severe. These clinical variations, independent of the causative mutation are also described in human JEB.

A canine model for *in vivo* gene therapy of junctional epidermolysis bullosa

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Our aim is to perfect somatic gene therapy of inherited skin diseases. Junctional epidermolysis bullosa (JEB) is a heterogeneous group of genodermatoses characterized by disruption of the dermo–epidermal junction, and blistering and erosions of the skin consequent to minor trauma. Using this model system, we demonstrated that the transfer of the cDNA coding for the α3 chain of laminin-5 (the major adhesion ligand of basal keratinocyte) into α3-null JEB keratinocytes regenerates artificial epithelia assembling the prototypic network of the adhesion macromolecules absent in the JEB skin. However, the effect of a possible host immune response to the curative transgene product must be assessed in an immunocompetent animal model. To this purpose we characterized a breed of German Short-haired Pointers with littermates affected by a mild form of JEB caused by the homozygous missense mutation 1514C → T in the laminin-5 α3 cDNA (5.1 kb). Phenotypic reversion of the canine JEB stem cell keratinocytes by transfer of a retroviral MMLV-based vector expressing the wild-type laminin α3 cDNA shows enhanced adhesion, proliferation and clonogenic potential of the transduced keratinocytes demonstrating that the wild-type α3 polypeptide successfully competes with the endogenous counterpart to generate functional laminin-5 molecules. We have reconstructed canine epithelia *ex vivo* using the genetically modified keratinocytes. These results, enable us to use these large animals as a suitable model system for perfection of JEB gene therapy and provide the opportunity of validating *ex vivo* as well as *in vivo* gene therapy procedures in a clinical setting.

From canine chromosome maps to the identification of genes responsible for genetic diseases: example of laminins

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The identification of a gene implicated in a genetic disease requires, first, the availability of informative pedigrees and second, knowledge of the genome of interest. Formerly, molecular biology methods consisted of isolation of the protein followed by cloning of the corresponding gene. Nowadays, genetic strategies aimed at searching genes using wide genome scan approaches are feasible and very promising, owing to the recent availability of canine genomic maps. The dog (canis familiaris), with 300 well-established pure breeds, appears to be a powerful resource for dissecting the molecular basis of genetic diseases and traits. With this aim we constructed a dog genomic map using the radiation hybrid strategy. We also identified 800 dog gene markers and 800 microsatellite markers that were placed on the last map. Since 1998, we have produced four maps with an increasing number of markers, the last version harbouring 3000 markers localized on the 38 + X, Y chromosomes of the dog. Using this map, 'genetic linkage' analyses and 'gene candidate' approaches can be conducted to localize genes of interest. Collaboration with others provided pedigrees of 'Braque Allemand' dogs affected by junctional epidermolysis bullosa and identification of a mutation in laminin-5 α3 (LAMA3). The three laminin 5 genes have been mapped to canine chromosome 7 (CFA7) and their positions are in accordance with the localization of the orthologous human genes. The identification of microsatellite markers close to those genes allows genetic linkage studies to be performed on disease segregating pedigrees. Moreover, on the available map, several genes for collagens, keratins, etc. are localized, constituting good candidates for other genodermatoses. Because of the breed specificity of these diseases, the search for the genes responsible in each breed will help the understanding of the molecular basis of genodermatoses in dogs and humans. Dog genome maps are available at: www-recomgen.univ-rennes1.fr/doggy.html

Canine model for gene therapy of recessive dystrophic epidermolysis bullosa (RDEB)

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Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited autosomal skin disorder caused by mutations in collagen VII, the major component of the dermal anchoring fibrils. RDEB is characterized by skin blistering, abnormal healing, contractures and cancers. No conventional treatment is yet available. Until now, gene therapy for RDEB has been difficult because of the large size of the collagen VII cDNA and the absence of an animal model suitable for preclinical trials. We identified a family of inbred Golden Retriever dogs presenting with mild RDEB. Immunohistological analysis of the skin and mucosa detected an intracellular retention of collagen VII. We isolated and sequenced the whole of canine collagen VII cDNA (9.2 kb). The search for mutations in the RDEB dogs identified a 5716G → A transition leading to glycine substitution in the collagenous domain of the protein. The wild-type canine collagen VII cDNA was cloned in a retroviral MMLV vector and transduced in collagen VII-null keratinocytes. Immunological analysis of the transduced cells showed synthesis and secretion of collagen VII molecules of the expected size. The triple-helical conformation of the collagenous domain of the recombinant procollagen VII was assessed using assays of limited proteolysis. The transduced keratinocytes demonstrated a reversal of the hypermotility phenotype characteristic of RDEB cells. Deposition of the recombinant molecules at the basement membrane was assessed in organotypic cultures constructed using RDEB fibroblasts and the reverted keratinocytes. Immunofluorescence analysis clearly showed incorporation of the recombinant collagen VII at the dermal–epidermal junction. These results show that: (i) MMLV-vectors can accommodate relatively large cDNA; (ii) collagen VII transgene delivery to RDEB keratinocytes can produce a population of corrected cells; and (iii) RDEB dogs offer the possibility of testing therapies based on collagen VII gene replacement in immune competent hosts.

Absence of laminin 5 causes junctional epidermolysis bullosa in the Belgian horse

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Recent achievements in the genetic correction of keratinocytes isolated from patients with junctional epidermolysis bullosa (JEB) have brought somatic gene therapies within reach. Because gene therapy protocols require preclinical validation in animals, we elucidated the genetic basis of the hereditary junctional mechanobullous disease in the Belgian horse, a condition characterized by blistering of the skin and mouth epithelia. Immunofluorescence analysis showed absent expression of the γ2 chain of laminin 5 and designated *Lamc2* as the candidate gene. Comparative analysis of the nucleotide sequence of the γ2 cDNA isolated by RT–PCR amplification of total RNA purified from the epithelium of a JEB foal and a healthy control disclosed a homozygous base pair insertion (1368insC) in the affected animal. Mutation 1368insC results in a downstream premature termination codon and is predicted to cause absent expression of the laminin γ2 polypeptide. Our results also show that: (i) horse JEB genetically corresponds to the severe Herlitz form of JEB in man; (ii) the amino acid sequence and structure of the horse laminin γ2 chain are virtually identical to the human counterpart; (iii) the moderate eruption of skin blisters in the affected animals with respect to human H-JEB patients correlates with the protection provided by hair suggesting that hairless strains of animals with recessive skin disorders would be the best models for *in vivo* gene therapy approaches of skin blistering diseases. Finally affected foals are a convenient source of epithelial cells from tissues that cannot be obtained from human JEB patients.

Immunohistochemical and molecular analysis of cutaneous lesions in distemper, parvovirosis and canine herpes virosis

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Distemper, canine herpes virus and parvovirus diseases have numerous major clinical signs. This study discusses skin lesions diagnosed in five cases of viral disease among different pups. Cases 1 and 2 were two Labrador pups, a 3-month-old female and a 4-month-old male suffering from distemper. Case 3 was acute-onset parvoviral disease in a 4-month-old male Rottweiler. Case 4 was a 2-month-old female Brittany spaniel with both parvovirus and herpes virus infections. Case 5 was a 5-week-old female Shar-Pei that died of canine herpes viral disease two days after diagnosis. Both necroscopy and histopathological examinations of skin were done. Molecular analysis (PCR or RT-PCR) and immunohistochemistry with the specific monoclonal antibodies were performed on the skin biopsies. In the five cases, skin and mucosal eruptive disease with erythemateous lesions were the main cutaneous signs. The histopathological findings were similar: apoptosis of epidermis and lymphocytic exocytosis. Both lesional (cases 1, 3, 4, 5) and healthy skin (case 2) showed viral antigen particles in the different epidermal layers using PCR or RT-PCR analysis and immunostaining. The association between parvovirus and canine herpes virus (case 4) is unusual.

	Lesions	Histopathology	PCR / RT–PCR	Immunostaining
Case 1	facial erythema naso-digital hyperkeratosis	colloid bodies satellitosis multinuclear keratinocytes	+	viral Ag lesional skin and foot pads canine distemper virus
Case 2	papular-pustules foot pads atrophy	apoptosis satellitosis	+	viral Ag lesional and healthy skin distemper virus
Case 3	gastro-enteritis skin ulcers	colloid bodies satellitosis	+	viralAg:lesional skin parvovirosis
Case 4	scaly crusts vulvar erosions	apoptosis satellitosis	+	viral Ag mixed: parvovirosis/herpes
Case 5	conjunctivitis vaginitis pneumonia	colloïd bodies satellitosis	+	viral Ag:canine herpes virosis

Canine leproid granuloma: a South African perspective

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Canine leproid granuloma, first reported in Africa in 1973, has now been recorded in Australia, New Zealand, Brazil and North America. In the original survey conducted by Malik *et al*. in Australia, 45 cases were identified from 1990 to 1997. Larsson and others reported 12 cases in Brazil from 1990 to 2000, while Foley *et al*. recently published a report on seven cases in North America. From 1998 to 2001 we documented 47 cases of canine leproid granuloma from the Kwazulu-Natal province. The situation in other provinces of South Africa is not currently known. Clinical presentation in all cases was of single or multiple nodular granulomatous cutaneous lesions. In the vast majority of cases (> 85%), anatomical predilection sites were the pinnae and head. Staffordshire Bull Terriers and Boxers appear to be over represented in our survey. Histopathology was characterized by nodular to coalescing granulomatous inflammation consisting of a mixture of large reactive macrophages, lymphocytes, plasma cells and fewer neutrophils. Giant cells were rare to absent in our material. Formalin-fixed tissue blocks from 24 cases, were sent to Canada for molecular analyses. Genomic DNA was extracted and PCR analysis of mycobacterial 16*S* rRNA and dna J genes were performed. Amplified products from selected 16S rRNA-specific assays were sequenced. Thus far, the following matches from GenBank have been matched; unidentified Corynebacterineae (×2) and *Propionibacterium* acnes (×3). A *Propionibacterium* sp. specific PCR was developed and 20 of 22 were positive. Currently, the zoonotic potential of the 'mycobacteria' involved in canine leproid granuloma, is considered to be extremely low. Of concern to us in Kwazulu-Natal is the high incidence of HIV in our human population (> 20% of the population are seropositive) and the potential risk of zoonotic spread from a dog population with a significant incidence of canine leproid granuloma.

Oral eosinophilic granuloma in two German Shepherd dogs

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Oral eosinophilic granuloma (OEG) in dogs is a rare entity classified as an idiopathic disease. It is described in Siberian Husky and Cavalier King Charles and a genetic predisposition is suspected. Other hypotheses of the causes of OEG are trauma and hypersensitivity. The aim of this study is to describe OEG in two unrelated German Shepherd dogs. Case 1, Laika, a female German Shepherd dog, 2 years old, was presented for moderate dysphagia of few days duration. Case 2, Nanà, a female German Shepherd dog, 6 years old, was asymptomatic. The oral lesions were observed during a routine control for her atopic dermatitis. In both cases there was an ulcerative reddish plaque-like lesion in the palate vault. A complete blood count (CBC), bacterial and micotic culture, cytological and histological examinations were performed. CBC was normal and fungal cultures were negative. Cytological examination was characterized by epithelial cells, a few neutrophils, eosinophils and some extracellular bacteria. Histopathological examination was suggestive of eosinophilic granuloma with flame figures. Oral administration of prednisone 1 mg kg^{-1} day^{-1} for three weeks was curative and there was no recurrence during a follow-up that lasted several years. In case 1 a possible traumatic role was suspected, whereas in Case 2 hypersensitivity may have played a role. A genetic role could not be excluded in this breed. To the authors' knowledge, OEG had not been reported previously in German Shepherd dogs.

New protocol to treat demodicosis in dogs with milbemycin oxime: preliminary results

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New pharmacokinetics studies show that weekly administration of 3 mg kg^{-1} of milbemycin oxime achieve minimum plasma concentrations similar to those obtained by daily administration of 0.5 mg kg^{-1}. Furthermore, single doses of 5 mg kg^{-1} were well tolerated in safety studies. The objective of this study was to use this data on milbemycin (INTERCEPTOR®) for the treatment of demodicosis in dogs after the clinical cure had been obtained using the daily regimen. Seventeen dogs, aged from 4 to 132 months were included in the study. The demodicosis was associated with furunculosis in three dogs and with cellulitis in six dogs. Dogs were treated daily during the meal at the mean dosage of 1 mg kg^{-1} (range: 0.63–1.642 mg kg^{-1}) until clinical cure, and then followed by a weekly dose of 3 mg kg^{-1} until the parasitological cure occurred (two negative skin scrapings at 1 month interval). Clinical cure was obtained in all 17 dogs (100%). Parasitological cure was obtained in 16 of 17 dogs (94.1%). Relapses at 4 months were observed in 5 dogs (31.25%). Slight transient diarrhoea following each administration was noticed in 2 dogs. The mean time to obtain clinical cure was 3.6 ± 1.3 months (range: 1–5 months) and mean total duration of the treatment was 6.78 ± 1.98 months (range: 3–9.5 months). These results are comparable with those obtained previously with daily administrations of 0.5–1 mg kg^{-1} until complete parasitological cure. This new protocol with milbemycin for demodicosis results in a reduction of treatment cost of 13–40% depending on the weight of the dog. However, mild transient side effects (e.g. diarrhoea) cannot be excluded at this dose rate.

A clinical study on the use of Lufenuron in small animal dermatophytosis: a pilot study

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Dermatophytosis is a common skin disease in small animals. Lufenuron has been described as an alternative treatment for fungal infections. The aim of this study was to evaluate the efficacy of Lufenuron used as sole treatment against dermatophytosis in small animals. Thirty-three small animal veterinary clinics participated in this clinical study. A total of 352 pets with dermatophytosis was selected. The clinical diagnosis was confirmed by positive fungal cultures. Animals were treated with Lufenuron as a sole treatment. Follow-up fungal cultures were obtained and the animals were declared cured when the fungal cultures were negative. Two hundred and fifty-four cats (72%) and 98 dogs (28%) were treated with Lufenuron. In 23 clinics all cases received 60 mg kg^{-1} Lufenuron. In the remaining clinics the animals were treated with dosages ranging between 50 and 100 mg kg^{-1}. The majority of cases were treated twice with a mean interval of 19 (± 5.8) days. Mean time to clinical recovery was 25.5 (SD 14.8) days with a minimum of 7 days and a maximum a 60 days. Mean time to mycological recovery (negative cultures) was 34.7 (SD 16.4) days with a minimum of 12 days and a maximum of 90 days. No adverse drug reactions were reported. This treatment was convenient, safe and without adverse reactions. Mycological recovery was reached ≈ 10 days after clinical recovery. In the authors' opinion this therapy can be an alternative safe treatment to Griseofulvin in small animal dermatophytosis.

A prospective study of bacterial skin disease in dogs: 191 cases

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Bacterial pyoderma is one of the most frequent canine cutaneous diseases in veterinary practice but there are few prospective studies. The aim of the study was to better evaluate the history, clinical signs and microscopy data of canine bacterial pyoderma in a prospective multicentred study. One hundred and ninety-one dogs were included. The diagnosis of bacterial pyoderma was based on visualization of compatible skin lesions and the demonstration of pus and/or abnormally high numbers of bacteria on cytology. For each case, historical information (age, sex, breed), type, severity and extent of clinical lesions (papules, pustules, fistulae, etc.), and the results of cytological examination were recorded. There was no sex predilection (males 59.5%, females 40.5%). Various breeds were represented (Labrador Retrievers 10.99%, cross-breeds 8.38%, German Shepherd 7.33%, West Highland Terrier 6.81%, Poodle 5.76%, bulldog 4.71%). One hundred and seventy-four dogs (91.1%) were pure breed. Eighty-six dogs (46.74%) were short-haired vs. 98 dogs (53.26%) with long hair. Folliculitis was the most common type of pyoderma (67.37%), followed by furunculosis (26.84%), impetigo (12.1%) and cellulitis (7.9%). The most frequent lesion was erythema (92.67%), followed by pustules (81.15%), papules (79.06%), alopecia and epidermal collarettes (61.78%). The abdominal area was the most frequent lesional zone (136 cases, 71.2%). Cytological examination revealed bacteria in all cases (cocci: 93.7%, rods: 0.5%, mixed infection: 5.2%), associated with degenerated (73.8%) or nondegenerated (12.6%) neutrophils. In 23 dogs (12%), a concomitant infection with *Malassezia pachydermatis* was noted. This prospective study confirms data from the literature and provides new insights into the epidemiology, clinical signs and cytological characteristics of bacterial pyoderma in dogs.

This study was sponsored by Pfizer Animal Health.

Discriminant diagnostic criteria for the clinical diagnosis of canine FAD

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The diagnosis of flea allergy dermatitis (FAD) in the dog is based on clinical signs, allergy testing and response to flea control. However, the diagnostic value of allergy tests is quite controversial and the delay between consultation and response to flea control can be quite long. The aim of this study is to evaluate the possibility of using simple diagnostic criteria of FAD for epidemiological study. The protocol used was derived from protocols used for the assessment of diagnostic criteria of human and canine atopic dermatitis. We added to this method the observation of the response to flea control at 6 weeks. All animals presented for pruritus were included in the study. Diagnosis was made by each clinician and the diagnosis of FAD confirmed by response to flea control at 6 weeks. After this delay dogs were included in the FAD group or the pruritic dermatitis without FAD group. In order to make efficient comparisons between each group, the same number of dogs was included in each. In one case this was not possible, and so the results of this centre were not taken into account. During the consultation an exhaustive questionnaire based essentially on the clinical history, response to treatments and way of life was used. A complete clinical examination was made and lesion type and localization noted. C2 was used to estimate discrimination between groups for each criteria. The Youden test was calculated to estimate the intrinsic diagnostic value of each criterion. The total number of dogs included was 39 in the FAD group and 38 in the other group.

- Highly sensitive criteria (> 90%): low back lesions or pruritus.
- Highly specific criteria (> 85%): Good response to flea control, lower back lesions or pruritus, tail base lesions, tail lesions, living with cat(s).
- Highly discriminant criteria ($\chi^2 > 15$): in the FAD group: tail or tailbase lesions, lower back lesions, good response to previous flea control, previous good response to flea control; in the non FAD group: perioral lesions, pododermatitis, bilateral otitis externa.
- Three criteria are highly efficient in the diagnostic of FAD: lower back lesions ($Y = 0.8$), lower back pruritus ($Y = 0.7$), tailbase lesions ($Y = 0.6$). Because these criteria are not independent, only one is used: lower back lesions (highest Youden index).

The presence of lesions of the lower back in a dog presented for a pruritic dermatitis allows a diagnosis of FAD to be made with a risk of error of 10%. The use of such criteria for diagnostics must be validated in a multicentre international study.

Intramuscular use of medetomidine sedation for intradermal skin test in 435 dogs

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The aim of this study was to determine the suitability of intramuscular use of medetomidine for intradermal skin test (IDST) at a dose of 20 mg kg^{-1}, and its effects on wheal and flare responses to intradermal injection of standard allergens, positive and negative control. Thirty healthy dogs with no evidence of current skin disease and 405 atopic dogs were sedated with medetomidine at a dose of 20 mg kg^{-1} intramuscularly. Dogs were skin tested 15 min after the sedative injection, using 15 standard allergens Greer®, 12 standard allergens Artuvetrin®, positive and negative controls. The mean subjective and objective scores were determined for each allergen, positive and negative controls. Statistical analysis of wheal diameter in all dogs was performed and the necessity of physical restraint to obtain lateral recumbency was evaluated. There was no statistically significant difference between mean scores for positive and negative controls in the two groups, whereas there were significant differences between the mean scores for allergens between healthy and atopic dogs, as expected. The duration of sedation was adequate for all dogs; immobilization without restraint was good to excellent in 70% of dogs; in 30% moderate physical restraint was needed during IDST. Intramuscular use of medetomidine for IDST in dogs does not seem to suppress wheal and flare responses to injected allergens. Compared with an intravenous administration, the intramuscular administration route is easier in clinical practice and does not change the time or quality of sedation.

Dermal microdialysis: a new technique to investigate mediator release during an allergic reaction in canine skin

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Dermal microdialysis is an *in vivo* technique, based on the dialysis principle, that allows the collection of samples from the extracellular water compartment of the skin, and continuous monitoring of mediator release during an inflammatory cutaneous process. This study was designed to set up microdialysis in canine skin and to evaluate the usefulness of this technique in the investigation of the early response of an allergic process after an intradermal antigenic challenge. The study was performed in four Beagle dogs hypersensitive to *Ascaris suum*. A pair of microdialysis fibres of 5 and 3000 kDa cut-off were inserted into the dermis of the dog trunk under general anaesthesia. The fibres were perfused with phosphate-buffered saline (PBS), and the dialysate collected for 30 min before challenge (negative control). 20 μL of purified antigen of *A. suum* (Asc S 1, 0.1 mg mL^{-1}) was then injected intradermally between the fibres. Histamine concentration and total protein content were assessed from the dialysate samples collected from the 5 and 3000 kDa cut-off fibres, respectively, every 2 min for 40 min. Before challenge, the histamine concentration in the dialysate was undetectable. A maximum concentration of 480 nM was detected 4 min after antigen injection. This decreased to a minimum of 50 nM at 36 min post antigenic challenge, illustrating rapid metabolism of this mediator in the skin. Protein concentrations rose from a baseline of 62 mg mL^{-1} to a maximum of 810 mg mL^{-1} 10 min after the injection and persisted longer than histamine. We have shown, for the first time in dogs, that using dermal microdialysis it is possible to quantify the histamine and protein production within the wheal during an allergic reaction in the skin. This technique is useful not only to study the release of mediators, but also to evaluate the efficacy of pharmacological compounds in the control of inflammatory and allergic processes in the skin.

Increase of cyclosporin (CyA) oral bioavailibility after administration of grapefruit juice in the dog

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Grapefruit juice is able to change the pharmacokinetic parameters of a variety of drugs metabolized primarily by cytochrome P_{450} 3A such as cyclosporin (CyA). Because of both the high cost and the poor oral bioavailability of CyA, a co-administration with ketoconazole is widely used in veterinary practice. However, some side effects of this treatment have been recorded. In a three-phase crossover study, three male dogs were administered 100 mL of water (control), with 100 mL of liquid grapefruit juice, or with 10 g of frozen–dry (FD) grapefruit juice (equivalent to 100 mL of liquid grapefruit juice) with 100 mL of water, followed after 2 h by an oral single dose of CyA (15 mg kg^{-1}). Blood samples were collected after 0.08, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10 and 24 h. CyA analyses were performed with a TDX method (Abbott, IL, USA). Liquid and FD grapefruit juice pre-administration increased the C_{max} of CyA by ≈ twofold and the AUC of 35 and 39%, respectively, whereas T_{max} and $T_{1/2}$ were unaffected. Both 10 g of FD grapefruit juice and 100 mL of commercial liquid grapefruit juice significantly increased plasmatic concentrations of CyA in dogs. These findings suggest that FD grapefruit juice tablets co-administered with CyA could be used to increase CyA bioavailability, and reduce therapy cost with negligible side effects and ameliorate the palatability problem of grapefruit juice in dogs.

	CyA	GFJ + CyA	FD-GFJ + CyA
AUC (ng h mL^{-1})	7465 ± 926	10065 ± 1390*	10396 ± 1604*
C_{max} (ng mL^{-1})	1118 ± 134	1963 ± 562*	2314 ± 573*
$T_{1/2}$ (h)	1.94 ± 0.2	1.73 ± 0.9	1.54 ± 0.68
T_{max} (h)	1.5	1.8 ± 0.3	1.6 ± 0.6

GFJ, grapefruit juice.

Usefulness of one hypoallergenic diet in the management of atopic dermatitis in dogs

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Canine atopic dermatitis (CAD) is a multifactorial pruritic disease. It has been suggested that control of this dermatitis may be helped with the use of hypoallergenic diets. The aim of this study was to evaluate the interest of one such diet in atopic dogs not suffering from food allergy/intolerance. Twenty dogs diagnosed with CAD were included (history and clinical signs compatible with this diagnosis following Willemse's and Prélaud's criteria; at least one positive intradermal test to house dust mites; no response to a scabicidal treatment; absence of *Malassezia* dermatitis or pyoderma; no response to a home cooked food trial during at least 6 weeks). Each dog was randomly assigned to one of two groups: group A received the hypoallergenic diet (Iams F/P, Eukanuba Veterinary Diets) for 4 weeks, followed by a home-cooked fish and potato diet for 4 weeks, group B received the home-cooked diet first followed by the hypoallergenic diet. Dogs were scored at days 0, 30 and 60 by the same investigator, using a modified CADESI. Pruritus was evaluated by the owner using a visual analogue scale. Sixteen dogs completed the study (eight in group A, eight in group B). CADESI scores were reduced when dogs were fed the hypoallergenic diet compared with the home-cooked diet (respectively 45/57 in group A and 55/42 in group B). Pruritus was also improved with the hypoallergenic diet (21%, group A; 17% group B). The use of an hypoallergenic diet may help control pruritus and/or skin lesions in atopic dogs. This could be linked to a higher level of essential fatty acid in commercial diets and/or an improved digestibility.

The effect of omega-3 fatty acids on canine atopic dermatitis a double-blinded, placebo-controlled study

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The objectives of this study were to evaluate the influence of supplementation with flax oil and a commercial product containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in a placebo-controlled, double-blinded study on the clinical symptoms of atopic dogs. Twenty-nine dogs with nonseasonal atopic dermatitis were supplemented with 1000 mg flax oil capsules, a commercial product (3V Caps) containing 180 mg of EPA and 120 mg of DHA/capsule or mineral oil as a placebo at 1 capsule 5 kg^{-1} day^{-1} in a randomized, double-blinded trial. Dogs were evaluated by owners and clinicians using a clinical scoring system. Overall daily intake of omega-3 and -6 fatty acids was calculated for each patient before and after supplementation. Clinical scores of the three groups before and after supplementation were compared and correlated to the daily intake of fatty acids and their ratios. The dogs in the treatment groups responded significantly better than the dogs receiving placebo (mean improvement in clinical scores 5, 9 and 15% for the dogs on placebo, flax oil capsules and 3V Caps, respectively). Two dogs treated with 3V Caps and one dog on flax oil capsules went into complete remission with no other medication. All three dogs deteriorated within one week after cessation of supplementation and improved again once the treatment was restarted. There was no correlation between total omega-3 or omega-6 intake or omega-3 : omega-6 ratio and clinical scores or their improvement during the trial. Based on these results, fatty acid supplementation as performed in this study is useful in the treatment of canine atopic dermatitis.

Skin pH, thickness, hydration, elasticity, and trans-epidermal water loss in adult cats kept in group housing situations

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The objective of this study was to obtain data on typical biophysical parameters in group-housed adult cats. A second objective was to determine if there were gender or age effects on skin parameters measured. Fifty cats were selected and a 7×7 cm section of hair was shaved on the left dorsal side of the lumbar region one day prior to the biophysical measurements. Cats were evaluated for skin pH, thickness, hydration, elasticity and transepidermal water loss (TEWL) over a 2-day period. Temperature and humidity were kept constant in the group housing and evaluation room. The table below shows the means, standard deviations and range of values for the parameters measured. Sixty-three percent of the cats measured had skin thickness values between 1.25 and 2.0 mm. Fifty-two per cent of cats had hydration values between 4 and 6, whereas 72% of cats had a pH value between 6.0 and 6.6. The majority of cats had skin elasticity values between 0.25 and 0.40. TEWL values were very variable. Skin hydration was higher in male cats than female cats ($P < 0.10$) and to be positively correlated with age ($P < 0.01$). Skin thickness increased with age in healthy cats. All skin parameters were similar to values reported previously. There was a trend for a positive age correlation for hydration, which has not been reported previously.

Variable	Mean	SD	Minimum	Maximum
Skin thickness (mm)	1.738	0.568	1.093	3.640
Hydration	5.220	2.099	3.000	10.667
pH	6.379	0.348	5.567	7.500
Elasticity	0.353	0.164	0.147	0.879
TEWL (g h m^{-2})	7.998	1.657	3.600	11.500

Skin pH, thickness, hydration, and trans-epidermal water loss in kennel dogs

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The objective of the study was to obtain data on typical skin biophysical parameters for dogs in our kennel and to compare these results with published values. A second objective was to determine if there were breed (B), sex (S), gonadal status (G) or age (A) effects on the skin parameters measured. Fifty-one dogs were selected for biophysical measurements. A 7 × 7 cm section of hair was shaved on the left dorsal side of the lumbar region prior to measurement of skin pH, skin thickness, skin hydration, skin elasticity and transepidermal water loss (TEWL) over a 3-day period in May. Breeds included in the study were Labradors, Beagles and Fox Terriers. The table below shows means, standard deviations, the range of values for the skin parameters measured and effects found to be significant ($P < 0.05$) by analysis of variance. Labradors had thicker skin and higher skin elasticity than Fox Terriers or Beagles. Males had thicker skin than females. Neutered Labradors had thinner skin than intact Labradors. Female Beagles had higher skin pH than male Beagles. Neutered Beagles had higher skin pH than intact Beagles. Female Labradors had higher skin elasticity than male Labradors, female Fox Terriers and female Beagles. Neutered females had higher skin elasticity than neutered males. Some skin parameter values were observed to differ according to breed, sex, gonadal status or age.

Variable	Mean	SD	Minimum	Maximum	Significant effects
Skin thickness (mm)	3.64	1.03	1.68	6.38	B, S
Skin hydration	20.27	7.94	6.33	41.33	A*B
Skin pH	8.49	0.84	5.7	9.77	B*S, B*G
Skin elasticity	0.56	0.14	0.28	0.94	B, B*S, S*G, B*S*G
TEWL (g h m^{-2})	13.21	5.36	6	28	–

Corneometric assessment of seasonal variations of skin hydration of 124 normal South African dogs

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Skin hydration is a relatively unexplored field of veterinary dermatology. The objective of this study was to measure skin hydration of normal dogs to establish baseline values for of South on different cutaneous areas African dogs and to compare the findings of winter vs. summer. A corneometer was used to measure skin hydration (*C* values) in 62 dogs in summer and 62 in winter, using 4 replicates on 20 different cutaneous sites. For statistical analyses, an ANOVA was performed with the following variables: season, sex, age, weight, length and thickness of hair coat, activity and mode of life. Then, for the significant variables, multiple comparisons were performed using a Newman–Keuls test. Right and left aspects of the 20 sites were pooled in 11 different cutaneous areas: periorbital ($C = 5.4$); external and internal ear pinnae ($C = 6.2$ and $C = 16.8$, respectively); chin ($C = 8.6$); axillae ($C = 8.0$); inguinal ($C = 12.7$); palmar and dorsal aspects of front ($C = 5.4$) and hind ($C = 5.1$) feet; and ano-genital ($C = 5.9$). Every cutaneous area was less hydrated in winter than in summer ($P < 0.0001$ for 7 on 11), except the internal ear pinna which was more hydrated in winter ($P < 0.0001$). Total skin hydration was also lower in winter ($C_{mean} = 6$) than in summer ($C_{mean} = 7.5$), $P < 0.0001$. Comparing the various cutaneous areas, chin, axillae and particularly internal ear pinnae and inguinal areas were more hydrated than the other cutaneous areas measured. These findings show that the skin of South African dogs is less hydrated in winter than in summer. It might then be useful to use topical products (shampoos and lotions) that hydrate the skin of dogs more frequently in winter. Any correlation between variously hydrated cutaneous areas and preferential areas for the development of certain dermatoses, e.g. atopy, impetigo and chin pyoderma may be studied further.

Efficacy of combined topical therapy with keratoregulating shampoo and lotion in the management of keratoseborroeic disorders associated with *Malassezia* proliferation in dogs

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The present study was designed to evaluate the clinical and antimicrobial benefit of combined keratoregulating shampoo and lotion therapy (Sebomild P®, Virbac) in the management of *Malassezia*-associated keratoseborrhoeic disorders (KSD) in dogs under field conditions. Fourteen dogs presenting with clinical signs of KSD and abnormally high cutaneous *Malassezia* populations were studied. *Malassezia* overgrowth was confirmed by microscopic examination of cutaneous smears. Exclusion criteria were: associated pyoderma, severe concomitant otitis, parasitic dermatitis and dermatological treatment in the previous week. The dogs were bathed using the keratoregulating shampoo (containing piroctone olamine and ammonium lactate) once a week for 3 weeks while the lotion (with piroctone olamine and salicylic acid) was sprayed all over the body twice a week, the day after the shampoo and 3 days later. No other treatment was administered to the dogs. Clinical scores (KSD, pruritus, erythema, excoriation and lesion extent) and *Malassezia* counts recorded at cytology were graded on a 5-point scale according to severity, before treatment (D1) and at the end of the treatment period (D20 ± 1). Dogs included in the study showed moderate to marked KSD associated with large *M. pachydermatis* populations. Atopy was a frequent underlying disease (57% of the cases). Significant reductions of all clinical scores and *M. pachydermatis* counts were recorded over the 3-week treatment period ($P < 0.01$). Mean clinical scores were reduced by more than 50% while yeast counts were reduced by more than 75%. Combined use of Sebomild P® shampoo and lotion is highly beneficial in the management of moderate to marked keratoseborrhoeic disorders and helps reduce the frequently associated *M. pachydermatis* overgrowth. The antimicrobial, keratoregulating and cleansing effects of the shampoo are prolonged and enhanced by the regular use of the leave-on lotion in the intervals between baths.

Feline phaeohyphomycosis caused by *Phyalophora verrucosa*

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Phaeohyphomycosis is the collective name for a group of opportunistic dematiaceous fungi characterized by the development of cutaneous and subcutaneous infections. The purpose of this study is to report a case of *Phyalophora verrucosa* infection in a domestic cat. A Domestic Short Hair, 8-year-old, male, indoor/outdoor cat was presented for a black, ulcerated, sessile mass on its left auricular pinna, draining a black–brownish fluid. Cytological, histopathological examination and fungal cultures of the mass were carried out. Haematological analyses, serology for FeLV and FIV and urine analyses were performed. Cytological examination showed inflammatory cells and brown septate fungal hyphae. The cultured material on a dermatophyte test medium/Sabouraud, showed colonies of *Phyalophora verrucosa*. Histopathological examination, stained with haematoxylin & eosin (HE) and periodic acid Schiff (PAS), revealed a granulomatous dermatitis with numerous pigmented fungal elements. The cat tested positive for FIV. Blood count showed mild anaemia. Biochemical and urine analysis showed evidence of chronic renal failure. The owner declined full surgical excision of the left pinna so Itraconazole (10 mg kg^{-1} day^{-1}) as oral medication and local flushing with miconazole were initiated. After two months and three weeks no improvement was detected and a new granuloma developed. The cat was euthanased because of the poor general conditions associated to chronic renal failure. Feline phaeohyphomycosis may be secondary to a cat bite or wound contamination. In the case reported here FIV infection and renal failure enhanced the fungal infection. Medical therapy was unsuccessful as reported previously by other authors, so surgery must be considered the therapy of choice.

Dermatophytosis due to *Microsporum persicolor* in dogs: a retrospective study of 14 cases (1990–2000)

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The prevalence of dermatophytosis due to *Microsporum persicolor* (MP) is suggested to be between 2 and 10% of canine dermatophytosis. The purpose of this study is to report 14 canine cases of dermatophytosis due to *Microsporum persicolor* between 1990 and 2000. Standard mycological examinations were performed. Definitive diagnosis was based on positive fungal culture (DTM or Sabouraud middle) and microscopic identification. In eight cases, biopsies were performed. Classical antifungal agents were used: griseofulvin, ketoconazole or enilconazole. Age at onset varied between 2 and 10 years. Hunting breeds were over-represented (11/14), particularly terriers (six Fox, two Jack Russell). One dog had regular contact with a guinea pig. Lesions were always facial (14/14), particularly on the dorsum of the muzzle (12/14), but could have other localizations (limb, neck). We especially observed alopecia (11/14), erythema (11/14), scales (11/14) and crusts (11/14). Pruritus could be moderate (6/14) or severe (1/14), but was absent in seven dogs. Wood's lamp and hair examination were always negative. Macroscopic and microscopic aspects of fungal cultures were characteristic of MP. Histopathology showed lesions of interface lichenoid dermatitis, lymphoplasmocytic infiltrate, perifolliculitis and folliculitis. Hyphae without arthrospores were observed in superficial keratin in only five cases (haematoxylin & eosin and periodic acid Schiff). Clinical cure was achieved in all the dogs (mean duration of treatment: 49 days). A follow-up of one year for eight dogs showed two recurrences after 3 and 6 months. In both cases, new contact with rodents was confirmed (hunting dogs). This study confirms the findings of previous studies: the importance of anamnesis (contact with rodents), facial lesions and good prognosis with treatment. An interface lichenoid dermatitis was systematic in our study but mycological culture was necessary for the definitive diagnosis.

A case of leishmaniasis with histological findings of sebaceous adenitis in an Akita dog

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Canine leishmaniasis (CL) is a multisystemic disease, endemic in Portugal, caused by the protozoon parasite *Leishmania infantum*. Sebaceous adenitis (SA) in dogs is an uncommon skin disease. The Akita dog breed has a high predisposition for SA. The aetiology is unknown, but apart from a genetic predisposition, an immune-mediated pathogenesis may also influence the onset and course of SA. A 5-year-old, noncastrated, male Akita dog, presented with a 6-month history of intense skin scaling, mainly on the dorsal midline, and moderate hair loss. There was no evidence of associated pruritus. An enzyme-linked immunosorbent assay (ELISA) test for leishmaniasis was negative. The dog was treated with oral essential fatty acids and propylene glycol/glycerine baths. Three months later the scaling was still very intense and had spread to involve the head. A rib bone marrow biopsy did not reveal *Leishmania*. A skin biopsy was performed. The skin biopsy showed peri-adnexial inflammatory infiltrates, mainly lymphocytes, macrophages and plasma cells, associated with focal destruction of sebaceous glands. Inside the macrophages some material that resembled *Leishmania* was seen. The histopathological diagnostic was pyogranulomatous peri-adnexial dermatitis with sebaceous adenitis probably associated with leishmaniasis. A *Leishmania* PCR blood test was positive. The haematological and biochemistry profiles were within normal limits. Treatment with antimoniate of *n*-metil glucamine (100 mg kg^{-1} subcutaneously for 40 days with a middle pause of 10 days) and allopurinol (20 mg kg^{-1} twice daily, until a negative PCR) was started. No other treatment was given. After 2 months the scaling was significantly better. The PCR remained positive. Six months later scaling was cured but the PCR was still positive. Histopathological findings of CL can resemble SA. PCR is an important tool in seronegative dogs. More studies are needed to establish whether the breeds of dogs that are predisposed to SA are also predisposed to the exfoliate form of CL.

Idiopathic mucosal fibropapilloma of the penis in a dog

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Fibropapillomas of viral origin are frequent in dogs and at least five syndromes are recognized. The aim of this study was to report a case of idiopathic mucosal fibropapilloma of the penis in a dog. A 6-year-old, male spitz dog was presented with an inguinal mass and urinary problems. Physical examination showed the presence of neoplasia inside the prepuce. Complete blood count cells and biochemical profile were normal. The dog was anaesthetized to perform cytological and histopathological exams. Surgical excision of the preputia skin was necessary to show the neoplasia. This revealed an ulcerated peduncolated and cauliflower-like mass of 6 cm of diameter arising from penile mucosa. Cytological examination was performed and impression smears were suggestive of an inflammatory process. A fine needle aspirate revealed a homogenous population of keratinic epithelial cells with rare nuclear and cytological atypia. Histopathological examination was suggestive of a fibropapilloma. The samples did not show any evidence of ballooning degeneration (koilocytosis) or basophilic intranuclear inclusion bodies. Electronic microscopy and viral detection failed to reveal Papillomavirus. The neoplasia was identified as idiopathic fibropapilloma of penis mucosa. Viral papillomas are frequent in dogs and generally affect young animals. Idiopathic fibropapillomas seem to be related to an 'aspecific' reaction more than to a neoplastic process. In this case, the electron microscopy, viral and histopathological examinations failed to reveal any viral agent. To the authors' knowledge this is the first report of a idiopathic mucosal fibropapilloma of the penis.

Epizootic alopecia in maedi-visna

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Sheep viral diseases induce some specific symptoms (e.g. foot and mouth disease, blue tongue). Muco-cutaneous lesions are often found: vesicles, ulcers, crusts. In the case of maedi-visna, the main symptoms involve the respiratory tract (and the nervous system) but cutaneous signs are not reported. In the space of 15 days, a 30% mortality was recorded in a flock of transhumant cross-breed Andorran sheep grazing in the coastal area of the Herault region in France. The animals affected were adults and showed major respiratory disorders, anorexia and nervous symptoms. In adults, the development lasted from 6 to 7 days. In all cases, an extensive, symmetrical, trunk-located, nonpruriginous alopecia of the fleece began along the animal's back. First, the wool became felt-like or matted, and then fell out, before the animal died or made a poor recovery. In the context of foot and mouth disease, a certain number of specimens were taken in order to ensure an accurate diagnosis. Toxicological, microbiolgical, serological and necroscopy examinations were carried out. The conclusion reached following the anatomo-pathological examination was acute interstitial pneumonia with diffuse lymphocytic infiltration in adult animals, and in lambs the conclusion was synovitis and pulmonary oedema with grey pneumonia of the apical lobes. The serological test for maedi-visa was significantly positive in both the cases for which an autopsy was performed and in more than 60 adult animals of the 350 in the flock subjected to general screening. Alopecia was severe, accompanied by a moderate atrophy of hair follicles and erythema without any specific type of lesion. In sheep, localized or generalized alopecia accompanying an infection is not a rare occurrence. It may be associated with blue tongue, scrapie, rabies or Aujeszky's disease. Pruritus is to be found in these last three instances.

Bovine cutaneous schwannoma

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A 9-year-old female, half-breed cow had multiple subcutaneous, oval, grey, painful nodules about 1–2 cm in diameter. The clinical signs were difficult walking and pyometra. The nodules were bilaterally located on the neck, shoulder and chest areas and had a firm consistency and smooth surface. Some of nodules were removed and examined histopathologically. Microscopically, there were two patterns; Antoni type A and B. The immunohistochemical study was positive for S-100 protein but negative for epithelial membrane antigen and schwannoma was diagnosed.