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Idiopathic mucosal penile squamous papillomas in dogs

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Fibropapillomas of viral origin are frequent in dogs, and at least five clinical syndromes are recognized. This study reports the clinical and pathological features of 10 canine idiopathic mucosal squamous papillomas localized on the penis. Ten male dogs of various breeds (Pekingese, Yorkshire terrier, Boxer, Spitz, Giant Schnauzer, Bracco Italiano), aged between 6 and 13 years, were presented for urinary problems associated with a genital mass inside the prepuce. Cytology and surgical biopsy samples were taken under anaesthesia. In all cases, surgical resection of the preputial skin showed ulcerated cauliflower-like masses arising from the penile mucosa, ranging in diameter from 2 to 8 cm. Impression smears were indicative of an inflammatory process, while fine needle aspiration revealed epithelial cells with rare nuclear and cytological dysplasia. Histopathologically, the epidermis showed normal differentiation with elongated rete ridges slanted towards the periphery of the lesion. Viral cytopathic effects such as ballooning degeneration or basophilic intranuclear inclusion bodies were not present. Immunohistochemistry and PCR failed to reveal papillomavirus. The neoplasias were defined as idiopathic mucosal penile squamous papillomas. Viral papillomas are clinically classified as cutaneous papillomas, cutaneous inverted papillomas, multiple pigmented papular cutaneous papillomas, multiple pigmented plaques and multiple papillomas. Viral papillomas usually affect young dogs, whereas the idiopathic squamous papillomas described arose in middle-aged or older dogs. This new recognized group seems to be related to a 'nonspecific' reaction rather than to a neoplastic process of viral origin. The neoplasms appeared to be larger than those previously reported on other mucosal and cutaneous sites.

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Immune responses to *Staphylococcus aureus* and *Psoroptes ovis* in sheep infected with *P. ovis* sheep scab mite

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Sheep scab is typically a severe, debilitating, exudative dermatitis accompanied by exuberant crust formation and intense pruritus. It is caused by the highly contagious astigmatid mite *Psoroptes ovis*. In sheep, lesions caused

by *P. ovis* may become colonized by *Staphylococcus aureus*. The present study compares clinical signs, lesional area and the immune response to *P. ovis* and *S. aureus* in *P. ovis*-infested sheep with and without secondary *S. aureus* infection. No differences were detected in the clinical signs or lesional areas in the *S. aureus*-positive and *S. aureus*-negative sheep. However, 6 weeks after infestation an IgG but not IgE isotype antibody response to *S. aureus* was detected in the *S. aureus*-positive but not in the *S. aureus*-negative group of sheep. This response targeted *S. aureus* antigens with molecular weights of approximately 36, 38, 50 and 65 kDa. In addition, 6 weeks after infestation, an IgE response to *P. ovis* was detected in the *S. aureus*-positive but not in the *S. aureus*-negative group of sheep. This study shows that *S. aureus* provoked a significant immune response in sheep infested with *P. ovis*.

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Comparison of a corticoid agent with a mixture of antifungal, antibiotic and corticosteroid agents for the treatment of allergic otitis in dogs

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The study aims to test the tolerability and efficacy of a combination of antibiotic–antifungal and corticoid agents in comparison with a corticoid agent used alone for the treatment of acute allergic erythematous-ceruminous otitis externa (AECOE) in dogs. The combination product (Aurizon®, Vetoquinol, containing marbofloxacin, dexamethasone and clotrimazole) was compared with the corticoid agent (Percutalgine® gel, Besins International, dexamethasone) for topical treatment of AECOE in a randomized and blinded clinical study. Twenty dogs with AECOE were included. In each case one ear was treated with Aurizon® solution, the other ear with Percutalgine® gel. The substances were applied to the external ear canal once daily for 7 days. Efficacy and tolerability were evaluated daily by the owner using a visual analogue scale and at inclusion and after 8 days by a clinician using a three-step otitis score (erythema, cerumen, auditopodal reflex). In addition, smears were analysed to identify and quantify the causative pathogens (cocci and/or *Malassezia* yeasts). Both medications were well tolerated by the patients. The inflammation score improved rapidly in both groups. A statistically significant difference was noted for the quantity of cerumen on days 4–7 ($P < 0.05$), and a statistically significant improvement of cytological scores was noted for the combination product ($P < 0.05$). Aurizon® appears to be well tolerated and more effective than therapy with a corticoid agent alone for the treatment of AECOE.

This study was funded by Vetoquinol, France.

Occurrence and antimicrobial susceptibility patterns of small animal pathogens isolated from skin and ear infections in Portugal (2001–05)

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Skin infections and otitis externa therapy failure is often because of antimicrobial drug resistance. A total of 531 clinical isolates were recovered from dogs and cats included in the FMV Antimicrobial Surveillance Program between January 2001 and December 2005. Sensitivity testing was performed according to National Committee for Clinical Laboratory Standards veterinary guidelines (M31-A2). The majority of pathogens were Gram-positive cocci (64.8%). In skin infections, the most frequent bacteria isolated were: *Staphylococcus* spp. (62.3% of which 72.1% were *Staphylococcus intermedius*), *Enterococcus* spp. (8.7%), *Streptococcus* spp. (7.2%), *Escherichia coli* (6.5%), *Pseudomonas* spp. (5.1%), and *Proteus* spp. (4.4%). In otitis externa the most frequent isolates were: *Staphylococcus* spp. (44.1% of which 61.8% were *S. intermedius*), *Pseudomonas* spp. (21.1%), *Proteus* spp. (13.2%), *Streptococcus* spp. (7.6%), *E. coli* (3.8%), and *Enterococcus* spp. (1.5%). Antimicrobial sensitivity was tested. Staphylococcal resistance was high for amoxicillin (57.1%) and important for clindamycin (12.0%). Sensitivity remained high for fluoroquinolones and oxacillin (only three coagulase-negative staphylococci harbouring the *mecA* gene). Penicillin and ampicillin remained active antimicrobial agents against streptococci and enterococci. Emerging gentamicin and streptomycin resistance was noted in enterococci (five strains HLSR and one strain both HLGR and HLSR). No vancomycin resistant enterococci were detected. No ESBL-producing strains of Enterobacteriaceae were found. *Proteus* isolates were sensitive to amoxicillin and fluoroquinolones but the opposite was true for *E. coli*. Ceftazidime and imipenem showed an excellent spectrum of activity (> 90% susceptibility) against *Pseudomonas*.

This study was self-funded.

Multiple epidermal cysts in a 4-year-old stallion

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Follicular cysts are uncommon in the horse and are usually classified according to the differentiation of their epithelial lining into infundibular, isthmus-catagen, matrical and hybrid forms. Previous reports described follicular cysts as usually solitary, with predisposition to affect the head and distal limbs. This case report describes a case of generalized multiple epidermal cysts. A four-year-old, chestnut, Spanish purebred stallion was presented with multiple cutaneous masses that had developed progressively in the previous 2 years. Lesions were nonpruritic and did not respond to changes in the diet. On clinical examination, more than 100 nodules and papules were seen, distributed over the trunk, neck, head and limbs. Lesions varied from 0.5 to 3 cm in diameter, were firm, well-circumscribed and nonadherent to subcutaneous tissues.

Five nodules were removed for histopathology. Microscopically, the lesions were simple follicular cysts (infundibular and isthmus-catagen) with squamous epithelium and keratin filled cavities. Follicular cysts are uncommon in the horse and the presentation of more than 100 cysts with a generalized distribution has not been previously reported. Dermoid and follicular cysts can occur simultaneously in the same animal. Although all nodules examined were follicular cysts, dermoid cysts cannot be excluded as there were too many nodules for surgical treatment, and the owner declined further biopsies.

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Selamectin spot-on formulation to treat sarcoptic mange in seven rabbits: a case report

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Traditional treatments for rabbits with sarcoptic mange are of low to moderate efficacy or potentially toxic. Seven mixed-breed rabbits from the same litter, aged 5 months and of both sexes, were admitted with a 2-month history of partial anorexia, progressive weight loss and pruritic dermatitis. History indicated a contagious skin disease as both parents had had the same type of lesions and the owner had developed a pruritic papulocrustous exanthema 4 months earlier. Dermatological examination showed alopecic areas with erythema and severe crusting over the face, muzzle, eyelids, pinnae, paws and external genitalia. Skin scrapings from all affected rabbits contained numerous sarcoptid mites. Two rabbits were also scrape positive for *Leporacarus gibbus* fur mites. Histopathology, revealed orthokeratotic hyperkeratosis, mites embedded within the stratum corneum, papillated or regular epidermal hyperplasia and superficial mononuclear dermatitis with scarce heterophils. A selamectin spot-on formulation (Stronghold® Pfizer) was applied between the shoulders, twice within 30 days at the dosage of 8–14 mg kg⁻¹ BW. Examination of the rabbits on the 15th, 30th and 45th day after the beginning of treatment showed a progressive resolution of both skin lesions and pruritus, a spectacular improvement of the appetite and body condition and no obvious side-effects. Clinical and parasitological cure, achieved within 30 and 45 days, respectively, and not accompanied by adverse reactions, makes selamectin a good choice for the treatment of leporine sarcoptic mange.

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Localization of oestrogen and progesterone receptors in canine skin

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Sex steroid hormones are important in the regulation of skin and hair follicle development and function. In the dog,

steroid hormones are thought to play a role in endocrine alopecia and the immunohistochemical localization of androgen and oestrogen receptors in different body sites has been reported recently. In this report, we immunolocalized oestrogen receptors (ER- α) and progesterone receptors (PR) in skin biopsies from seven different body sites obtained from seven nonlesional dogs. Receptor expression was determined immunohistochemically using commercially available polyclonal antibodies. In each sample the distribution of ER- α and PR in the epidermis, sebaceous glands, apocrine glands and hair follicles was determined. Only nuclear reactivity was considered positive. Samples from canine uterus were used as positive control.

Oestrogen receptors. Weak expression was seen in the perineal epidermis of one dog. Sebaceous glands at all body sites of all dogs stained but with different intensity and frequency of positive cells. Apocrine glands lacked ER α staining although isolated positive cells were seen without a regular pattern between dogs or body sites. Hair follicles from all body sites and all dogs consistently expressed ER- α . All showed similar numbers of positive cells and intensity of nuclear staining. Progesterone receptors. No PR were seen in apocrine glands. In the epidermis and other skin appendages, weak nuclear staining was seen in a few cells. No significant difference was found between body sites.

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Immunohistochemical characterization of a sebaceous gland carcinoma in a gerbil (*Meriones unguiculatus*)

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In humans, sebaceous carcinoma is characterized by expression of cytokeratins (CKs) such as AE1 (CKs 10, 14, 16, 19), LP34 (CKs 1, 5, 6, 8, 10, 14, 18), CK 8 and CK19. The tumour is also immunopositive for epithelial membrane antigen (EMA) and negative for carcinoembryonic antigen (CEA). Nuclear accumulation of p53 protein and increased expression of c-erbB-2 have also been noted and correlated with a worse prognosis. So far, canine sebaceous carcinomas have been immunostained for only 34 β E12 (CK 1, 5, 10, 11). Sebaceous gland carcinoma is a common neoplasia in male gerbils, but, to date, there are no reports of its immunohistochemical properties. This report links the histopathological and immunohistochemical features of a sebaceous carcinoma from a 4.5-year-old male gerbil. We studied the immunoreactivity profiles for p53 protein, CEA, EMA, c-erbB-2 (HER2/neu), cytokeratin 14, AE1/AE3 (CKs 1–8) and Ki-67 (MIB-1). The tumour was diffusely positive for p53, EMA, c-erbB-2, cytokeratin 14 and AE1/AE3, and focally positive for CEA. Hyperplastic areas near the tumour did not stain for p53, CEA or c-erbB-2. Ki-67 staining frequency in the tumour (labelling index 71.5), compared to hyperplastic areas (labelling index 19.4), indicates increased proliferation of the tumour. These findings suggest that p53 and c-erbB-2 alterations (mutations) are present in sebaceous gland carcinoma in gerbils.

This study was self-funded.

Prevalence and susceptibility of pathogenic bacteria responsible for skin and soft tissue infections in pets between 1994 and 2004

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Marbofloxacin is a fluoroquinolone developed for cat and dog treatment and marketed in Europe since 1995. Vetoquinol set up a European survey programme in France, Germany, UK, Italy, the Netherlands, and Belgium to assess marbofloxacin activity against pathogenic strains isolated from skin and soft tissues. Between 1994 and 2004, 1249 pathogenic strains were collected: 437 from cats and 947 from dogs. The two major cat infection sites were skin (51%) and wounds (45%). *Pasteurella multocida* was the main pathogen isolated (67.5% from dermatological and 15% from wound infections). Cat abscess isolates were all strains of *P. multocida*. The main pathogenic strain isolated from dogs was *Staphylococcus intermedius* with the next most frequent isolate *Pseudomonas aeruginosa* strains (16.5%). Marbofloxacin MIC testing in a referenced laboratory according to Clinical Laboratory Standards Institute guideline M31-A showed that marbofloxacin activity was good against the two main cat and dog pathogens (*P. multocida* and *S. intermedius*) with 97–100% sensitivity. No change over time was observed with a MIC90 between 0.023 and 0.095 μ g/mL against *P. multocida* strains and between 0.221 and 0.416 μ g/mL against *S. intermedius* strains. Marbofloxacin remains a good treatment option for skin and soft tissue infections in dogs and cats.

This study was funded by Vetoquinol, France.

Efficacy of a 0.0584% hydrocortisone aceponate spray in the treatment of pruritic inflammatory skin disease in dogs

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The efficacy of 0.0584% hydrocortisone aceponate (HCA) spray (Virbac SA) was compared with a 0.125% prednisolone acetate, 0.15% hexetidine and 2% benzocaine spray (registered control product), in reducing clinical signs of pruritic inflammatory skin disease in dogs. One-hundred and five dogs with pruritic dermatitis of known or suspected allergic basis (atopic dermatitis, flea allergy dermatitis, acute moist dermatitis, contact allergy, undetermined allergic pruritus) were included in a multicentric randomized clinical trial in France and Belgium. Fifty-four dogs were sprayed s.i.d. for 7 days with the HCA solution, while 51 were sprayed b.i.d. for 7 days with the control solution (D0–D6). No other antiallergic treatment was allowed. On D0, D7 and D14, pruritus and skin lesions were scored by investigators. At baseline, pruritus was graded as marked in 88.6% of dogs. Erythema (98.1% of dogs) and excoriations (68.6%) were the most frequent signs reported. On D7, pruritus was reduced by 75.6% and 70.3% in the HCA and control group, respectively ($P > 0.05$). The lesion score decreased significantly from D0 to D14 in both groups, with

a more rapid decline at D7 with HCA ($P < 0.05$). The mean reduction of the lesion score on D7 was 71.7% with HCA and 60.1% with control. No dog relapsed on D14 in the HCA group, whereas two dogs relapsed in the control group. Both treatments were well tolerated. In this clinical trial a new topical diester corticosteroid, HCA, was effective in reducing pruritus and skin lesions in dogs with pruritic dermatitis.

This study was funded by Virbac, France.

Canine leishmaniasis: atypical skin lesions observed in the Ebro Valley Veterinary Teaching Hospital

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Canine leishmaniasis is a common disease in Spain with a prevalence of 7–10% in the Aragón region. Skin lesions are the most common manifestation of this disease. The purpose of this abstract is to review the literature and to describe our experience with atypical skin lesions in canine leishmaniasis seen in Ebro Valley Teaching Hospital/Aragón Region. Classical symptoms include exfoliative dermatitis with characteristic light scales, nonpruritic symmetrical alopecia starting on the head and mainly around the eyes, dry generalized or localized seborrhoea, cutaneous ulcers and onychogryposis. Less common manifestations are nodules, sterile pustular dermatitis, depigmentation of the external nares, and inoculation chancres. Atypical manifestations such as forelimb nodular dermatofibrosis without renal involvement, papular dermatitis, or atypical localizations of nodules or cutaneous ulcers have been reported. We report granulomatous lesions on a bitch's teat as a single manifestation of *Leishmania infantum*, a granulomatous lesion on the penis, a granulomatous lesion on the tongue, an ulcerative-crusting dermatosis affecting both forelimbs, kerion-like lesion in puppy and papulo-nodular lesions in abdominal area in a litter of rottweilers. In geographical endemic areas, we think it is necessary to include leishmaniasis in the differential diagnosis of almost all skin disease. We encourage clinicians to look for underlying leishmaniasis in difficult-to-diagnose and poorly responsive skin lesions even if serology for antileishmania antibodies is negative.

This study was self-funded.

Unusual presentations of cutaneous leishmaniasis

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The diagnosis of canine leishmaniasis is usually based on detection of antileishmania antibodies in serum, alteration of protein electrophoresis and compatible clinical signs. We present three atypical cutaneous presentations of leishmaniasis: one of nodular cutaneous leishmaniasis, one of granulomatous conjunctival plus papular cutaneous lesions and a third of pustular lesions. These presentations have not been described previously without serological evidence of leishmaniasis and with normal protein electrophoresis analysis. All were, finally, diagnosed by microscopic

identification of leishmania amastigotes in skin biopsies. Treatment with allopurinol and levamisol induced remission. New forms of canine cutaneous leishmaniasis are being diagnosed. The diagnosis is not easy and skin biopsy is necessary. Prognosis is favourable as there is no systemic involvement, and the response to treatment is excellent. These patterns of cutaneous leishmaniasis could represent an immunocompetent reaction specific for leishmania infection. Recently, it has been suggested that lesions in these patterns of cutaneous leishmaniasis could be caused by the own presence of the parasite rather than the immunological response. The simple cutaneous lesions could represent the inoculation site of the parasite as in humans. It is important to identify these new forms of leishmaniasis and investigate the immunological response of the host.

Skin lesions in goats caused by *Chorioptes caprae* and *Microsporium nanum*

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We describe an unusual case of skin lesions in goats caused by parasite *Chorioptes caprae* and dermatophyte *Microsporium nanum*. In a large flock of goats, a few animals developed nonfollicular papules and crusts extended into areas of alopecia and erythema. These were mainly on the lower parts of the hind legs. Mites were demonstrated in skin scrapings after boiling in 10% KOH. The isolated parasites were identified morphologically as *Chorioptes caprae*. Mycological culture on Sabouraud-dextrose agar supplemented with chloramphenicol and cycloheximide and microscopic morphological characteristics enabled identification of *M. nanum* which is resistant to cycloheximide and produces macroconidia that are solitary.

Chorioptes caprae is quite common in goats and an important surface-living mite. Pruritus, excoriation and secondary bacterial infection occur commonly, but fungal infection less frequently. Typical locations are skin on the lower limbs, inguinal region or scrotum. *Microsporium nanum* is a geophilic and zoophilic fungus, frequently causing chronic non-inflammatory lesions in pigs and a rare cause of ringworm in humans and animals other than pigs. The role of *M. nanum* in our case was unclear but the presence of keratinolytic enzymes that are produced by this dermatophyte undoubtedly made the infection worse so treatment with both antiparasitic and antimycotic drugs was required. The present report is, to our knowledge, the first to describe *M. nanum* infection in goats also affected by mites.

This study was self-funded.

A novel papillomavirus identified in multiple skin neoplasms in twin goats

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Papillomaviruses (PV) have rarely been found in caprine neoplasms, and never in multiple coexisting tumours. We

describe a novel papillomavirus in multiple neoplastic lesions in two adult twin goats. Anatomico-histopathological, immunohistochemical and ultrastructural investigations characterized the neoplasms as two ocular squamous cell carcinomas, an ocular sarcoma, an ocular fibrosarcoma, a cutaneous malignant melanoma, multiple cutaneous fibropapillomas and squamous cell carcinomas. No viral particles were detected by ultrastructural investigations. Immunohistochemical staining was positive for papillomavirus antigen (L-1) only in the ocular squamous cell carcinomas. Immunohistochemistry for HSP 27 and HSP 73, involved in PV assembly, showed increased expression in the tumoral cells. Polymerase chain reaction (PCR) with several sets of degenerate and consensus primers for PV indicated the presence of amplified product. Sequence searching/alignment in the data bank showed homologies to human, chimpanzee, and bovine PV. Our sequences showed also homology, but not identity, to a recently sequenced papillomavirus found in healthy goat. New sets of primers were optimized to detect both goat PV. PCR with these primers seems to indicate a low copy number of PV in the samples. *In situ* hybridization showed nuclear positivity exclusively in the neoplastic goat skin. Multiply primed rolling-circle amplification (RCA) demonstrated the presence of circular DNA sequences referable to a DNA papillomavirus. This study indicates the existence of different PV in caprine species, which may be implicated in the development of some tumours in sibling animals.

This study was self-funded.

Disk diffusion test for prediction of *mecA*-mediated resistance in staphylococcal small animal isolates from skin and ear infections

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Recently, the Clinical and Laboratory Standards Institute (CLSI) adopted the use of the Cefoxitin Disk Diffusion Test (CDDT) for the prediction of *mecA*-mediated resistance to oxacillin in staphylococci. The aim of this study was to access if the CDDT was able to predict *mecA*-mediated oxacillin resistance in *Staphylococcus* of animal origin. A total of 242 clinical strains (155 *Staphylococcus intermedius*, 15 *Staphylococcus aureus* (coagulase-positive, CoPS); and 72 coagulase-negative staphylococci, CoNS) isolated from skin ($n = 75$; 31%) and ear ($n = 167$; 69%) infections in dogs and cats were collected. These isolates were submitted to the CDDT according to the CLSI breakpoints (resistant/susceptible) of $= 19 \text{ mm}/ = 20 \text{ mm}$ for CoPS and $= 24 \text{ mm}/ = 25 \text{ mm}$ for CoNS. CLSI veterinary oxacillin breakpoints (M31A-2) were applied. All CoPS ($n = 170$) were both oxacillin and cefoxitin susceptible. Among CoNS, only three strains previously described by us as harbouring the *mecA* gene were oxacillin resistant and detected by the CDDT. One *Staphylococcus schleiferi* had zone diameters of 15 mm to oxacillin and 31 mm to cefoxitin. If human oxacillin breakpoints were used, this isolate would have been incorrectly classified as resistant but correctly as susceptible by CDDT. Whether veterinary or human oxacillin

breakpoints should be used remains a debatable issue. The CDDT was able to correctly predict the presence of *mecA*-mediated oxacillin resistance in all the three *mecA*-bearing strains. Thus, the CDDT is preferred to the oxacillin DD test because it is easier to read and provides equivalent detection of oxacillin-resistant staphylococci of animal origin.

This study was self-funded.

Aspergillus niger dermatitis in a cat

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Aspergillus spp. are ubiquitous fungi as soil and vegetation saprophytes and a component of normal skin hair coat and mucosal flora in animals. To date, cutaneous *Aspergillus* infection has not been reported in cats. A 3-year-old cat has been lame for 1 year. A cutaneous nodule on the right metacarpal footpad presented as a large abscess with draining tracts. Extensive necrosis affected underlying muscle. The pre-scapular lymph node was enlarged. Systemic signs were anorexia, lethargy and weight loss but not pyrexia. Systemic antimicrobial therapy combined with antibacterial shampoo was ineffective. Retrovirus serological tests gave a positive result for feline immunodeficiency virus. Cytological examination of direct smears indicated pyogranulomatous inflammation but no fungal elements. Histopathology showed diffuse pyogranulomatous panniculitis. Deep biopsies were cultured on Sabouraud dextrose agar at 27 °C and 37 °C and a grossly dark colony grew, which was identified as *Aspergillus niger*. The owner refused amputation of the distal limb and the cat was euthanized. Permission for necropsy was refused. Retrovirus-positive cats sometimes have deep fungal skin infections but *A. niger* is very uncommon.

This is study self-funded.

Demodicosis due to *Demodex injai* in dogs: eight cases

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Demodex injai is a species of *Demodex* mites, first described in 1996. The aim of this study is to report demodicosis caused by this mite in dogs in three private referral practices in France. The eight infected dogs included four West Highland white terriers, a Scottish terrier, a Yorkshire terrier, a Fox terrier and a Epagneul Tibétain. Age at diagnosis varied between 1 and 7 years (mean 4.2 years); there were four females and four males. Skin lesions were always localized on the dorsum. They consisted of erythema (8/8), scaling (8/8), greasy seborrhoea (7/8), diffuse alopecia (6/8) and otitis externa (6/8). Pruritus was always present and was severe in four of eight cases. The mites were readily detected in deep skin scrapings which showed between two and 24 adult long-bodied *D. injai*. Skin biopsies in two of eight cases showed sebaceous gland hyperplasia (2/2), interface

dermatitis (1/2) and two adult mites in a hair follicle (1/2). Cytological examination in all cases showed bacteria (8/8) and *Malassezia* (5/8). Miticidal therapy with moxidectin (3/8), ivermectin (3/8) (0.4 mg kg⁻¹ per day per os) or milbemycin oxime (2/8) (2 mg kg⁻¹ per day per os) was successful after 2–9 months (average 5 months).

This study was self-funded.

Genetic parameters for allergic eczema in Icelandic horses

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Allergic eczema is common in Icelandic horses outside Iceland, reflecting hypersensitivity to bites of *Culicoides*, which is absent from Iceland. We have already shown that the prevalence and severity of allergic eczema are significantly higher among adult horses exported from Iceland to Sweden (26.2%) compared to Swedish-born horses (6.7%). The present study estimates genetic parameters for allergic eczema among 825 Icelandic horses born in Sweden between 1991 and 2001. All were descended from 33 sires, each selected for having more than 50 offspring.

Owners classified clinical signs of allergic eczema as unaffected, mild, moderate or severe eczema. Eight per cent of horses had clinical signs of allergic eczema and offspring of dams suffering from eczema had a higher risk of developing eczema ($P < 0.05$). Variance components were estimated using REML animal model, including fixed effects of age and geographical area of the horses. The heritability for allergic eczema was estimated at 0.19, indicating that at least 19% of the variation in severity of allergic eczema can be explained by differences in the genetic background as assessed by pedigree analysis. Some geographical areas were significantly associated with greater severity of the eczema ($P < 0.05$). It is concluded that genetic selection could decrease the disease in Swedish-born horses.

Comparison of parasitic density between flank, dorsal muzzle and footpad skin in dogs with symptomatic canine leishmaniasis (*Leishmania infantum*)

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In canine leishmaniasis, the density of parasites in the skin may be important for the infection of sandflies. Physical trauma has been hypothesized to result in the accumulation of *Leishmania*-infected inflammatory cells in the dermis. The aim of this study was to compare the parasitic density

in three body sites: flank (FL; control site), dorsal muzzle (DM; sandfly feeding site), and footpads (FP; mechanical stress sites). Parasites were visualized with indirect immunofluorescence in biopsies obtained from 15 dogs with symptomatic leishmaniasis and their density was counted in the upper and lower dermis. The severity of macroscopic lesions (exfoliative dermatitis for FL, DM and digital hyperkeratosis for FP) and inflammatory infiltrate was scored from 0 (absent) to 3 (severe) using a 0–10 visual analogue scale. No difference in parasitic density was seen between the control (FL) and tested sites (DM, FP) or between the upper and lower dermis, apart from the FP where it was higher in the superficial dermis (paired *t*-test, $P = 0.03$). No linear correlation could be found between parasitic density and severity of the macroscopic lesions or inflammatory infiltrate, except for the lower FP dermis (Pearson's product moment correlation coefficient $r = 0.562$, $P = 0.036$). Selective accumulation of the parasite to favour transmission to sandflies was not demonstrated in the superficial dermis of the DM nor was mechanical stress on the FP associated with increased parasitic density. There was no relevant correlation between severity of cutaneous lesions, inflammatory infiltrate and parasitic density in the selected body sites.

Feline viral plaques and bowenoid *in situ* carcinomas: a clinical, histological and virological study of feline pigmented plaques

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In contrast to exophytic warts caused by papillomavirus (PV), feline viral plaques (VP) are hyperpigmented and flat. Bowenoid *in situ* carcinomas (BISC) are also hyperpigmented plaques. Results of a previous study in which up to 47% of feline BISC samples were positive for PV-antigen by immunohistochemistry indicated that feline VP could be a precursor of feline BISC and that BISC could be virally induced. The aim of this study was to record the clinical, histopathological and virological features of 26 cases of feline dermatoses that were clinically described as pigmented plaques and in which a histological diagnosis of viral plaque and/or BISC was made. Clinical data of all cases were recorded. Histological evaluation was made using standard criteria and cases were classified in one of the three following groups: VP, BISC or BISC + VP. PV group-specific antigen was detected immunohistochemically with a polyclonal rabbit antiovine PV antiserum. Of the eight cases in the VP group, seven were deemed positive for PV-antigen expression. All nine cats in the VP + BISC group were also positive. Conversely, only one out of nine BISC cats was positive. The presence of both VP and BISC lesions in some cats and the high detection rate of PV-antigens in the VP and VP + BISC groups suggest that both conditions might have the same viral cause. The lower rate of antigen detection in the BISC group indicates the possibility of another cause or a loss of viral expression during carcinogenesis.

ALIAmides in veterinary dermatology: an update on mechanism of action and clinical use in dogs and cats

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ALIAmides are the synthetic analogues of endogenous fatty acid amides accumulating in mammalian tissues as the result of injury, presumed to prevent excessive damage, and whose parent molecule is palmitoylethanolamide. ALIAmides behave in a cannabimimetic fashion and are able to down-modulate mast cell degranulation by a mechanism called ALIA (autacoid local injury antagonism). The present work provides evidence of the use of Palmidrol (the international non-proprietary name of palmitoylethanolamide) and its topically active congener Adelmidrol in some canine and feline skin diseases in which mast cells play a pivotal role. Densitometric and morphometric analyses of skin biopsies from feline eosinophilic disorders and open canine skin wounds showed that treatment with Palmidrol and Adelmidrol led to an increase in the granular mast cell density, thereby suggesting a decrease in degranulation. In canine skin wounds, Adelmidrol induced a significant increase in re-epithelialization and enhanced elastic fibre density. Clinically, oral administration of Palmidrol reduced clinical signs and lesions in cats with eosinophilic disorders and delayed the development of clinical signs in a model of canine atopic dermatitis. These studies substantiate the supposed mechanism of action of ALIAmides and suggest their use in canine and feline skin diseases sustained by mast cell hyper-reactivity.

The effect of oral cyclosporin on acute reactions following oral allergen challenge in a spontaneous canine model of food allergy

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Twenty Maltese × beagle dogs with known sensitivity to oral corn were divided into two equal groups: A and B. For 6 weeks, A received oral cyclosporin (Atopica, Novartis Animal Health) at 5 mg kg⁻¹ daily, and B, a placebo hypromellose capsule. After 2 weeks of therapy all dogs were fed 400 mg kg⁻¹ corn. This was repeated at weekly intervals for three additional challenges. Cutaneous manifestations of allergic disease were assessed using CADESI three times weekly and a daily pruritus index. Blood samples were collected from all dogs at intervals during the study to measure corn-specific IgE, leucocyte histamine release and circulating CD4 +/CCR4 + T lymphocytes. Data were analysed using unpaired *t*-tests and significance was set at 5%. An acute allergic response was demonstrated 2–9 days post-challenge but was not statistically different between A and B. No statistically significant differences between A and B were noted for the

immunological parameters measured. CCR4/CD4+ T-lymphocyte expression increased with repeated challenge. Leucocyte histamine release in response to allergen was variable (0–48% of total cell histamine). Serum corn-specific IgE was detectable but did not change during the study in any dogs. In conclusion, the administration of daily oral cyclosporin to dogs with spontaneous food allergy did not abrogate the acute clinical response to repeated, intermittent oral allergen challenge, nor did it affect immunological parameters associated with the acute allergic reaction. However, this study did not address the use of cyclosporin as a therapy for food allergic dogs exposed chronically to low-dose allergen.

Sugar inhibition of adherence by *Staphylococcus intermedius* to canine corneocytes

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Adherence is an established prerequisite for microbial colonization and subsequent invasion. The aim of this study was to determine the anti-adhesive properties of a variety of sugars molecules including various monosaccharides, fructooligosaccharide (FOS) and polyalkylglucoside (PAG) against *Staphylococcus intermedius*. Three strains of *S. intermedius* were cultured from clinical cases of canine pyoderma. Corneocytes were collected from the inner aspect of the pinna using an adhesive disc (D-Squame®). A 0.5 mL bacterial suspension in PBS (or sugar PBS solution) was placed over the corneocyte layer and incubated in moist chambers. After incubation (45 min) the corneocytes were washed and stained. Adherent staphylococci were quantified using image analysis. The assay and counting methods were validated prior to sugar studies. Each of the three *S. intermedius* strains was used in each of six dogs and the anti-adhesive effect of the test sugar was calculated as a percentage of the adherence without sugar. All monosaccharides studied and the oligosaccharide FOS failed to reduce the adherence by *S. intermedius*. Polyalkylglucoside did show anti-adhesive properties. The reduction in adherence varied with the strain of staphylococcus tested and ranged from 29.4–62.4%. On average PAG as a 1% solution was able to reduce adherence by *S. intermedius* to canine corneocytes by 47.7%. This study suggests that certain polysaccharides such as PAG may have therapeutic potential in the treatment of staphylococcal infections.

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Malassezia spp. overgrowth in allergic cats

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Malassezia dermatitis has been described as a marker of serious disorders in cats, including neoplasia, and has been

anecdotally recorded in allergic cats. Sixteen allergic cats with *Malassezia* spp. overgrowth averaged 7.68 years in age; six were castrated males, nine spayed females and one cryptorchid male. Eleven were domestic cats, two Siamese and three Persian cats. All patients had a history of pruritic dermatitis of more than 2 months duration and parasitic dermatitis had been previously ruled out in all of them. All cats were otherwise healthy and the 13 of 16 tested for evidence of feline leukaemia virus and feline immunodeficiency virus infection were all negative. At dermatological examination, multifocal areas of alopecia, erythema and greasy adherent brownish scales were observed. Cytological examination of affected areas revealed *Malassezia* overgrowth with or without bacterial infection. Azole therapy alone was prescribed in six cats, azole and antibiotic therapy in eight cats, and azole with both antibiotics and anti-inflammatory therapy in two cats. After 3–4 weeks of treatment, substantial reduction of pruritus and skin lesions was observed in all the 10 cats treated with combined therapy and in five of the six cats treated solely with azoles. Following a diagnostic protocol for allergic skin disease, atopic dermatitis was diagnosed in 15 cats and adverse food reaction in one cat. The favourable response to treatment with antifungals alone suggests that, as in dogs, *Malassezia* may be partly responsible of both pruritus and cutaneous lesions in allergic cats.

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Immunohistochemical detection of hyaluronic acid and CD44 in shar-pei dogs with mucinosis

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Cutaneous mucinosis (CM), a syndrome characterized by deposition of mucin in the dermis, is relatively common in Chinese shar-pei. Hyaluronic acid (HA) is the most common molecule noted to accumulate in both human and canine mucinosis. CD44, a transmembrane glycoprotein, is the principal cell receptor for HA, and is necessary for its catabolism. CD44 is expressed in epithelial and mesenchymal skin cells. A dermal accumulation of HA has been reported in transgenic mice with a specific CD44 expression defect and in several human diseases with mucin deposition (e.g. myxoid dermatofibroma). This study aimed to identify the dermal mucin and correlate this with CD44-expression. Skin biopsies from 15 dogs, 10 shar-peis (five with CM and five without), and five from other breeds, were stained with Alcian Blue (AB)-PAS and HA-binding protein (HABP), with and without previous hyaluronidase treatment. CD44 was detected immunohistochemically. The dermal mucin of dogs with CM was strongly stained with AB and with HABP. This staining disappeared when the sections were pretreated with hyaluronidase, demonstrating that the staining corresponded to HA. Staining was almost absent in biopsies from normal dogs and faint in biopsies from normal shar-pei. In all skin sections CD44 staining was strong in the basal and spinous layers of epidermis, follicular epithelia, sebaceous ducts and sweat glands. No correlation was observed between CD44 intensity of expression and HA deposition.

Identification of the TRPV1 vanilloid receptor in canine keratinocyte primary cell cultures

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In the last few years, study of the endocannabinoid system has intensified because of its potential involvement in anti-inflammatory and analgesic responses. Recently, it has been hypothesized that endocannabinoids could be closely related to another key system of endogenous regulation, the endovanilloid system. Evidence to support this hypothesis is that the endocannabinoid anandamide (ANA) is active on the main vanilloid receptor, TRPV1; palmitoylethanolamide (PEA), a cannabimimetic compound, and enhances ANA TRPV1-mediated effects and that CB receptors and TRPV1 are coexpressed by neuronal and epithelial cells. An interaction of the two systems on mutual control mechanisms of pain and inflammation is argued. The endovanilloid system has not been yet investigated in veterinary medicine. This pilot study aimed to identify the expression and functionality of TRPV1 in epithelial cells of dogs. Keratinocyte primary cultures were obtained from epithelial biopsies; cells were plated and expanded to obtain aliquots of cells necessary to perform the binding assay. The specific binding demonstrated the presence of a measurable concentration of TRPV1 (1240 ± 120 fmol/mg protein) in keratinocyte cell membranes. Competition assays, using selective agonists and antagonists, indicated TRPV1 receptor characteristics. The functionality of TRPV1 in primary cultures of canine epithelial cells was evaluated by measurement of labelled Ca²⁺ influx. The involvement of endocannabinoid and endovanilloid systems in controlling pain and inflammation and the demonstration of the vanilloid receptor in dog epithelium indicate the potential for therapeutic development of related compounds in veterinary dermatology and odontostomatology.

A placebo-controlled, blinded study on the clinical efficacy of a single *Mycobacterium vaccae* injection in dogs with atopic dermatitis

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Immunodysregulation is important in the development of atopic dermatitis (AD) in dogs. Decreased activity of regulatory T cells (Treg) is key to evolving immunodysregulation in humans. Murine studies have revealed induction of Treg by *Mycobacterium vaccae*, and clinical studies in human patients with allergic conditions have shown promising results. The objective of this study was to investigate reduction in pruritus and skin lesions in canine AD as measured by CADESI scores when given a single injection of a killed *M. vaccae* suspension or placebo. According to strict selection criteria, 60 dogs with clinical signs of AD were enrolled in the study. Power analysis revealed that with 30 dogs per group a 30%

difference in CADESI scores, with a standard deviation < 30%, can be detected with a 96% chance. The dogs were monitored monthly for 3 months. Overall, treatment with *M. vaccae* failed to reduce CADESI scores significantly compared to placebo. However, there were statistically significant differences between dogs with initial CADESI scores < 60 ($n = 41$: 19 dogs receiving treatment; 22 dogs treated with placebo). After 3 months, the difference between the treatment and placebo groups in log CADESI scores, owner pruritus scores and overall benefit were statistically significant ($P = 0.0276$, 0.037 , and 0.0067 , respectively). In contrast, with initial CADESI scores > 60, there were no significant differences. These results indicate efficacy of treatment with *M. vaccae* in dogs with mild but not severe AD.

Coagulase gene variability in *Staphylococcus aureus* isolated from animals

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Coagulase activity is a distinctive characteristic of pathogenic *Staphylococcus aureus*. Thus, far the coagulase gene (*coa*) has been detected in all *S. aureus* studied regardless of coagulase phenotype. Variability within *coa* has been applied to genotype *S. aureus* isolated from human infections using restriction fragment length polymorphism analysis. For this study, polymerase chain reaction (PCR) was used to amplify a variable region of *coa* from *S. aureus* clinical veterinary isolates, and *coa* sequence information was compared to methicillin resistance and staphylococcal cassette *mec* (SCC*mec*) type. *Staphylococcus aureus* was isolated from horses (5), cats (5), dogs (6), and a bird and identified by the bacteriology service at the University of Tennessee College of Veterinary Medicine. Nine of the isolates were resistant to methicillin based on the Kirby–Bauer disc diffusion test. The SCC*mec* types of these isolates were determined by multiplex PCR. They included type V (3), type II (5), and type IVa (1). The region of the coagulase peptide studied occurred in shorter (4) and longer (13) forms. All of the isolates with the shorter coagulase peptides were susceptible to methicillin. The coagulase peptides segregated into three major clusters based on amino acid comparisons. Two clusters contained only sequences from equine and avian methicillin susceptible isolates. Subdivisions within the cluster containing all of the methicillin-resistant organisms and two susceptible isolates did not definitively segregate based on patient species, methicillin resistance status, or SCC*mec* type. Thus, *coa* typing may be a valuable tool for fingerprinting *S. aureus*; however, these patterns do not appear linked to methicillin resistance gene profiles.

Exfoliative erythema multiforme associated with herpesvirus 1 infection in two European cats

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Case 1: A 2-year-old female domestic short-haired cat was referred with a 1-week history of extensive scaling and alopecic dermatitis with a firm cardboard-like texture skin. The cat had concomitant ocular and upper respiratory tract

signs. It was lethargic and reluctant to move. Case 2: A 6-year-old male domestic short-hair cat was referred with a 2-week history of extensive scaling dermatitis with large sheets of exfoliated stratum corneum trapped in the remaining hair coat. The cat was lethargic, dehydrated and had lost weight but did not present any ocular or respiratory signs. Nevertheless, the owner reported a bout of sneezing and conjunctivitis 2 months previously. Feline leukaemia virus and feline immunodeficiency virus tests were negative. Complete blood counts, serum biochemistry profiles, thoracic radiographs and ultrasonographic examination of thorax and abdomen showed no abnormalities. Deep and superficial skin scrapings did not show any pathogens. Histopathology of skin biopsies showed a lymphocytic interface dermatitis, severe hydropic degeneration of basal cells and numerous apoptotic keratinocytes with lymphocyte satellitosis at all levels of the epidermis. Feline herpes virus type 1 was found by polymerase chain reaction in skin, nasal and ocular discharges for case 1 and in a conjunctival brush for case 2. Supportive care was required for some weeks with spontaneous recovery 5 months later.

A randomized, double-blinded, placebo-controlled study on the efficacy of a new antifungal (compound MNLP1242) against *Microsporum canis* dermatophytosis in experimentally infected guinea pigs

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The plant product derivative MNLP1242 (MNLpharma Ltd, Aberystwyth, Ceredigion, UK) has been shown to have *in vitro* antifungal properties. This phenyl acetylene class substance was tested as a topical therapeutic agent in experimental *Microsporum canis* infection in guinea pigs. Sixteen animals were infected and randomly allocated into four equivalent separated groups. From day 9 post-infection, they received once daily 0.5 mL of placebo for 7 days (group 1) or 1 mL enilconazole 0.2% for 7 days (group 2) or 0.5 mL of compound MNLP1242 0.2% for 3 or 7 days (groups 3 and 4). Clinical lesions and fungal persistence on the skin were monitored blind twice weekly and weekly, respectively, for 4 weeks from start of treatment. The clinical score was calculated by grading erythema, crusts, alopecia and scales. The mycological score was calculated by counting the number of *M. canis* colonies obtained by the tooth brushing method. The four groups were compared using a linear model, fitting treatment, time and interaction between treatment and time. Correlations between repeated measurements on the same animal were modelled using the MIXED procedure of the SAS software. Least square means were used for contrasts, and results were assumed significant when associated *P*-values were < 0.1. Treatment with MNLP1242 for 7 days (group 4) induced a significant reduction in the clinical score at day 27 ($P = 0.09$), and a significant reduction in the mycological score at all sampling dates. The results indicate that compound MNLP1242 is an effective antidermatophytic agent in this model.

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