Veterinary Dermatology 2004, **15** (Suppl. 1), 20–40

Free Communication Abstracts
Session 1: FUNGAL AND BACTERIAL DISEASES

FC-1

Comparative efficacy of lufenuron and itraconazole in a guinea pig model of cutaneous *Microsporum canis*

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During the last few years, reports have appeared claiming that lufenuron diminished or even cured dermatophyte infections in cats and dogs. As these observations have a rather anecdotal character leading to some ambiguity in the literature, it was decided to test lufenuron in a generally accepted animal model for dermatomycotic infection. The test was carried out in guinea pigs artificially infected with *Microsporum canis* on scarified dorsal skin and orally treated with lufenuron (Program™). The efficacy of up to five doses of 80 mg/kg was assessed 7 and 14 days after the start of treatment. All animals failed to show any improvement in skin lesions as compared to the vehicle-only treated animals. Clinical symptoms taken into account were scaling, crust formation, erythema, and exudation. Neither the number of treatments (one or five) nor the dose range (40 or 80 mg/kg) made any difference. Itraconazole, tested earlier under identical circumstances, resulted in a clear and consistent improvement at day 7 of the infection at a dose of 15 mg/kg, given either in one dose or spread over several days. The absence of antimycotic activity of lufenuron in this established animal model constitutes a significant element in the discussion on the antifungal potency of lufenuron and supports the fact that there is, as yet, no evidence that benzoylphenyl urea derivative compounds have an effect on chitin synthesis in fungi.

Funding: J&J Pharmaceutical Research and Development, Janssen Animal Health.

FC-2

The role of endogenous protein inhibitors of dermatophyte proteolytic activity

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Microsporum canis and *Trichophyton mentagrophytes* produce different proteolytic enzymes necessary for their growth and progression. In general, enzymatic activity can be tightly regulated by various mechanisms, of which endogenous inhibitors are of great importance. Considering the fact that an imbalance between proteases and their inhibitors has been implicated in the pathogenesis of several diseases, the aim of our work was to investigate the production and role of protease inhibitors produced by fungi. Both dermatophytes were demonstrated to produce protein as well as low-molecular-weight inhibitors of different proteases. Protein inhibitors were isolated from culture medium and cytosolic extracts obtained from mechanically disintegrated mycelia by affinity chromatography on CM-Sepharose 4B or by HPLC separation in the case of low-molecular-weight inhibitors. The size of the purified proteins was analysed by SDS-PAGE, and their immunogenic properties by immunoblotting. Their inhibitory properties were determined by inhibition of trypsin, papain, and cathepsins B and L. Temperature and pH stability of the low-molecular-weight inhibitors was established as well. From our data, it is evident that both protein and low-molecular-weight inhibitors of cysteine proteases have been isolated from dermatophyte fungi. Our data represent the basis for further studies of the role of cysteine protease inhibitors in the pathogenesis of dermatophytoses and their influence on the host immune response.

Funding: Ministry of Education, Science and Sport, Republic of Slovenia.

FC-3

Immunity to bovine trichophytosis

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Bovine trichophytosis is a mycotic cutaneous disease caused mainly by the dermatophyte *Trichophyton verrucosum*. Cell-mediated immunity (CMI) is reported to play a crucial role in defense against the disease. The objective of this study was to investigate the immune response developed after administration of a vaccine containing a live immunogenic culture of *T. verrucosum*. For the detection of CMI, we used an antigen-specific lymphocyte proliferation test measured by both ^{3}H-thymidine incorporation and flow cytometry, and specific antigen-induced production of IFNγ. We also tested specific antibody production by an indirect ELISA test. After vaccination by either the subcutaneous, intramuscular, or intradermal route, animals developed solid *in vitro* CMI 1 month after revaccination. $CD4^{+}$ lymphocytes were the basic cell population activated by *Trichophyton* antigen *in vitro*. IgM and IgG antibody responses were detected from postvaccination days 3 and 7, respectively. Animals expressing higher levels of CMI displayed higher ratios of IgG2:IgG1 (a Th1 pattern of immune response). Using the challenge test, protection was higher after intramuscular > subcutaneous > intradermal routes of administration. We conclude that administration of a vaccine containing a live immunogenic culture of *T. verrucosum* leads to the development of protective immunity against bovine trichophytosis, and that the results of our laboratory tests correlated well with the results of challenge tests.

Funding: Ministry of Agriculture of the Czech Republic.

FC-4

Canine serum immunoreactivity to *Malassezia pachydermatis* is influenced by the phase of growth *in vitro*

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Western immunoblotting studies of canine sera using *Malassezia pachydermatis* extracts have shown that infected dogs commonly have antibodies that recognize multiple antigens. However, reported patterns of immunoreactivity vary between different laboratories. Since culture duration influences the antigenic composition of lipid-dependent *Malassezia* when probed with human sera, we investigated whether the *in vitro* growth phase of *M. pachydermatis* influences immunoreactivity to canine sera. Extracts of *M. pachydermatis* CBS1879 grown in Sabouraud's liquid medium at 37°C for 2, 4, 6, 8 and 10 days were prepared by mechanical disruption, centrifugation, dialysis and lyophilization. Yeast growth phase was assessed by sequential colony counts and optical density measurements. Patterns of IgG immunoreactivity in high ($n = 3$) and low ($n = 3$) titre sera were compared using extracts prepared at each time point by SDS-PAGE and western immunoblotting. Protein bands of 62 and 49 kDa were recognized by all sera, and five sera recognized 98 and 68 kDa bands. Proteins of 188, 66, 58, 57, 38, 28 and 17 kDa were only recognized by high titre sera. All high titre sera recognized more bands in exponential phase (day 2) extracts when compared with decline phase (days 8–10) extracts, and two of these sera showed most bands in stationary phase (days 4–6) extracts. Bands of 62 and 57 kDa were primarily detected in exponential and early stationary phase extracts. Antigenic variation in extracts of *M. pachydermatis* prepared during different growth phases might explain discrepancies between previous laboratory studies of immunity to this yeast.

Funding: Government of Malaysia.

FC-5

Unsaturated transferrin inhibits the growth of *Malassezia pachydermatis in vitro*

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Transferrin, an iron-binding protein that transports iron to mammalian cells, may contribute to innate immunity to fungal pathogens, primarily by limiting microbial access to iron. We investigated whether unsaturated (apo)-transferrin had an inhibitory effect *in vitro* on *Malassezia pachydermatis*, an important opportunistic cutaneous yeast pathogen of dogs. *M. pachydermatis* strains were grown at 32°C in medium containing canine or bovine apo-transferrin at concentrations of 0.7, 1.3, 2.7 and 5.3 mg/mL. Optical density (OD_{492}) was measured daily until growth was stationary (day 6) in saline-control wells and then compared between treatments by ANOVA and Tukey's tests. Bovine and canine transferrin inhibited ($P < 0.01$) yeast growth at all concentrations tested. Using bovine transferrin, the mean OD ± SE for 10 strains were: saline, 0.55 ± 0.19; 0.7 mg/mL, 0.33 ± 0.05; 1.3 mg/mL, 0.18 ± 0.03; 2.7 mg/mL, 0.18 ± 0.04; 5.3 mg/mL, 0.16 ± 0.03. Using canine transferrin, the mean OD ± SE for seven strains were: saline, 0.79 ± 0.03; 0.7 mg/mL, 0.37 ± 0.07; 1.3 mg/mL, 0.27 ± 0.04; 2.7 mg/mL, 0.24 ± 0.03; 5.3 mg/mL, 0.20 ± 0.03. For both bovine and canine transferrin, inhibition by 5.3 mg/mL exceeded ($P < 0.05$) that of 0.7 mg/mL. Since both bovine and canine apo-transferrin inhibit the growth of *M. pachydermatis in vitro*, serum transferrin may contribute to innate immunity to *M. pachydermatis* in dogs and cattle. The spongiotic dermatitis reaction seen histologically in many dogs with *Malassezia* dermatitis may promote the accumulation of transferrin at infection sites.

Funding: Self-funded.

FC-6

Four isoforms of exfoliative toxin produced by *Staphylococcus hyicus* abolished immunofluorescence for desmoglein 1 in pig skin

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Exudative epidermitis is an acute, often fatal skin disease in piglets caused by infection with *Staphylococcus hyicus*. Clinical and histopathological manifestations of exudative epidermitis are similar to those of a human blistering skin disease, staphylococcal scalded skin syndrome, in which cleavage of desmoglein (Dsg) 1 by exfoliative toxins produced by *S. aureus* results in blister formation in the upper part of the epidermis. The aim of this study was to determine whether the four types of exfoliative toxin produced by *S. hyicus* (ExhA, ExhB, ExhC and ExhD) are able to affect swine Dsg1. We first generated recombinant toxins of ExhA, ExhB, ExhC and ExhD by an *E. coli* expression system. To determine the pathogenic activity of these recombinant toxins, we injected them subcutaneously into 3- to 4-week-old piglets. After administration of all four recombinant toxins, local exfoliations developed at the inoculation sites of the piglets' skin at 21–48 h postinjection. To further assess whether these Exhs affect swine Dsg1, we incubated cryosectioned pig skin with Exhs *in vitro* and subsequently stained Dsg1 and Dsg3 by immunofluorescence with human pemphigus foliaceus sera and anti-Dsg3 monoclonal antibody (5H10), respectively. The cell surface staining of Dsg1 was abolished by incubation with any of the four isoforms of Exh, whereas that of Dsg3 was not affected at all. These findings indicate that all four types of exfoliative toxin produced by *S. hyicus* (ExhA, ExhB, ExhC and ExhD) can cause skin exfoliation in pigs with exudative epidermitis, presumably by digestion of Dsg1.

Funding: Ministry of Education, Culture, Sports, Science and Technology of Japan.

Session 2: CONGENITAL/INHERITED DISEASES

FC-7

Acral mutilation and analgesia due to hereditary sensory neuropathy in 13 French spaniels

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The purpose of this study was to describe a new hereditary sensory neuropathy in French spaniels resulting in analgesia and progressive mutilation of the distal extremities. Thirteen French spaniels (six females, seven males) from five different litters with acral mutilation were identified. Clinical signs were first noted between 3.5 and 12 months of age and typically consisted of sudden excessive and intense licking and biting of toes and/or footpads of one or several feet. This generally progressed rapidly into swollen digits, paronychia, footpad ulceration, and occasionally fracture and osteomyelitis. If the affected dogs were unrestrained, autoamputation of claws, toes and footpads usually resulted. However, even in the more severe cases, affected dogs walked and ran on their mutilated feet without any evidence of lameness, pain or ataxia, and they allowed wound care without evidence of pain or discomfort. Dogs were otherwise healthy, with the exception of frequent secondary bacterial infections of the wounded feet. This disorder was clinically very difficult to manage, and the majority of the dogs were euthanized within days to months of diagnosis. None of the sires or dams of the affected dogs reported here were clinically affected. There was no apparent sex predilection. An autosomal recessive mode of inheritance was strongly suspected. The clinicopathological findings, the early age of onset and the disease progression in affected French spaniels were very similar to those reported for hereditary sensory neuropathy in German short-haired pointers, English pointers and English springer spaniels.
Funding: Self-funded.

FC-8

Microarray analysis of differential gene expression between genetically normal, heterozygous and homozygous affected Norfolk terrier dogs with a heritable keratin 10 defect

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A heritable cornification defect resulting from a keratin 10 mutation has been identified in Norfolk terrier dogs. Affected dogs have marked scaling and hyperpigmentation, and develop erosions following mild trauma. The morphologic lesions are similar to epidermolytic hyperkeratosis in humans. The mutation is recessive, and heterozygous dogs lack cutaneous lesions. A diagnostic test for heterozygosity has been developed. Total RNA was extracted from 6-mm skin biopsy samples from two genetically normal, two affected and two heterozygous dogs. Reverse transcription with P^{33}-labeled dCTP generated a cDNA probe that was hybridized to a commercially available cDNA array designed for dermatologic research. This array contained approximately 4400 human cDNAs, and cross-hybridization strategies were developed to allow canine cDNA to hybridize to the membrane. The average background intensity was subtracted from the raw intensity value for each gene. Genes with a corrected value less than one were eliminated from further analysis. Each gene was then normalized to the median intensity of the remaining genes. A gene was considered upregulated or downregulated if its relative expression was greater than two or less than two, respectively. Fifty-three genes were upregulated in both heterozygous and affected dogs over normal. Five genes were downregulated in affected dogs when compared to normal. No downregulated genes were identified in heterozygotes. No differential regulation was apparent between affected and heterozygous dogs. These results indicate that despite a normal phenotype, the decreased keratin 10 production associated with heterozygosity leads to many of the same alterations in gene expression identified in affected dogs.
Funding: Morris Animal Foundation, AAVD Postdoctoral Fellowship.

FC-9

Coat funk in Alaskan malamutes

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The objective of this study was to determine if a condition called coat funk by Alaskan malamute breeders has clinical, microscopic, haematologic, serum chemistry, urine and/or hormonal changes that allow affected dogs to be distinguished from normal dogs. Alaskan malamute breeders provided five dogs that met the breed standard for a normal pelage and five dogs considered to have coat funk. All animals were given complete physical examinations, and skin biopsies were collected. In addition, clipped hair was examined by optical-based fibre diameter analysis. The skin biopsy samples were analysed by descriptive and morphometric analyses. Complete blood counts, serum chemistry profiles, urinalyses, and endocrine evaluations (thyroid profiles, urine cortisol:creatinine ratios, somatomedin C levels, and pre- and post-ACTH stimulation concentrations of cortisol and adrenal sex hormones) were performed on all dogs. Clinically, affected dogs had varying degrees of involvement of the dorsal neck, tail and trunk. This was the only consistent difference noted between normal and affected dogs. In spite of the detailed analyses used in this investigation, the procedures failed to always separate normal from affected dogs and precluded separating coat funk from other hair loss diseases of Alaskan malamutes. This could be due to the lack of real differences between the two groups, failure of the procedures to allow separation of the two groups, or small sample size. Larger numbers of dogs followed over time are needed to better define the clinical and microscopic features of this disease and its related hair loss.
Funding: The Alaskan Malamute Club of America.

FC-10

Adult-onset hair loss in Chesapeake Bay retrievers: a clinical and histological study

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Hair loss in Chesapeake Bay retrievers has been increasingly recognized by breeders in recent years. Anecdotal reports suggest an endocrine disorder or follicular dysplasia as the underlying cause, but no scientific study has been done to investigate the underlying problem. A prospective study was carried out in collaboration with the American Chesapeake Club. Affected dogs were recruited into the study. Routine dermatological and hormonal (blood and urine) tests, and skin biopsies were performed. Ten dogs (age 1.5–10 years), seven females (two spayed) and three males (two neutered), were included in the study. All dogs had mild or severe hair loss affecting the lateral ventral chest, flanks, rump and thighs. Affected dogs were clinically healthy. Hormonal tests revealed normal thyroid hormone panels, insulin-like growth factor-1 levels, and urinary cortisol:creatinine ratios in samples collected for ten consecutive days. In six of 10 dogs, an adrenal hormone panel showed slight or moderate increased values pre- and/or post-ACTH stimulation of cortisol (three of six), 17-hydroxyprogesterone (five of six), androstenedione (three of six), estradiol (two of six) and progesterone (six of six). The major histopathologic changes resembled canine flank alopecia and follicular dysplasia with pronounced infundibular hyperkeratosis, mild follicular atrophy, and occasional melanin clumping with dystrophic hair shafts. Chesapeake Bay retrievers suffer from a type of hair loss that is likely related to an abnormal production of adrenal sex hormone. Further studies are currently underway to determine if there is a heritable basis for this disease and to evaluate therapeutic options.
Funding: University of Pennsylvania.

FC-11

Histopathology of alopecia X

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Dogs with endocrinopathy have been reported to have hair follicles with excessive trichilemmal keratinization (flame follicles). These follicles have been described in cases clinically diagnosed as hypothyroidism, hyperadrenocorticism, hyposomatotropism and sex hormone abnormality. Breed variation in the incidence of these follicles has also been described. Recently, endocrine alopecias have been better defined, and a number of these conditions (previously known as congenital adrenal hyperplasia, pseudo-Cushing's disease, castration-responsive dermatosis and adult-onset hyposomatotropism) are now grouped under the name alopecia X. In this prospective study, a group of 24 spitz-type dogs, which included 15 Pomeranians, had an extensive hormonal work-up. This included thyroid and adrenocorticotrophic hormone stimulation tests and sex hormone assays. Histopathology of the skin specimens collected from alopecic and clinically normal skin indicated features common to many endocrinopathies such as surface and infundibular hyperkeratosis and comedone formation. However, catagenization with flame follicle formation was a prominent feature of skin specimens from 22 dogs. This feature was so marked in 20 of these specimens that it was considered to be diagnostic for alopecia X when the specimens were evaluated in a blinded manner. The other two specimens could not be differentiated reliably from hyperadrenocorticism. Histopathology is a useful diagnostic procedure to support a diagnosis of alopecia X in dogs.
Funding: Self-funded.

FC-12

Diagnostic value of skin biopsy in feline symmetric alopecia

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Feline symmetric alopecia (SA) is classified aetiologically as idiopathic or self-induced, the latter due to allergic or psychogenic causes. It is characterized clinically by truncal alopecia not accompanied by skin lesions. Aetiology-specific histopathological findings of SA have been reported. Idiopathic SA is characterised by follicular atrophy and telogenization, allergy-induced alopecia by mild epidermal hyperplasia and superficial perivascular dermatitis (SPVD), and psychogenic-induced alopecia by normal skin. The objective of this study was to assess the diagnostic value of histopathological examination to establish the aetiology of feline SA. Eighteen skin biopsies from cats with clinical histories suggestive of self-induced SA were retrospectively evaluated. Nine control cats with normal hair and skin were included in the study. Sixty-five per cent of 1230 and 72% of 1208 hair follicles evaluated in alopecic and control cats, respectively, were in the resting phase. Hairs and hair follicles with morphology resembling what is defined as trichomalacia in human trichotillomania were observed in two study cats. Mild epidermal hyperplasia was observed in two alopecic cats, and mild lymphocytic SPVD was present in 72% of alopecic and 66% of control cats, respectively. Results obtained in this study showed that high numbers of resting follicles are common in cats with normal hair and cats with self-induced SA. Consequently, their presence cannot be considered a diagnostic criterion of idiopathic SA. In addition, because SPVD was common in normal feline skin, it might not be suggestive of allergy-induced alopecia. Therefore, skin biopsy is not recommended to define the aetiology of feline SA.
Funding: Self-funded.

Session 3: NEOPLASTIC DISEASES

FC-13

Molecular markers in canine T-cell lymphoma

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The objectives of this study were to review and correlate results of the clinical, histological and immunohistochemical features of different types of canine T-cell lymphoma (CTCL) according to the World Health Organization Classification of 2002. Further, we intended to define criteria for the diagnosis of these distinct clinicopathologic entities and to determine the prevalence of molecular markers of therapeutic efficacy and thereby prognosis. Skin samples of 10 cases of cutaneous canine lymphoma were evaluated as follows: histological preparations utilizing H&E, immunohistochemistry for CD3 and CD79α, and retinoid acid receptors for RAR and RXR isomers. Results of these examinations were correlated with available clinical data. The diagnoses included: mycosis fungoides (five cases), pagetoid reticulosis (three cases), and nonepitheliotropic type (two cases). All cases were CD3 + CD79α–, RARα +, RXRα +, RXRγ +; nine cases were RARβ +; and nine were RXRβ +. The presence of RARγ was not detected. CD3 positivity demonstrated T-cell lineage. Differentiation of these three types of CTCL requires careful histological and cytological review as well as immunohistochemical evaluation. Clinically, nonepitheliotropic types often present with diffuse or multifocal cutaneous involvement; epitheliotropic types are less well defined and signs vary with stage of the disease. The presence of retinoid receptors suggests that, as in humans, the use of specific ligands may be effective in the treatment of canine cutaneous T-cell lymphomas. Further updating of the World Health Organization system is required to better define the characteristic features of canine CTCL to permit more accurate diagnosis and prognosis, and thus effective treatment.

Funding: Self-funded.

FC-14

Clonality studies of feline cutaneous lymphocytosis

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A previous study described cutaneous lymphocytosis (CL) in 23 cats. The process resembles cutaneous pseudolymphoma in humans, a heterogeneous group of benign reactive proliferations of well-differentiated lymphocytes in the skin of humans. Morphological and immunophenotypic characteristics do not offer reliable criteria to accurately predict the clinical outcome of feline CL or pseudolymphoma in humans. Presence of clonal cell populations is more consistent with a neoplastic process. In a previous study, feline CL lesions (20 cats) were evaluated for clonality using PCR, and only two cats had monoclonal T-cell populations. Because false-negative results may occur, the purpose of this study was to repeat the PCR using a revised primer set based on analysis of additional feline T-cell receptor γ (TCRγ) sequences. DNA was isolated from 29 skin lesions and six internal organs of 20 cats. DNA integrity was assessed by glyceraldehyde-3-phosphate dehydrogenase PCR. Polymerase chain reaction clonality was performed using the revised primer set specific for feline TCRγ, and duplicate samples were evaluated. The PCR products were assessed by heteroduplex analysis. Clonal rearrangement of TCRγ was detected in 14 cats (24 of 35 tissues: 21 of 29 skin lesions and three of six internal organs); eight of these cats are still alive and six were euthanized. Monoclonal populations were seen in three of five cats that had involvement of internal organs. These findings indicate that feline CL is best considered as a slowly progressive process which may be reactive, but often evolves into a low-grade indolent lymphoma.

Funding: George H. Muller Fund for Research in Dermatology.

FC-15

Immunohistochemical detection of c-kit in canine cutaneous mast cell tumours

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The objectives of this study were to evaluate the immunohistochemical expression of c-kit by mast cells in canine mast cell tumours (MCT) and to correlate this expression with the tumour grade. Cutaneous MCT from 60 dogs were graded histologically (Patnaik grading system), and immunohistochemical staining for c-kit was performed. Evaluation criteria were the percentage of labelled cells, the homogeneous/mixed character, the intensity (0–3) and the location of labelling, either cell membrane or cytoplasmic (diffuse, granular, or near the nucleus). For grade I tumours (20 dogs), c-kit expression was detected in 100%: 80% showed homogeneous labelling, and 90% contained 80–100% labelled mast cells. Labelling was detected on the cell membrane (65%) and near the nucleus (75%). For grade II tumours (20 dogs), c-kit expression was detected in 100%: 61% showed homogeneous labelling and 96% contained 80–100% labelled mast cells. Labelling was detected on the cell membrane (74%) and close to the nucleus (74%). For grade III tumors (20 dogs), c-kit expression was detected in 100%: 71% showed homogeneous labelling, 21% contained 10–50% labelled mast cells, 21% contained 50–80% labelled cells, and 54% contained 80–100% labelled cells. Most of the labelled cells had a poorly labelled membrane (75%), with labelling near the nucleus being undetectable or low in 50% of the tumours. Other cytoplasmic labelling was detected in 21% of the tumours (10% of grade I and 13% of grade II). Results suggest that c-kit can be used as a reliable marker for canine MCT, notably for undifferentiated MCT.

Funding: National Veterinary School of Toulouse.

FC-16

Clinical and immunological effects of Newcastle disease virus vaccine on bovine papillomatosis

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Newcastle disease virus (NDV) has antineoplastic and immunostimulatory properties, and it is currently being clinically tested in anticancer therapy. In order to analyse the immunostimulatory effects of NDV on bovine papillomatosis, we inoculated 14 cows subcutaneously with an attenuated vaccine containing the LaSota strain of NDV (LS-NDV). Four cows with papillomatosis served as controls. Serum samples were collected from each animal 1 h preinoculation and 7 and 21 days postinoculation. In inoculated cows on days 7 and 21, the mean antibody titres were $\log_2$ 2.43 ± 0.92 and 5.57 ± 0.72, respectively, by haemagglutination inhibition, and the mean levels of TNFα were 5.80 ± 4.19 and 5.39 ± 2.66 ng/mL, respectively, by WEHI-164 cytotoxicity assay. Significant differences between inoculated and control animals were evident for antibody titres on day 21 and clinical scores on day 60. A correlation was evident between the TNFα activities and clinical scores on day 21. The clinical observations at day 60 showed that the papillomas in five cows had completely resolved (36%), one animal had no alterations on clinical appearance of the tumour (7%), and papillomas in eight cows had regressed (57%). In conclusion, these results demonstrated that inoculation of LS-NDV vaccine stimulates an antibody response and a limited increase in TNFα activity and may enhance clinical recovery in bovine papillomatosis.
Funding: Scientific Research Council of Akdeniz University.

FC-17

Cutaneous papillomatosis and carcinomatosis in the highly endangered Western Barred Bandicoot

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The Western Barred Bandicoot (WBB) is a highly endangered marsupial, only existing in the wild in Western Australia. Conservation efforts using captive breeding programmes to prevent the extinction of the WBB are being hampered by a progressively debilitating, wart-like syndrome in captive and wild WBB. Multicentric proliferative lesions have been observed predominately over the face and feet of captive and wild populations of WBB. Introduction of apparently healthy, wild WBB into captive colonies of WBB affected with the skin lesions has been associated with the subsequent development of the skin lesions in the introduced bandicoots. Grossly and histologically, the smaller skin lesions resemble papillomas, whereas the larger lesions demonstrate malignant transformation into carcinomas. Chlamydial organisms have been detected in association with the lesions; however, their role in the pathogenesis of the lesions is unknown. Rarely, large intranuclear inclusion bodies have been observed in proliferative epithelium under light microscopy, suggesting a viral aetiology. Papilloma, polyoma and herpes viruses have all been reported in association with neoplastic transformation of cutaneous epithelial structures in a variety of vertebrate species. Preliminary examination of skin lesions from five WBB has utilized light microscopy, transmission electron microscopy, indirect immunohistochemistry for papillomavirus, and PCR tests for murine polyoma virus, human papilloma virus and conserved herpes virus sequences. None of these tests has definitively identified an aetiological agent. Further research is being undertaken using degenerate PCR primers targeting highly conserved regions of genes from papilloma, polyoma and herpes viruses to further investigate the possibility of a viral aetiology.
Funding: Self-funded.

Session 4: IMMUNE-MEDIATED DERMATOSES

FC-18

Pemphigus foliaceus in 97 dogs

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Medical records of 97 dogs with pemphigus foliaceus were evaluated. The average age of onset was 6.3 years (range 0.5–16 years). Crusts were the most common lesions in 79 dogs; pustules were observed in 36 dogs. No gender predisposition was identified. The trunk was the most commonly involved area (51 dogs), followed by the inner pinnae (46), dorsal muzzle (37), footpads (32), periocular area (26), outer pinnae (23) and planum nasale (23). Facial involvement only was noted in 15 dogs. Of the 48 dogs in which cytology was recorded, concurrent infections were identified in 32 dogs, acantholytic cells were seen in 37 dogs, numerous neutrophils in 35 dogs, and numerous eosinophils in eight cases. Final control of the disease was achieved with: glucocorticoids (24 dogs); azathioprine (9); chlorambucil (1); aurothioglucose (1); a combination of glucocorticoids and azathioprine (31); glucocorticoids and aurothioglucose (2); tetracycline/doxycycline and niacinamide (8); prednisolone, tetracycline and niacinamide (1); fatty acid supplementation (2); and tacrolimus (1). One dog was completely tapered off drugs and stayed in remission. Average time to improvement was 6 weeks, and average time to remission was 9.3 months. Forty-three dogs were followed for <12 months, and 12 of these were euthanized: eight for other diseases and four due to a lack of response or adverse effects of treatment. In 54 dogs, the follow up was >12 months; four of these dogs were euthanized (one due to an unrelated cause, one due to neoplastic disease and two related to pemphigus foliaceus).

Funding: Self-funded.

FC-19

Autoantibodies against extracellular domains of desmocollin 1 are not involved in canine pemphigus foliaceus

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Pemphigus foliaceus (PF) is an autoimmune skin disease of dogs and humans. In human PF, the autoimmune target has been identified as desmoglein (Dsg) 1, a desmosomal cell–cell adhesion molecule, whereas the target in canine PF has not been identified. Many studies have suggested the possibility of Dsg1 being the target molecule. However, the pathological characteristics are similar to those of SPD type IgA pemphigus in humans, the target molecule of which is desmocollin (Dsc) 1. In this study, we attempted to clone canine Dsc1 and investigated whether canine PF sera could recognize Dsc1. Total RNA was purified from the muzzle skin of a healthy dog, and the cDNA of Dsc1 was amplified by RT-PCR. Sequence analysis showed that the open reading frame of the extracellular domain of Dsc1 consisted of 695 amino acids and shared 82 and 79% amino acid identities with human and mouse Dsc1, respectively. The recombinant extracellular domain of Dsc1 was expressed by transient transfection with CHO cells. We used six canine PF sera: four sera that were positive in the indirect immunofluorescence test, two sera that were negative, and two normal canine sera. Immunoprecipitation-immunoblotting analysis revealed that no canine PF sera or normal sera showed affinity for recombinant Dsc1. Although the tested sera were limited, the results suggest that no autoantibodies against the extracellular domain of Dsc1 were involved in the pathogenesis of canine PF and that another cell surface molecule may be involved.

Funding: Self-funded.

FC-20

Efficacy of griseofulvin for juvenile cellulitis in dogs

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Juvenile cellulitis is an uncommon idiopathic granulomatous and pustular disorder with submandibular lymph node involvement that usually occurs in puppies. Currently, large doses of glucocorticoids are the treatment of choice. Griseofulvin is a fungistatic antibiotic that also has immunomodulatory properties and is occasionally used in human patients with idiopathic inflammatory skin disorders. The purpose of this study was to investigate the efficacy of griseofulvin in the treatment of juvenile cellulitis in dogs. Six dogs with juvenile cellulitis were investigated. The diagnosis was based on both clinical features and standard diagnostic procedures, and fungal disorders were carefully ruled out. These dogs were treated with griseofulvin (14.2–34 mg/kg orally, twice daily) without any other treatment. On the basis of clinical observation, the efficacy of griseofulvin was evaluated as excellent (complete resolution within 2 weeks), good (complete resolution within 4 weeks), fair (>4 weeks for complete resolution), and poor (unresolved). Two cases were evaluated as excellent, and four cases were good. All cases were completely resolved within 3 weeks. No adverse reactions were recognized in any of the dogs. In this study, griseofulvin appeared to be effective in the treatment of canine juvenile cellulitis. Although the aetiology remains uncertain, it is postulated that griseofulvin could induce down-regulatory signals within the lesions. Further investigation is needed to understand the clinical effects of griseofulvin.

Funding: Self-funded.

FC-21

Efficacy of topical tacrolimus ointment for treatment of plantar fistulae in German shepherd dogs

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The objective of this open pilot study was to evaluate the efficacy of topical tacrolimus ointment for treatment of plantar fistulae in German shepherd dogs. Seven dogs (four males, three females) were included. All subjects had a 6-month to 2-year history of plantar fistulae involving the plantar aspect of two to four metatarsi/metacarpi. No other skin lesions were present and the dogs appeared otherwise healthy. Before treatment with tacrolimus, all dogs received antibiotics for 4–8 weeks. Hair was clipped to visualise the lesions. The presence of erythematous papules, oedema and fistulae was recorded for each foot. All dogs served as their own controls. Dogs with four legs involved had one front and one hind leg treated. Dogs with two to three feet affected had only one foot treated. Tacrolimus 0.1% ointment (Protopic®) was applied twice daily onto the site of lesions. Partial improvement of treated lesions was seen in all cases within 3 weeks. After 6 weeks, treated lesions were in complete remission in four dogs, while the other three subjects had palpable but invisible lesions. Signs had not improved on the untreated legs. Follow-up varied between 4 months and 2 years. Lesion remission persisted in six dogs with the intermittent application of tacrolimus. Adverse effects of treatment were not seen. In conclusion, the application of topical tacrolimus seems to provide a safe and effective treatment option for plantar fistulae in German shepherd dogs.

Funding: Self-funded.

FC-22

Doxycycline therapy in 10 cases of feline plasma cell pododermatitis: clinical, haematological and serological evaluations

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Various treatments have been described for feline plasma cell pododermatitis. Doxycycline has been reported recently as an effective medical option. Plasmacytic pododermatitis was diagnosed clinically and histopathologically in 10 cats. Blood samples for haematological, biochemical and immunological evaluation were taken before treatment. Cats were administered doxycycline at a dosage of 10 mg/kg/day for 40 days and re-evaluated at 30 and 60 days. Biochemical evaluation was repeated at both rechecks. All were Domestic Short-haired cats, eight males (five neutered) and two females (one spayed), ranging in age from 6 months to 8 years. Duration of lesions prior to consultation ranged from 1 to 48 months. Lesions were present in multiple footpads in all cases and consisted of swelling and softening (100%), erythema (50%), exfoliation (60%) and ulceration (20%). Haematological and serological findings included: thrombocytopenia (70%), leucocytosis (40%), lymphopenia (30%), and mild to pronounced hypergammaglobulinaemia (100%). Four of nine cats were FIV positive, 9/9 were FeLV negative, and 4/4 were negative for leishmaniosis. Response to treatment was as follows: complete remission within 30 days (one cat), complete remission within 60 days (four cats), more than 50% improvement in lesions (four cats), no improvement (one cat), and lost to follow up (one cat). Hypergammaglobulinaemia was still present in five of nine cats at day 60. Therapy with doxycycline at 10 mg/kg/day resulted in a high improvement rate in cats with plasma cell pododermatitis. Thrombocytopenia, a previously unreported haematological finding, was detected in the majority of the cats.

Funding: Italian Society of Veterinary Dermatology, Merial.

FC-23

Feline plasma cell pododermatitis: a retrospective study of 26 cases

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Feline plasma cell pododermatitis is a rare dermatological condition exclusively described in cats. Our objective was to report a retrospective study of 26 cats with plasma cell pododermatitis. Neutered males were overrepresented (19 cases), and 25 cases were Domestic Short-haired cats. Age at time of diagnosis was 6 months to 12 years. The primary complaint was painful lameness (22 cases), poor condition (two cases) or excessive salivation (two cases). Lesions were present primarily in the metatarsal and metacarpal footpads and rarely in the digital footpads. One, two or four feet were involved. Clinical signs included swelling (35 footpads), softening (36 footpads), exfoliation (19 footpads), ulcers (nine footpads) and abscesses (nine footpads). Two cats had plasma cell stomatitis, and general signs were observed in all cases. Sixteen cats were FIV positive and one cat was FeLV positive. Histopathological findings were characterized by a superficial and deep perivascular lymphoplasmacytic dermatitis (two cases), an almost pure diffuse plasmacytic dermatitis (15 cases, 10 FIV positive), a diffuse plasmacytic dermatitis with lymphocytes grouped in follicular structures (four cases, all FIV positive) and a granulomatous inflammation (five cases, one FIV positive). Plasmacytic infiltration was also observed in one FIV-positive case within the kidneys, liver and lungs. Five cases were positive for FIV-PCR performed from lesional skin biopsies. Two cats were euthanized. Twenty-four cats were treated by surgical excision with no relapse for 1–5 years. Feline plasma cell pododermatitis can be considered a cutaneous reaction pattern with multiple causes, and FIV infection may be a predisposing factor.

Funding: Self-funded.

Session 5: CONGENITAL/INHERITED DISEASES AND SKIN BIOLOGY

FC-24

Risk factors for atopic dermatitis in a Swedish population of insured dogs

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Studies into the epidemiology of canine atopic dermatitis (CAD) are in great demand. Estimates of the prevalence and incidence of CAD are commonly based on hospital studies where no reference population is defined. Such studies tend to overestimate the disease frequency due to referral bias and a higher proportion of complicated cases at secondary care centres than in the general population. The aim of this paper was to present better estimates of the incidence of CAD. The Swedish dog population offers unique opportunities to study the epidemiology of CAD due to several characteristics: a large proportion of dogs are purebred, fleas and flea allergies are rare, and a secondary database of disease records is available through an insurance company that covers approximately 30% of all Swedish dogs. By accessing insurance-claims records for the years 1995–2000, the true incidence rate of CAD was estimated as 10 cases per 10,000 dog years at risk. Univariate analysis showed that the incidence was the same across genders. Additionally, large differences in the risk of being diagnosed with CAD existed among breeds. In this study, breeds with the highest risk were the bull terrier (88 cases/10,000 dog years at risk), Staffordshire bull terrier (58/10,000), West Highland white terrier (51/10,000), Welsh terrier (50/10,000) and boxer (50/10,000). Decreased risk was observed among sighthounds; no cases were recorded among the Borzoi, Saluki and Whippet breeds. A proportional hazards (survival) model was developed in order to take sex, breed, age and geographical region into account in a multivariate analysis.

Funding: Swedish University of Agricultural Sciences, The Foundation for Research.

FC-25

A novel ulcerative nasal dermatitis of Bengal cats

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The purpose of this report was to describe a unique dermatitis affecting Bengal cats in Sweden. Forty-eight Bengal cats were presented to DjurAkuten between 1999 and 2003. Six cats (four males and two females) exhibited a unique dermatosis characterized by fissures, crusts, erosions and ulcers limited to the nasal planum. No other skin or internal lesions were noted, except for a heart murmur in one cat. The age of onset varied between 4 months and 1 year. The skin disease did not appear to be contagious. Fungal cultures were negative in four subjects, and haematology and serum chemistry results were unremarkable in two cats. In one patient, histopathology revealed marked epidermal parakeratosis and crusting, and a moderate mononuclear and neutrophilic dermatitis with periadnexal plasma cells. Five cats were treated with antibiotics without noticeable improvement in nasal lesions. Prednisolone administration resulted in complete remission in one cat and partial, short-term improvement of lesions in another. Topical application of salicylic acid improved lesions in one of two cats. Finally, tacrolimus ointment led to a rapid decrease in lesions in four patients. Follow-up varied between 4 months and 3 years. One cat remained in complete remission with prednisolone, partial improvement was maintained in one cat with salicylic acid, and marked improvement of lesions persisted with topical tacrolimus in four patients. The cause of this unique dermatitis remains elusive. The occurrence of skin lesions in one breed suggests a heritable cause. The response of lesions to immunomodulators suggests of an immune pathogenesis.

Funding: Self-funded.

FC-26

Junctional epidermolysis bullosa in a Charolais calf with deficient expression of integrin $\alpha6\beta4$

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Hereditary junctional epidermolysis bullosa (JEB) is a rare mechanobullous disease associated with defective expression of the hemidesmosome/anchoring filament proteins laminin-5, collagen XVII and integrin $\alpha6\beta4$. The condition is characterized by extensive blistering of the skin caused by splitting of the lamina lucida of the basement membrane (BM) in the squamous epithelia and by extracutaneous manifestations. Congenital JEB has been sporadically reported in cattle, but the BM protein associated with the genetic defect has not been identified. We report a case of spontaneous JEB in a 2-week-old female Charolais calf. Clinical lesions observed at birth were acral, auricular and oral erosions and ulcers, and onychomadesis of the four feet. General signs included anorexia, apathy, emaciation and marked cutaneous pain that justified rapid euthanasia. Histopathological examination revealed a dermo-epidermal separation of the BM zone without keratinocyte cytolysis. Antigen immunomapping of frozen skin sections using antibodies to the major components of the human cutaneous BM revealed an intralamina lucida cleavage of the skin, which confirmed the diagnosis of JEB. In skin samples from the affected calf and wild type controls, the immunoreactivity of laminin-5 and collagen XVII was comparable, while expression of integrin $\alpha6\beta4$ was strongly reduced in the proband. These are the first results identifying *itg* $\alpha6$ and *itg* $\beta4$ as the candidate genes affecting cattle in JEB. They also suggest that altered expression of integrin $\alpha6\beta4$ in ruminants is not associated with pyloric atresia – a complication always observed in humans suffering from altered synthesis of integrin $\alpha6\beta4$.

Funding: Self-funded.

FC-27

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

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Junctional epidermolysis bullosa (JEB) is a mechanobullous skin disease associated with mutations in the basement membrane components of the dermo-epidermal junction. This condition is a suitable prototype for proving the feasibility of a gene therapy approach to genodermatoses. As successful genetic treatment requires immunocompetent animal models to study the possible host immune response to the transgene, we have characterized a breed of short-haired pointers suffering from a mild form of JEB. These animals exhibit skin blistering and erosions following minor trauma. Immunohistochemical analysis of the skin biopsies and immunoprecipitation of spent medium from cultured JEB keratinocytes showed reduced expression and secretion of the α3 chain of laminin 5 (α3, β3, γ2), the major adhesion ligand of basal keratinocytes. The search for genetic mutations detected a homozygous insertional mutation (4818 + 207 ins6.5 kb) in intron 35 of the *lama3* gene. Consequently, keratinocytes from dogs with JEB secrete reduced amounts of wild-type laminin 5, and adhesive properties of the keratinocytes are compromised. Retroviral transfer of wild-type dog α3 cDNA into JEB keratinocytes enhanced secretion of laminin 5 in the extracellular matrix and restored the adhesion, differentiation and proliferative capacity of the transduced cells. Transplantable fibrin-based skin equivalents made with transduced JEB keratinocytes and grafted onto SCID mice generated normal cohesive and stratified epithelia and showed stable localized deposition of laminin 5 at the dermo-epidermal junction. Our results form the basis for preclinical assays of gene therapy on a unique immunocompetent animal model for an inherited skin disease.
Funding: DEBRA (UK) Foundation, Association Francaise Contre les Myopathies.

FC-28

Ultrastructural markers expressed in cultured canine dendritic cells and laminated bodies found in Langerhans cells: similarities and differences

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Ultrastructural markers that consist of coated vesicles, regularly laminated bodies, pleiomorphic inclusions and paracrystalline structures have been described in canine cutaneous histiocytoma (CCH), a cutaneous tumour of Langerhans cell origin. These microstructures are also observed in congenital self-healing histiocytosis, a human Langerhans cell tumour. We have observed for the first time ultrastructural markers in canine dendritic cells (DC) that share some of the features of the microstructures described in CCH. Canine DC were generated *in vitro* from adherent peripheral blood mononuclear cells. Morphological analyses and ultrastructural and functional studies showed that these cells display typical DC features. After 1 week of *in vitro* culture of adherent peripheral blood mononuclear cells in the presence of canine IL-4 and human GM-CSF, a great proportion of the cells displayed typical cytoplasmic processes (optical and electronic microscopy) and expressed CD14 and MHC class II molecules (cytometric analysis). In addition, allogeneic mixed lymphocyte reactions were performed to assess the ability of these cells to stimulate the proliferation of allogeneic lymphocytes. Compared to monocytes/macrophages, these cells were able to stimulate allogeneic lymphocyte proliferation very strongly, as is reported for DC in other species. Ultrastructural analysis of these DC revealed the presence of cytoplasmic organites, dense granules of variable sizes containing periodic microstructures. These results were obtained for the three different breeds tested. The microstructures previously described in Langerhans cells (e.g. in CCH) share some similarities with periodic microstructures (membrane, laminated aspect, cytoplasmic localization), but not the typical periodic aspect seen in DC.
Funding: National Veterinary School of Nantes.

FC-29

Distribution of γδ-T lymphocytes in normal canine skin

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The exact function of T cells with the γδ-T-cell receptor is still poorly understood and their participation in different skin diseases is largely unknown. The goals of this study were to investigate the distribution of γδ-T cells in normal canine skin and mucocutaneous junctions, and to evaluate their frequency in different body locations. Skin samples were collected from 10 dogs. From each dog, 10 different skin regions were sampled. Two random 30 μm cryosections were used for immunohistochemistry. Each tissue was evaluated for the presence of T cells, αβ-T cells and γδ-T cells. The numbers of positive cells per volume of tissue were determined in the epidermis and superficial dermis of each sample using a 42-point square grid overlay. Accurate thickness of the tissue section was measured with an A2 axis stage micrometer. Results were analysed statistically and 1000 cells/mm^3 were used as the threshold value. Independent from body location, the vast majority of γδ-T cells were observed in the epidermis. Significant occurrences of epidermal γδ-T cells were found in 50% of samples from the anorectal skin and eyelid, and in 25% of samples from the lip, interdigital skin and pinna. However, <1000 cells/mm^3 were observed in the axilla, trunk, inguinal skin, tongue and footpad samples. This study reveals information about the distribution of γδ-T cells in the skin and, hence, may provide new insights into the prevalence of these cells in skin diseases preferentially developing in certain body locations.
Funding: Leukocyte Antigen Biology Laboratory, University of California-Davis.

Session 6: ALLERGY – ALLERGENS AND CYTOKINES

FC-30

Presence of dust mites in the environment of dust mite-sensitized atopic dogs

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Dust mites (DM) are the most common offending aeroallergens in atopic dogs. The aim of this study was to compare the DM load of households with atopic dogs (Group A, $n = 8$) that had positive intradermal test reactions to *Dermatophagoides farinae*, *D. pteronyssinus, Acarus siro, Lepidoglyphus destructor* and/or *Tyrophagus putrescentiae* to the DM load of households with nonatopic dogs (Group B, $n = 4$) and of nonpet households (Group C, $n = 8$). Group A dogs presented with perennial pruritus, were free of pathogenic mites and fleas, did not respond to an elimination diet, and fulfilled the diagnostic criteria of atopic dermatitis. All Group B dogs tested intradermally negative and had no dermatological problems. Dust samples were vacuum collected in a standardized fashion from the human (all groups) and dog mattresses (Groups A and B) or from the couch (Group C) four times, once for each season of the year. The presence of DM was assessed with a commercial test (Acarex test) and stereoscopically. At least one DM was found in all Group A houses. The DM load was not significantly different between the seasons or the three animal groups. The sensitivity of the Acarex test was significantly lower than that of stereoscopic examination ($P < 0.001$). In conclusion, the environmental DM load was similar between atopic and nonatopic dogs, the presence of dogs in a household didn't increase DM numbers, and stereoscopy was more sensitive than the Acarex test for the detection of DM.
Funding: Self-funded.

FC-31

Prevalence and characterization of house dust mites and house dust mite allergens collected from the bedding, skin and hair coat of dogs in southwest England

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The house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* are commonly used in diagnostic serological and intradermal tests for canine atopic dermatitis in the UK. However, there are few studies that have characterised the exposure of UK pet dogs to these mites. Objectives of this study were to determine the prevalence of mites and to characterise the mite species on skin, hair coat and bedding of pet dogs. A population of nonhospitalized pet dogs for which bedding was available for analysis was recruited irrespective of dermatological status ($n = 34$). Dust samples ($n = 68$) were collected from both dogs and their beds using a standardised vacuuming technique. Mites were identified using accepted morphological criteria. House dust mite allergen concentrations were assayed using a standardised ELISA for Der p 1 and Der f 1. Mites were identified in 15 of 68 samples (22%): *D. pteronyssinus* alone in three of 68, *D. pteronyssinus* in combination with storage or unclassified mites in five of 68, and storage or unclassified mites alone in seven of 68. *Dermatophagoides farinae* was not identified in any samples. Der p 1 allergens were detected in 37 of 68 samples (54%), and Der f 1 in four of 68 samples (5.9%). Contrary to studies elsewhere in Europe and North America, these findings support studies of human asthma patients in the UK where exposure to *D. pteronyssinus* is common, but rare to *D. farinae*. Given the high prevalence of positive intradermal and serological reactions to *D. farinae* in atopic dogs, further investigations are warranted to clarify potential cross-reactivity with other mite allergens.
Funding: PetPlan Charitable Trust, Pfizer Animal Health.

FC-32

Identification of airbourne moulds in stables over a 2-year period

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Fungal moulds are well recognised as a trigger for allergic disease in people. Good correlation is recognized between rising levels of airbourne moulds and allergic reactions to these moulds. This study was designed to evaluate the levels and types of airbourne moulds in different stable types over a 2-year period. Four stables were chosen within an equine hospital environment. Shavings were used as bedding in all stables throughout the period. The stables were selected as four different constructions with different outlooks and different ventilation, although they were all within the same establishment. Each month throughout the investigation period, a Sabouraud's medium petri dish was mounted vertically on the wall of each stable at a height of 1.5 m and left uncovered for 30 min. The temperature and general weather conditions were recorded. The dish was submitted to the laboratory for culture. A variety of different moulds were identified. These included *Penicillium* spp., *Alternaria* spp., *Aspergillus* spp., *Mucor* spp. and *Candida* spp. The different stable constructions grew different numbers of moulds, but the same type on each occasion. Cold icy weather tended to favour growth of *Penicillium* spp. *Aspergillus* spp. was identified most commonly when conditions were cold and damp in the autumn. Mould growth was sparse in the spring, but tended to rise in mid-summer and into the autumn.
Funding: Self-funded.

FC-33

Expression of IL-12 receptor $\beta 2$ gene in atopic dogs

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Atopic dermatitis (AD) is very common in dogs, but its pathogenesis is not yet fully understood. It has been suggested that a Th2-dominant status may be associated with the occurrence of canine AD. IL-12 is thought to be important for the differentiation of Th1 cells. The IL-12 receptor $\beta 2$ (IL-12R$\beta 2$) gene is considered to play a critical role in signal transduction and is attracting attention as one of the causative genes of AD in humans. The purpose of this study was to investigate the relationship between IL-12R$\beta 2$ gene expression and canine AD. The canine IL-12R$\beta 2$ gene was cloned by RT-PCR and its nucleotide sequences were determined. Canine IL-12R$\beta 2$ showed 76.8% homology at the amino acid level with human IL-12R$\beta 2$, and its structural motifs were well conserved. cDNA with a 91 bp deletion including the transmembrane region was also cloned, which consequently produced a frame shift and an early stop codon. The deletion region corresponded to exon 14 of the human IL-12R$\beta 2$ gene on chromosome 1. The expression of deleted canine IL-12R$\beta 2$ mRNA in phytohemagglutinin-stimulated peripheral blood mononuclear cells was examined in seven healthy dogs and 11 AD dogs. Both deleted and intact mRNAs were expressed at constant ratios in healthy and AD dogs. The results indicate that the deletion of the transmembrane region is not associated with the occurrence of AD, and that the expression of the deleted mRNA may be constitutive and produced by alternative splicing.

Funding: Self-funded.

FC-34

Quantitative real-time RT-PCR for the measurement of feline cytokine mRNA expression in skin of normal cats and cats with allergic skin disease

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Feline allergic skin disease is thought to be associated with dermal infiltration of Th2 lymphocytes and the synthesis of associated cytokines. In this study, real-time RT-PCR assays were developed to measure feline IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p35 and p40), IL-18, TNFα, TGFβ, IFNγ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in the skin of healthy control cats and in the lesional and nonlesional skin of cats with allergic skin disease. Total RNA was extracted from skin biopsies using the RNeasy Mini Kit with on-column and in-solution DNase digestion steps. Improm-II reverse transcriptase and random hexamers were used to synthesise cDNA. Real-time PCR was carried out using an iCycler IQ system, and gene-specific primers were designed to span an exon/exon junction of each cytokine gene. Taq-Man probes were used to add specificity to the system. Messenger RNA from the housekeeping gene GAPDH was used for normalisation of the cytokine threshold cycle. The 11 cytokine mRNA transcripts quantified were present at varying levels, but there were no apparent differences in expression among normal, nonlesional and lesional skin. TGFβ represented the most abundant transcript while IL-4, IL-5, IL-6, IL-10, IL-12, IL-18 and TNFα were present at levels approximately 1000-fold less. IL-2 and IFNγ represented the least abundant templates with no detectable copies in most RNA samples. This quantitative analysis of cytokine mRNA expression in feline skin biopsies has suggested that there is not a simple Th2 bias in the lesional skin of cats with allergic dermatopathies.

Funding: RCVS West Scholarship, Novartis Animal Health.

FC-35

Real-time evaluation of cytokine and protease expression in flea-allergic and nonallergic dogs

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Flea bite allergy is a skin disorder with a complex immunopathogenesis. Th2 cytokines and proteases have been hypothesized to play a role in the pathogenesis. The aims of our study were to evaluate the cytokine and protease expression in skin biopsies and peripheral blood mononuclear cells (PBMC), the histopathological response, and the response to intradermal tests (IDT) in sensitized nonallergic and sensitized allergic dogs. Twenty dogs were exposed to fleas once a day for 4 days. Before flea exposure and after the last flea exposure, an IDT was performed, skin biopsies were taken, and PBMC were isolated. Skin biopsies and PBMC were stimulated with various substances, and real-time RT-PCR was performed using primers for tryptase, chymase, IL-4, IL-5, IL-13, TNFα and IFNγ. Intradermal tests and histopathological examination of biopsies revealed much stronger reactions in the allergic group. Before flea exposure, mRNA expression of chymase, TNFα, IFNγ, IL-5 and IL-13 in biopsies of allergic dogs was higher compared to nonallergic dogs, whereas in PBMC only the expression of IFNγ was higher. After flea exposure, the difference in mRNA expression between allergic and nonallergic dogs was not as striking. In allergic dogs, stimulation of PBMC with flea antigen resulted in a higher expression of IL-4 and IL-13, whereas in biopsies only the expression of IL-4 was higher. Our results demonstrate that even without the presence of antigen, the pattern of cytokine expression differs between allergic and nonallergic dogs. Furthermore, these results clearly demonstrate that the inflammatory response is much stronger in allergic dogs.

Funding: Novartis SA.

Session 7: ALLERGY – SYMPTOMATIC THERAPY

FC-36

The use of immunostimulatory bacterial DNA sequences in allergen-specific immunotherapy of canine atopic dermatitis

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The purpose of this study was to evaluate a combination of immunostimulatory bacterial DNA sequences and allergen-specific immunotherapy for the treatment of canine atopic dermatitis. Seven dogs with nonseasonal atopic dermatitis diagnosed by history, clinical signs and exclusion of differential diagnoses were included. All dogs had been on allergen-specific immunotherapy for at least 12 months with incomplete responses, were on additional antipruritic therapy and showed residual pruritus. Pruritus was marked by the owner on a visual analogue scale, lesions were determined by a clinician using the Canine Atopic Dermatitis Extent and Severity Index (CADESI), and concurrent medications were recorded before entering the study and after 14 weeks of treatment. Peripheral blood mononuclear cells were isolated and cultured; canine cytokine message for IFNγ, IL-4, TNF and IL-10 was quantitated using RT-PCR. A mixture of allergen extract and liposome-DNA complexes was injected intradermally at the beginning of the study and after 2, 4, 6, 10 and 14 weeks. CADESI, pruritus and medication scores, and cytokine messages at the beginning and end of the study were compared with a paired *t*-test. There were significant improvements in pruritus scores ($P = 0.0277$). Reductions in medication scores and CADESI were not statistically significant. IL-4 production decreased significantly ($P = 0.0428$); decreases in other cytokines were not significant. Although the number of dogs in this pilot study was small, the results warrant further investigation of a combination of immunostimulatory bacterial DNA sequences and allergen-specific immunotherapy for the treatment of canine atopic dermatitis.

Funding: Self-funded.

FC-37

Use of recombinant omega interferon therapy in canine atopic dermatitis: a pilot study

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It has been postulated that atopic dogs show a predominant Th2-type response associated with overexpression of IL-4 and increased secretion of IgE. In a pilot, open uncontrolled study, 20 dogs with confirmed atopic dermatitis were given Virbagen Omega®, a feline recombinant omega interferon (rfeIFN-ω), to ascertain whether this type-1 cytokine could modulate the Th2-dominated cytokine response. Dogs received rfeIFN-ω as a monotherapy at the dosage of 1 MU/kg subcutaneously, three times per week for 3 weeks. Flea control and the use of nonmedicated shampoo were maintained during the study. Lesional and pruritus indices for canine atopic dermatitis (LICAD and PICAD, respectively) were assessed before, after treatment (day 21) and 3 weeks post-treatment (day 42) using a scoring system based on the extent and severity of skin lesions (erythema, excoriation and lichenification scored on 12 anatomic areas) and pruritus (scored on six anatomic areas). Two dogs were excluded, one for an erythematous reaction and another for relapsing folliculitis. After rfeIFNω therapy (day 21), both LICAD and PICAD were significantly improved ($P < 0.01$). By day 42, mean LICAD and PICAD had decreased by 60 and 51%, respectively, compared to baseline values. Adverse reactions to rfeIFN-ω were rare and inconsistent. This study suggests that rfeIFN-ω may be useful in the control of canine atopic dermatitis. However, further work is needed to confirm efficacy and define the optimal dosage through a long-term randomized controlled trial.

Funding: Virbac SA.

FC-38

Efficacy of conjugated linoleic acid and black currant seed oil in the treatment of canine atopic dermatitis: a double-blinded, randomized, placebo-controlled study

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Conjugated linoleic acid (CLA) has shown inhibitory effects on histamine release, eicosanoid production and pruritus in laboratory rodents. Its use in canine atopic dermatitis (AD) has not been reported. The aim of this study was to assess the efficacy of CLA, black currant seed oil (BSO) or a combination of both compared to placebo in dogs with AD. A further aim was to evaluate changes in fatty acid metabolism induced by these treatments. Twenty-four dogs with AD (no response to food trials and negative intradermal tests for fleas) were randomly allocated to four groups. Each group was treated orally daily for 2 months in a blinded manner with 1 mL/10 kg CLA, 1 mL/10 kg BSO, 1 mL/10 kg CLA + 1 mL/10 kg BSO, or 1 mL/10 kg sugar syrup (placebo). Serum was obtained for polyunsaturated fatty acid analysis on days 0, 30 and 60. Owners assessed pruritus with a visual analogue scale, and veterinarians evaluated lesions with the Canine Atopic Dermatitis Extension and Severity Index on days 0, 30 and 60. The best clinical results occurred with BSO alone and were particularly evident in half of the animals treated. However, global improvement was not significant with any of the treatments. Serum levels of di-homo-gamma-linolenic acid markedly increased in BSO-treated dogs, which may be related to the clinical improvement. Conjugated linoleic acid at the dosage used in this study does not seem to be helpful in canine AD, whereas BSO may be helpful in a selected population of dogs with AD.

Funding: NBF Lanes.

FC-39

Successful management of canine atopic dermatitis using a plant extract: a randomized, double-blinded, placebo-controlled trial

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This study evaluated the efficacy of Phytopica™, a proprietary blend of standardised plant extracts, in canine atopic dermatitis (AD). One hundred twenty dogs with perennial AD were recruited on the basis of history and clinical signs, and a positive intradermal allergen test or rFcϵRIα serology to perennial allergens. Other pruritic dermatoses were eliminated by antimicrobial treatment, skin scrapings, *Sarcoptes* serology, flea control and a 6-week food trial. Exclusion criteria included antimicrobial therapy within 7 days, antihistamines within 14 days, oral/topical glucocorticoids or cyclosporin within 28 days, and parenteral glucocorticoids, essential fatty acids or immunotherapy within 56 days of entry into the study. Dogs [minimum Canine Atopic Dermatitis Extent and Severity Index (CADESI) = 25] were randomly allocated to receive placebo, 100, 200 or 400 mg/kg Phytopica™ daily for 12 weeks. Their CADESI was assessed every 4 weeks. A modified intention-to-treat population was analysed. The mean reductions in CADESI scores at the end of treatment compared to baseline were 4.4% (100 mg/kg; $n = 30$), 23.4% (200 mg/kg; $n = 29$, $P < 0.01$), 8.5% (400 mg/kg; $n = 29$) and 3.9% (placebo; $n = 29$). For more severely affected dogs (minimum CADESI ≥ 50 at baseline), there was significant reduction in mean CADESI score (29.3%, $P = 0.038$) only in the 200 mg/kg treatment group ($n = 14$). In conclusion, this study demonstrates that Phytopica™ is an effective nonsteroidal treatment for canine AD.

Funding: Phytopharm plc.

FC-40

Efficacy of combined topical therapy with antiallergic shampoo and lotion for the control of signs associated with atopic dermatitis in dogs

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This study evaluated the benefit of Allermyl® shampoo and lotion in alleviating signs of atopic dermatitis in dogs. After treatment of previous infections, 35 dogs diagnosed with atopic dermatitis according to Prelaud's criteria were included in the study. Three of the dogs improved on prior elimination diet trials, but signs were not completely controlled. The dogs first underwent a 3-week antiparasitic therapeutic trial period (permethrin spray once every 7 days and acaricidal medication). Dogs whose lesions and pruritus did not improve entered a 3-week topical antiallergic treatment period (Allermyl® shampoo and lotion alternately every 3 days). No other treatment was allowed. Erythema, excoriation and lichenification were graded at 12 different sites spanning the whole body surface according to an extent-severity scale (0–10) to calculate an aggregate lesional index score (LICAD: 0–360). Pruritus was similarly evaluated on six body areas according to a frequency-intensity scale (0–10) to calculate an aggregate pruritus index score (PICAD: 0–60). Twenty-nine dogs completed the trial. While LICAD and PICAD did not decrease significantly over the control antiparasitic-treatment period, both indices decreased significantly after 14 days of Allermyl® therapy (repeated measures ANOVA, $P < 0.05$). Median (95% confidence interval) lesional and pruritus index reduction were 55% (36–77%) and 58% (42–77%), respectively, within 3 weeks of antiallergic topical treatment. Almost half the dogs (48.3%) were markedly improved (>50% score reduction) with Allermyl®. Obvious clinical benefit (≥30% score reduction) was documented in 75.9% of dogs.

Funding: Virbac SA.

FC-41

A prospective pilot study on the use of cyclosporin on feline allergic diseases

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The efficacy of cyclosporin in canine atopic dermatitis has been well documented, but reports on its use in feline allergic dermatoses are scarce. The aim of this study was to evaluate the efficacy of cyclosporin in cats affected by pruritus and eosinophilic dermatoses. Ten otherwise healthy cats were selected. All cats had no response to a hypoallergenic diet trial, had not been treated with short-acting corticosteroids or antihistamines for at least 2 weeks, had not been treated with long-acting steroids for 2 months, and had been treated with endo- and ectoparasiticidal drugs with no improvement 2 weeks before entering the study. All cats were pruritic and had erythema, seven had alopecia, five had an eosinophilic plaque, three had facial pruritus, one had miliary dermatitis, and one had an eosinophilic granuloma. Cyclosporin was administered daily at the dosage of 5 mg/kg for 1 month. Owners assessed pruritus by means of a visual analogue scale, and veterinarians evaluated cutaneous lesions on days 0 and 30 by a newly developed index called Feline Eosinophilic Granuloma, Eosinophilic Plaque, Extension and Severity Index (FEGEPESI), similar to the Canine Atopic Dermatitis Extension and Severity Index (CADESI). Fifty percent of the cats had a reduction of pruritus ≥ 50%, and in 50% of the animals, the lesions improved or disappeared completely. However, overall pruritus and FEGEPESI scores did not improve significantly. In conclusion, cyclosporin was helpful in symptomatically treating about half of the cases of feline pruritus and eosinophilic dermatitides.

Funding: Novartis.

Session 8: PARASITES AND EAR DISEASE

FC-42

Sarcoptic mange epidemic in a cat population

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Worldwide, sarcoptic mange in cats is seldom reported, and then only in sporadic individual cases. We describe an epidemic in a household with a dog and 25 cats. From September 2002, the dog was repeatedly treated with ivermectin for sarcoptic mange. The diagnosis was confirmed by skin scrapings. Fifteen months later, cats from the same household were diagnosed with severe sarcoptic mange. Twenty-one of the cats were euthanized and necropsies were performed. Skin samples were taken from all cats from different body sites for histology, and skin scrapings were examined for ectoparasites. Samples for bacterial and dermatophyte culture were taken from six cats. Smears for cytology were made from lesions on four cats with severe mange. Sera from 21 cats and the dog were analysed for specific antibodies to *Sarcoptes scabiei*. Molecular characterizations of six individual mites were done. Large numbers of *S. scabiei* were isolated from the infected skin of most of the cats. Two-thirds of the cats showed skin lesions compatible with chronic sarcoptic mange. Macroscopically, internal organs exhibited no obvious pathology. Yeast organisms and coccoid bacteria were found in the smears; penicillinase-negative *Staphylococcus aureus* was isolated from all samples and *Malassezia pachydermatis* was identified from four cats. *Sarcoptes scabiei* was seen histologically in all cats showing chronic skin lesions. No other ectoparasites were found. All analysed cats had specific antibodies against *S. scabiei*. Twenty-one cats tested negatively for FeLV and FIV. The mites had DNA sequences identical to *S. scabiei* from naturally infected dogs and Swedish wildlife.
Funding: Self-funded.

FC-43

Feline demodicosis: a retrospective study of 12 cases

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Feline demodicosis is rarely reported compared to canine demodicosis. The objective of this report was to describe a retrospective study of 12 cases of feline demodicosis. Neither sex nor breed predisposition were observed. The age of diagnosis was between 5 months and 13 years. *Demodex cati* was identified in nine cases and *Demodex gatoi* in three cases. Cutaneous lesions were localized in 10 cases, generalized in two cases, and present on the face (8 cases), neck (5), shoulders (1), trunk (2) and limbs (1). Pruritus was mild in four cats and moderate in two cats. Dermatological lesions were characterized by alopecia (10 cases), erythema (7), scaling (4), erosions (4), ulcers (2), crusts (2), comedones (2), seborrhea oleosa (2), papules (1), pustules (1) and hyperpigmentation (1). Ceruminous otitis was noted in two cats. Pyoderma was noted in four cases. An underlying disease was identified in eight cats: FeLV infection (two cats), FIV infection (two cats), FeLV/FIV infections (one cat), diabetes mellitus (one cat), iatrogenic Cushing's syndrome (one cat), and diabetes mellitus associated with iatrogenic Cushing's syndrome (one cat). In two cats, an infestation by *Demodex cati* was identified at lesional sites of multicentric squamous cell carcinoma *in situ*. Different drugs were used in nine cases: crotamiton (two cases), amitraz (six cases), milbemycin oxime (one case). Therapeutic results were variable and related to the underlying disease.
Funding: Self-funded.

FC-44

The therapeutic effect of selamectin and ivermectin regimens in canine sarcoptic mange

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Sarcoptic mange is a contagious, parasitic and pruritic skin disease of the dog caused by the *Sarcoptes scabiei* var. *canis* mite. The aim of this study was to compare the efficacy of ivermectin and selamectin in dogs with sarcoptic mange. The study included 120 naturally infested dogs. Several skin scrapings were collected from each dog. Microscopic evidence of at least one adult mite or its eggs was considered to be diagnostic (inclusion criteria). The dogs were divided into two groups of 60 animals each. Group A dogs were given injectable ivermectin (Ivomec®) at 300 μg/kg subcutaneously every 15 days for three treatments; Group B dogs were treated with selamectin spot-on (Stronghold®) at 6–12 mg/kg twice monthly. Clinical evaluation was conducted on days 15, 30 and 45, and parasitologic evaluation by skin scrapings on days 30 and 45. On day 30, all dogs in Group B had negative skin scrapings (100%) with no evidence of cutaneous lesions. Only 58 of 60 dogs in Group A had negative skin scrapings (96%), but the two positive dogs became negative on day 45. These results provide preliminary evidence that there is no significant difference between the therapeutic effects of ivermectin and selamectin in the management of sarcoptic mange, with both treatments being highly efficacious.
Funding: Self-funded.

FC-45

Mites as newly emerging disease pathogens in rodents and human beings

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Different mite species generally found on animals may temporarily also infest humans. Consequently, these arthropods may be responsible for pruritic skin reactions that are often misdiagnosed. Mite dermatitis caused by the tropical rat mite *Ornithonyssus bacoti* occurs in several small mammals and rodents under tropical and temperate climatic conditions. According to various observations in Germany, *O. bacoti* appears in wild rodents more frequently than previously thought. In most cases, symptoms of mites are recognized only when they attack humans, but the diagnosis of rat mite dermatitis requires identification of the parasite, which is more likely to be found in the environment than on the hosts' skin itself. Here, five different outbreaks from Bavaria are reported. A clinical example is the case of a 23-year-old medical student and several other residents inhabiting a rat- and mouse-infested house in Munich. The arthropods originally came from an Italian restaurant and surrounding facilities. Mites were found in large numbers in the students' flat. The patient was suffering from severe itching and papular urticaria. He consulted a dermatology clinic complaining of a pruritic dermatitis of 2-weeks duration. Dermatitis was misdiagnosed as allergy and treatment with an anti-inflammatory agent was unsuccessful. Eradication of rodents and treatment of the house with a pyrethroid were performed to prevent reinfestation. *Ornithonyssus bacoti* is a periodic haematophageous parasite and spends a relatively short time on the host. Causal therapy with antiparasitic agents on human patients is not necessary. If indicated, treatment should be symptomatic.

Funding: Self-funded.

FC-46

Methicillin-resistant *Staphylococcus intermedius* organisms from the vertical ear canal of dogs with end-stage otitis externa

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Methicillin-resistant staphylococci (MRS) have been isolated from the ears of dogs with otitis externa; however, the specific location of the sample and the condition of the ear were not disclosed. The purpose of this study was to identify MRS from the vertical ear canal in dogs with end-stage otitis externa. Swab samples were obtained from one vertical ear canal of 26 dogs undergoing a total ear canal ablation and bulla osteotomy. Swab samples were routinely processed for bacterial culture. No topical or oral antimicrobial agents had been administered within 24 h of obtaining the sample. Testing for oxacillin (methicillin) resistance was performed with the disk diffusion test (DD) and confirmed with the oxacillin screen agar test (OSA) for staphylococcal organisms. Twenty-three staphylococcal organisms [21 *Staphylococcus intermedius* (SI), 2 coagulase-negative staphylococci (CNS)] were isolated from 17 dogs. On the DD, 15 organisms were susceptible (13 SI, two CNS), one was intermediate (SI), and seven were resistant (all SI) to methicillin. On the OSA, one SI that was intermediate on DD was susceptible, and of the seven that were resistant on DD, five SI were susceptible and two SI were resistant to methicillin. Both methicillin-resistant *S. intermedius* (MRSI) were susceptible to chloramphenicol, polymyxin B and trimethoprim-sulfadiazine. In dogs with end-stage otitis externa, MRSI may be an important pathogen. Had OSA not been performed to confirm MRS, five of seven staphylococcal organisms would have been erroneously reported as resistant to certain antibiotics, which may have affected antibiotic selection and treatment of the otitis.

Funding: Bayer Animal Health, The Ohio State University Canine Research Fund, American College of Veterinary Dermatology, Ohio Animal Health Foundation.

FC-47

Comparison of four different types of stain in ear cytology

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Swab cytology is one of the most useful and rapid methods to assess the presence of external infections in ear canals. Smears are generally stained with Romanowsky-type stains, with or without prior heat fixation. The aim of this study was to compare four different ways of staining ear cytology swab samples. Eight animals with ceruminous or purulent otitis externa were selected. Cotton swabs were introduced in a total of 12 ear canals, and four cytologic samples on four different slides were obtained from each swab. Glass slides were air-dried and randomly identified with the number of the ear canal and the letters A, B, C or D. Slides marked with A were heat-fixed and stained with Dip Quick®; slides marked with B were stained without heat fixation. Slides marked with C were heat-fixed and dipped in the blue colour only; slides marked with D were stained in the blue colour only without prior heat fixation. Ten high-power fields (oil immersion) were evaluated for each slide, and keratinocytes, yeasts, bacteria and neutrophils were counted. A statistical comparison was performed with parametric (Student's *t*-test applied after verifying the normal distribution of the data) and non-parametric tests (Wilcoxson's and signs' tests). There were no significant differences between the four staining methods ($P \leq 0.05$). The authors conclude that heat fixation does not improve the cytological evaluation of ear swab samples and propose a one-step dip in the blue colour only as a rapid method of staining cytological samples from ear canals.

Funding: Self-funded.

Session 9: ALLERGY – DIAGNOSIS AND ALLERGY TESTING

FC-48

Reliability of the Canine Atopic Dermatitis Extent and Severity Scoring Index

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Objective assessment of canine atopic dermatitis severity is very difficult and only a few scoring systems have been developed. The most commonly used is the Canine Atopic Dermatitis Extent and Severity Scoring Index (CADESI), adapted from the human SCORAD. Despite wide use of this score in clinical trials, no validation has been performed to our knowledge. The aim of this study was to determine the reliability of the CADESI in clinical practice. First, a set of 28 pictures taken from dogs diagnosed with atopic dermatitis was scored by six different investigators for three items: erythema, lichenification and excoriation. Next, 23 dogs with clinical signs compatible with atopic dermatitis were graded by two investigators using the CADESI. Erythema, lichenification and excoriation were assessed on 39 areas. With the pictures, significant correlations (Spearman's r, $P < 0.05$) were found for each combination of investigators for erythema and lichenification, but only in 10 of 15 combinations for excoriation. Interobserver agreement ranged between poor and fair ($0.221 <$ Cohen's $\kappa < 0.508$, mean $= 0.395$). For the living animals, significant correlations ($P < 0.0001$), but poor interobserver agreement, were found for the three items ($\kappa_{erythema} = 0.366$, $\kappa_{lichenification} = 0.385$, and $\kappa_{excoration} = 0.226$). A significant correlation ($P < 0.05$) was found for each location, and interobserver agreement varied between very poor and good ($0.16 < \kappa < 0.66$). These results suggest that erythema and lichenification are reliably assessed, but that grading excoriation is more difficult. Also, the assessment of severity varied depending on the site studied.
Funding: Self-funded.

FC-49

Feline Dermatitis Extent and Severity Index: a pilot study

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Feline dermatology lacks a reliable clinical scoring system. A Canine Atopic Dermatitis Extent and Severity Index (CADESI) is widely used. Cats, however, develop well-defined reaction patterns: symmetrical alopecia (SA), eosinophilic plaque (EP), miliary dermatitis (MD), and head and neck dermatitis (HND). This might preclude comparisons between cats with different lesions of a similar aetiology. A pilot study was therefore undertaken using a modified CADESI termed the Feline Dermatitis Extent and Severity Index (FeDESI). Cats with SA ($n = 11$), EP ($n = 11$), MD ($n = 5$) and HND ($n = 3$) were recruited. The aetiologies included atopic dermatitis ($n = 8$), adverse food reactions ($n = 2$), flea allergic dermatitis ($n = 8$), psychogenic alopecia ($n = 2$) and idiopathic ($n = 10$). Erythema, excoriation and alopecia were graded 0 (normal), 1 (mild), 3 (moderate) or 6 (severe) at 42 body sites to give a final score from 0 to 756. The clinical grading was repeated 2–4 h later for each cat. All cats were assessed by the same investigator and the two scores were blinded. The mean (SD) scores were: SA 50.6 (18.4) and 52.8 (21.8); EP 63.9 (21.5) and 64.4 (19.0); MD 47.2 (17.4) and 43.4 (24.0); HND 57.3 (23.2) and 58.0 (29.5). There were no significant differences between different lesions or between the two scores for each lesion. The proposed FeDESI is therefore an accurate and reliable way for a single investigator to objectively grade the severity of feline skin lesions.
Funding: Self-funded.

FC-50

Results of repeat intradermal testing in 135 dogs

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The usefulness of repeat intradermal testing (IDT) was assessed in 135 dogs in which initial IDT results were inconsistent with the history or when response to treatment was unsatisfactory. The interval between repeat IDT ranged from 8 days to 7 years. Repeat IDT was pursued: when a seasonal history was inconsistent with initial IDT results (45); response to immunotherapy (18) or flea treatment (3) was unsatisfactory; an elimination diet was unsuccessful (66); or for miscellaneous reasons (3). Fifteen dogs that had no reaction on first or second IDT were diagnosed with cutaneous food reaction (9), idiopathic seborrheic dermatitis (2), scabies (1), hypothyroidism (1), or an unidentified seasonal pruritus (1). The signs of the final dog in this group resolved with long-term antibiotic therapy. Twenty-two dogs that had negative initial IDT results developed one or more positive reactions on the repeat IDT and of these, 14 were started on immunotherapy. Forty-four dogs with initial positive IDT results developed at least one additional significant reaction on repeat IDT leading to initiation or reformulation of immunotherapy in 34 dogs. Thirty-three dogs with initial positive IDT results had the same number of reactions on repeat IDT, but reactions were not identical and prompted reformulation of immunotherapy. Twenty-one dogs with initial positive results had fewer reactions on repeat IDT. In conclusion, these results provide evidence that repeat IDT is worthy of consideration in dogs with atopic dermatitis when initial IDT is negative, a seasonal history is inconsistent, or response to treatment has been suboptimal.
Funding: Self-funded.

FC-51

Comparison of intradermal test and antigen-specific IgE test in 22 cases of feline allergic dermatitis

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The usefulness of the intradermal test (IDT) and the serological allergy test (SAT) for detecting antigen-specific IgE in allergic cats has not yet been established. In this study, we compared the results of IDT with those of SAT and evaluated the clinical usefulness of the two tests for detecting possible allergens in allergic cats. IDT and SAT using eight antigens were performed on 22 cats with intense pruritus after excluding ectoparasites and performing diet elimination tests. Approximately 50% of the cats reacted to at least one allergen by either IDT or SAT, and 36.4% of the cats reacted on both IDT and SAT. In contrast, seven healthy cats did not show any reactions on IDT or SAT. The most commonly detected allergen in both tests was house dust mites (IDT, 36.4%; SAT, 40.9%). Five cats reacted to one allergen and the others reacted to more than one allergen with IDT. Three cats reacted to one allergen with SAT. The following percentage agreement between the results of the two tests was calculated: house dust mites (86.4%), cat fleas (63.6%), grass mix (86.4%), common mugwort (81.8%), cat epithelia (90.9%), ragweed (86.4%), Japanese cedar (90.9%), and plantain (81.8%). The overall mean percentage agreement was 83.5%. In summary, the present study showed good agreement between IDT and SAT for cats, and SAT may be more sensitive than IDT, but less specific for detecting sensitized allergens.

Funding: Self-funded.

FC-52

Cross-reactivity between house dust, sarcoptic and storage mites in dogs with atopic dermatitis

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In this study, sera from 43 dogs that previously tested positive to different mites with IgE antibodies were used for immunoblotting to screen for common allergen bands. The use of gradient gels allowed the search for small (< 20 kD) and large (up to 250 kD) proteins. Anticanine-IgE detection antibody (D9) was used for immunostaining. The mites showed species-specific allergen patterns; yet, we had reason to believe that several proteins might be conserved among mites and cause cross-reactivity. Often observed bands for *Acarus siro* had sizes of approximately 43, 45, 54, 63, 71 and 96 kD; for *Dermatophagoides farinae*, 55, 68, 96, 98, 104 and 114 kD; for *Dermatophagoides pteronyssinus*, 98 kD; for *Lepidoglyphus destructor*, 45, 46, 55 and 107 kD; for *Sarcoptes scabiei*, 98 and 104 kD; and finally for *Tyrophagus puterescientiae*, 55, 66, 68, 70, 89 and 98 kD. We have shown that cross-reactivity can lead to false-positive results if serological tests or intradermal tests are carried out with total mite protein extracts.

Funding: Laupeneck AG.

FC-53

Serologic allergy testing in horses with insect hypersensitivity

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Serologic allergy testing (SAT) results in horses have not been consistent with manifestations of hypersensitivity or intradermal test (IDT) results. The purpose of this study was to determine whether the level of agreement between insect-specific IgE values (as measured by a commercial polyclonal antihorse IgE-based ELISA) and manifestation of disease or IDT was improved by shifting the positive/negative threshold from 200 (positive) to 150 (borderline) or by the utilization of insect type-specific cut-off points. The study included a population of 37 healthy horses and 40 Icelandic horses suffering from insect hypersensitivity. The study was conducted during the fall when affected horses showed acute clinical signs. IDT was performed as standard procedure using extracts of black ant, mosquito, horsefly, deerfly, housefly, and *Culicoides variipennis*. Insect-specific IgE levels in horse sera were measured by an equine ELISA. Sensitivity and specificity was 98 and 3% (SAT versus insect hypersensitivity) and 97 and 4% (SAT versus IDT), respectively. A shift of the threshold value from 200 to 150 increased sensitivity, but did not improve the level of agreement between IDT and SAT ($\kappa = 0.04$, slight agreement). Circulating allergen-specific IgE levels were not significantly different for the incidence and size of positive skin reactions or the onset and severity of insect hypersensitivity for any insect tested. Therefore, the utilisation of insect type-specific cut-off points did not increase the level of agreement between SAT and IDT or clinical data.

Funding: Self-funded.

Session 10: ALLERGY AND THERAPY

FC-54

Evaluation of IgE-mediated late-phase reactions in the skin of normal placebo- and prednisolone-treated dogs: cellular, cytokine and chemokine responses

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IgE-mediated late-phase reactions can be induced in the skin of normal and atopic dogs by intradermal injections of anti-IgE antibody. The histology of these reactions is very similar to that of naturally occurring atopic dermatitis. To characterize the cellular, cytokine and chemokine responses in the skin of placebo- and prednisolone-treated dogs, normal beagles received either placebo or 0.5 mg/kg prednisolone twice daily for three days prior to intradermal injection of polyclonal rabbit anti-canine IgE. Eight-millimetre punch biopsy skin samples were taken before injection and at the injection sites after 6, 24 and 48 h. Histological and immunohistochemical examination revealed a rapid cellular influx. Eosinophil and neutrophil numbers increased from <1 to 61.4 ± 14.1, and from 7 to 62.2 ± 10.8 cells/mm^2, respectively, within 6 h after injection, and remained moderately elevated 48 h later. The numbers of CD1c+, CD3+ and CD4+ mononuclear cells were also increased by 6 h. Taqman analysis demonstrated 2.5- to 72-fold increases in mRNA expression for IL-13, IL-5, MCP (CCL2), RANTES (CCL5) and TARC (CCL17). Levels of mRNA for IL-2, IL-4, IL-6, and IFNγ remained negligible. Prednisolone administration suppressed the influx of neutrophils and eosinophils, and the expression of IL-13, CCL2, CCL5 and CCL17 (33, 97, 58, 86, 73 and 90%, respectively), as well as the influx of CD1c+ and CD3+ cells. These data document the cytokine and chemokine response to anti-IgE injection and demonstrate the anti-inflammatory effect of prednisolone.

Funding: Schering-Plough Animal Health.

FC-55

Efficacy of low-dose immunotherapy in the treatment of canine atopic dermatitis: a prospective, double-blinded study

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There are anecdotal reports of increased effectiveness of allergen-specific immunotherapy (ASIT) with doses of vaccine lower than that recommended by the manufacturers. However, no controlled studies have been carried out. The aim of this prospective, double-blinded study was to evaluate whether induction and maintenance with low-dose (LD) ASIT resulted in a different success rate compared with the standard dose (SD). Twenty-seven dogs with confirmed atopic dermatitis were allotted by block randomisation to two groups. One ($n = 13$) received SD ASIT; the other ($n = 14$) received LD ASIT (1/10 of the SD) following the same frequency protocol. Cases were graded at 0, 3, 6 and 9 months for clinical signs using a modified Canine Atopic Dermatitis Extent and Severity Index (mCADESI) and for pruritus using a 0–5 descriptor scale. There were no significant differences between the groups in the pruritus and mCADESI scores ($P > 0.155$) at the end of the study. Changes in pruritus ($P > 0.920$) and mCADESI ($P > 0.296$) scores from the beginning to the end of the study were similar in both groups, with pruritus scores in both groups not changing during the study ($P > 0.052$). However, significant reductions in mCADESI scores were seen in both groups ($P < 0.032$). Six dogs achieved a final pruritus score of 0, six dogs achieved a reduction in pruritus score, and 15 did not improve or worsened. There was, therefore, no evidence that LD ASIT was more effective than the standard protocol.

Funding: Petsavers, British Small Animal Veterinary Association, Wellcome Trust.

FC-56

Nodular and non-nodular focal alopecia related to drug injections: a retrospective study of 32 dogs

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Cutaneous manifestations of idiosyncratic adverse drug reactions are considered uncommon. These manifestations are the result of unexpected drug effects, independent of dose, and are not related to the known mechanism of action of the drug. The pathogenesis is not well understood and may occur through immunologic or nonimmunologic mechanisms. Several clinical cutaneous manifestations of drug reactions are described: anaphylaxis, immune-complex formation with or without vasculitis, allergic contact dermatitis, erythema multiforme and injection-site reactions. Thirty-two dogs were recorded with focal nodular and non-nodular alopecia. Skin biopsies were taken from 31 cases for histopathological examination. Cytology was done in the remaining case. Based on clinical information and dermatopathological findings, they were divided into five groups. Group 1 included 17 toys dogs (10 French poodles) with non-nodular circular alopecia associated with rabies vaccination. Group 2 dogs showed a nodular focal panniculitis and included five dogs (three French poodles); three had received a multivalent vaccine and the other two a rabies vaccine. Group 3 dogs included three females (two Maltese terriers) with circular alopecia related to an injectable progestin. Group 4 included three dogs (a Pekinese, a Shih Tzu and a French poodle) that had received glucocorticoid injections. Group 5 was a miscellaneous group of four dogs; two dogs had received injectable ivermectin, another ketoconazole, and there was no clear indication that the fourth dog had received a drug injection.

Funding: Self-funded.

FC-57

Interspecies differences in the percutaneous permeation of substances with various lipophilicities

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In veterinary medicine, the percutaneous permeation of drugs has relevance to therapeutic and forensic problems. It is commonly investigated in Franz diffusion cells using the excised skin of different mammalian species. Drug permeation characteristics through skin are considered to be significantly influenced both by the biochemical constitution of the stratum corneum and by the number and depth of hair follicles. Drug absorbance characteristics thus show a marked interspecies variance. This complicates the comparison of results obtained in different studies using different species. We examined the species influence on permeation parameters of three test compounds with different lipophilicities (benzoic acid, caffeine and testosterone) in Franz diffusion cells under standardized conditions. While testosterone was found to have the highest permeation coefficient, the permeation coefficients of benzoic acid and caffeine were lower and similar in all species. As expected, the absolute permeabilities showed marked interspecies differences, but the order of permeabilities of the test compounds was conserved in all species. The intraindividual coefficient of variation showed no significant difference between the examined species; the interindividual variation was significantly less marked in bovine skin than in other species. We conclude that the skin of different species can be used for the examination of skin permeation. However, results are transferable to other species only if they were obtained under standardized test conditions.

Funding: German Federal Ministry of Education and Research.

FC-58

A new method for measuring canine transepidermal water loss

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Many studies have focused on the relationship between barrier damage and human atopic dermatitis. Currently, evaluating human skin barrier function depends on measuring transepidermal water loss (TEWL) by Evaporimeter. However, applying this method clinically to dogs is impractical due to the influences of air turbulence and vapour from the hair coat. To minimize these influences, we developed and evaluated a new method for measuring TEWL using a closed chamber system. Dry air was injected into a closed chamber placed on the dog's skin surface, and the vapour content of recovered air was detected with a quartz crystal sensor as a frequency variance. At first, a frequency decrease attributable to hair coat vapour was observed, and then the frequency gradually increased and became constant. The steady state value was used to calculate TEWL. It was unnecessary to restrict a dog's movement to control independent environmental effects. Transepidermal water loss was measured in 18 beagles with normal skin at seven different anatomical sites. Results confirmed it was possible to measure TEWL without clipping hair at the inguinal site.

Funding: Kao Corporation.

Session 11: WOUND HEALING

FC-59

Mast cell morphometry of cutaneous wounds treated with an autacoid gel: a placebo-controlled study

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Mast cells (MC) are pivotal in wound healing. Adelmidrol, a synthetic autacoid, can down-regulate MC degranulation and potentially accelerate healing and improve cosmetic outcome. This study evaluated an Adelmidrol gel on MC in experimental wounds. Two rows of six 5-mm wounds were created in 10 beagles and allowed to heal; one row was treated daily with therapeutic gel and the other with vehicle only. Treated and control 8-mm samples of wounds were taken after 1, 2, 4, 8 and 14 days, fixed in formalin and paraffin embedded. Four-micrometer sections were stained with toluidine blue for MC counts and densitometry. Counts (MC/mm^2) were obtained from areas lateral to and within the ulcer/granulation tissue, and in deep dermis/panniculus. A decreasing trend in MC counts occurred throughout days 1 (3.8 ± 11.3), 2 (4.3 ± 14.9) and 4 (4.3 ± 9.9) compared to day 0 (5.0 ± 10.6), followed by an increase at day 8 (6.0 ± 12.3) and a significant increase at day 14 (10.0 ± 18) compared to days 1, 2 and 4 in granulation tissue and perifollicular areas ($P < 0.05$) and deep dermis/panniculus ($P < 0.01$). No difference in MC counts was detected between treated and control wounds. Preliminary data from two dogs showed granulation density was higher in treated wounds from day 2 (119.1 ± 27.4 vs. 69.4 ± 14.2) until day 14 (92.4 ± 22.1 vs. 67.9 ± 12.9). In conclusion, MC populations seem to change during wound healing. The autacoid gel did not alter MC numbers, but decreased degranulation.

Funding: Royal Veterinary College, Innovet.

FC-60

The role of a fresh oleaster leaf preparation in healing of experimental wounds in calves: a histopathologic study

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Oleaster (*Elaeagnus angustifolia*) is a small tree that grows throughout Iran. Using smashed tree leaves combined with olive oil is proposed in one ancient Iranian text as a good remedy for wound healing. Our objective was to evaluate this remedy experimentally. The study was carried out in two female 10-month-old Holstein calves. An area of 10 × 10 cm on each side of the thorax was surgically prepared and infiltrated with local anaesthetic. Eight uniform skin wounds were created in two rows with an 8-mm biopsy punch on each prepared area. Each side of an animal was used to evaluate one treatment (Groups 1–4). To make the mixture, fresh oleaster leaves (50 gm) were smashed with a pestle and mixed with olive oil (25 gm). The wounds were treated for 7 days. The wounds of Groups 1–3 were rinsed daily with normal saline, after which the mixture of oleaster and olive oil (Group 1) or only the olive oil (Group 2) was applied. In Group 4, no treatment was applied. All wounds were left open. On day 8, skin biopsies were taken from the wounds for histopathologic study. Group 1 wounds all showed mild granulation tissue, scab formation and complete re-epithelialization. In Group 2, there was mild granulation tissue, massive scab formation and minimal re-epithelialization. In Groups 3 and 4, mild granulation tissue, severe scab formation and very limited re-epithelialization were observed. We conclude that a fresh oleaster leaf preparation can be an effective remedy for wound healing.
Funding: Self-funded.

FC-61

The role of a liquorice preparation in healing of experimental wounds in calves: a histopathologic study

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Liquorice, a derivative of the root of *Glycyrrhizia glabra,* has been proposed as a treatment for gastric ulcers. Here, a preparation of liquorice combined with sesame oil was evaluated in experimental wound healing. The study was carried out in two female 10-month-old Holstein calves. An area of 10 × 10 cm on each side of the thorax of the animals was surgically prepared and infiltrated with local anaesthetic. Eight uniform skin wounds were created in two rows with an 8-mm biopsy punch on each prepared area. Each side of an animal was used to evaluate one treatment (Groups 1–4). To make the mixture, powdered and sieved liquorice (16 g) was mixed with sesame oil (36 g) to make a suspension. The wounds were treated for 7 days. The wounds of Groups 1–3 were rinsed daily with normal saline, after which the mixture of liquorice and sesame oil (Group 1) or only the sesame oil (Group 2) was applied to the wounds. In Group 4, no treatment was applied. All wounds were left open. On day 8, skin biopsies were taken from the wounds for histopathologic study. In Group 1, seven wounds (87%) showed moderate granulation tissue, mild scab formation, and complete re-epithelialization. In Group 2, there was massive scab formation with no granulation tissue and minimal re-epithelialization. In Groups 3 and 4, mild granulation tissue, severe scab formation and very limited re-epithelialization were observed. We conclude that a preparation of liquorice and sesame oil can be an effective remedy for wound healing.
Funding: Self-funded.

FC-62

Pale sulphonated shale oil exhibits antimicrobial and tissue repairing effects in wound healing

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A proliferation-promoting effect on keratinocytes as well as increased generation of the wound-healing factor PDGF$\alpha\beta$ could be observed *in vitro* in the presence of diluted aqueous solutions of pale sulphonated shale oil. Concomitantly, pale sulphonated shale oil exhibits antimicrobial properties against all infectious agents that are relevant in wound infection and delayed healing, including methicillin-resistant *Staphylococcus aureus*. Since most antiseptic substances and local antibiotics cause delayed wound healing, pale sulphonated shale oil has special significance in this field. For this reason, a project for development and approval of a medicinal veterinary product for wound healing in horses according to European Community procedures has been started. No approved product for horses is currently available in Europe. In a dose-finding study, a hydrogel formulation containing 20% pale sulphonated shale oil as the active pharmaceutical ingredient was found to be most efficient in wound healing. In a study on target-species tolerance in horses, this formulation proved to be well tolerated in long-term topical application. Case studies from the clinical efficacy trial show a broad profile of action of pale sulfonated shale oil.
Funding: ICHTHYOL-Gesellschaft.