Microneedling as a successful treatment of alopecia X in two Pomeranians

S. STOLL*, C. DIETLIN† and C. S. NETT-METTLER‡
*Stolldesign, Muotathal, Switzerland
†Hautarzt Zentrum, Liestal, Switzerland
‡Tierärztliche Spezialistenklinik Hünenberg, Switzerland

Alopecia X is a well-recognized syndrome of hair cycle arrest, of unknown cause, typically occurring in Pomeranians and other plush-coated dogs. It is characterized by symmetrical, noninflammatory alopecia without concurrent systemic signs. Currently recommended treatments show variable and often only partial clinical success. In humans, microneedling has been used in cosmetics and various medical treatments to induce the self-healing potential of the skin. It was hypothesized that superficial mechanical skin trauma with a microneedling device would induce generalized and permanent hair regrowth in alopecia X. Two four-year-old female spayed Pomeranian siblings were selected with clinical and histological changes compatible with alopecia X and in which neither melatonin, deslorelin nor topical minoxidil resulted in substantial or permanent hair regrowth. Under general anesthesia the sterile microneedling device (Dermaroller MC925; Dermaroller GmbH, Wolfenbüttel, Germany) was rolled over alopecic areas with moderate pressure vertically, horizontally and diagonally until microbleeding was achieved. Five weeks after microneedling, hair re-growth was observed in both dogs followed by reduction of hyperpigmentation of the affected skin. After 12 weeks 90% of the coat had regrown and skin hyperpigmentation had started to subside. No adverse effects were observed. Coat quality had not deteriorated at the 12 months follow-up. This is the first report of successfully using microneedling to induce almost 100% hair regrowth in two dogs diagnosed with alopecia X. Microneedling may provide an alternative to conventional therapy for alopecia X and possibly other hair cycle arrest disorders. A larger case series is on the way to further elucidate the long-term success of microneedling.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

© 2015 ESVD and ACVD, Veterinary Dermatology, 26, 297–313.

Use of oclacitinib for the treatment of acral lick dermatitis in a dog: a case report

A. VERCELLI* and L. CORNEGLIANI*
*Ambulatorio Veterinario Associato, Torino, Italy

A seven-year-old male mixed breed dog was referred for evaluation and treatment of acral lick dermatitis that had not responded to oral glucocorticoids and anti-depressive drugs. Firocoxib at 5 mg/kg daily for 6 months led to a 70% clinical improvement of skin lesions. After 9 months of firocoxib administration, the dog exhibited persistent vomiting despite concomitant anti-acid therapy and this drug was then withdrawn. Clinical examination revealed bilateral alopecic, erythematous, hyperpigmented and crusted lesions of 5-cm diameter on the dorsal carpus. Complementary tests were negative for parasitic infections; radiological and neurological examinations failed to reveal any associated problem. A complete blood count and biochemical profile were unremarkable. Cytology revealed secondary bacterial infection, which was treated with enrofloxacin at 5 mg/kg/daily for 4 weeks. Oclacitinib was then started at 0.4 mg/kg twice daily for two weeks and then once daily because of its activity as a pruritogenic cytokine inhibitor. After seventy days of therapy, the skin lesions’ diameters were reduced by 50%. Finally, skin lesions completely regressed after another 4 months of oclacitinib administration. Treatment was continued for 1 year. Complete blood count and serology were performed every 6 months during oclacitinib administration and parameters remained within the reference range. Oclacitinib might represent a safe alternative systemic treatment for pruritus in cases of acral lick dermatitis not responsive to other therapies or when adverse effects of treatment are unacceptable.

Sources of funding: Self-funded.

Conflicts of interest: None declared.
Characterization of the pro-inflammatory and pruritogenic transcriptome in experimental canine acute atopic skin lesions

T. OLIVRY*, †, J. S. PAPS*, K. E. LINDER†, ‡, C. PEREDO§, D. MAYHEW¶, L. YOON**, D. RAJPAL¶ and J. COTE-SIERRA§
*Department of Clinical Sciences, and College of Veterinary Medicine, Raleigh, NC, USA
†Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC, USA
‡Department of Population Health and Pathobiology, College of Veterinary Medicine, Raleigh, NC, USA
§Stiefel, GlaxoSmithKline, Research Triangle Park, NC, USA
¶Computational Biology, Target Sciences, GlaxoSmithKline, King of Prussia, PA, USA
**Safety Assessment, PTS, GlaxoSmithKline, Research Triangle Park, NC, USA

Determining the activated inflammation and pruritus pathways in skin lesions from patients with atopic dermatitis (AD) is compromised by the inability to precisely assess the age of lesions, thereby affecting the analysis of mediators that are time-dependent. The objectives of this study were to characterize the inflammatory transcriptome of experimental acute canine AD lesions. Biopsies were collected 6, 24 and 48 h after epicutaneous application of Dermatophagoides farinae house dust mites to eight mite-sensitized Maltese beagle atopic dogs. We extracted mRNA from skin biopsies and the transcriptome was assessed using a dog-specific microarray. Gene expression of mite-challenged biopsies was compared to that of unchallenged specimens (1.5-fold change, 0.05 false discovery rate-corrected P-values). Acute canine atopic lesions had a significant upregulation of genes encoding pro-inflammatory (e.g. IL6, TNF), T-helper-(Th)2 (e.g. IL4, IL5, IL13, IL31, IL33 and others), Th9 (IL9) and Th22 (IL22) cytokines, as well as Th2 chemokines (e.g. CCL5, CCL17 and others). Surprisingly the expression of thymic stromal lymphopoietin (TSLP) was downregulated. There was also significant upregulation of genes encoding other known pruritogenic proteins and pathways, such as cathepsin S (CTSS), neutromedin-B (NMB), nerve growth factor (NGF), leukotriene-synthesizing enzymes (ALOX5, ALOX5AP, LTA4H), and the mast cell proteases chymase (CMA), trypstatine and mastin. In conclusion, acute canine atopic skin lesions exhibit an upregulation of immunological and pruritogenic pathways that are very similar to those seen in human atopic skin. The successful treatment of AD lesions is likely to require inhibiting multiple rather than single pruritogenic and pro-inflammatory pathways.

Sources of funding: Stiefel, GlaxoSmithKline, Research Triangle Park, NC, USA

Preliminary assessment of percutaneous (prick) test in dogs

C. LORENTE* and P. RUIZ*
*Centro Dermatologia Veterinaria ADERVET, Madrid, Spain

Skin prick testing (SPT) is the standard method used to assess IgE hypersensitivity to allergens in atopic humans. The objective of this study was to assess the potential use of SPT in atopic dogs to identify allergen-specific hypersensitivity. SPT was performed without sedation on the inner thigh of 11 healthy dogs and 34 dogs with atopic dermatitis using prick lancets (Entaco; Worcestershire, UK). Glycerinated histamine (5 mg/mL) and saline solution (Diavet; Madrid) were used as positive and negative controls, respectively. Healthy dogs were then tested percutaneously with Dermatophagoides farinae, D. pteronyssinus, Tyrophagus putrescentiae, Acarus siro, Cuspressus arizonicus, Cynodon dactylon, Phleum pratense and Olea europaea at concentrations of 0.01 mg/mL, 0.1 mg/mL, 1 mg/mL and 10 mg/mL (Diavet, Spain). None of the healthy dogs developed any reaction to any allergen. The allergen concentration of 10 mg/mL was selected for testing the 34 atopic dogs; 19 (56%) developed a wheal to at least one of the allergens tested within 7 minutes of SPT. Allergen-specific serum IgE was assessed in 25 of the 34 atopic dogs; of these, 3 dogs were negative on SPT and serology, 16 were positive on SPT and serology, and 6 were SPT negative and positive on serology. SPT was found to be an easy and rapid technique that could be used in outpatients. The positive reactions obtained in more than half of dogs with atopic dermatitis, but in none of the healthy dogs, suggests that SPT should be investigated further as a means to identify allergens for specific immunotherapy formulations.

Sources of funding: Diavet, Spain.

Conflicts of interest: None declared.

Stem cell-associated marker expression in canine hair follicles

N. M. GERHARDS*, B. SAYAR†, F. C. ORIGGI†, A. GALICHET†, E. J. MÜLLER†, M. WELLE†, 1 and D. J. WIENER†
*Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
†Institute of Animal Pathology, Vetsuisse Faculty, Dermfocus, University of Bern, Bern, Switzerland
†Center for Fish and Wildlife Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Functional hair follicle (HF) stem cells (SCs) are crucial to maintain the constant recurring growth of hair. In mice and humans, SC subpopulations with different biomarker expression profiles have been identified in discrete anatomic compartments of the HF. The rare studies investigating canine HF SCs have shown similarities to biomarker expression profiles of mouse and human SCs. The aim of our study was the assessment of the mRNA and protein expression levels of SC-associated markers in...
dogs. In a series of experiments we demonstrated: (i) the mRNA expression of the presumptive SC markers CD34, Sox9, keratin 15, LGR5, nestin, LGR6 and CD200; (ii) the presence of CD34, Sox9, keratin 15, Nestin and LGR6 proteins in whole skin lysates by Western blot using validated antibodies; and (iii) by immunohistochemistry the location of CD34 [lower two-thirds of the isthmic outer root sheath (ORS) and entire ORS in anagen and telogen, respectively], Sox9 (innermost layer of the ORS in anagen), keratin 15 (entire ORS in the lower half of the isthmus in telogen and basal cell layer of the ORS in the upper isthmus in anagen), LGR5 (secondary germ in telogen) and nestin (dermal papilla and connective tissue sheath in anagen) in the canine HF.

Our results provide the basis for future functional studies investigating the canine HF SC compartment to assess possible SC-associated alterations in different diseases, such as alopecia or skin cancer, and to provide new insights into the molecular pathways involved in human follicular SC activation and regulation.

Sources of funding: European Society of Veterinary Dermatology and Spezialisierungskommission of the Vetsuisse Faculty, University of Bern.

Conflicts of interest: None declared.

Effects of sphingolipid extracts and glycosaminoglycans on morphological structure and lipid content in an in vitro canine skin model

S. CERRATO*, L. RAMIÓ-LLUCH*, P. BRAZÍS*, D. FONDEVILA†, S. SEGARRA‡ and A. PUIGDEMONT§

*UNIVET, Barcelona, Spain
†Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona, Barcelona, Spain
‡R&D Veterinary Division, Bioibérica S.A., Barcelona, Spain
§Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Barcelona, Spain

Ceramides are essential stratum corneum sphingolipids that play a key role in maintaining cutaneous barrier function. Skin barrier defects in both canine and human atopic dermatitis have been associated with decreased ceramide levels (especially CER[NP], CER[EOS] and CER[EOP]) and morphological stratum corneum alterations. The aim of this study was to investigate changes induced by sphingolipid extracts and glycosaminoglycan treatments on morphological structure and lipid composition in a canine skin model. The canine skin model was developed by seeding keratinocytes onto fibroblast-embedded collagen type I matrix at an air–liquid interface. Skin equivalents were cultured and supplemented with two different sphingolipid extracts (#1, with higher sphingomyelin content; and #2, with higher levels of ceramides), glycosaminoglycans or vehicle for 14 days. Quantitative lipid assays were performed using ultra-performance liquid chromatography/mass spectrometry. Differences between groups were analysed by Student’s t-test for paired data. The ultrastructural morphology of the skin equivalents was examined by transmission electron microscopy. Sphingolipid extract #1 induced significant increases in total ceramides, CER[NS], CER[NP], CER[NH], CER[AS], CER[AP], CER[EOS] and CER[EOP]. Ultrastructural analysis revealed an increase of lipid lamellar-related structures in the stratum corneum. No significant changes were observed in any other study group. In conclusion, sphingolipid extract #1, despite lower ceramide levels, stimulated *de novo* endogenous production of ceramides (particularly the subclasses reduced in atopic dermatitis). Thus, it may contribute to the formation of a well-organized stratum corneum and is a potential therapeutic tool for improving skin barrier function in canine atopic dermatitis.

Sources of funding: This work was supported in part by Bioibérica S.A.

Conflicts of interest: S.S. is an employee of Bioibérica S.A.

Evaluation of *Staphylococcus pseudointermedius* and incidence of biofilm production in healthy dogs

C. OUSCHAN*, J. CSOKAI* and E. MUELLER*

*LABOKLIN, Labor für Klinische Diagnostik GMBH & CO. KG, Bad Kissingen, Germany

We evaluated the frequency of *Staphylococcus* (*S.*) *pseudintermedius* on healthy dog skin and investigated whether the bacteria is able to produce a biofilm. Biofilm production by *S. pseudintermedius* has been documented in human and veterinary medicine, but its frequency and role in canine skin diseases is poorly understood. Swab samples were taken from the ventral abdomen (*n* = 36) and the paws (interdigital space; *n* = 36) of different clinically normal dogs with no history of skin problems. The bacteria were cultured and *S. pseudintermedius* was isolated. Congo red agar plates consisting of brain–heart infusion broth (37 g/L), sucrose (50 g/L), agar (10 g/L) and Congo red (0.8 g/L) were used to determine bacteria with the ability to form biofilm. This method is easy and fast to perform but less sensitive than other tests. Positive and negative controls as well as streak-and-dot inoculation were used for easier interpretation of the colour. In the abdomen group, 19.4% (7 of 36) were positive for *S. pseudintermedius*, in the paw group 36% (13 of 36). In contrast to the literature we found generally higher counts of staphylococci on the paws than on the abdomen. Interestingly 100% from the abdomen group (7 of 7) and 85% from the paw group (11 of 13) were able to produce biofilm. The study shows that *S. pseudintermedius* is not that common on dog skin, but frequently able to form biofilms. We present a rapid and inexpensive method for the detection of biofilm producers.

Sources of funding: Laboklin.

Conflicts of interest: The authors are employees of Laboklin.
Restructuring effect of phytosphingosine-containing shampoo and mousse on the cutaneous barrier in five atopic dogs: preliminary results of a field study

O. FANTINI*, C. ZEMIRLINET, M. BELLARD†, E. LATI§, L. PENO-MAZZARINO§, E. GONTIER¶, E. OLLIVIER† and D. PIN*

*Dermatology Unit, VetAgro Sup Lyon Campus, Marcy L’Étoile, France
†Ceva Santé Animale, Libourne, France
§Laboratoire BIO-EC, Longjumeau, France
¶Bordeaux Imaging Center, Electronic Imaging Department, University of Bordeaux, Bordeaux, France

Previous studies have shown alterations in the skin lipid organization and composition in atopic dogs. The aim of this study was to evaluate the effect of a phytosphingosine-containing shampoo and mousse (Douxo® Calm, Ceva Santé Animale) on the defective skin lipid barrier in such dogs. Five dogs from different breeds clinically diagnosed with atopic dermatitis according to Favrot’s criteria, with a maximum Canine Atopic Dermatitis Extent and Severity Index (CADESI)-04 score of 40 on Day 0 (D0) and stabilization of skin condition for at least 3 months, were included after rigorous flea control. Dogs were shampooed on D0, D8 and D15 and treated with the mousse on D3, D6, D10, D13, D17 and D20. Measurement of the skin hydration rate by a corneometer (Corneometer© CM825, Courage & Khazaka; Cologne, Germany), tape-stripping for chemical analysis and skin biopsies all from the lateral aspect of the thorax for structural analysis of the stratum corneum (SC) lipids by electron microscopy were performed on D0 and D21. Skin hydration rate [11.2 (± 5.6) to 39.4 (± 41.7)], total cholesterol (cholesterol and cholesterol esters) [1737 (± 1010) to 3957 (± 2074) µg/g protein], as well as total ceramides (especially hydroxylated ceramides) [52 (± 15) to 75 (± 30) µg/g protein] increased (no significant differences). Blind analysis of electron microscopy images revealed a slight to marked increase in SC lipid bilayer thickness together with improved ultrastructural arrangement. The results indicate the potential effect of this combination treatment with phytosphingosine-containing shampoo and mousse on the barrier function of the epidermis in canine atopic dermatitis

Sources of funding: Ceva Santé Animale.

Conflicts of interest: Douxo® Calm is sold by Ceva Santé Animale. C.Z., M.B. and E.O. are employees of Ceva.

Assessment of the influence of weekly shampooing of dogs on acaricidal efficacy of a dinotefuran-permethrin-pyriproxyfen topical ectoparasiticide

M. VARLOUD*, E. HODGKINS† and R. KOCHANOWSKI‡

* Ceva Santé Animale, Libourne, France
† Ceva Animal Health Polska, Warszawa, Poland
‡ Ceva Animal Health, Lenexa, KS, USA

This study was conducted to assess the influence of weekly shampoo on the persistency of acaricidal efficacy of a topical ectoparasiticide combining dinotefuran, permethrin and pyriproxyfen (DPP, Vectra® 3D) on dogs. Forty mixed-breed dogs (11.0–20.8 kg body weight; 10–55 mm hair length) were allocated to five groups of eight dogs: a control group and four DPP treated groups shampooed for approximately 10 min with 180 mL of either a low foam shampoo with Yucca extract, a soothing shampoo containing chitosanide and colloidal oatmeal, an antimicrobial shampoo with benzoyl peroxide, sulphur and salicylic acid, or an antiseptic/anti-inflammatory shampoo containing chlorhexidine gluconate and fatty acids. Dogs in the treated groups were administered 3.6 mL of DPP on Day 0. Shampoos were performed on days 6, 13, 20 and 27. Dogs were infested with 100 unfed adult ticks (Rhipicephalus sanguineus) on days –2, 7, 14, 21 and 28. Viability, attachment and engorgement status of ticks were assessed by combing dogs 48 h after treatment or infestation. Arithmetic and geometric means of live or engorged ticks were calculated. Comparisons between treatments were performed by ANOVA. The mean live or engorged tick numbers differed between the treated and control groups on each assessment day (P < 0.05). For each shampoo, the efficacy of DPP remained >87% and >70% for 2 and 3 weeks after administration, respectively. After one month, the antimicrobial shampoo had the least influence on efficacy (>62%). Weekly shampooing of treated dogs reduces the acaricidal efficacy of DPP and may require re-treatment every 2 weeks.

Sources of funding: This study was funded by Ceva.

Conflict of interest: All of the investigators are employees of Ceva.

Antimicrobial activity of pomegranate extract against canine skin pathogens

L. RAMIO*, S. CERRATO*, L. AROSEMINA†, M. A. CALVO†, P. BRAZÍS* and A. PUIGDEMONT†

*UNIVET, Barcelona, Spain
†Department of Animal Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain
‡Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Barcelona, Spain

The prevalence of antibiotic resistance has increased interest in the antimicrobial activity of natural compounds. Punicalagins, the active bioflavonoids present in pome-
granate extract, have been shown to exert antimicrobial, anti-oxidant and anti-inflammatory effects. The aim of this study was to investigate the antimicrobial properties of pomegranate extract against the common canine skin pathogens (*Malassezia spp.* and *Staphylococcus spp.*) by determining the minimum inhibitory concentration (MIC). *Malassezia* and meticillin-resistant and -sensitive *Staphylococcus* isolates were obtained from dogs with yeast overgrowth or superficial pyoderma. Serial dilutions of pomegranate extract in tryptone soy broth medium ranging from 50 to 0.1 mg/mL were incubated with 0.5x10⁵ CFU/mL of each isolate for 30 h at 37°C. The MIC for each isolate was defined as the lowest concentration of the pomegranate extract that inhibited microbial growth. Differences between the mean MICs were analyzed by ANOVA and Tukey’s multiple comparison post hoc tests. The MIC values of the pomegranate extract were 3.2 ± 1.8 mg/mL for meticillin-sensitive *Staphylococcus* strains and 4.8 ± 1.6 mg/mL for meticillin-resistant *Staphylococcus*, and there was no significant difference between the two *Staphylococcus* groups. Inhibition of *Malassezia* was obtained at a MIC of 12.5 ± 0.0 mg/mL. These results suggest that pomegranate extract may represent a new nonantibiotic antimicrobial strategy for managing dogs with skin infections.

**Sources of funding:** Self-funded.

**Conflicts of interest:** None declared.

**Overexpression of FoxP3-expressing CD4⁺CD25⁺ regulatory T cells in peripheral blood from patients with canine atopic dermatitis and correlation with disease severity**

C. FAVROT*, V. HAUCK*, P. HÜGLI*, N. FISCHER*, A. ROSTAHER* and M. MELI*

*Vetsuisse Faculty, University of Zürich, Switzerland*

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases in humans and dogs. Human and canine AD share numerous similarities. Regulatory T cells (Tregs) are essential controllers of the immune homeostasis and have been shown to play a key role in human AD. However, the reports of the frequencies of Tregs in human atopic patients are conflicting. At this time only two studies have assessed Tregs numbers in atopic dogs. The aim of this work was to explore the role of Tregs in the pathogenesis of canine AD by assessing the number of circulating Tregs in healthy and atopic dogs and to determine whether this frequency correlates with patient age, gender, disease severity or pre-treatment. Thirty-five atopic and fourteen healthy dogs were involved in the study. Peripheral blood mononuclear cells were stained with monoclonal antibodies (anti-CD4, anti-CD25, anti-FoxP3) and evaluated by flow cytometry. Tregs were phenotypically identified as T cells triple positive for CD4, CD25 and FoxP3. The percentage of circulating CD4⁺CD25⁺FoxP3⁺ Tregs in atopic patients was significantly increased compared to healthy dogs (mean 2.1% versus 1%, *P* = 0.002) and correlated with disease severity [Hill’s Pruritus Scale: 48%, *P* = 0.003; Canine Atopic Dermatitis Extent and Severity Index (CADESI)-04 (clinical score): 34%, *P* = 0.044]. Our data suggest that as in humans, CD4⁺CD25⁺FoxP3⁺ Tregs may play an important role in the pathogenesis of canine AD. Additionally, there is an association between Treg frequency and disease severity. Further investigation is required to improve our understanding of the role of Tregs in atopic dogs.

**Sources of funding:** Self-funded.

**Conflicts of interest:** None declared.

**Evaluation of lactoferricin in vitro bactericidal activity on strains selected from dogs with pyoderma**

A. CORONA*, A. VERCELLI*, N. BRUNI† and L. CORNEGGLIANI*

*Ambulatorio Veterinario Associato, Torino, Italy†Istituto Profilattico e Farmaceutico Candioli & C. S.p.A., Beinasco, Torino, Italy*

Lactoferricin (LC) is a peptide able to inhibit growth and to prevent biofilm formation in some bacteria. The aim of this study was to evaluate the *in vitro* activity of LC against *Staphylococcus intermedius* group (SIG), *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* strains isolated from dogs with pyoderma. The minimum inhibitory concentrations were evaluated using a microtitre plate dilution method. The bacterial inoculums in log-phase growth were prepared in brain heart infusion broth (BHI) with a turbidity of 0.5 McFarland, corresponding to 10⁵ to 10⁶ cells/mL. The LC solution was diluted in alcohol (prior studies showed that the alcohol exerted no antimicrobial activity) to achieve final concentrations of 7.3%, 5.5%, 3.7% and 2.2% for each bacterial isolate. Appropriate negative and positive controls were included. The plates incubated at 37°C for 24 h, after which 10 μL aliquots from each well were incubated on blood agar at 37°C for a further 24 h. Inhibition was defined as the lowest concentration of LC that prevented growth in the microtitre wells and blood agar. LC showed bactericidal activity against all the bacterial isolates at 7.3% and 5.5% concentrations, but bacterial growth was evident at 3.7% and 2.2%. These results show that LC exhibits *in vitro* bactericidal activity against Gram-positive and Gram-negative bacteria associated with canine pyoderma. Lactoferricin is a potential novel topical antibacterial treatment that could be used instead of conventional antiseptics and/or antibiotics to treat skin infections. However, *in vivo* studies are needed to confirm these results.

**Sources of funding:** Self-funded.

**Conflicts of interest:** One of the authors is employed by the producer of the active ingredient.
Cost evaluation of home-cooked and extensively hydrolysed diets during an elimination trial: a randomized prospective study

M. C. CADIERGUES*, A. MULLER†, E. BENSIGNOR‡, D. HÉRIPRET§, L. YAGUIYAN-COLLIARD¶ and I. MOUGEOT**

*INP-ENVT, Toulouse, France
†Clinique vétérinaire Saint Bernard, Lomme, France
‡Clinique vétérinaire de la Boulais, Cesson-Sévigné, France
§Centre Hospitalier Vétérinaire Pommery, Reims, France
¶Unité de Nutrition, ENVA, Maisons-Alfort, France
**Royal Canin SAS, Aimargues, France

Customized recipes are designed and recommended by veterinarians as elimination diets to diagnose adverse food reaction (AFR) in dogs. These home-cooked diets (HCD) are sometimes perceived to be cheaper than commercially-available hydrolysed protein-based diets. The objective of this French prospective multicentre study was to compare the daily cost of elimination diets performed using either HCD or an extensively-hydrolysed diet (EHD). Sixty-two dogs were randomized to be fed either a balanced nutritionist-designed HCD (28 dogs) or a low molecular weight poultry feather EHD (Anallergenic, Royal Canin; Aimargues, France; 34 dogs). Investigators reported all feeding expenses paid by the owners (grocery store bills for the HCD group; commercial diet prices for the EHD group). At the time of inclusion, there were no significant differences between groups in either age of the dogs or their body weight. Similarly, there was no significant difference between groups regarding the duration of the elimination diets. The daily cost of the HCD, standardized for a 20-kg dog, was significantly higher (mean: 3.60 €/day, range: 1.14 to 8.50 €/day) than that of the EHD (mean: 2.60 €/day, range: 1.75 to 3.63 €/day) (Student’s t-test; P = 0.004). In conclusion, in France HCD are more expensive than feeding an EHD as an elimination diet. As a result, the low molecular weight Anallergenic EHD might offer a cost-saving choice over HCD when screening dogs for suspected AFR.

Sources of funding: Royal Canin, Aimargues, France covered all the costs for the cooked/hydrolysed diets.

Conflicts of interest: I.M. is an employee of Royal Canin.

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Control of fly strike dermatitis in dogs with a topically applied combination of imidacloprid and permethrin: a prospective open-label controlled clinical trial

E. CASTILLA-CASTANO*, C. MANDIN-CABARET†,‡, C. PRESSANTI* and M. C. CADIERGUES*

*Dermatology Unit, Toulouse Veterinary School, Toulouse, France
†Preventive medicine Unit, Toulouse Veterinary School, Toulouse, France
‡Rescue Center of the Society for the Protection of Animals of Toulouse, Toulouse, France

The aim of this study was to investigate the efficacy of a combination of imidacloprid (10%) and permethrin (50%) (ADVANTIX®, Bayer HC AH; Lyon, France) applied topically as a spot-on, for the treatment of canine fly dermatitis. The study was an open-label controlled study and one-month follow-up. Fifteen dogs, from the same animal sanctuary, with active pinnal lesions of fly dermatitis, received a single application of the solution on the cranium and the base of the ears on Day (D)0. Five dogs, from the same sanctuary and similarly affected, served as nontreated controls. No other therapeutic or hygienic measures were taken. Lesional score was based on extent, alopecia, crusts, scales, erosions/ulcers and lichenification, each assessed on a 0–4 scale. Evaluation was performed on D0, D14 and D30. No adverse event was recorded. In control dogs, lesional scores were maintained on D14 and D30, mean ± SD: D0 7 ± 1.4, D14 6.6 ± 3.4 and D30 8.6 ± 5.4. Lesional scores of the treated dogs were reduced from D0 9.9 ± 2.5 by 59% on D14 to 4.1 ± 2.8 and 80% on D30 (P < 0.05) to 1.9 ± 1.5. The combination imidacloprid–permethrin proved safe and helpful in the management of canine fly dermatitis and confirmed previous results in preventing Stomoxys calcitrans from taking a blood meal on dogs during a period of 29 days after a single application. It could also be suggested as a preventive measure with a monthly application during the fly season.

Source of funding: This study was partly funded by Bayer HC AH, Puteaux, France.

Conflicts of interest: E.C.-C., C.M.-C. and C.P. have no conflict of interest to declare. M.C.C. has previously participated in clinical studies and has given lectures that were funded by Bayer HC AH.
Control of idiopathic nasal hyperkeratosis in dogs with a mixture of essential oils and essential fatty acids (DERMOSCENT BIO BALM®): a randomized, double-blinded, placebo-controlled clinical trial

M. CATARINO*, C. PRESSANTI†, P. MIMOUNI† and M. C. CADIERGUES*

* Dermatology Unit, Toulouse Veterinary School, Toulouse, France
† Center of Canine Reproduction in South-west of France (CRECS), Isle Jourdain, France

The study aim was to investigate the efficacy of a balm containing a mixture of essential oils and essential fatty acids for the treatment of idiopathic nasal hyperkeratosis in dogs.

The study was a placebo-controlled, randomized, double-blinded, clinical trial with parallel group design and two-month follow-up. Dogs with clinically diagnosed idiopathic nasal hyperkeratosis were randomized to receive a daily topical application of Dermoscent Bio Balm (LDCA, France) (BB) or placebo (aqueous gelling agent with preservatives, PB). The main outcome variables were lichenification (score 0 to 4), dryness (0–1), softness (0–2) and extent (0–4). Subjective owner satisfaction index was a secondary variable. Evaluation was performed on days 0, 30 and 60, at least 12 h after application. Response to treatment was assessed as the change from baseline to each examination day for each criterion. Intention-to-treat data were analysed. Forty seven dogs were enrolled of which 39 completed the study. French (26 of 47) and English (7 of 47) bulldogs predominated. No major adverse events occurred during the study period. On Day 60, changes from baseline for lichenification, extension, softness and total score were –31.2%, –18.3%, –72.8% and -36.8% in group BB (23 dogs) and -11.9%, 2.3%, -42.1% and -14% in group PB (16 dogs), respectively. The total score was significantly improved on Day 60 in group BB compared to group PB (Mann-Whitney U-test, \( P = 0.0016 \)). Of 21 owners, 17 were satisfied in group BB, 8 of 15 owners in group PB (\( P = 0.14 \), Fisher’s exact test). Dermoscent Bio Balm proved safe and helpful in the management of canine idiopathic nasal hyperkeratosis.

Source of funding: This study was partly funded by LDCA, Castres, France

Conflicts of interest: M.C.’s veterinary thesis was partly supported by LDCA. C.P. and P.M. have no conflict of interest to declare. M.C.C. is consultant for LDCA.

Mannan-conjugated allergoids of Dermatophagoides farinae increase their uptake by canine dendritic cells

I. SORIA CASTRO*, C. M. DÍEZ RIVERO*, J. LÓPEZ-RELANO*, B. CASES ORTEGA*, A. MAS-FONTAO† and J. ÁLVAREZ†

*R&D Department, Inmunotek SL, Madrid, Spain
† R&D Department, Alergovet SL, Madrid, Spain

Allergoids are glutaraldehyde modified allergenic extracts with decreased ability to react with canine-IgE antibodies. Dendritic cells (DCs) are involved in the uptake and presentation of allergens to T cells. It has been seen that human DCs show a better uptake of Mannan-conjugated allergoids than native allergens and nonconjugated allergoids. This work studied the uptake by monocyte-derived DCs from dogs (canine DCs). Canine CD14+ monocytes were purified from peripheral blood of five dogs using magnetic anti-CD14-beads. This cell population was differentiated into canine-DCs in 6-d cultures with hGM-CSF, dog-IL-4 and hFlt-3L. Assays of allergen uptake were performed with native, allergoid and mannan-conjugated allergoid derived from the same Dermatophagoides farinae (DFA) batch. All three allergen preparations were labelled with Alexa Fluor® 488-M and incubated with canine DCs. Uptake was measured after 30 min at 37˚C by flow cytometry in Mean Fluorescence Intensity units (MFI). Confocal microscopy was employed to visualize allergen uptake. MFI units for each dog were normalized by its corresponding MFI value of native allergen (NorMFI). Student’s t-test was used for statistical evaluation of NorMFI averages. All three DFA preparations were taken up by canine DCs as assessed by flow cytometry and corroborated by confocal microscopy. However, the uptake of mannan-conjugated allergoid by dog DCs was much higher (NorMFI = 170.85) than that seen for the native allergen (NorMFI = 100; \( P = 0.012 \)) or the nonconjugated allergoid (NorMFI = 110.67; \( P = 0.0071 \)). Conjugation of DFA allergoids with mannan increases their uptake by canine DCs supporting its use in novel vaccines for veterinary immunotherapy.

Sources of funding: Self-funded.

Conflicts of interest: I.S-C., C.M.D-R., J.L-R and B.C. are employees of Inmunotek S.L.A. A.M-F. and J.A. are working at Alergovet S.L.

Repeated oral dose tolerance in dogs treated concomitantly with ciclosporin and oclacitinib for three weeks

A. PANTERI*, †, G. STREHLAU†, R. HELBIG† and K. DOUCETTE‡

* Elanco Centre de Recherche Santé Animale, St Aubin, Switzerland
† Elanco Animal Health, Basel, Switzerland
‡ Elanco Animal Health, Greenfield, IN, USA

Ciclosporin (Atopica, Elanco Animal Health; Basel, Switzerland) and oclacitinib (Apoquel, Zoetis; Florham Park, New Jersey) were administered to 15 dogs treated concomitantly with ciclosporin and oclacitinib for three weeks.

Repeatability of DFA allergoids using flow cytometry

OPEZ-RELA*, C. M. DEL RÍO‡, C. M. DÍEZ RIVERO*, J. LÓPEZ-RELANO*, A. MAS-FONTAO† and J. ÁLVAREZ†

*R&D Department, Inmunotek SL, Madrid, Spain
† R&D Department, Alergovet SL, Madrid, Spain
‡ Elanco Centre de Recherche Santé Animale, St Aubin, Switzerland

To assess the uptake by canine dendritic cells of DFA allergoids with different modifications.

DFA allergoids were conjugated with mannan or a mixture of fatty acids (FA). Allergoid modified with mannan (MFI = 840.16) incorporated 1.4 times more allergen than the native allergoid (MFI = 598.86). Mixture of fatty acids increased the allergen uptake (MFI = 1348.90) compared to native allergen (MFI = 100; \( P = 0.0005 \)). Conjugation with mannan increased allergen uptake by canine dendritic cells of DFA allergoids, suggesting a possible application for vaccines.
Cytology scores determined by a validated semiquantitative scoring system for bacteria (cocci, rods), yeasts, inflammatory cells and keratinocytes were compared between groups (Student’s and Welch’s t-tests; Tukey’s test). Cytology scores were correlated with semiquantitative bacterial culture scores (Spearman’s rank correlation). The frequency of existing lip folds and all scores except keratinocytes were higher in dogs with cheilitis than in controls (all \( P < 0.001 \)). The swab technique showed lower \( (P < 0.05) \) mean counts of cells and microbes compared to direct impression smears and tape samples in both groups. Cytology and bacterial culture were poorly correlated, with tape impressions showing the highest correlation \( (r_s = 0.38, P = < 0.001) \). Dental plaques/calculus and feeding habits did not influence the development of cheilitis. Direct and tape impression samplings are recommended for the lip area. While low scores of microbes were present in the majority of dogs, high scores, inflammatory cells and lip folds indicated cheilitis. Dental health may not affect the skin in this area.

**Sources of funding:** The study was founded by the German Society for the Advancement of Kynologic Research (Deutsche Gesellschaft zur Förderung Kynologischer Forschung) and Synlab Services GmbH.

**Conflicts of interest:** None declared.

### High molecular-weight proteins in hydrolysed dog foods

O. ROITEL*, D. MAURICE*, G. DOUCHIN*, S. JACQUENET*, B. BIHAIN*, C. FAVROT† and N. COUTURIER*

*Genclis SA, Vandœuvre-lès-Nancy, France
†Clinic for Small Animal Internal Medicine, University of Zurich, Switzerland

The diagnosis of food allergy relies on an elimination diet. One approach consists of using hydrolysed foods where the cleaved parent proteins are too small to react with the allergen-specific IgE that may be present in the dog serum. The goal of this study was to assess whether the molecular weight of peptides present in three different hydrolysed foods (Royal Canin Anallergenic (Royal Canin; Aïmargues, France), Purina HA (Nestlé Purina PetCare; Meaux, France) and Hill’s z/d Low Allergen (Hill’s Pet Nutrition; Sophia-Antipolis, France)) matches with the producers’ claims and if peptides are recognized by IgE. Electrophoresis of food extracts was carried out on two batches for each food and proteins were visualized using Coomassie blue and silver nitrate to increase the sensitivity of the detection. Western-blot analyses were carried out to assess the sensitization profile. In one instance, mass spectrometry analysis was conducted to identify

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**Clinical, cytological and microbiological findings from the lower lip in healthy dogs and dogs with cheilitis, and evaluation of three sampling techniques**

M. DOELLE*, K. WOLF†, A. LOEFFLER†, V. KOSTKA§ and M. LINEK*

*Tierärztliche Spezialisten, Hamburg, Germany
†Institute of Veterinary Physiology, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
‡Department of Clinical Science and Services, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, UK
§Synlab.vet GmbH Labor Hamburg, Geesthacht, Germany

Cheilitis is common in dogs, associated with many skin diseases and often complicated by microbial infections. We assessed clinical, cytological and microbiological findings from the lower lip in dogs with cheilitis and controls and evaluated three sampling techniques in order to optimize diagnostic procedures.
the protein. In all three hydrolysed foods, we detected the presence of several high molecular-weight (HMW) proteins ranging from 15 kDa to 60 kDa. IgE immunoblotting revealed that these HMW proteins were targeted by circulating dog IgE. By mass spectrometry (76% coverage), we identified one of these HMW proteins as the intact form of granule-bound starch synthase 1 protein from maize, a component of the kibbles. Our study demonstrates that even highly hydrolysed hypoallergenic foods may still contain immunoreactive HMW proteins. Further studies are mandatory to determine the clinical relevance of these findings. These HMW proteins may impact the success rates of some elimination diets.

**Sources of funding:** Self-funded.

**Conflicts of interest:** None declared.

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**RNA-Seq analysis of equine papillomavirus type 2 associated squamous cell carcinoma tissue samples identifies affected biological processes and potential marker genes**

S. RAMSAUER*,†, K. TOBLER*, M. ACKERMANN† and C. FAVROT*

*Dermatology Department, Vetsuisse Faculty, Zurich, Switzerland
†Institute of Virology, Vetsuisse Faculty, Zurich, Switzerland

There is strong evidence that Equine Papillomavirus type 2 (EcPV2) is a crucial factor in the development of equine penile squamous cell carcinomas (SCC). To date, few satisfactory therapeutic options to control this condition have been proposed. It will be important to unravel the pathomechanisms associated with the cancer transformation to uncover potential marker genes. We therefore collected penile skin biopsy samples from five horses with EcPV2-positive penile SCCs and from three EcPV2-negative horses with no penile lesions for transcriptome profiling by RNA-Seq. The sequencing reads were mapped to EcPV2 and the horse genome. Mapping reads to the viral genome confirmed expression of viral genes in EcPV2-infected samples. Mapping reads to the host genome revealed 1957 genes differentially expressed in SCC samples.

The differentially expressed genes were evaluated to identify key biological processes and potential marker genes. Significant regulation of cell cycle, DNA replication, extracellular matrix interaction and focal adhesion was recognized by KEGG pathway analysis. In addition, effects on RNA processing and stress response were detected by Gene Ontology-term analysis for biological processes. To detect markers we compared the hundred highest upregulated genes to other cancer gene expression studies. Matrix metalloproteinase 1 and interleukin 8 were defined as potential marker genes for the development of EcPV2-associated SCC. These results provide a basis to study the disease in more detail, and might support the development of new methods for early diagnosis and improving the prognosis.

**Sources of funding:** Clinomics.

**Conflicts of interest:** None declared.

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**Comparison of two plating procedures of samples obtained by toothbrush technique to diagnose feline dermatophytosis**

D. DI MATTIA*, M. MONACO*, A. FONDATI* and A. PEANO†

*Vetetinaria Cetego, Roma, Italy
†Dipartimento Scienze Veterinarie – Università di Torino, Torino, Italy

The toothbrush technique is described as an effective way of collecting material for fungal cultures, yet there are scant details on how to transfer material to plates. This study compared a toothbrush pressed onto agar-medium surface (A) with a toothbrush pressed plus hair-scales entrapped in the bristles, manually removed and transferred onto the agar (B). The study was conducted on 25 cats housed in a cattery with a history of recurrent dermatophytosis. A new toothbrush was combed for three minutes over each longitudinal half of each cat’s haircoat. Samples were cultured on Mycobios Selective Agar (Biolife; Milan, Italy) blindly using procedure A or B. Cultures were incubated at 25˚C and examined daily for two weeks. Colony forming units (CFUs) of dermatophytes and nondermatophytic contaminant moulds (NDM) and the percentage of plate surface covered by NDM were recorded. Twenty cats were positive for *Microsporum canis*. For these animals, CFU ranges were as follows. Procedure A: *Microsporum canis* 1–20 (median 2.5); NDM 0–50 (median 5.5); percentage of plate invaded by NDM 41% (mean value). Procedure B: *Microsporum canis* 0–6 (median 2); NDM 0–50 (median 6); percentage of NDM 60% (mean value). Visualization/identification of *Microsporum canis* was considered very easy (A: 35% of cases; B: 5%; P = 0.04, Fisher’s exact test); easy (A: 40%; B: 35%); difficult (A: 20%; B: 15%); very difficult (A: 5%; B: 35%); impossible (A: none; B: 10%). This study provides evidence that cultures should be performed by pressing toothbrushes on the agar without plating hair-scales.

**Sources of funding:** Self-funded.

**Conflicts of interest:** None declared.

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**A pilot study on the use of medical grade honey in the management of canine otitis externa**

E. MARUHASHI*, B. S. BRAZ*, T. NUNES* and A. M. LOURENÇO*

*CIISA/Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

Existing evidence regarding the use of honey in medicine suggests that it may play a key role in healing and infec-
The concurrent presence of different Demodex species in one cat has been reported anecdotally, but those reports were mainly based on morphological characteristics of the Demodex mites to determine the different species. All three feline Demodex species have distinct genotypes, hence molecular analysis can be used for species differentiation. A 17-year-old FIV-infected domestic short hair cat was presented with nonpruritic otitis externa. Swab samples were sent for culture and susceptibility testing, as well as for biocidal activity of MGH. MGH yielded rapid clinical improvement, with 70% of dogs achieving clinical cure between days 7 and 14 and over 90% on Day 21. Although most dogs required longer time periods to achieve cytological cure in comparison with clinical cure, all dogs demonstrated decreases in numbers of micro-organisms on cytology. MGH showed antimicrobial properties against fungal and bacterial agents. MGH had beneficial effects in both clinical and laboratory settings in the management of OE. Larger controlled studies are required to further assess MGH for otitis externa.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

Morphological variability of *Demodex cati* in an FIV-positive cat

E. R. L. TAFFIN*†, E. CLAEREBOU†, S. CASAERT† and S. VANDENABEELE*†

*Department of Small Animal Medicine and Clinical Biology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
†Department of Virology, Parasitology & Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

The concurrent presence of different Demodex species in one cat has been reported anecdotally, but those reports were mainly based on morphological characteristics of the Demodex mites to determine the different species. All three feline Demodex species have distinct genotypes, hence molecular analysis can be used for species differentiation. A 17-year-old FIV-infected domestic short hair cat was presented with nonpruritic alopecia and comedones, involving the head and neck. Superficial and deep skin scrapings of the lesions were obtained. Microscopic examination revealed a morphologically very heterogeneous population of Demodex mites. Micrometry results showed a broad range of body lengths (92.68–245.94 μm) and the authors theorized that three different *Demodex* species might be present in the skin lesions of this cat. Therefore, sequence analysis of the 16S rRNA gene of the sampled mites was performed. Samples appeared 100% identical to the *Demodex cati* sequence as deposited in GenBank (X193759), but a similarity of only 79.2% was found when compared with *D. gatoi* (JX881921) and 74.4% when compared with *D. felis* (KF052995). Molecular analysis thus identified all observed morphological forms as *D. cati*. These results show that morphology alone is not diagnostic for species specification, but that it has to be combined with molecular analysis. Feline demodicosis is often associated with underlying disease and it has been suggested that an altered immune response may influence size variation of *Demodex* mites. Further research is required to assess factors that induce species polymorphism, but these results suggest that immunodeficiency caused by FIV infection may contribute to this phenomenon.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

Allergen-specific immunotherapy with pullulan-conjugated major HDM allergen

R. SUZUKI*, T. TSUKUI*, T. OSUMI†, M. SAKAGUCHI‡ and H. TSUJIMOTO§

*Animal Life Science Laboratory, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan
†Pigeon Animal Care Hospital, Saitama, Japan
‡School of Veterinary Medicine, Azabu University, Kanagawa, Japan
§Graduate School of Agricultural and Life Science, the University of Tokyo, Tokyo, Japan

We have produced a recombinant house dust mite (HDM) allergen Der f 2 using a silkworm–baculovirus expression system, which was conjugated to pullulan so that IgE-binding activity was reduced and IgG production was improved. In a clinical trial 143 HDM-sensitized Canine Atopic Dermatitis (CAD) dogs were used. We divided the dogs into four groups; four, five and six injections of Der f 2-pullulan, and the control group. Administrations were conducted subcutaneously once weekly. Pre-treatment values of the Canine Atopic Dermatitis Extent and Severity Index (CADESI) in these groups were 167.6 ± 168.2, 124.9 ± 132.5, 160.1 ± 170.6 and 125.1 ± 114.8, respectively. Post (four weeks after the final injection) values were 102.0 ± 137.7, 60.6 ± 121.9, 82.3 ± 124.3 and 66.3 ± 97.8, respectively. Significant decreases were observed in groups given four, five or six injections (P < 0.05, Wilcoxon signed-rank test). Pre-treatment values of pruritus scores were 2.6 ± 1.2, 2.5 ± 1.1, 2.7 ± 1.1 and 2.6 ± 1.0, and post values were 2.0 ± 1.3, 1.8 ± 1.0, 1.9 ± 1.2 and 2.0 ± 1.0, respectively. Pruritus scores also decreased in groups given five or six injections (P < 0.05, Wilcoxon signed-rank test). As evidence for immune induction, serum Der f 2-specific IgG significantly increased in groups given five or six injections (P < 0.05, paired Student’s *t*-test). Furthermore, about 70% of dogs given six injections maintained remission (no additional medications and no pruritus or lesions) for at least one year after the final injection. These results indicate efficacy of Der f 2-pullu-
Ian in HDM-sensitized dogs. This agent was approved by the Japanese Ministry of Agriculture, Forestry and Fisheries as “Allermune HDM®” (Nippon Zenyaku Kogyo, Fukushima, Japan) in 2014.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

Profile of lymphocyte subpopulations and cytokine concentration in peripheral blood of dogs with atopic dermatitis

K. DEMBELE*, A. MAJEWSKA#, M. GAJEWSKA† and A. PROSTEK†
*Department of Small Animal Diseases with Clinic, Faculty of Veterinary Medicine, Warsaw University of Life Sciences-SGGW, Warsaw, Poland
†Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences-SSGW, Warsaw, Poland

This study aimed at determining the profile of peripheral blood lymphocytes and plasma levels of cytokines in dogs with atopic dermatitis (AD). Twenty dogs with AD and eight healthy dogs were selected. Lymphocyte subpopulations were determined using flow cytometry, and the levels of cytokines were determined by ELISA. The comparisons between atopic and healthy dogs were performed with unpaired Student’s t-test with a 5% level of significance. The mean percentage of lymphocytes was similar in both groups (AD: 45.2%, healthy: 42.7%). The mean percentage of CD3+ T- and B-lymphocytes (LSM 11.425 antibody) was significantly lower in atopic than in healthy dogs (T cells: 25.7% versus 35.9%; B cells: 3.8% versus 6.2%). The mean percentage of CD4 helper T-lymphocytes was significantly lower in AD than healthy dogs (16.9% versus 25.2%). The opposite was seen for the percentage of CD8+ cytotoxic T-lymphocytes (AD: 19.8%; healthy: 14.7%). The plasma concentrations of IL-4 and IL-13 (Th2 cytokines) were significantly elevated in dogs with AD compared to healthy dogs (IL-4: 147.0 versus 134.8 pg/mL; IL-13: 259.5 versus 105.5 pg/mL). Although Th1 cytokines IFN-γ and IL-2 were detected only in a few plasma samples, TNF-α was detected in all animals, and its level was significantly increased in dogs with AD compared to healthy dogs (96.0 versus 58.2 pg/mL). These results confirm a Th2-predominant immune response in dogs with AD. The higher percentages of cytotoxic T cells in atopic dogs should be explored further.

Sources of funding: grant No. N N308 575940 from the National Science Centre of Poland.

Conflicts of interest: None declared.

Efficacy of fluralaner for the treatment of canine demodicosis

J. KARAS-TECZA* and J. DAVIDOWICZ†
*Dermatology Clinic For Dogs and Cats “Dermawet”, Warsaw, Poland
†Veterinary Clinic “Brynów”, Katowice, Poland

Canine generalized demodicosis is a common and often very severe skin disorder. At present no universal and fully effective therapy of this disease is known. The aim of this study was to evaluate the efficacy of fluralaner (Bravecto, MSD Animal Health; Madison, NJ, USA) in the treatment of demodicosis in dogs. Client-owned dogs (n = 163) of different breeds and both sexes with generalized demodicosis confirmed by deep skin scraping and/or hair plucking were included. Animals were divided into two age groups at presentation for the treatment: group one, 2–18 months (62.6%) and group two, over 2 years of age (37.4%). Dogs were treated with fluralaner (25 mg/kg) orally, twice three months apart. No other treatment against demodicosis was used. Individuals with secondary pyoderma additionally received cefalexin (30 mg/kg). Skin scraping and/or hair plucking were performed 1, 2 and 3 months after the first fluralaner administration. Obtained results suggest that fluralaner may be an effective, safe and convenient treatment option for dogs with generalized demodicosis. The number of dogs with negative scrapings after one month was significantly higher in group one compared with group two (P < 0.001, χ² test).

Sources of funding: Self-funded.

Conflicts of interest: None declared.

House dust mites provide enzymes that disrupt the epithelial integrity

K. OIDA*, †, L. EINHORN%, †, I. HERRMANN%, S. VRTALA¶, †, Y. RESCH¶, L. PANAKOVA¶, G. HOFSTETTER*, H. MATSUDA†, A. TANAKA‡ and E. JENSEN-JAROLIM*, †
*Comparative Medicine, Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria
†Laboratory of Veterinary Molecular Pathology and Therapeutics, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan
¶Division of Comparative Immunology and Oncology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria
%Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria
Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria

Abstracts
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Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria

**Christian Doppler Laboratory for the Development of Allergen Chips, Medical University Vienna, Vienna, Austria

†Laboratory of Comparative Animal Medicine, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

With a lifestyle change of pet animals, house dust mites (HDMs) are considered an important source of allergens in dogs as well as in humans. It is accepted that epithelial barrier dysfunction or disruption may be the initial event before sensitization in allergic diseases. We investigated here the barrier disruption potential of HDM in vitro using whole body extract from Dermatophagoides pteronyssinus. Because various kinds of enzymes in HDM extract have been reported, their impact on the extracellular matrix (ECM) was analysed. Protease activities and their biochemical properties were assessed by gel zymography. N-acetyl-glucosaminidase activities were colourimetrically detected using a chromogenic substrate. Finally, to clarify that the extract elicits an allergic inflammation, a degranulation assay was performed with canine mast cells and sera from dogs with allergic dermatitis. Regardless of the presence of Ca and Zn ions, the HDM extract degraded casein (detection for serine and cysteine protease activity) and gelatin gels (for collagenase activity), indicating that these proteases do not belong to the matrix metalloprotease family. The optimal pH for proteolysis was pH 7.69; proteolysis occurred in a wide temperature range. The extract also exhibited N-acetylglucosaminidase activities which can degrade proteoglycans of the ECM. Moreover, the extract triggered IgE-dependent degranulation of canine mast cells. Exposure to HDM may influence the disruption of the epithelial barrier in dogs, especially when the pH of the skin surface is elevated due to inflammation or detergents when HDM may deliver allergens deep into the skin surface.

Sources of funding: This work was supported by Austrian Science Fund project SFB F4606-B19, F4602-B19 and W1205-B09 (MCCA-PhD program).

Conflicts of interest: None declared.

The transverse histomorphometry of alopecia in curly coated retrievers from UK and Sweden

R. BOND*, K. VARJONEN*,1, A. HENDRICKS*, Y. CHANG† and H. BROOKS BROWNLIE‡,2

*Department of Veterinary Clinical Sciences, Royal Veterinary College, Hatfield, U.K.
†Research Office, Royal Veterinary College, Hatfield, UK
‡Department of Pathology and Pathogen Biology, Royal Veterinary College, Hatfield, UK
1Present address: UC Davis School of Veterinary Medicine, Davis, USA
2Present address: School of Veterinary Sciences, University of Bristol, Langford, UK

We have shown that young adult curly coated retrievers (CCR) that are otherwise healthy often present with alopecia and “frizzy” coat quality changes that may wax and wane, often in association with reproductive cycles. Changes were characterized in routine (vertical) histopathological sections (14 dogs) by infundibular hyperkeratosis (28 of 32 sections), low-grade pigment clumping (17 of 32) and subjective, subtle telogenization of hair follicles. Because in humans, transverse sectioning of biopsies (“Headington technique”) is essential for full definition of follicular abnormalities, a transverse morphometric analysis of biopsies from affected and unaffected sites was conducted in these dogs. The density of compound follicles (CF/mm2), numbers of follicles/CF (HF/CF), and anagen/telogen plus catagen (A/T) ratio for each CF were compared in transverse skin sections through the lower isthmus area from unaffected, “frizzy” and alopecic areas in 14 dogs using a mixed model statistical analysis (SPSS v20, significance P < 0.05). CF density (mean CF/mm2 ± SE) in healthy (3.5 ± 0.3), frizzy (2.9 ± 0.4) and alopecic (3.4 ± 0.2) areas were comparable (P = 0.47) and did not vary between anatomical site (P = 0.14). HF/CF (mean ± SE) in frizzy areas (17.2 ± 1.1) exceeded (P = 0.01) those of unaffected (14.6 ± 0.9) and alopecic (14.4 ± 0.8) areas. Mean ±SE A/T ratios in alopecic areas (0.7 ± 0.2) were lower (P > 0.0001) than those of frizzy (1.1 ± 0.3) and unaffected (1.4 ± 0.2) areas. Transverse sections were pivotal in unequivocally determining that the alopecia seen in CCR is associated with telogenization but not reduced CF density or HF/CF. This method should be routinely adopted for studies of hair follicle disorders in veterinary species.

Sources of funding: BSAVA PetSavers.

Conflicts of interest: None declared.

Treatment of canine perianal fistulas with an intralesional injection of human mesenchymal, embryonic-derived stem cells (hE-MSCs)

L. FERRER*, A. LAM*, E. B. FALK*, C. ZEWE*, M. MEOLA† and A. HOFFMAN†

*Department of Clinical Sciences, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA
†Laboratory of Regenerative Medicine, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA

A previous study demonstrated that intralesional injections of autologous bone-marrow derived stem cells were helpful as adjunctive therapy for perianal fistulas in dogs. In this pilot trial we investigated the efficacy and safety of injecting human embryonic-derived stem cells (hE-MSCs) in the fistulas, as a complement to the ciclosporin. Six dogs with a diagnosis of perianal fistulas that responded only partially to ciclosporin were recruited (1–6 fistulas/dog). The dogs were maintained on the same dose of ciclosporin and the SC treatment was used with the aim of accelerating the healing and reducing the dose of ciclosporin. On Day 0, under sedation, the lesions were recorded and 20 million hE-MSCs of high purity were injected. On Day 48, the wounds were monitored and the animals were followed-up for 1 year. Overall, 3/6 (50%) of the wounds were resolved on Day 48. At 1 year, 4/6 (66.6%) dogs were free of fistulas and 2 dogs were ambulatory with significantly reduced pain scores. This study provides evidence that hE-MSCs might be a safe and effective treatment for perianal fistulas in dogs.

Sources of funding: BSAVA PetSavers.

Conflicts of interest: None declared.
immunomodulatory potency (hemangioblasts, Advanced Cell Technology; Worcester, MA, USA) were injected in and around the fistulas. After the injection of the MSCs, the fistulas were sealed with fibrin (Evicel®, Omrix Biopharmaceuticals; Israel). The patients were examined one week, and 1, 2, 3 and 6 months post-injection (PI) of the hE-MSCs. The dose of ciclosporin was adjusted according to the clinical improvement. No adverse effects were observed on any of the six dogs. At three months PI all six dogs were free of fistulas and at 6 months PI four dogs were free of fistulas and two presented with a single small fistula. The mean doses of ciclosporin were 8.22 mg/kg daily and 3.59 mg/kg daily at Day 0 and 6 months PI, respectively ($P = 0.036$). The intralesional injection of hE-MSCs is a safe and effective complementary treatment in dogs with perianal fistulas.

**Sources of funding:** Advanced Cell Technology Inc, Massachusetts, US, covered all expenses of the pilot trial and provided the stem cells.

**Conflicts of interest:** None declared.

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**Pharmacokinetic determinations of cefalexin (Rilexine®) in plasma and skin after repeated oral administration to dogs**

C. NAVARRO*, N. BERNACHON* and M. SILLON†
*Medical department, Virbac, Carros, France
†R&D, Virbac Carros, France

This study determined the pharmacokinetics of cefalexin (Rilexine®, Virbac; Carros, France) in plasma and skin after oral administration to seven beagle dogs at 15 mg/kg twice daily over a 7-day period. Blood samples were taken at various times on Day (D)3 and D7. Skin samples were collected on D3 at 2 and 5 h, and on D7 at 2, 4, 8 and 10 h after each dose. The cefalexin level was determined by HPLC. The limit of quantification was determined at <0.50 μg/g for a 50 mg aliquot. The minimum effective skin concentration was determined to be >1 μg/g. Plasma levels increased until a mean tmax of approximately 2 h after dosing was achieved. The mean Cmax was about 20 μg/mL and the mean AUC was about 80 μg h/mL. Mean elimination t1/2 and MRT were respectively 2 and 4 h, and mean volume of distribution was 0.5 l/kg. Skin levels were above the minimum effective concentration at all sampling times in all animals, except 10 h after the first daily dosing on D7 (means D3, 2 h 2.19 and 5 h 1.34; d7 2 h 5.98, 4 h 3.50 and 8 h 1.26 μg/g). After oral administration at 15 mg/kg twice daily, cefalexin is rapidly absorbed with effective therapeutic concentrations in serum. It is well distributed in the tissues and sufficient concentrations of cefalexin are achieved in the skin, mimicking that of the serum but at a lower level depending upon protein binding.

**Sources of funding:** Virbac.

**Conflicts of interest:** Authors are employees of Virbac.

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**Cutaneous macroscopic and microscopic lesions caused by Pennella sp., Crassicauda sp. and Phyllobothrium delphini in Cetaceans**

S. FERRO*, D. DAL COLLO†, S. MAZZARIOL* and C. CENTELLEGHE*
*Department of Comparative Biomedicine and Food Science, University of Padua, Italy
†Freelance practitioner, Venice, Italy

Recently skin changes have been considered valuable indicators of health in free-ranging cetaceans and several studies have been performed worldwide using photo-ID. The aim of the study was to describe the macroscopic parasitic lesions and their less-known correlated microscopic patterns. In this study skin samples collected from 67 cetaceans (35 *Stenella coeruleoalba*, 20 *Tursiops truncatus*, 4 *Grampus griseus*, 4 *Balaenoptera physalus*, 3 *Physeter macrocephalus* and 1 *Ziphius cavirostris*), found stranded along the Italian coastline in the period 2011–2015, were examined macro- and microscopically. Parasitic cutaneous lesions were reported in 29 animals: *Pennella* sp. (18), *Phyllobothrium delphini* (9) and *Crassicauda* sp. (5). *Pennella* sp. was visible at external examination, while histological lesions revealed epidermal, dermal and subcutaneous focal necrosis associated with an intense neutrophilic infiltrate, sometimes involving the underlying muscle. *Phyllobothrium delphini* appeared macroscopically as round cysts in the blubber or abdominal musculature. Histologically the larvae were lined by a fibrous capsule with a thin internal rim of scant inflammatory cells. *Crassicauda* sp. was found as roundworms in the fascia between the deep blubber and the subcutaneous muscle. Microscopically there was a mild infiltrate around the parasite or around the fistula. In conclusion, our study described the macroscopic and microscopic aspects of several parasitic cutaneous lesions, which should be part of a systematic observation of skin lesions in live animals and part of routine sampling in dead animals.

**Sources of funding:** Self-funded.

**Conflicts of interest:** None declared.

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**Junctional epidermolysis bullosa in French Charolais cattle is due to a homozygous deletion encompassing exons 17 to 23 of the integrin beta 4 gene**

M. MOSCA*, P. MICHOTT†, O. FANTINI*, R. BRAQUE§, A. ALLAIS-BONNET†, R. SAINTILAN†, C. GROHS†, J. BARBIERI†, L. GENESTOUT¶, C. DANCHIN-BURGE**, J.-M. GOURREAU††, D. BOICHARD†, A. CAPITAN†,‡ and D. PIN*
*VetAgro Sup Campus vétérinaire de Lyon, Marcy l’étoile, France
†INRA, domaine de Vilvert, Jouy-en-Josas, France
‡ALLICE, Paris, France

Abstracts
In humans and other mammalian species, junctional epidermolysis bullosa (JEB) is associated with mutations in genes encoding proteins of the hemidesmosome anchoring filament complex (ITG6, ITGB4, COL17A1, LAMA3, LAMB3 and LAMC2). Recently, a large deletion in the integrin beta 4 encoding gene ITGB4 was found to cause JEB in German Charolais cattle. Charolais calves, born from consanguineous matings in the same French herd, presented at birth with exungulation, erosions and ulcers of the carpal and tarsal joints, fetlocks, ears, eyelids, muzzle, oral cavity and tongue. Histopathological examination revealed subepithelial clefting without keratinocyte cytolyis. Staining with Periodic acid Schiff showed that splitting occurred between basal keratinocytes and the basement membrane. Clinical and histopathological examinations were suggestive of JEB. Sequencing of the entire genome of an affected calf identified a homozygous deletion that includes exons 17 to 23 of ITGB4. Genotyping of another unrelated case and of six parents of affected calves revealed a perfect association between this mutation and the presumed genotypes of the individuals. In addition, the identification of the same deletion in German Charolais with JEB further supports the causality of this mutation. Screening of 6,870 Charolais genotyped for genomic selection enabled the identification of 44 individuals carrying a 5.6Mb haplotype shared with JEB calves around ITGB4. Interestingly, none of them carried the causative deletion suggesting that this mutation occurred recently. In conclusion, the identification of the mutation in Charolais cattle will permit the identification of healthy carriers and their withdrawal from the breeding pool.

Sources of funding: Apis Gène funded the sequencing of the genomes.

Conflicts of interest: None declared.

Pemphigus foliaceus in a cow

M. MOSCA*, O. FANTINI*, S. DRUART†,
A. ARNOULT†, D. LEDOUX*, C. BECKER* and D. PIN*

* VetAgro Sup Campus vétérinaire de Lyon, Marcy l’étoile, France
† Cabinet vétérinaire, Rebais, France

A 3-year-old Montbeliard cow was presented with a 6-month history of generalized chronic pruritic dermatitis. A few weeks after parturition, erythema, thick crusts, alopecia and lichenification developed in a bilaterally and symmetrical pattern on the flanks and dorsum before becoming generalized. The mucous membranes were not affected. The cow lived with 40 other Montbeliareds that had no dermatological lesions. Skin scrapes and a dermatophyte culture were negative. Bacterial culture showed a polymicrobial population with only coagulase negative Staphylococci. Skin cytology revealed numerous bacteria, intact neutrophils and rare isolated, round, acantholytic keratinocytes. Dermatopathology revealed orthokeratotic hyperkeratosis, crusting, irregular acanthosis, and subcorneal and intraepidermal pustules containing neutrophils and acantholytic keratinocytes. Acantholytic pustules were also present within the outer root sheath of the hair follicles. Using direct immunohistochemistry, intercellular immunoglobulin deposits were observed in lesional skin sections in the spinous and granular layers of the epidermis. Finally, an exfoliative pyoderma was ruled out based on the combination of epidemiological (6-month duration), clinical (thick adherent multilaminar crusts), cytological, microbiological and histopathological findings. The diagnosis of pemphigus foliaceus (PF) complicated with bacterial overgrowth was made as a consequence. Systemic glucocorticoid and antibiotic therapy enabled a complete remission of skin lesions. Although PF is the most common antibody-mediated autoimmune skin disease in dogs, cats, horses and goats, it has never been described in cattle. In this cow, PF had similar clinical and histopathological features to those seen in other animal species; PF should be included in the differential diagnosis of pruritic generalized crusted dermatoses in cattle.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

Activity in vitro of chlorhexidine alone and in combination with miconazole or tromethamine-EDTA against canine meticillin-resistant and susceptible staphylococci

S. M. CLARK*, A. LOEFFLER*, A. WILSON*,
Y.-M. CHANG†, V. SCHMIDT‡ and R. BOND*

* Department of Clinical Sciences & Services, Royal Veterinary College, Hatfield, UK
† Research Office, Royal Veterinary College, Hatfield, UK
‡ School of Veterinary Science, University of Liverpool, Neston, UK

Emerging multidrug-resistance has heightened interest in topical treatments as alternatives to systemic antibacterial therapy in canine superficial pyoderma. We therefore determined the in vitro minimum inhibitory concentration (MICs) of shampoo components in a collection of Staphylococcus pseudintermedius (SP) from northern (NUK) and south-east U.K. (SEUK). MICs of chlorhexidine (CH), miconazole (M), tromethamine-ethylenediaminetetra-acetic acid (TE, 16:1) and combinations of CH:M (1:1) or CH:TE (80:16:1) were determined \( n = 196 \): NUK: 49 meticillin-resistant (MRSP) and 50 meticillin-susceptible (MSSP); SEUK: 48 MRSP, 49 MSSP) using agar dilution (CLSI VET01-A4). Mueller-Hinton plates contained two-fold dilutions of each drug or combination (512 to 0.03 mg/L). MICs were compared (drug\*region\*bacterial type) with a linear mixed-effect model after log2 transformation (SPSS v20, significance
An in vitro assay to assess the killing effect of three silicone products on Demodex canis

B. MIGNON* and A. SIEG†
*Department of Parasitic and Infectious Diseases, Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liège, Liège, Belgium
†Application Engineering and Technical Service - Healthcare Industries, Dow Corning Europe, Seneffe, Belgium

Several silicone-based products are an efficient alternative to neurotoxic ectoparasiticides for the treatment of Pediculus capitis infestations in humans. Demodex spp. are other arthropods involved in several human and canine skin manifestations for which treatment can be problematic. The objective of this pilot study was to develop an in vitro test to assess the potential killing effect of silicones on Demodex mites. Demodex canis mites were collected from a dog with generalized demodicosis, suspended in paraffin oil and introduced in wells from cell culture plates filled with either a low molecular mass linear polydimethylsiloxane containing silica, or paraffin oil as negative control (all products from Dow Corning, MI, USA).

Products were tested in duplicated wells containing 16 to 70 living mites initially. Plates were incubated at 32°C and regularly observed under the microscope to determine the mortality rate (%) of mites. After 90 min contact with all three silicone products, the mortality rates ranged from 69% to 91% and were significantly higher than the mortality rate of mites exposed to paraffin oil (6%, P < 0.05). The percentage of mortality increased with time, reaching 100% after 3 to 12.5 h contact, depending on the silicone product. The high molecular mass polymer was the most effective in rapidly killing the mites. In conclusion, our model based on D. canis appears suitable for evaluating in greater depth the in vitro killing effect of silicone-based products against Demodex mites.

Source of funding: Dechra Veterinary Products Limited.

Conflict of interest: Dechra Veterinary Products have previously collaborated with and funded teaching, clinical and research activity at the Royal Veterinary College.

Killing of canine strains of Staphylococcus pseudintermedius and Escherichia coli by cefovecin, doxycycline and pradofloxacin

J. M. BLONDEAU* and S. SHEBELSKI*
*Department of Clinical Microbiology, Royal University Hospital and University of Saskatchewan, Saskatoon, SK, Canada

We compared killing by cefovecin, doxycycline and pradofloxacin against clinical canine isolates of Staphylococcus pseudintermedius (n = 3) and Escherichia coli (n = 3). Approximately 10^5–10^6 colony forming units per millilitre (cfu/mL) of each strain were exposed to minimum inhibitory (MI), mutant prevention (MP), maximum serum (C_{max}) and maximum tissue (T_{max}) drug concentrations (DC). The log_{10} (and % kill) reduction in viable cells was recorded following 0.5, 1, 2, 4, 6, 12, 24 h of drug exposure. Experiments were conducted in triplicate and results averaged. For S. pseudintermedius, pradofloxacin (0.13–1.94 log_{10}, 16–98%) killed more organisms by 4 h than did cefovecin (growth-0.76 log_{10}, 51%) or doxycycline (growth-0.21 log_{10}, 36%) at MP DC. For C_{max} and T_{max}, pradofloxacin killed more bacteria at 1 h (0.2–1.46 log_{10}, 26–95%) than cefovecin (growth-0.14 log_{10}, 21%) or doxycycline (growth-0.03 log_{10}, 8%). For pradofloxacin, 48–95% of bacteria were killed in the first 0.5 h of drug exposure at T_{max} DC (growth 6% kill for cefovecin and doxycycline). For E. coli, pradofloxacin killed 30– >99% (0.07–4.5 log_{10}) bacteria by 4 h versus cefovecin (growth-1.74 log_{10}, 98%) or doxycycline (growth-0.71 log_{10}, 76%) at MP DC, and pradofloxacin killed more cells at 0.5 and 1 h than other drugs exposed to C_{max} and T_{max} DC (0.95–4 log_{10}, 61– >99% versus growth-0.32 log_{10}, 45% for cefovecin and growth-0.12 log_{10}, 24% for doxycycline).

Pradofloxacin rapidly killed S. pseudintermedius and E. coli at clinically relevant bacterial densities and drug concentrations. These observations may have important implications for optimal treatment and durations of therapy for dermatological infections.

Sources of funding: Funded, in part, by an unrestricted research grant from Bayer Animal Health.

Conflicts of interest: None declared.
Clinical pattern of sarcoptic acariosis in dogs (Sarcoptes scabiei) in comparison to other pruritic skin diseases

P. BOURDEAU*, †, A. TONUS* and V. BRUET*, †
*LUNAM, Oniris, Dermatology, parasitology, mycology unit, Nantes, France
†LUNAM, Oniris, UPSP 5304, Animal pathophysiology and functional pharmacology, Nantes, France

Sarcoptic acariosis (SAc) is a common differential diagnosis of pruritic dermatoses. We present here a study on the pattern of SAc in dogs compared to canine atopic dermatitis (CAD), flea bite hypersensitivity (FBH) and food intolerance/allergy (FIA). Cases included were seen in clinics with information collected in a GCP approach (period 1993–2013). Diagnoses were based on epidemiology, clinical aspects, identification of parasites and therapeutic efficacy. Distribution of lesions in SAc was detailed (19 body areas). Dogs included were (a) SAc (n = 202), (b) CAD (n = 601), (c) FBH (n = 498) and (d) FIA (n = 22). The lesions of SAc were most frequently seen on the ventrum (74.3%), pinnae (68.6%), elbows (67.1%), face (54.3%), legs (forelegs 51.4%, hindlegs 50%), trunk (50%) and other body areas less commonly. The distribution differed significantly from that of other skin diseases: CAD – elbows (11.5%, P < 0.0001), ventral neck (12%, P < 0.0001), lumbar (17.6%, P < 0.0001), thighs/groin. (26.3%, P < 0.0001), pinnae (40.1%, P < 0.0001), hind legs (4.8%, P < 0.0001) and ventrum, face, sternum, thorax, ano-genital and tail (P < 0.01–0.0001); FBH – ventrum (14.5%, P < 0.0001), face (15.7%, P < 0.0001), hind legs (22.1%, P < 0.0001) and less commonly lumbar [a] 48.6% versus (c) 78.9%, P < 0.0001), ano-genital [a] 28.6% versus (c) 77%, P < 0.0001); and FIA – outer pinnae [a] 45.7% versus (d) 9.1%, P = 0.002); forelegs [a] 75.7% versus (d) 45.5%, P = 0.016) and less frequently ano-genital [a] 28.6 versus (d) 72.7%, P = 0.0004), inner pinnae [a] 38.6 versus (d) 77.3%, P = 0.003) and interdigital areas (P < 0.05). Based on these results, a suggestive lesional pattern of SAc is proposed to include the ventrum, elbows, pinnae, face and legs.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

The clinically normal skin of dogs with leishmaniosis

L. ORDEIX*, †, L. ALBORCH*, A. DALMAU†, M. OSSOŠ and L. SOLANO-GALLEGO*
*Departament de Medicina i Cirugia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain
†Servei de Dermatologia, Ars Veterinaria, Barcelona, Spain
‡Mediterrani Veterinaris, Reus, Spain
§Consultori Veterinari, Fals de Poblet, Spain

Clinical pattern of sarcotic acariosis in dogs (Sarcoptes scabiei) in comparison to other pruritic skin diseases along with the presence of Leishmania amastigotes. The objective of the study was to evaluate the histopathological pattern and to demonstrate Leishmania in lesional and macroscopic nonlesional skin from dogs with leishmaniosis. Seventeen Leishmania infantum seroreactive dogs with clinical leishmaniosis were included and two biopsies (lesional and nonlesional skin) were taken from each dog. Ten healthy, noninfected beagle dogs were also enrolled as controls. Biopsies were processed for routine haematoxylin and eosin (H&E) staining and Leishmania immunohistochemistry. The study demonstrated microscopic lesions in 10 of 17 (58.8%) nonlesional samples that appeared healthy. The inflammatory pattern observed ranged from perivascular to interstitial mainly in the superficial and mid-dermis (perifollicular). The intensity of the dermatitis was mild to moderate and always more prominent in lesional skin. Macrophages with lymphocytes and plasma cells were the predominant cells. In macroscopically nonlesional samples, the presence of parasites was demonstrated in 4 of 17 (23.5%) and in 8 of 17 (47%) by routine H&E and immunohistochemistry, respectively. Seven of 17 (41.2%) macroscopically nonlesional samples and all samples from controls dogs were histologically normal and did not show Leishmania. Amastigotes were noted in 9 of 17 (53%) and in 17 of 17 (100%) lesional skin samples by routine H&E and immunohistochemistry, respectively. The nonlesional skin of dogs with leishmaniosis frequently shows microscopic lesions and harbours the parasite, demonstrated by routine H&E staining and, more often, by immunohistochemistry. This finding may represent a diagnostic challenge for dermatological conditions in endemic areas of canine leishmaniosis.

Source of funding: European Society of Veterinary Dermatology Research Grant 2012.

Conflicts of interest: None declared.

A case of cutaneous Dirofilariosis due to a cutaneous vessel microthrombi by microfilariae of Dirofilaria repens

L. PANÁKOVÁ*, B. MIKLOŠOVIČOVÁ†, S. BETTENAY†, E. REINBACHER* and K. SILBERMAYR§
*Clinical Unit of Internal Medicine Small Animals, University of Veterinary Medicine, Vienna, Austria
†Veterinary Clinic Vetteam, Pezinok, Slovakia
§Department of Pathobiology, Institute of Parasitology, University of Veterinary Medicine, Vienna, Austria
§Tierdermatologie, Deisenhofen, Germany

Dirofilariosis caused by Dirofilaria repens is an emerging zoonotic parasitic disease mainly associated with skin nodules. Pruritic dermatitis has been reported and the condition can be asymptomatic. We present a dog with unusual clinical manifestations associated with microthrombi due to D. repens microfilariae. A 7-year-old male 53 kg dogo Argentino from South-West Slovakia was presented with nonpruritic, erythematous dermatitis of the
caudo-medial thighs of 5 months duration. The skin changes had spread from one hind limb to the other and into the periphery despite 4 weeks treatment with oral cefalexine. Moderately erythematous widespread dermatitis of the caudo-medial thighs with irregular linear crusts predominantly at the edge of the lesions did not blanch with diascopy. Superficial skin cytology revealed few degenerated neutrophils. Skin biopsies revealed “filaria-like” structures within superficial thrombotic vessels. Low numbers of microfilaria were observed on the blood smears and PCR from the EDTA blood was negative for D. immitis, but positive for D. repens. The lesions resolved within 6 weeks of microfilaricidal treatment with 2 tablets of milbemycin oxime/spinosad 1620 mg/27 mg (Trifexis, Elanco Animal Health; Basingstoke, UK), given three times at 14-day intervals. Eight weeks subsequent to the successful microfilaricidal treatment three 1–2 cm big cutaneous nodules with adult dirofilaria were detected and surgically excised. This is a unique case of the cutaneous Dirofilariosis caused by microthrombi due to D. repens microfilaria. Classical presentation with nodules was discovered only later in the course of the disease. This case highlights a potential pathogenicity of Dirofilariosis with an unusual clinical presentation.

Sources of funding: Self-funded.

Conflicts of interest: None declared.

Anaphylactic and severe systemic toxic reaction caused by the stings of 28 hornets (Vespa crabro crabro) in a dog

N. TARPATAKI*, Z. DUDÁS-GYÖRKI*, N. KUBIK* and C. S. PRIXENZKY†
*Department of Internal Medicine, Szent István University, Budapest, Hungary
†Department of Animal Breeding, Nutrition and Laboratory Animal Science, Department of Animal Breeding and Genetics, Faculty of Veterinary Sciences, Szent István University, Budapest, Hungary

A 4-year-old intact male miniature schnauzer (8.6 kg) was attacked by at least 28 hornets (Vespa crabro crabro). It collapsed and was presented in shock within an hour. At the hospital the dog was not able to stand, was apathic with bradycardia (70/min), tachyypnoea (60/min), haematuria and multiple painful erythematous papules. The blood examination showed elevated white blood cells (32.8 G/L) with neutrophilia (99%) and left shift. There were mild-to-moderate elevated ALT, ALKP, LDH and severely decreased globulin fraction (12 g/L). There was proteinuria (total protein/creatinine ratio = 3.1), blood and haemoglobin in the urine. Thoracic radiographs initially showed an interstitial pattern and atelectasis in the right medial lobe; pulmonary oedema developed on the third day. In ICU, intravenous fluid therapy, amoxicillin-clavulanic acid, methylprednisolone, heparin, furosemide, butorphanol, famotidine, omeprazole, Vit C, Vit E, acetylcysteine and Hepa-Pet Plus® were administered. Skin lesions changed to elevated plaques with craters. After one week of intensive monitoring and therapy the dog recovered.

Sources of funding: Self-funded.

Conflict of interest: None declared.