Keratinocyte differentiation and cornification abnormalities in hereditary nasal parakeratosis in Labrador retrievers

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Hereditary nasal parakeratosis (HNPK) is an inherited disorder affecting Labrador retrievers. The main clinical feature is a nonpruritic, parakeratotic hyperkeratosis of the nasal planum, but the exact mechanism leading to the clinical signs is currently unknown. Very recently, HNPK has been associated with a specific mutation affecting the SUV39H2 gene, which encodes for histone H3-K9 methyltransferase-2. Histones and their modifications influence chromatin structure, thereby resulting in transcriptional alterations of affected genes. The biological effect and relevance of the mutated SUV39H2 gene in Labrador retrievers with HNPK remains unknown. The aim of this study was the phenotypic characterization of the altered keratinocyte differentiation and cornification process in affected Labrador retrievers having a mutation of the SUV39H2 gene. Formalin-fixed biopsies of the nasal planum of dogs with HNPK (n = 6) and nonaffected control dogs (n = 6) were collected and screened by immunofluorescence for the presence and distribution of selected epidermal proliferation and differentiation markers, including Ki-67, involucrin, loricrin, desmogleins 1 and 2 (DSG 1/2) and keratins K1, K10 and K14. Ki-67 staining results suggested that epidermal proliferation was not enhanced. Immunostaining for K14, K1, K10 and DSG1/2 was similar between affected and control biopsies. In contrast, the expression of loricrin and involucrin was altered in biopsies from affected dogs. Our study results suggest that mutations in the SUV39H2 gene cause abnormal terminal differentiation of nasal planum keratinocytes.

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Chitin and lipopolysaccharide modulate innate immune responses of the canine keratinocyte cell line CPEK

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Toll-like receptors (TLRs) play a central role in the cutaneous innate immune system. Chitin is a component of several pathogens, including ectoparasites and fungi, and it can be recognized by the cutaneous innate immune system. Lipopolysaccharide (LPS) is found in the outer membrane of Gram-negative bacteria and it is known to elicit strong immune responses. We hypothesized that exposure to chitin and LPS would activate innate immune responses of keratinocytes by modulating TLR expression and chemokine secretion. The CPEK canine cell line was cultivated and then stimulated with sonicated chitin (0.2 and 2 mg/mL) and with LPS (10 and 100 μg/mL). The expression of TLRs 1–9, interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) was measured after 3, 24 and 48 h. Nonstimulated CPEK cells (control cells) expressed TLRs 1–6 and TLR-9 and did not express TLRs 7, 8 and 10. Chitin induced a marked increase in the expression of TNF-α and slightly downregulated the expression of IL-8. At high concentrations, chitin strongly induced expression of TNF-α and was toxic to keratinocytes at 48 h. Lipopolysaccharides upregulated the expression of TLR4 and TNF-α and slightly downregulated the expression of IL-8. At high concentrations, chitin strongly induced expression of TNF-α and was toxic to keratinocytes at 48 h. Lipopolysaccharides upregulated the expression of TLR4 and TNF-α and downregulated the expression of IL-8. These results suggest that canine keratinocytes can recognize chitin and LPS, which appear to modulate the innate immune response. Upregulation of TLR-4, as observed in other species, seems to play a central role in this process.

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Cutaneous larva migrans in an immunocompromised dog with a multiple nematode infection

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Angiostrongylus vasorum is a nematode classically responsible for respiratory, neurological and gastrointestinal signs and/or bleeding in dogs. Skin lesions associated with this parasite are very unusual. We report here one case of cutaneous larva migrans compatible with this diagnosis. A 3-year-old female Weimaraner was referred for acute lesions on the nose, ear pinnae and one foot. The dog was otherwise healthy but had been under long-term treatment with glucocorticoids and azathioprine for aseptic meningitis for 2 years. Clinical signs included erythema, alopecia, papules and raised plaques with hyperkeratosis on the bridge of the nose and the ears pinnae and alopecia, swelling and perionyxis on one foot. No pruritus was reported. Biochemistry was unremarkable except for increased alkaline phosphatase. A complete blood count revealed a mild leukocytosis without eosinophilia. Histopathological examination demonstrated numerous dermal pyogranulomas with eosinophils centred around parasitic elements (200–300 μm long) compatible with larvae of A. vasorum. A Baermann’s test demonstrated the presence of numerous larvae of A. vasorum. Coproscopic examination also demonstrated eggs of Uncinaria stenocephala and Eucoleus boehmi. A few days later the dog developed respiratory distress and lethargy. Chest radiographs showed an alveolar and interstitial opacity compatible with angiostrongylosis. The dog was treated with fenbendazole (Panacur; Intervet, Beaucoze, France) 20 mg/kg/day for 3 weeks. A marked improvement of the skin lesions was reported 5 days after the first dose. To the best of our knowledge, this is the first case of a dog infested with larvae of A. vasorum that presented initially with only skin lesions.

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Certifect-triggered pemphigus foliaceus in dogs: clinical, histological and immunological characteristics

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A recently launched topical ectoparasiticide containing fluralaner and S-methoprene (Certifect; Merial, Duluth, GA, USA) has been associated with the development of an acantholytic pustular dermatitis similar to that of Promeris-triggered pemphigus foliaceus (PF). Our objectives were to describe the clinical and immunological features of this PF-like cutaneous adverse drug reaction (CADR). Twenty dogs with a probable or definitive (Naranjo scale) diagnosis of PF-like CADR were identified between May 2012 and February 2013. Most dogs were middle-aged or older (median, 9 years) and of large size (median, 24 kg). In six dogs (30%), the PF-like lesions remained confined to the site of application, while 14 dogs (70%) exhibited lesions at distant sites. One or two applications of Certifect were sufficient to trigger PF-like lesions in seven (35%) and six (30%) dogs, respectively. Systemic signs were reported in eight dogs (40%), all with lesions extending to sites distant from application areas. Tissue-bound antikeratinocyte IgG were detected in the lesional epidermis of 8/18 (44%) cases by direct immunofluorescence, while serum antikeratinocyte IgG were detected in 9/14 (64%) cases by indirect immunofluorescence. Autoantibodies were found to target canine desmoglein-1 in 11/14 dogs (79%), but not canine desmoglein-1, by indirect immunofluorescence on transfected cells. These immunological findings were similar in cases with localized and distant disease. In conclusion, Certifect is capable of triggering the development of an acantholytic pustular dermatitis that is a close clinical, histological and immunological match for Promeris-triggered PF and naturally occurring autoimmune PF in dogs.

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Evaluation of pruritic reflexes used for the diagnosis of flea-related dermatoses in dogs

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The purpose of this study was to evaluate the usefulness of induced pruritus reflexes on previously identified pru-
ritic areas to differentiate dogs with flea infestation (FI, abdomen) or flea-bite hypersensitivity (FBH, dorsolumbar area) from those with skin diseases not related to fleas. Two body areas were selected for a maximum of 30 s by the clinician: the umbilicus and the dorsolumbar area. A reflex was considered positive if the scratching triggered a pedalling motion, a labial motion, licking or chewing. This study included 191 dogs: 90 with flea-related dermatoses (FRD; 31 FBH and 59 FI) and 101 dogs with skin diseases not due to fleas (nonflea-related dermatoses; NFRD). Umbilical and dorsolumbar reflexes were positive in 52 (58%) and 47 dogs (52%) with FRD, respectively; both were negative in 27 of these dogs (30%). Umbilical and dorsolumbar reflexes were positive in five dogs each (5%) with NFRD; both were negative in 92 of these dogs (91%). The positivity of either umbilical or dorsolumbar reflexes, or of both, was not correlated specifically to FI or FBH, but to FRD as a group. For the diagnosis of FRD, a positive reflex at either the umbilicus or the dorsolumbar area had a sensitivity of 77%, a specificity of 87%, a positive predictive value of 87% and a negative predictive value of 77%. In conclusion, umbilical and dorsolumbar reflexes appear useful in helping to diagnose FRD in the absence of visible fleas or of classic lesions of FBH.

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Specific increased level of peripheral blood CD34+ cells in dogs with canine atopic dermatitis

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The bone marrow might be involved in human atopic diseases, as shown by the specific release of CD34+ cells into the peripheral blood. The purpose of this study was to determine whether a specific increase of CD34+ cells was also seen in atopic dogs. Three groups of dogs were included: those with nonfood-induced atopic dermatitis (NFIAD; 27), healthy dogs (13) and dogs with nonallergic inflammatory diseases (16). Dogs with NFIAD were selected after fulfillment of Favrot’s criteria with the exclusion of other pruritic dermatoses, rigorous flea control and after no improvement following a hypallergenic diet trial. Healthy dogs did not have any history or clinical signs of cutaneous or systemic diseases. Blood samples were obtained from all dogs, and CD34+ cells were stained with phycoerythrin-conjugated anti-canine CD34 and enumerated using a flow cytometer. Kruskal-Wallis, Mann-Whitney and nonparametric Spearman’s rank correlation tests were used to analyse the data. Numbers of peripheral CD34+ cells in dogs with NFIAD (median, 1.7) were statistically higher than in dogs with other nonallergic inflammatory diseases (median, 1.0; \( P = 0.01 \)) or in healthy dogs (median, 0.9; \( P = 0.009 \)). In dogs with NFIAD, a correlation was not noted between the numbers of CD34+ cells and the lesional (CADESI-03) or the pruritus (visual analog scale) scores. Results of this study suggest a possible involvement of CD34+ cells in dogs with NFIAD. These observations are consistent with those seen in human atopic dermatitis, although the role of these cells in the disease itself remains unclear.

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A case of generalized verrucosis associated with papillomavirus 9 infection in a dog

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A 7-year-old female American Staffordshire bull terrier was presented for generalized alopecia and recurrent demodicosis. Physical examination revealed pyrexia, depression and peripheral lymphadenopathy. Skin lesions consisted of generalized alopecia, hyperpigmentation, multiple dark exophytic warts and slightly prominent raised plaques affecting mainly the abdomen and coalescing on the distal limbs. Deep scrapes on alopecic areas demonstrated numerous Demodex canis mites. Histopathological examination of the exophytic lesions revealed a prominent granular layer with large keratohyalin granules. Keratinocytes had a pale cytoplasm and oval hypocromic nuclei with marginated chromatin. Eosinophilic intracytoplasmic aggregates and intranuclear viral inclusions were visible; mitoses were frequently observed. An infection with canine papillomavirus 9 (CPV9) was confirmed by PCR. The dog was treated orally with ivermectin (0.5 mg/kg once daily; Ivermectil, Vileuraube, France) and interferon-α (60 IU/day; Roferon-A; Roche, Boulogne, France). The demodicosis underwent remission within 4 months, but no improvement was noted for the papillomatosis. To our knowledge, this is the second isolation of CPV9 infection in a dog but the first report of this unusual clinical presentation. We propose to define this condition generalized verrucosis. This term was employed previously to describe a dog with a diffuse oral CPV infection that spread to the skin. In humans, however, generalized verrucosis is used for widespread cutaneous papillomavirus infections with up to thousands of lesions across the body; it is generally observed in patients with acquired immunodeficiency status or congenital immunodeficiencies. In our case, an immunodeficiency was suspected.
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because of coexisting demodicosis, but it remained unproven.

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The use of deslorelin to promote hair regrowth in dogs with alopecia X

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Alopecia X affects dogs such as Nordic breeds, Pomeranians and miniature poodles. Its pathogenesis is not completely understood; it may be different from breed to breed. Treatment with hormones, mitotane, neutering and triostane has given inconsistent results. Deslorelin (Suprelorin; Virbac, Bury St Edmunds, UK) is a nonsteroidal, peptide-based contraceptive implant containing a GnRH agonist, licensed for the induction of temporary infertility in healthy, noncastrated adult male dogs. Whether it exerts any role on the hormonal receptors at the skin/hair follicle levels is unknown. Our aim was to study whether the deslorelin implant promoted hair regrowth in dogs with alopecia X. Three chow chows, two keeshonds, one Chihuahua, one Pomeranian and one toy poodle were diagnosed with alopecia X, after ruling out other causes of alopecia by performing routine dermatological tests, an adrenal–gonadal and thyroid hormonal evaluation and skin biopsy. All dogs received a subcutaneous implant of deslorelin (4.7 mg per dog), and all treated dogs showed a progressive and profuse regrowth of hair within 2–4 months. Adverse effects were not noted, other than a decreased testicular size in intact males. Our findings suggest that deslorelin could be used to promote hair regrowth in dogs with alopecia X. This therapeutic approach may provide a more cost-effective treatment, as it appeared to be well tolerated in this group of dogs. Further studies are required to determine the long-term efficacy and safety of deslorelin for treatment of alopecia X in dogs.

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A cross-sectional survey of leishmaniosis in clinically normal and sick cats in Greece with indirect immunofluorescence antibody test and enzyme-linked immunosorbent assay

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Cats living in areas where canine leishmaniosis is endemic may become exposed to the parasite and develop anti-Leishmania antibodies. The aims of the present survey were to evaluate a population of clinically normal cats and cats with various diseases living in an endemic area for the presence of anti-Leishmania IgG and IgM, to compare the results of indirect immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) for anti-Leishmania IgG and to investigate for possible associations between seropositivity to Leishmania spp. and several possible risk factors. Fifty clinically normal cats and 50 cats with various diseases were screened for anti-Leishmania IgG by IFAT and ELISA and for anti-Leishmania IgM by IFAT. Cut-off values for either test were established using serum samples from 25 clinically normal cats and 50 sick cats from a nonendemic area (TX, USA). Low levels of anti-Leishmania IgG were detected by IFAT in 10/100 (five clinically normal and five sick cats) and by ELISA in 1/100 (one IFAT-negative clinically normal cat), whereas IgM antibodies were present in a single clinically normal cat. Seropositivity for Leishmania was not associated with signalment, living conditions or health status or with seropositivity to feline leukaemia virus, feline immunodeficiency virus, feline coronavirus, Toxoplasma gondii and Bartonella henselae. The low serum levels of anti-Leishmania IgG and the discordant results between IFAT and ELISA may challenge the validity of using serology in epidemiological studies in cats. The reasons for this discordance in serological results must be explored further.

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**Conflict of interest:** None declared.
Selection of an efficacious dosing regimen of oclacitinib (Apoquel®; Zoetis) for the control of atopic dermatitis in client-owned dogs using visual analog scale and CADESI scores

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Oclacitinib inhibits the function of multiple pro-inflammatory and pruritogenic cytokines that are dependent on Janus kinase (JAK) enzyme activation. Oclacitinib preferentially inhibits JAK1-dependent cytokines. The efficacy of three doses and regimens of oclacitinib for the control of canine atopic dermatitis compared with placebo was evaluated. Fourteen dermatologists enrolled 220 dogs with a history of chronic nonseasonal atopic dermatitis. Dogs were randomly allocated to one of the following four treatment groups: T01, placebo; T02, oclacitinib 0.4–0.6 mg/kg twice daily from day 0 to 14 followed by once daily until day 112; T03, oclacitinib 0.4–0.6 mg/kg once daily from day 0 to 112; and T04, oclacitinib 0.2–0.3 mg/kg once daily from day 0 to 112. Treatment success was defined as a ≥2 cm reduction from baseline for owner visual analog scale score for pruritus or by a ≥50% reduction from baseline CADESI-02 scores. For pruritus assessments on days 28, 56, 84 and 112, the three oclacitinib-treated groups showed a larger percentage of treatment success than the placebo-treated controls. Within the oclacitinib-treated groups, T02 had a larger percentage of treatment success than the placebo-treated controls. Within the oclacitinib-treated groups, T02 had a larger percentage of treatment success than the placebo-treated controls. The twice-daily/once-daily dosing regimen (T02) was selected for later clinical trials.

Source of funding: Pfizer Animal Health, now Zoetis.

Conflict of interest: All authors are current or former employees of Zoetis, formerly Pfizer Animal Health.

The effect of flea treatment on the efficacy of oclacitinib (Apoquel®; Zoetis) for the treatment of pruritus associated with canine allergic dermatitis

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Oclacitinib inhibits the function of multiple pro-allergic, pro-inflammatory and pruritogenic cytokines that are dependent on Janus kinase (JAK) enzyme activity. Four hundred and seven client-owned dogs with moderate-to-severe owner-assessed pruritus and a presumptive diagnosis of allergic dermatitis were enrolled. Dogs were randomized to receive either oclacitinib at 0.4–0.6 mg/kg orally twice daily or an excipient-matched placebo. A 10.0-cm-long visual analog scale (VAS) was used by owners to assess pruritus severity from days 0 to 7. Treatment success was defined as achieving a ≥2 cm reduction from baseline VAS score on at least 70% of the study treatment days assessed (i.e. day 1 to day 7). Treatment success on day 7 was compared between dogs receiving flea control or not on day 0, but regardless of whether fleas were present at that time. Sixty-five of 407 dogs (16%), [27/204 (13%) placebo treated and 38/203 (18%) oclacitinib treated] received flea products on day 0. On day 7, the percentage of treatment success for oclacitinib-treated dogs was approximately the same whether or not they were treated for fleas (63 versus 67%). In contrast, initiating flea treatment in placebo-treated dogs on day 0 doubled the percentage of treatment success (52%) compared with dogs not treated for fleas (26%). In summary, adding flea treatment on day 0 did not appear to impact the efficacy of oclacitinib for pruritus control in dogs with allergic dermatitis, but it could explain up to half of the pruritus reduction observed in placebo-treated dogs.

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Conflict of interest: All authors are current or former employees of Zoetis, formerly Pfizer Animal Health.
Canine nasal dermatitis: histopathological and immunopathological features of discoid lupus erythematosus and leishmaniosis

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In areas where canine leishmaniosis (CanL) is endemic, the most important clinical differential diagnoses for nasal planum erosive–ulcerative dermatitis in dogs are discoid lupus erythematosus (DLE) and CanL. The objective of this study is to compare histopathological and immunopathological features of nasal biopsies from dogs with DLE and CanL, both diagnosed on the basis of compatible clinical signs, histopathology results and response to treatment. Furthermore, CanL was confirmed through the demonstration of intralesional Leishmania by immunohistochemistry using a standard protocol and a polyclonal anti-Leishmania spp. antibody. Sections of paraffin-embedded samples from 14 cases of DLE and seven cases of CanL were stained with haematoxylin and eosin. Additionally, serial sections were immunostained for T lymphocytes (CD3), B lymphocytes (CD20) and macrophages (Mac387), with positively stained cells counted in the dermis using image analysis software. Superficial band-like and perivascular mononuclear cell-rich inflammation with basal cell damage was observed in both DLE (13/14) and CanL (6/7). A nodular-to-diffuse superficial and/or deep mononuclear cell-rich infiltrate was seen only in CanL (4/7). CD20-positive cells predominated over both CD3- and Mac387-positive cells in both DLE and CanL. The number of dermal Mac387-positive cells was higher in CanL compared with DLE. In conclusion, a band-like lymphoplasmacytic dermatitis with basal cell damage, a pattern suggestive of chronic DLE, was also found commonly in nasal biopsies from dogs with leishmaniosis. As a result, where CanL is endemic, the presence of Leishmania should be investigated by immunohistochemistry in samples showing a histopathological pattern suggestive of DLE.

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Orodental diseases and dermatological disorders are highly associated in pet rabbits: a case–control study

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Dermatomial disorders (DDs) and orodental diseases (ODDs) are a major source of morbidity in pet rabbits. Additionally, ODD has anecdotally been associated with cutaneous disorders. The purpose of this study was to analyse the possible association between DDs and ODDs, in particular whether ODDs increased the risk of DD development in pet rabbits. Medical records of 222 pet rabbits seen in 2010 in 20 private veterinary clinics in and around Naples (Italy) were retrospectively evaluated. Records of rabbits diagnosed with DDs were selected. The frequencies of ODDs and other variables were evaluated in rabbits with and without DDs using logistic regression. Rabbits seen during the same time period without a diagnosis of DDs were included as controls. The prevalence of DD was 28% (63/222) and that of ODD was 23% (51/222). There was a significant association between

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Leporacarus gibbus infestation in client-owned rabbits and their owner

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Leporacarus gibbus is an uncommonly reported rabbit fur mite infesting laboratory and pet rabbits. It usually causes subclinical infection and only rarely pruritic dermatitis. Two pet rabbits, living in the same household, presented with moderate scaling, erythema, pruritus and alopecia. The lesions were located mainly on the neck in both rabbits. A pruritic papular dermatitis was present on the owner’s arms and legs. Parasitological examination of the rabbits’ skin and fur revealed L. gibbus. Skin cytology and fungal culture were negative for bacterial and dermatophyte infections, respectively. Both rabbits were treated with a single application of a spot-on formulation of 1% moxidectin and 10% imidacloprid (Advocate; Bayer Animal Health, Leverkusen, Germany). The environment was also disinfected with a miticide. After treatment, the clinical signs of these two rabbits improved markedly, and the lesions on the owner’s arms disappeared. Leporacarus gibbus dermatitis in humans has been reported only once in the UK. The main lesions described here included small cutaneous papules on the owner’s arms and legs. Due to its zoonotic potential, even though it is uncommon, L. gibbus should always be considered as a possible differential diagnosis in pet rabbits when their owners are exhibiting a papular dermatitis.

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DDs and ODDs; rabbits diagnosed with ODDs were 63 times more likely to be diagnosed with DDs in comparison to rabbits without ODDs (odds ratio 63; 95% confidence interval 23.9–170.2; \( P < 0.0001 \)). Rabbits with ODDs appear to be at greater risk of developing cutaneous disorders. Although coat condition and hair quality can be influenced by many biological and environmental factors, ODD should be carefully considered as a possible underlying condition in rabbits with DDs. Further prospective studies are needed to evaluate a causal association and pathological factors.

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Identification of three different *Demodex* species in cats using a novel PCR assay

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Demodex cati and *Demodex gatoi* are considered to be the two *Demodex* species of cats. However, several reports have also identified *Demodex* mites morphologically different from these two species in cats. DNA amplification/sequencing has been used effectively to identify *Demodex* mites in humans and dogs. The goals of this investigation were to develop a PCR technique to identify feline *Demodex* mites and to use this technique to investigate the prevalence of *Demodex* in cats. *Demodex cati* mites were obtained from a 16-year-old domestic short hair cat with bilateral ceruminous otitis. *Demodex gatoi* mites were obtained from a 2-year-old Cornish rex cat with pruritic dermatitis. *Demodex* mites, classified morphologically as a third species, were obtained from a 3-year-old domestic long hair cat with partial alopecia. DNA was extracted, and a 301 or 331 bp fragment of the 16S rDNA was amplified and sequenced. Sequences of *D. cati* and *D. gatoi* mites shared 100% identity with those published in GenBank. The sequence of the third unnamed species was different, as it exhibited only 79 and 77% identity with the *D. gatoi* and *D. cati* sequences, respectively; this confirmed that it was a distinct *Demodex* species. Further hair samples from 11 cats were taken from 10 skin locations. Three cats were positive for *Demodex* DNA. In one case, the sequence corresponded to that of *Demodex canis*. A larger epidemiology study is underway, but preliminary results indicate that at least three *Demodex* species can be found in cats, and that *D. canis* mites can be found occasionally on feline skin.

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A retrospective study on the prevalence and causative allergens of food-induced atopic dermatitis in France

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Cutaneous adverse food reactions (CAFRs) are known to trigger flares of atopic dermatitis (AD) in some dogs. The aim of this retrospective study was to determine the prevalence of CAFRs in dogs with nonseasonal AD (i.e. food-induced AD; FIAD) in a French specialty clinic. From July 2009 to January 2012, 578 dog files were randomly selected. Canine AD was diagnosed in 336/578 dogs (58%) based on Favrot’s criteria. After exclusively feeding a commercial hydrolysed diet (CHD; z/d ULTRA; Hill’s Pet Nutrition, Sophia-Antipolis, France) for 8–10 weeks, 123/336 dogs (37%) were determined by the dermatologist to be in full remission. Of these, 36/123 dogs (29%) were exclusively fed the CHD and remained in remission for at least 1 year (presumed FIAD). Fifty of 123 dogs (41%) underwent selected food challenges that triggered pruritus (confirmed FIAD). Positive challenges were found for zero, one, two, three or four food items in 12/50 (24%), 22 (44%), 11 (22%), three (6%) and two (4%) cases, respectively. Beef, chicken, lamb or pork meats, dairy products, rice and wheat were the offending allergens in 16/50 (32%), 13 (26%), 10 (20%), eight (16%), seven (14%), three (6%) and one (2%) dog, respectively. In this group of French dogs with nonseasonal AD, the prevalence of confirmed and presumed FIAD was 11% (38/336 dogs) and 14% (48/336 dogs), respectively. Regrettably, approximately one-third of owners did not agree to follow the restrictive part of the diet with complete provocative food challenges to confirm the diagnosis of FIAD.

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Prevalence of papillomavirus EcPV2 in clinically healthy horses in Switzerland

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The development of several genital disorders in horses, including penile papillomas and squamous cell carcino-
mas, has been proposed to be dependent on an infection with equine papillomavirus type 2 (EcPV2). However, little is known about the prevalence of this virus. Therefore, the aim of this study was to determine the geno- and seroprevalence of EcPV2 in clinically healthy horses in Switzerland. Cytobrush samples from the penis or vulva and serum samples were collected from 50 horses displaying neither skin or mucous membrane pathology nor severe signs of other diseases. To determine the genoprevalence of EcPV2, DNA was extracted from the cytobrush samples and tested for viral DNA with a PCR assay that amplifies a 338 bp fragment of the E7/E1 region of the viral genome. To determine the seroprevalence of this virus, an enzyme-linked immunosorbent assay was designed to detect specifically antibodies against the major capsid protein (L1) of EcPV2. The EcPV2 DNA was amplified by PCR, and further sequencing confirmed viral identity in 9/50 horses (18%). Antibodies against EcPV2 were detected alone in 31/50 horses (62%), while viral DNA and EcPV2-specific antibodies were found together in 4/50 horses (8%). This high seroprevalence suggests that EcPV2 is circulating intensely in the Swiss equine population, whereas active infections seem to be less common. The discrepancy between geno- and seroprevalence indicates different stages of infection in the tested population.

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Conflict of interest: None declared.

Oclacitinib (Apoquel®, Zoetis) is a novel Janus kinase inhibitor that has activity against canine pro-allergic and pro-inflammatory cytokines

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Cytokine dysregulation can orchestrate a variety of cellular and molecular changes that lead to chronic conditions, including allergy, in dogs. Many cytokines thought to trigger such changes bind receptors that activate Janus kinase (JAK) enzymes. The objective of this study was to determine whether the novel JAK inhibitor oclacitinib could reduce the activity of a variety of cytokines thought to induce many of the clinical signs associated with allergic conditions in dogs. Using isolated enzyme systems and in vitro human or canine cell models, the potency and selectivity of oclacitinib was evaluated against individual JAK family members as well as cytokines dependent on JAK activation. Oclacitinib inhibited JAK family members by 50% at concentrations (IC50) ranging from 10 to 99 nmol/L and did not inhibit a panel of 38 other non-JAK kinases (IC50 values >1000 nmol/L). Oclacitinib was most effective at inhibiting JAK1 (IC50 = 10 nmol/L). Oclacitinib also inhibited the function of JAK1-dependent cytokines involved in allergy and inflammation [ interleukin (IL)-2, IL-4, IL-6 and IL-13], as well as those that cause pruritus (IL-31) at IC50 values ranging from 36 to 240 nmol/L. Oclacitinib had minimal activity against JAK2-dependent cytokines involved in haematopoiesis [erythropoietin and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF); IC50 >1000 nmol/L], and it did not inhibit other JAK2-dependent cytokines involved in innate immune responses (IL-12 and IL-23; IC50 >3000 nmol/L). These results demonstrate that oclacitinib selectively inhibits JAK1-dependent cytokines involved in allergy, inflammation and pruritus. As a result of this widespread yet role-specific anti-cytokine activity,
oclacinib is likely to be effective in treatment of clinical signs of allergic diseases in dogs.

Source of funding: Pfizer Animal Health, now Zoetis.

Conflict of interest: all authors are current or former employees of Zoetis, formerly Pfizer Animal Health, or employees of Pfizer.

Evaluation of patch testing with proteins, carbohydrates and commercial foods for diagnosis of canine adverse food reactions

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The gold standard for diagnosing canine adverse food reactions (AFRs) remains a dietary restriction–provocation trial. Recently, patch testing with food allergens was reported to be helpful for choosing ingredients for an elimination diet. The aim of this study was further to evaluate patch testing with proteins, carbohydrates and commercial foods in dogs. In 25 dogs suspected of AFRs, patch testing was performed with raw and cooked meat (n = 16), salmon and carbohydrates (n = 11) and with their own commercial foods (n = 4); the median number of patches per dog was 21 (range 17–30). After 48 h, skin reactions were evaluated. In each dog, patch test results were compared with the outcome of sequential oral food challenges. Overall, the sensitivity of patch testing was 78% and its specificity 82% (138 comparisons patch versus challenges). For proteins (meats and salmon), the sensitivity was 100% and specificity 69% (62 comparisons), for carbohydrates 70 and 83%, respectively (49 comparisons). For commercial foods, the sensitivity was low (22%) but the specificity highest (100%; 27 comparisons). A positive patch reaction was observed to both raw and cooked proteins in 93% of cases and to raw proteins in the others; there were no reactions solely to cooked meats. In conclusion, patch testing seems a useful tool to predict food antigen reactivity, especially against meat proteins, with the majority of positive reactions being seen against raw proteins.

Source of funding: Royal Canin.

Conflict of interest: C. Johansen’s residency is supported by Royal Canin; C. Mariani is an employee of this company; in the last 5 years, R. Mueller has obtained funding, lectured or consulted for Royal Canin.

First report of straelensiosis in cats and unique features of the canine disease in Israel

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Straelensia cynotis is a parasitic larva that causes nodular dermatitis in outdoor dogs. To date, the disease has been documented in dogs in France, Spain and Portugal. Here, we report the first cats infested with this parasite, as well as some unique aspects of canine straelensiosis in Israel. Two cats and 10 dogs were diagnosed with straelensiosis between February 2003 and January 2012 in Israel. Both cats exhibited erythematous macules and nodules on the abdomen; one was extremely pruritic, while lesions were incidentally detected during neutering in the other cat. The histopathology of feline straelensiosis appeared similar to that of the canine disease. Clinical signs in dogs included multiple small erythematous or crusted papules scattered over the head, dorsum and limbs. Additionally, this disease was associated with severe pruritus in 6/10 dogs (60%). Treatment consisted of different combinations of drugs, including systemic avermectins and topical insecticides or acaricides. All animals experienced a complete resolution of clinical signs with any therapeutic regimen used; amitraz was the most effective treatment, because it led to fast (median, 5 weeks) and complete resolution of signs in the five dogs that received it. In 4/5 dogs the treatment consisted of 1:200 amitraz dips every 4–7 days, while the last dog wore an amitraz collar. In summary, we report that cats are also susceptible to straelensiosis, which causes erythematous ventral lesions in this species. Furthermore, in Israel, canine straelensiosis is often very pruritic and appears to respond best to amitraz.

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Abstracts

Case–control risk factor study for meticillin-resistant Staphylococcus pseudintermedius infection in dogs and cats in Germany

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Meticillin-resistant Staphylococcus pseudintermedius (MRSP) has emerged as a highly drug-resistant pathogen. Although often isolated from outpatients in veterinary clinics, there is concern that MRSP could follow a similar epidemiology to meticillin-resistant Staphylococcus aureus (MRSA), which is an important nosocomial pathogen for humans. Our objective was to identify risk factors for MRSP infections in dogs and cats in Germany. Clinical data from cases of MRSP (n = 150) and meticillin-susceptible Staphylococcus pseudintermedius (MSSP) controls (n = 133) 6 months prior to staphylococcal isolation were analysed by multivariable logistic regression. The identity of all MRSP isolates was confirmed genotypically through related phenotypic resistance was assessed by agar dilution screening, including erythromycin, kanamycin, streptothricin and trimethoprim. Amongst mecA-positive SP, two genetically distinct types were found: strain ST71 possessed a Tn5405-like transposon, carrying five resistance genes (aphA, sat, aadE, ermB and dfrG); Tn916 (tetM) and IS1272 (aac-aph) were characteristic for other types. Resistance to β-lactam antibiotics correlated with the presence of mecA, while the multidrug-resistant phenotype (13% of MSSP and 88% of MRSP) was associated with the presence of the Tn5405-like transposon (P < 0.001). Despite the seemingly sudden emergence of MRSP, at least two different MRSP genetic backgrounds (ST71 and ST118) have evolved independently in Europe. Each has unique transposon and resistance profiles, involving four distinct MGEs. These MGEs in MRSP are a risk for horizontal gene transfer into the human pathogen Staphylococcus aureus and are a public health concern.

Source of funding: ESVD research grant.

Conflict of interest: None declared.

Genetic insights into the emergence of multidrug resistance in meticillin-resistant Staphylococcus pseudintermedius

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Staphylococcus pseudintermedius (SP) is a common canine pathogen, and most infections are treated successfully with antimicrobials. However, meticillin-resistant strains (MRSP) with a multidrug-resistant phenotype have recently emerged. In MRSP infections, all classes of clinically relevant antimicrobial agents may be ineffective, and zoonotic infections can occur. Our objectives were to identify mobile genetic elements (MGEs) responsible for the multidrug-resistant phenotype of MRSP. Antimicrobial resistance genes, putative MGEs and their genomic location were identified by sequencing and analysing five MRSP genomes using the Illumina HiSeq platform. A collection of 60 meticillin-susceptible (MS) SP and 64 MRSP from the UK and Germany were screened by PCR for the presence of MGEs, including mecA and the transposase of Tn5405. Related phenotypic resistance was assessed by agar dilution screening, including erythromycin, kanamycin, streptothricin and trimethoprim. Amongst mecA-positive SP, two genetically distinct types were found: strain ST71 possessed a Tn5405-like transposon, carrying five resistance genes (aphA, sat, aadE, ermB and dfrG); Tn916 (tetM) and IS1272 (aac-aph) were characteristic for other types. Resistance to β-lactam antibiotics correlated with the presence of mecA, while the multidrug-resistant phenotype (13% of MSSP and 88% of MRSP) was associated with the presence of the Tn5405-like transposon (P < 0.001). Despite the seemingly sudden emergence of MRSP, at least two different MRSP genetic backgrounds (ST71 and ST118) have evolved independently in Europe. Each has unique transposon and resistance profiles, involving four distinct MGEs. These MGEs in MRSP are a risk for horizontal gene transfer into the human pathogen Staphylococcus aureus and are a public health concern.

Source of funding: Royal Veterinary College.

Conflict of interest: None declared.
**Antimicrobial susceptibility monitoring of dermatological pathogens isolated from diseased dogs and cats across Europe (ComPath I, 2008–2010)**

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ComPath is the first pan-European antimicrobial susceptibility monitoring programme for pathogens isolated from diseased dogs and cats not recently treated with antimicrobials. Samples were collected from animals with skin, ear and soft tissue infections in 10 European countries. Aerobic bacteria were isolated and identified by standard biochemical methods in national laboratories (one strain per bacterial species per animal per owner). Minimal inhibitory concentrations (MICs) were determined for 14 commonly used antibiotics in a central laboratory by agar dilution according to CLSI M31-A3 standards. Results were interpreted using CLSI breakpoints where available. In total, 1182 canine and 226 feline strains were recovered. In dogs, *Staphylococcus pseudintermedius* (n = 556) susceptibility varied from 70–80% for penicillin, clindamycin and chloramphenicol to ≥91% for amoxicillin–clavulanic acid, ampicillin, oxacillin, gentamicin, enrofloxacin, marbofloxacin and orbifloxacin, and for *Staphylococcus aureus* (n = 45) from 49–58% for penicillin and amoxicillin to ≥91% for amoxicillin–clavulanic acid, oxacillin, gentamicin, clindamycin, chloramphenicol, enrofloxacin, marbofloxacin and orbifloxacin. For canine streptococci (n = 167), resistance against penicillin, amoxicillin–clavulanic acid, ampicillin, chloramphenicol, enrofloxacin, marbofloxacin and orbifloxacin was very low (≤2%). Canine *Escherichia coli* (n = 108) showed good susceptibility (≥86%), except to ampicillin, whereas resistance was frequently seen in *Pseudomonas* spp. (n = 173). Generally, susceptibility ranges for the feline isolates were comparable to those for dogs. In total, 44 staphylococci harboured a mecA gene, 36 from dogs and eight from cats. This is the first international antimicrobial susceptibility monitoring programme to use standardized methods and centralized MIC determination.

**Sources of funding:** Bayer, MSD, Novartis, Vetoquinol, Virbac and Zoetis.

**Conflict of interest:** All authors are full-time employees or consultants of the above-listed veterinary pharmaceutical companies.

In *vitro* activity of pradofloxacin against canine and feline pathogens recovered from skin infections in four European Union countries

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Pradofloxacin (Veraflox; Bayer Animal Health, Leverkusen, Germany) is a novel fluoroquinolone approved for the treatment of bacterial infections in dogs and cats. It has enhanced antibacterial activity compared with conventional fluoroquinolones, covering major Gram-positive and Gram-negative aerobic and anaerobic pathogens. The aim of this survey was to study the pradofloxacin susceptibility of common aerobic bacterial pathogens isolated from acute skin and soft tissue infections in dogs and cats across Europe. Pretreatment bacterial isolates from dogs and cats with skin infections were obtained from local diagnostic laboratories in Germany, Hungary, Sweden and the UK between 2007 and 2012. Minimal inhibitory concentration (MIC) values were determined by the agar dilution method (CLSI; M31-A3, 2008), and MIC\(_{50}\) and MIC\(_{90}\) were calculated. In total, 1049 isolates were tested, 77% from dogs and 23% from cats. The most frequently isolated species was *Staphylococcus pseudintermedius* (n = 469), followed by *Escherichia coli* (n = 90), *Streptococcus canis* (n = 84), *Pasteurella multocida* (n = 68), coagulase-negative staphylococci (n = 46), *Pseudomonas aeruginosa* (n = 38), *Staphylococcus aureus* (n = 33) and various minor species (n = 221). For the major species, MIC\(_{50}\) values ranged from 0.008 to 0.06 μg/mL, except for *S. canis* (0.125 μg/mL) and *P. aeruginosa* (0.25 μg/mL). The MIC\(_{90}\) values varied between 0.015 and 0.125 μg/mL, except for *P. aeruginosa* (0.5 μg/mL). There were no notable differences in MIC patterns among the four countries. In conclusion, the survey demonstrates high *in vitro* activity of pradofloxacin against major canine and feline pathogens, particularly Gram-positive bacteria, isolated from skin infections.

**Source of funding:** Bayer Animal Health.

**Conflict of interest:** The authors are employees of Bayer Animal Health.

Development of an enzyme-linked immunosorbent assay for the serodiagnosis of ringworm infections in cattle

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Several enzyme-linked immunosorbent assays (ELISAs) have been developed to evaluate antibody responses in dermatophytopsis of animals, but only few focused on detecting specific antibodies in cattle dermatophytosis. The goal of this study was to develop an in-house ELISA based on recombinant antigens for the serological diagnosis of cattle dermatophytosis.
Antigens consisted of available recombinant forms of either *Trichophyton rubrum* dipeptidyl peptidase V (TruDppV) or leucin aminopeptidase 2 (TruLap2), which are 98% identical to *Trichophyton verrucosum* orthologues. Sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive values (NPV) of both ELISAs were determined using field serum samples from 135 cattle with dermatophytosis, diagnosed by microscopy and PCR analyses, and from 55 healthy cattle without a history of dermatophytosis (negative controls). Differences between the optical density (OD) mean values obtained in both animal groups were highly significant, showing that our ELISAs can discriminate between infected and healthy animals (Mann–Whitney U-test; *P* < 0.0001). Using a cut-off point equal to the mean OD + 2SD of control sera, the ELISA detecting specific antibodies against DppV had the following performance: 90% Se, 93% Sp, 97% PPV and 78% NPV. The recombinant TruLap2-based ELISA exhibited 88% Se, 91% Sp, 96% PPV and 76% NPV. This is the first ELISA based on recombinant antigens to assess the immune response in bovine dermatophytosis. It could be useful in epidemiological studies and for the evaluation of vaccines and/or vaccination procedures.

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A pilot uncontrolled open study on the use of Oxalic (Medeor International) for treatment of sebaceous gland adenoma/hyperplasia in dogs

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Oxalic is a solution containing nitric acid, organic acids and metallic salts; it is proposed as a topical alternative to surgery for management of sebaceous gland adenoma/hyperplasia (SGAH). We report here the results of an open, uncontrolled trial to evaluate the efficacy of Oxalic (Medeor International, Braine-l’Alleud, Belgium) for treatment of SGAH in dogs. Healthy dogs of all ages with at least one verrucoid lesion typical of SGAH were included in the study. The presence of pre-existing local inflammation and the use of topical agents other than antiseptics or antibiotics were exclusion criteria. Oxalic was applied, following the manufacturer’s instructions, once on day 0; it was reapplied 14 days later if the clinical response was considered unsatisfactory. Treated lesions size (diameter and height) and subjective evaluation of treatment response were recorded at 2 week intervals. Twenty-nine dogs were included, and 35 SGAH lesions (mean size on day 0, 7.7 mm × 4.3 mm) were treated. Nine of 35 masses (26%) needed retreatment on day 14. On days 14 and 28, significant mean reductions of 34 and 71% (ANOVA, *P* < 0.001), respectively, for lesion diameter, and 61 and 86% (*P* < 0.001), respectively, for lesion height were observed. The owners and veterinarians subjectively evaluated the treatment response as excellent in 89 and 83% of dogs, respectively. The tolerance of Oxalic was reported as excellent by both owners and veterinarians in 97% of dogs. In summary, Oxalic appears to be an effective and well-tolerated topical alternative to surgical treatment for lesions of SGAH in dogs.

Source of funding: Oxalic was provided free of charge by Medeor International.

Conflict of interest: J. Fontaine consults for Medeor International.

Retrospective assessment of previous antibiotic therapy in dogs diagnosed with meticillin-resistant *Staphylococcus pseudintermedius* pyoderma

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The emergence of meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) has become a significant animal health problem. A recent study has indicated that previous antibiotic exposure is a factor in the development of canine MRSP pyoderma. The purpose of this study was to identify any association between prior antibiotic use and MRSP pyoderma in dogs presented to a veterinary teaching hospital. The medical records of canine MRSP and meticillin-susceptible *S. pseudintermedius* (MSSP) pyoderma diagnosed between January 2006 and November 2012 were reviewed. These included cases of deep or superficial, chronic or recurrent MRSP or MSSP pyoderma with at least a 12 month drug history prior to the diagnosis. Fifty-three MRSP cases and 45 MSSP cases met the inclusion criteria; 52/53 (98%) MRSP cases and 42/45 (93%) MSSP cases received at least one course of antibiotics prior to their diagnosis. The number of antibiotic prescriptions in MRSP cases (mean, 4.5) was higher than for MSSP (mean, 2.5; *P* < 0.0001). The number of different antibiotic classes prescribed in MRSP cases (mean, 2.5) was higher than in MSSP (mean, 1.9; *P* = 0.0086). The percentage of MRSP cases given β-lactam antibiotics (98%) was higher than that of MSSP cases (82%; *P* = 0.0066). Finally, the percentage of MRSP cases receiving concurrent anti-inflammatory therapy (e.g. glucocorticoid and ciclosporin) was higher (62%)
than that of MSSP (42%; \( P = 0.048 \)). These results suggest that the number of antibiotic prescriptions, exposure to multiple antibiotic agents or a certain class of antibiotics, and concurrent anti-inflammatory therapy may be associated with MRSP pyoderma.

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Conflict of interest: None declared.

Expression patterns of selected desmosomal, tight and adherens junction proteins in an experimental model of canine atopic dermatitis skin lesions

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In recent years, the stratum corneum was found to be important for providing a functional barrier against environmental allergens in humans and animals with atopic dermatitis (AD). Recently, the expression of intercellular epidermal tight junction proteins was shown to be reduced in human AD, thereby providing further evidence for defective epithelial permeability in this disease. We studied the expression of selected upper epidermal desmosomal, tight and adherens junction proteins in an experimental model of canine AD. Two types of control and house dust mite (HDM) extract-containing patches were applied to the skin of six Maltese–Beagle atopic dogs hypersensitive to HDM. Patches were left on for 48 h, and biopsies were collected 24 h after removal. Normal canine skin served as another control. Frozen skin sections were stained by indirect immunofluorescence for corneodesmosin (CDSN), desmoglein-1 (DSG1), desmocollin-1 (DSC1), claudin-1 (CLDN1) and E-cadherin (CDH1). Each expression pattern was assessed for its continuity on the entire epidermis of each section. The immunostaining of DSG1, DSC1 and CDH1 was intercellular and continuous in all control and HDM patches. In contrast, the immunoreactivity of CDSN and CLDN1 was discontinuous in 12/12 and 8/12 HDM patches, respectively, but in none of the control patches and normal skin (Fisher’s exact test, \( P < 0.001 \)). These observations suggest that HDM allergens, either via proteolytic digestion and/or because of induced allergic inflammation, might affect the integrity of corneodesmosomal and tight junction proteins. Ensuing intercellular junction alterations could promote further penetration of allergens through the epidermis.

Source of funding: Self-funded.

Conflict of interest: None declared.
Evaluation of the usefulness of Doppler blood flow in the diagnosis of canine cutaneous adverse food reactions

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A simple diagnostic tool that could indicate whether a diet is likely to be a flare factor in canine cutaneous adverse food reactions (CAFRs) would be very helpful. In this study, the range of the resistive (RI) and pulsatility indices (PI) in the cranial mesenteric and coeliac arteries were determined in eight healthy dogs fed various diets, and in 12 dogs with previously diagnosed CAFRs when fed appropriate hydrolysed or novel protein diets, as well as during dietary provocations. Resistive index and PI were calculated using Doppler ultrasonography with a microconvex 7.5 MHz probe. Each examination consisted of five ultrasonar assessments: preprandial and 20, 40, 60 and 90 min postprandial. Dogs with CAFRs showed a RI < 0.82, whereas healthy dogs had a RI > 0.82 in both mesenteric and coeliac arteries 60 min after the administration of the provocation diet (P < 0.005). There were no significant differences between groups at the other time points or when the hypoallergenic diet was fed. A significant difference in PI values was not found between groups. Using an RI < 0.82 cut-off in the mesenteric and the coeliac arteries after 60 min of dietary provocation in a group of 21 dogs diagnosed with nonfood-induced atopic dermatitis or CAFR yielded a test with 100% sensitivity, 76% specificity, 50% positive predictive value and 100% negative predictive value for the diagnosis of CAFR. These results suggest that Doppler ultrasonography might have some value in helping diagnose CAFRs in dogs.

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Conflict of interest: None declared.

Histopathological characteristics and expression of Toll-like receptor 2 in lesional skin of dogs with papular dermatitis due to Leishmania

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Papular dermatitis caused by Leishmania infantum (PDL) is considered a mild form (stage I) of canine leishmaniosis. It is associated with negative to low-positive antibody levels and specific cell-mediated immune responses, and with spontaneous resolution and a good prognosis. Dogs affected with PDL could mount a protective immune response useful to future studies. However, this condition is not fully characterized. The aim of this retrospective study was to define the histopathological pattern, the parasite load and the expression of Toll-like receptor 2 (TLR-2) in skin biopsies of dogs with PDL. Routine histology, as well as Leishmania and TLR-2 immunohistochemistry, was performed in skin biopsies from 11 patients and six healthy dogs from a nonendemic area. Moderate to severe granulomatous to pyogranulomatous dermatitis with several histopathological patterns was noted in all the PDL patients. The amastigote numbers ranged from 0 to 200 per high-power field (HPF; mean ± SD, 49 ± 76 amastigotes/HPF). Immunohistochemistry for TLR-2 was strongly positive in the hyperplastic epidermis overlying the dermal infiltrate. The mean number ± SD of TLR-2-positive mononuclear dermal cells was 26 ± 13 cells/HPF. Immunohistochemistry for TLR-2 in normal skin showed only scattered positive basal and suprabasal epidermal and epithelial follicular cells, positive grouped cells in the follicular istium and scattered positive perivascular mononuclear cells in superficial and mid-dermis. In conclusion, PDL exhibited a granulomatous to pyogranulomatous dermatitis, with a variable number of intraleSIONal amastigotes. The TLR-2 expression was increased in comparison to normal skin, as seen in human cutaneous leishmaniosis.

Source of funding: ESVD research grant.

Conflict of interest: None declared.

Intradermal injection of recombinant human type VII collagen restores collagen function in a canine model of dystrophic epidermolysis bullosa

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Dystrophic epidermolysis bullosa (DEB) is an incurable skin fragility disease due to mutations in the collagen VII (C7)-encoding gene. Collagen VII is the major component of anchoring fibrils in the basement membrane zone (BMZ). In our canine spontaneous recessive DEB (RDEB) model that reproduces the main features of human RDEB, we made one injection of purified recombinant human (rh)C7 intradermally into sites situated around lesions on the lips and ears on one side of the body. Saline injections were used as a control on the other side of the body. Skin biopsies taken 1, 2, 4 and 5 weeks after the injection were subjected to immunostaining and immunoelectron microscopy labelling with an anti-human C7 monoclonal antibody that does not cross-react with dog C7. Clinically, the injected sites were less inflamed.
and had no erosions after C7 injection compared with the same sites before injection or nontreated control sites. One week after intradermal injection, the injected rhC7 was incorporated into the affected dog’s BMZ, and it corrected dermo-epidermal separation. The localization of the injected rhC7 was confirmed within the BMZ by co-labelling the same sections of dog skin with a polyclonal antibody that recognizes both canine and human C7; immunoelectron microscopy further established that the injected rhC7 formed anchoring fibrils. This injected rhC7 remained incorporated into the dog’s BMZ for at least 5 weeks. This first study on protein-based therapy in a spontaneous large animal model establishes that intradermally injected human C7 can incorporate into the BMZ and restore C7 function.

**Sources of funding:** VetAgro Sup and Shire Human Genetic Therapies.

**Conflict of interest:** M. de Souza is an employee of Shire Human Genetic Therapies.

### Reproducibility of allergen-specific IgE assays and ensuing immunotherapy recommendations from four commercial laboratories

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Canine allergen-specific IgE assays in the USA are not subjected to an independent laboratory reliability monitoring programme. The objective of this study was to evaluate the agreement of diagnostic results and treatment recommendations of four serum IgE assays available commercially from four laboratories in the USA. Replicate serum samples from 10 atopic dogs were submitted to each of four laboratories in the USA (ACTT, Bio-medical Services, Austin, TX, USA; VARL Liquid Gold, Veterinary Allergy Reference Laboratory, Pasadena, CA, USA; Allercept, Hesca, Loveland, CO, USA; and Greer Aller-g-complete, IDEXX Laboratories, Westbrook, ME, USA). The interlaboratory agreement of standard regional panels and ensuing treatment recommendations were analysed with the kappa (κ) statistic to account for agreement that might occur merely by chance. Six comparisons of pairs of laboratories and overall agreement among laboratories were analysed for ungrouped allergens (as tested) and also with allergens grouped according to reported cross-reactivity and taxonomy. The overall diagnostic agreement between laboratories was only slightly better than expected by random guessing (κ = 0.14). No two laboratories displayed even moderate chance-corrected agreement (κ > 0.40) with each other. The overall agreement of the treatment recommendations was also poor (κ = 0.11). Altogether, 85% of ungrouped allergen treatment recommendations were unique to one laboratory or another. Our study results indicate that the choice of a specific commercial allergen-specific IgE assay is likely to have a major influence on the results obtained and ensuing treatment recommendations.

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

**Conflict of interest:** J. Plant is the owner of RESPIT, LLC.

### Development of a PCR technique specific for *Demodex injai* in biological specimens

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The identification of *Demodex injai* as a second *Demodex* species of dogs has opened new questions and challenges in the study of *Demodex*-host relationships. To advance our understanding of canine demodicosis, we developed a PCR technique with primers based on published genome sequences of *D. injai* from the GenBank. This technique amplified a 238 bp fragment corresponding to a region of the mitochondrial 16S rDNA of *D. injai*. The PCR was positive in DNA samples obtained from mites morphologically identified as *D. injai*, which served as positive controls, and also in samples from three cases of demodicosis in terrier dogs associated with proliferation of mites identified as *D. injai*. Samples of *Demodex canis* and *Demodex folliculorum* were consistently negative with this assay. Hairs with roots plucked from five sites in 19 healthy dogs were investigated for the presence of *D. injai* DNA. Two were positive, confirming that *D. injai* is also a normal inhabitant of canine skin. This sampling technique, however, probably underestimates the prevalence of *D. injai*, because these mites are suspected to live in the sebaceous glands and ducts. Skin samples from seven dogs with generalized demodicosis caused by *D. canis* were all negative with the *D. injai*-specific PCR, demonstrating that the mite proliferation in these dogs was species specific. This technique may be a useful tool in diagnosis as well as in epidemiology and pathogenesis studies.

**Source of funding:** ESVD-ECVD PhD scholarship grant.

**Conflict of interest:** None declared.
Toll-like receptor 2 is overexpressed in dogs with demodicosis, Malassezia dermatitis and cutaneous bacterial infection

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Toll-like receptors (TLRs) are transmembrane proteins that function as pattern-recognition receptors. Toll-like receptor-2 is activated by peptidoglycan of Gram-positive bacteria, yeast zymosan and bacterial lipoproteins, among other compounds. The aims of the present study were to investigate the expression of TLR-2 in normal canine skin (n = 9), canine demodicosis (n = 6), Malassezia dermatitis (n = 6) and bacterial infection (n = 4) using immunohistochemistry. Immunohistochemistry for TLR-2 was considered positive in the case of brown membranous and/or granular cytoplasmic specific staining of cells. Epithelial positivity was graded as absent (only scattered cells positive), mild (weak staining of groups of cells) or strong (strong staining of numerous cells). Normal skin showed scattered positive basal and suprabasal epidermal and epithelial follicular cells, with positive grouped cells in the follicular ostium and mononuclear cells mainly in perivascular superficial and mid-dermis. Toll-like receptor-2 positivity was observed in epidermal and follicular epithelium and in mononuclear inflammatory cells in all lesional samples, with the exception of one bacterial infection. Endothelial cells and fibroblasts were positive in 100, 75 and 50% of cases with demodicosis, bacterial infection and Malassezia dermatitis, respectively. Toll-like receptor-2 staining in the epidermis and hair follicles was graded as severe in 83, 75 and 50% of cases with demodicosis, bacterial infection and Malassezia dermatitis, respectively. Toll-like receptor-2 staining was associated with epidermal hyperplasia and/or spongiosis. In conclusion, these commensal micro-organisms seem to stimulate skin innate immunity, and TLR-2 appears to play a role in the inflammatory response in these cutaneous infections in dogs.

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Conflict of interest: None declared.

Canine epidermal tight junction proteins: comparison of their immunoreactivity in normal and experimental atopic canine skin

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Epidermal tight junctions have been well characterized in humans, and they appear to be involved in many skin diseases, such as atopic dermatitis. In dogs, there is no information regarding the implication of tight junctions in skin diseases such as atopic dermatitis. The aim of this study was to compare the expression and the distribution of zona occludens-1 (ZO-1), occludin and claudin-1 tight junction proteins in normal and experimental atopic canine formalin-fixed, paraffin-embedded skin. Biopsies from six experimentally sensitized atopic beagles were used; these dogs had been sensitized to house dust mites at a young age, and they were known to develop an atopic dermatitis-like pruritic dermatitis following allergen exposure. Samples were obtained prior to allergen challenge from clinically nonlesional skin. Skin specimens from nine healthy dogs without skin lesions were also obtained. Manual immunoperoxidase staining was used to study the immunoreactive pattern of ZO-1, occludin and claudin-1 in normal and experimental atopic canine epidermis. Biopsies from six experimentally sensitized atopic beagles were used; these dogs had been sensitized to house dust mites at a young age, and they were known to develop an atopic dermatitis-like pruritic dermatitis following allergen exposure. Samples were obtained prior to allergen challenge from clinically nonlesional skin. Skin specimens from nine healthy dogs without skin lesions were also obtained. Manual immunoperoxidase staining was used to study the immunoreactive pattern of ZO-1, occludin and claudin-1 in the nonlesional epidermis of both groups of dogs. Positive controls were healthy human skin samples. Immunoreactive patterns were blindly assessed by two investigators. Comparisons between groups were performed using the Wilcoxon–Mann–Whitney test. The mean expression score of claudin-1 was significantly lower in atopic skin compared with that of healthy dogs. Atopic dogs had a significantly lower expression of claudin-1 and ZO-1 along the membranes of the basal cells and a higher cytoplasmic staining for ZO-1 in the stratum granulosum than control dogs. Occludin expression remained similar between groups. These results suggest a possible defect in claudin-1 and ZO-1 expression in experimental canine atopic epidermis.

Source of funding: Self-funded.

Conflict of interest: None declared.
Breed differences in transepidermal water loss and pH among dogs with atopic dermatitis

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Canine atopic dermatitis (AD) is associated with changes in surface epidermal barrier in affected animals. This study evaluated the transepidermal water loss (TEWL) and the skin pH in atopic dogs from three different breeds: French bulldogs, cocker spaniels and Labrador retrievers, as well as that of normal control dogs. Transepidermal water loss was measured with a closed-chamber evaporimeter (Vapometer wireless; Delfin Technologies, Kuopio, Finland), and pH was measured with a pH meter (Mettler Toledo, Barcelona, Spain) on the nonlesional inguinal and axillary skin. Sixty dogs were evaluated: 29 with AD (12 bulldogs, nine cocker spaniels and nine Labrador retrievers) and 31 control dogs (11 bulldogs, nine cocker spaniels and 11 Labrador retrievers). French bulldogs with AD exhibited significantly higher inguinal and axillary pH values than control bulldogs (mean ± SD; 7.9 ± 0.2 versus 6.9 ± 0.2, P = 0.007; and 7.9 ± 0.2 versus 7.1 ± 0.3, P = 0.016, respectively). In contrast, atopic cocker spaniels tended to have an axillary pH lower than that of control cocker spaniels (6.9 ± 0.4 versus 7.8 ± 0.3, P = 0.063). For TEWL, bulldogs with AD had significantly higher axillary TEWL values than control bulldogs (19.3 ± 7.2 versus 11.4 ± 1.2 g/m²/h; P = 0.004); there were no further differences in TEWL values between other sites or groups. In conclusion, atopic French bulldogs exhibit significant differences in TEWL and pH values compared with atopic Labrador retrievers, cocker spaniels and normal dogs. These observations suggest that further investigations on skin barrier differences in different breeds of atopic dogs are warranted.

Source of funding: Affinity Petcare.

Conflict of interest: N. Sanchez and C. Torre are employees of Affinity Petcare.

Thyroid function in dogs with leishmaniosis due to Leishmania infantum before and during treatment

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Hypothyroidism may predispose to the development of canine leishmaniosis or it may appear during the course of leishmaniosis due to infiltration and destruction of the thyroid gland by infected macrophages. The main purpose of this study was to evaluate thyroid function through measurement of serum total thyroxine (TT4), free thyroxine (FT4) and canine thyroid-stimulating hormone (cTSH) concentrations in 36 dogs with leishmaniosis, before and after 2 and 4 weeks of treatment with allopurinol with or without meglumine antimonate. Before treatment, 27/36 (75%) dogs had serum TT4 concentrations below the lower limit of the reference interval, but only two dogs had concurrent serum FT4 concentrations below the lower limit of the reference interval and none had increased serum cTSH concentrations. During treatment, there were no significant changes in serum TT4 or FT4 concentrations, whereas a significant increase in serum cTSH was observed. Two dogs had decreased serum TT4 and FT4 but normal cTSH concentrations before treatment; two other dogs had decreased serum TT4 and increased cTSH, but normal FT4 concentrations during the treatment period. Although hypothyroidism could not be excluded definitively in these dogs, it was considered unlikely based on their overall hormonal profile, clinical presentation and response to treatment. In summary, hypothyroidism does not appear to be an important predisposing disease or a frequent complication of canine leishmaniosis. However, clinicians should be reminded that dogs with leishmaniosis may exhibit sick euthyroid syndrome, characterized by low TT4 levels, before treatment.

Source of funding: Self-funded.

Conflict of interest: None declared.

Dermoscopic features of dermatophytosis in 11 cats with Microsporum canis infection

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Scalp dermatoscopic has been shown to be a useful tool for the diagnosis of congenital and acquired hair shaft abnormalities in people, and the dermatoscopic characteristics of human tinea capitis have also been reported. The aim of this report was to describe the use of dermatoscopy in 11 cats with multiple patchy lesions due to Microsporum canis infection by using a conventional nonpolarized dermatoscope (Heine Delta 20; Heine Optotechnik, Herrsching, Germany). All cats were presented with multifocal

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Source of funding: Self-funded.

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Source of funding: Self-funded.

Conflict of interest: None declared.
alopecia and scales; 8/11 were European shorthaired, and the others were one Abyssinian, one British shorthair and one Persian; ages ranged from 2 to 194 months. Microsporum canis infection was confirmed by fungal culture in all cats. At a 10-fold magnification, the most common findings observed in circumscribed lesions of 9/11 cats (82%) were broken hair with a sharp slanted end, a homogeneous thickness and a variable amount of white-to-yellow greasy scales. Six of these nine cats (67%) were also positive by Wood’s lamp and microscopic examination. The remaining three were negative by Wood’s lamp, but microscopic examination of the broken, thickened hair seen with the dermoscope confirmed the presence of hyphae and spores along hair shafts. These first observations suggest that dermoscopy could be a useful screening test for M. canis-induced dermatophytosis in cats and, in cases where Wood’s lamp examination is negative, it might help in the selection of infected hairs for microscopic examination. This method is noninvasive, fast and relatively inexpensive, and it could be used easily in everyday clinical practice.

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Use of activity monitors to assess pruritus in an acute model of canine atopic dermatitis

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We developed a canine model of acute atopic dermatitis to evaluate the potential of compounds to treat pruritus and skin lesions induced in Dermatophagoides farinae (Df)-sensitized dogs. The aim of this study was to investigate the effectiveness of long-term recording activity monitors (AMs; Actical; Mini Mitter, Bend, OR, USA) to assess pruritus induced by allergen provocation. Twenty-eight Df-sensitized dogs were challenged on three consecutive days with a Df slurry applied to clipped skin on the abdomen. In two blinded crossover trials, dogs fitted with AMs were either treated with prednisolone (1 mg/kg once daily for 5 days, starting 1 day before challenge) or left untreated. The activity of dogs treated with prednisolone was significantly lower between midnight and 03.00 h and between 03.00 and 06.00 h compared with untreated dogs (repeated measures ANCOVA; P < 0.0001). To determine whether the recorded nighttime activity corresponded to observable pruritic behaviours (i.e. scratching, chewing, licking or rubbing), we compared AM and video recordings in four dogs for two periods (16.30–20.30 h and 00.00–03.00 h) from two nights before and every night during a Df challenge. The correlation between nighttime AM activity and time spent engaged in pruritic behaviours was highly significant (test of correlation coefficient versus zero; r = 0.57; P < 0.0001). In conclusion, determining nighttime activity with AMs after allergen challenge appears to be an objective and practical way to assess pruritus in this experimental model of canine atopic dermatitis.

Source of funding: Novartis Animal Health.

Conflict of interest: The first five authors are currently employed by Novartis Animal Health. P. Roosje is a consultant for this company.

Coprosopic detection and treatment of Demodex gatoi infestation in a Cornish rex cat in Austria

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We report herein the first observation of Demodex gatoi infestation in Austria and the use of coproscopy for mite detection. A 2-year-old Cornish rex cat – acquired as a kitten in the Czech Republic – was presented with pruritic dermatitis and alopecia. Skin scrapes revealed D. gatoi mites. Scrapes were negative on an asymptomatic housemate, a 3-year-old Thai cat that had never travelled abroad. Faecal floatation with sugar and zinc solutions permitted the detection of D. gatoi in both cats. Fewer mites were detected by coproscopy in the asymptomatic cat compared with the affected one. Both cats were treated with 250 µg/kg ivermectin (Ivomec; Merial, Lyon, France) orally every other day. After 3 months, treatment was stopped in the asymptomatic cat, as faecal examination was negative. The affected cat was treated for more than 4 months, as coproscopy remained positive for D. gatoi in spite of the cat not having visible skin lesions. At that time, ivermectin had to be stopped because the cat developed inappetence and hindleg ataxia. To confirm the identity of the mites as D. gatoi, PCR of the mite mitochondrial 16S rDNA gene was performed on the scrapes of the affected cat. This assay yielded a 325 bp DNA fragment, whose sequence was 100% identical to that of an American D. gatoi mite. This is the first report of demodicosis due to D. gatoi in Austria, and our observations suggest the validity of using coproscopy to detect mites in affected cats and nonaffected in-contact animals.

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Conflict of interest: None declared.
Beneficial effects of immunotherapy with *Gordonia bronchialis* on canine flea allergic dermatitis

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Flea allergy in dogs occurs in sensitized animals, and the skin lesions are thought to result from a T-helper 2 (Th2)-polarized response. As *Gordonia bronchialis* (Gb) has been identified as a promising candidate for immunomodulation of Th2 responses, our objective was to study whether killed suspensions of Gb could be used successfully to treat signs of canine flea allergic dermatitis (FAD). Following standard flea control, 31 dogs with FAD were randomly allocated to receive two intradermal injections of either 0.1 mL of a saline placebo (*n* = 15) or a 10 mg/mL suspension of Gb (*n* = 16) on day 0 and day 20. Skin lesions were scored using the CADESI-03, while pruritus was assessed using a visual analog scale (PVAS) on days 0, 20, 40 and 60. Twenty days after the second injection of Gb or placebo, the median relative decreases in CADESI-03 and PVAS from baseline were significantly greater in dogs injected with Gb (90 and 95% improvement, respectively) than with placebo (54 and 44%, respectively; Mann-Whitney U-test: CADESI-03, *P* = 0.0051; PVAS, *P* = 0.0011). Dogs injected with Gb had a five times higher chance of improvement compared with placebo recipients. Our observations suggest that injections of Gb could be helpful to reduce clinical lesions and pruritus associated with canine FAD.

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**Conflict of interest:** None declared.

Progressive tail necrosis in a rabbit colony

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Acral necrosis due to mycotoxin-contaminated feed has been described in cattle and swine, but not in rabbits. This report describes an outbreak of progressive tail necrosis in a rabbit colony where 15/103 rabbits (15%) were affected. Animals were kept in outdoor cages, on shavings and straw, and they were fed hay, pellets and water *ad libitum*. General clinical examination revealed no abnormalities apart from variable alopecia, scales, multifocal crusted erosions and ulcerations on the distal tails; other acral sites were not affected. Ischaemia was suspected, and two tails were amputated for histopathology. This revealed serocellular crusts, epidermal hyperplasia, superficial perivascular neutrophilic dermatitis, granulation tissue formation and muscle oedema with hylalinized fibres, without signs of vascular damage. Vasocostriction was likely, but vasculitis could not be excluded. There was no evidence of infection on clinical examination or on haematology or biochemistry profiles. The only environmental factor that could have triggered acral ischaemia was the cold temperature (down to 0°C). Toxin analysis of roughage did not reveal any abnormality, but the pellets contained markedly elevated levels of ergot alkaloids compared with 44 reference specimens. After withdrawal of the contaminated feed, the disease did not progress further, and all lesions eventually healed spontaneously. Acral necrosis due to ergotism has not been described in rabbits before, but a high susceptibility of this species to ergot alkaloids is already known. In conclusion, ergotism should be included in the differential diagnoses for progressive tail necrosis in rabbits.

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**Conflict of interest:** None declared.

Spontaneous alopecia in Lagotto Romagnolo dogs: a prospective questionnaire and a retrospective case study of Swedish dogs

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Alopecia affecting the trunk has been recognized in Lagotto Romagnolo dogs in Sweden. Our aim was to evaluate the prevalence of spontaneous alopecia in Swedish Lagotto Romagnolo dogs and to characterize this condition further.

Information was collected by questionnaires sent out to all members of the Swedish Lagotto Romagnolo Association. Furthermore, medical records from dogs reported to have alopecia were analysed retrospectively.

Information from 277 dogs (a response rate of 20%) belonging to Swedish Lagotto Romagnolo Association members was reviewed. Alopecia was reported in 68/277 dogs (25%). Owner information and the medical records reported a nonpruritic and noninflammatory alopecia. This most commonly presented as bilateral trunk alopecia that spared the head and extremities. The age of
onset was <4.5 years in 77% of cases. The hair loss usually started in the autumn/winter, and seasonal cycling was common (54%); a gender predisposition was not noticed. Alopecia onset or worsening of the condition was associated with oestrus. Affected dogs were otherwise healthy, with normal hormonal profiles. Histopathology revealed dilated, keratin-filled infundibula with fragmented hair shafts in infundibula. Telogen, sometimes pleomorphic, hair follicles often dominated, and sebaceous glands were mostly intact. Based on the collected information in Swedish dogs, this spontaneous alopecic dermatosis of Lagotto Romagnolo dogs appears to share similarities with both canine recurrent seasonal flank alopecia and follicular dysplasia of Irish water spaniels and Portuguese water dogs.

Source of funding: Self-funded.

Conflict of interest: None declared.

Fatty acid composition of lipids derived from isolated canine sebaceous glands and epidermis

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To date, studies of canine skin lipids have been performed using extracts lifted from the skin surface, thereby disguising relative contributions made by the two primary lipid sources, the epidermis and the sebaceous glands. By microdissection of small excised skin samples from adult Labrador retrievers (two males and two females), we separated the sebaceous and epidermal lipid components to identify the lipid classes and fatty acids (FAs) present. Following dissection, the purity and integrity of the skin components was checked via microscopy. Bligh and Dyer lipid extracts were then analysed using a combination of high-performance liquid, liquid, gas and thin-layer chromatography techniques. The major components of canine sebum were identified as triglycerides, wax esters and wax diesters. The predominant FAs present in sebum triglycerides were oleate = palmitate > stearate > linoleate, these four FAs contributed around 95% of all the FAs. In sebaceous wax esters, the pattern was similar, although behenic acid was now the fourth most abundant FA. After linoleate, linolenate was the second most abundant polyunsaturated FA in both fractions. The FA composition of epidermal lipids, detected as free FAs, as well as those integrated into ceramides and cholesterol esters, was similar to that of sebum, although it contained significantly more hydroxy-FAs and had a greater contribution of FAs with more than 20C (20 carbon atoms). Techniques used herein could be useful to understand better how changes in diet, microbiology and disease could interplay with the two primary lipid components that contribute to canine skin health and function.

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Proof-of-concept for the use of spectrophotometry to describe coat colour in dogs
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Coat colour in dogs is controlled by multiple factors that include genetics and nutrition. It is described using a variety of terms, and it would be valuable to have an objective means of expressing variations in hair colour. The Spectro Guide 45/0 (BYK-Gardner, Geretsried, Germany) is a hand-held spectrophotometer reading in the 400–700 nm spectrum. It provides quantitative colour information in three spectral ranges: white to black (L; 100–0); red–green (a; +120 to −120); and yellow–blue (b; +120 to −120). We tested the device for its ability to detect differences in three unpatterned canine coat colours: white, mid-brown and black (three dogs each). The spectrophotometer consistently discriminated between the three colours: there were distinct values for ‘L’ and ‘b’, and although ‘a’ overlapped between white and black, it clearly discriminated brown. Individual and all-colour coefficients of variation (CV) were lowest for the ‘L’ parameter (<5%), followed by ‘b’ and ‘a’ (both between 10 and 20%). Brown was detected with the lowest variation among all three parameters (CV < 10%), followed by white and black. Body site repeatability gave a CV < 10% across five distinct sites, with the exception of ‘a’ for the sternum, possibly due to reduced hair density. Hair length did not have a significant effect on measurements. The Spectro Guide 45/0 appears to be a valuable tool for objectively defining coat colour in dogs; larger studies with dogs of different coats and colours are now required.

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