22nd Annual Congress of the ESVD-ECVD, 13–15 September 2007, Mainz, Germany

Thursday, 13 September, 17:15

Reconstructed interfollicular feline epidermis as a model for the screening of drugs against *Microsporum canis*

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A fully differentiated reconstructed interfollicular feline epidermis (RFE) was recently developed *in vitro* and was demonstrated to be relevant for studying *Microsporum canis*–epidermis interactions. The aim of this study was to investigate whether RFE could be an adequate model for *in vitro* screening of drugs against *M. canis*. As a preliminary step, the minimum inhibitory concentration of miconazole nitrate against *M. canis* IHEM 21239 was determined to be 0.3 mg L^{-1} on Sabouraud's dextrose agar. Reconstructed feline epidermis grown at the air–liquid interface were cultured for 24 h in RFE culture medium without antibiotics but supplemented with miconazole (range 0.1–1 mg L^{-1}) or its solubilizing agent, dimethylsulfoxide. Then, RFE were inoculated in triplicates with 100 000 *M. canis* arthroconidia. Medium was replaced each day for five additional days. To evaluate fungal growth, RFE were processed for routine histopathology, three serial sections being performed across the block at 0.1 mm intervals. Results showed that dimethylsulfoxide had no effect on the growth of *M. canis* and cell viability while no fungal growth was detected in or on infected RFE in the presence of miconazole concentrations equal to or higher than 0.3 mg L^{-1} final concentration in the culture medium. This study demonstrates that RFE presents a good permeability to drugs included in the culture medium and is an adequate model for *in vitro* screening of drugs against *M. canis* and potentially against other skin pathogens.

Funding: Grant 3.4534.1 from FRSM.

Thursday, 13 September, 17:30

The concentration time course of pradofloxacin in the skin of dogs with and without pyoderma

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A third-generation fluoroquinolone, pradofloxacin (Bayer Animal Health, Germany), is being developed to treat bacterial infections in dogs. This study aimed at comparing serum and skin concentrations of pradofloxacin in dogs with pyoderma (affected and non-affected skin) and normal dogs. Twenty dogs with superficial and/or deep pyoderma and 20 normal dogs were included in the study. A complete blood count, biochemical profile and an initial aerobic skin culture were performed on all dogs with pyoderma. Pradofloxacin (approximately 3 mg kg^{-1} of body weight, per os) was administered daily to all dogs. Serum and skin biopsy specimens were obtained on day 0 prior to drug administration and on day 3 (prior to drug administration, 2 and 4 h post-drug administration, and in 10 of 20 normal dogs at 24 h post-drug administration). Samples and tissue specimens were assayed by high-performance liquid chromatography. Clinical efficacy for dogs with pyoderma was recorded at 4 weeks, and again at 6–12 weeks for dogs with deep pyoderma. Pradofloxacin tissue concentrations in lesional skin of dogs with pyoderma were greater than values for serum and nonlesional skin in the same group of dogs at 2 h post-drug administration as well as values for serum and tissue concentrations of normal control dogs. Clinical efficacy was noted as excellent for dogs with superficial and deep pyoderma within 4 weeks and 6–12 weeks, respectively. At a mean dosage of 3.7 mg kg^{-1} per os once daily, pradofloxacin adequately reached therapeutic tissue concentrations in dogs with pyoderma.

Funding: This study was self-funded.

Thursday, 13 September, 17:45

Shar-pei dogs with cutaneous mucinosis have high serum levels of hyaluronic acid

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Cutaneous mucinosis (CM) affects a high percentage of Shar-pei dogs. In a previous communication, we reported that such mucinosis is the consequence of deposition of hyaluronic acid (HA) in the dermis of affected animals. The aim of the present investigation was to correlate HA serum levels with the presence of CM in Shar-pei dogs, and to evaluate if this is the result of abnormal hyaluronan synthesis by dermal fibroblasts. Sera of 21 dogs were used. Sixteen were Shar-pei dogs, eight were affected by CM and eight were unaffected, while five were healthy dogs of other breeds. Serum HA concentration was determined using a competitive ELISA. A clear positive correlation was found between the degree of clinical mucinosis and serum HA concentration. In healthy dogs serum, the average HA level was of 234.8 μg L^{-1}, whereas in Shar-pei dogs without or with clinical signs of mucinosis, it was of 532.8 μg L^{-1} and 1500.7 μg L^{-1}, respectively. Furthermore, fibroblast cultures from two Shar-pei dogs with clinical

signs of CM were grown. Morphologically, fibroblasts from these dogs with CM were similar to those coming from other breeds although their growth rate was slower. These results suggest that CM of Shar-pei dogs is a systemic disease that probably is the consequence of a genetic defect in the regulation of HA synthesis.

Funding: This study was self-funded.

Friday, 14 September, 15:00

A pilot open trial evaluating the efficacy of low-dose antifolate therapy with aminopterin for treatment of canine atopic dermatitis

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In humans, antifolates have been used for decades for chemotherapy of neoplasia, but they are used currently at low doses for the treatment of immune diseases including psoriasis and rheumatoid arthritis. The objective of this proof-of-concept trial was to explore whether low doses of the antifolate aminopterin was safe and effective for treatment of canine atopic dermatitis (AD). Nine dogs with AD were treated with 0.01 mg kg^{-1} of aminopterin once weekly for 4 weeks. Before and after 2 and 4 weeks, clinicians graded skin lesions using the validated CADESI-03. At the same times, owners rated the intensity of pruritic manifestations using a visual analogue scale (PVAS). Global Scores (GS) combined CADESI and PVAS. Complete blood counts and serum chemistry panels were obtained weekly. Eight of nine dogs completed this trial, and one was withdrawn because of bacterial folliculitis. CADESI and GS values, but not PVAS numbers, were significantly lower after 4 weeks compared to pretreatment (ANOVA, $P < 0.05$). After 4 weeks, the median reduction in CADESI, PVAS and GS was 36%, 2.8 out of 10 points and 62%, respectively. After 1 month, a greater than 50% reduction from baseline CADESI and GS values was achieved in three of eight and six of eight dogs, respectively. Adverse effects and clinically relevant changes in blood counts and chemistry panels were not seen after aminopterin administration. Results of this preliminary trial suggest that low-dose antifolates may be beneficial and safe to treat canine AD. Future studies should aim at optimizing treatment protocols before verifying efficacy in randomized controlled trials.

Funding: NC State University Veterinary Teaching Hospital Clinical Innovation Grant.

Friday, 14 September, 15:15

A retrospective study of 21 cases of canine atopic-like dermatitis

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The revised nomenclature of allergic diseases recently defined two forms of canine atopic dermatitis (AD): AD *sensu stricto* and atopic-like dermatitis (ALD), the latter characterized by phenotypic AD with no evidence of hypersensitivity to environmental allergens. In the dog, this form of AD has not been reported yet. The objective of this study was to compare clinical and epidemiological data between dogs with AD and ALD. All dogs referred for evaluation of AD in 2006 were entered in this study. The diagnosis of AD was made according to Prélaud's criteria, elimination of other causes of pruritus and positive immediate intradermal tests (IDT) to aeroallergens. Canine ALD was diagnosed clinically as above, but dogs also had two negative IDT and low level of allergen-specific IgE, and signs had not responded to an eviction diet of 8 weeks. Sixty-one dogs were diagnosed with AD and 21 with ALD. French bulldogs were significantly more often diagnosed with ALD than with AD. Sex ratios, ages of disease onset, clinical scores, responses to steroids or anti-infectious treatment were identical in both diagnosis groups. Cyclosporine was significantly less effective in dogs with ALD (50% with good response) than in those with AD (92% with good response). In summary, French bulldogs appear predisposed to develop ALD, and this disease may be less responsive to cyclosporine than AD. As in humans with 'intrinsic, nonatopic' AD, we could document a lack of predisposition of female patients, a lack of influence of age of onset and a possible genetic predisposition.

Funding: This study was self-funded.

Friday, 14 September, 15:30

Monodose allergen-specific immunotherapy for treatment of canine atopic dermatitis: preliminary safety study

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Allergen-specific immunotherapy is used for treatment of canine atopic dermatitis (AD) with human medicine-based protocols administering progressive doses of allergenic extracts. A more simple protocol without a dose escalation phase would greatly simplify the practice of allergen-specific immunotherapy in the dog. Twenty-six dogs presented for non-seasonal AD were included in this study. The diagnosis of AD was based on exclusion of any other cause of pruritus and fulfilment of four of five major clinical diagnostic criteria. Immunotherapy formulation was based on immediate results of intradermal tests showing hypersensitivity to house dust mites. In this monodose protocol, cetirizine was prescribed at 0.25–0.5 mg kg^{-1} once daily for 15 days before the first injection of allergenic extracts and during 6 months thereafter. Other immune-modulating treatments were not administered. Immunotherapy injections consisted of 0.8 mL of alum-precipitated allergenic extracts (10 IR/mL, Allerbio, Varennes en Argonne, France) administered subcutaneously once monthly. Using this protocol, systemic or local reactions were not observed in any dogs either during the 3 h following allergenic extracts administration or during the ensuing days. In conclusion, this monodose protocol combined with cetirizine administration appears to be well tolerated in atopic dogs allergic to house dust mites. This protocol is simple and less

expensive than traditional or rush protocols. Safety and efficacy should be verified in larger trials before this regimen could be recommended for clinical practice.

Funding: This study was self-funded.

Friday, 14 September, 15:45

Immunostimulatory oligonucleotides modulate canine peripheral blood mononuclear cell activity *in vitro*

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Oligonucleotides rich in CpG exhibit potent immunostimulatory activity in humans and various animal species. These immunostimulatory sequences (ISS) interact with receptors on cells of both the innate and the specific immune system. Activation of cells involving Toll-like receptor-9 (TLR-9) promotes the development of a Th1 response *in vitro* and *in vivo* and prevents cytokine production by allergen-specific Th2 cells. TLR-9 activating ISS can be given alone or covalently linked to an antigen; both treatments result in inhibition of allergen-specific hypersensitivity in animal models. The effect of ISS on the immune system of dogs, as exemplified by their effect on *in vitro* cytokine production by canine peripheral blood mononuclear cells (PBMC), has not been determined. PBMCs of five healthy dogs were incubated for 6 h with ISS classes A, B and C known to elicit different effects on immune cells due to distinct structural characteristics. Subsequently, cytokine gene mRNA transcription was measured by quantitative polymerase chain reaction. Compared to negative controls (control oligonucleotide and medium), the transcription of IL-6, IL-10 and IL-12 mRNA was significantly elevated after stimulation with all three ISS. The expression of TNF-alpha mRNA increased significantly after stimulation with class B and C ISS, whereas only class C ISS resulted in enhanced expression of interferon-gamma mRNA. It is concluded that ISS have a modulating effect on PBMC of healthy dogs *in vitro* as in mice, primates and humans. Therefore, like in mice with experimentally induced allergic asthma, ISS may be a promising tools for modulation of atopic dermatitis in dogs.

Funding: University of Utrecht.

Friday, 14 September, 16:00

Cytological diagnosis of nodular dermatophytosis (kerion) in dogs: preliminary results

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Dermatophytosis is a disease with zoonotic potential, and as such, an early diagnosis is required. Wood's lamp examination and microscopic examination of hair plucks are diagnostic in 40–70% of cases. Fungal culture is reliable, but it takes longer and can yield false-negative results. In nodular dermatophytosis, these tests can be negative and histopathological examination with special stains (periodic acid–Schiff) is required. The aim of this preliminary study is to assess the usefulness of cytology in the diagnosis of canine nodular dermatophytosis. Ten dogs of different age, sex and breed with nodular lesions suggestive of dermatophytic kerion were selected. Wood's lamp examination, microscopic examination of hair plucks and fungal cultures were performed in all cases; impression smears from the nodular lesions obtained by squeezing the lesion were stained with a modified Romanowsky stain (Hemacolor). Wood's lamp examination was negative in all cases. Microscopic examination of hair plucks suggested dermatophytosis in four of 10 dogs. Fungal culture was positive for *Microsporum canis* in eight of 10 dogs. Cytological examination was suggestive of a pyogranulomatous lesion. Small fragments of hair shafts with arthrospores were easily detected in samples from all 10 dogs. In this study, cytology results were suggestive of dermatophytosis in all cases, while the other tests were not always diagnostic. Further studies should be performed to verify these findings in a larger group of dogs.

Funding: This study was self-funded.

Friday, 14 September, 16:15

Chronic radiant heat dermatitis in the dog: a report of four severe cases

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Chronic radiant heat dermatitis (CRHD) is a rare dermatological condition characterized by erythematous, reticular often-pigmented lesions resulting from repeated or prolonged exposure to infrared radiation. Our objective was to report four canine cases with severe CRHD. The mean age of affected dogs was 7.5 years (range 6–10 years). A breed predisposition was not observed. Heat sources included heat lamp (one case) or open fires (three cases). The duration of exposure to radiant head varied from 6 to 18 months. Concurrent hypothyroidism was diagnosed in two dogs that presented lethargy and obesity. Lesions of CRHD were on the lateral thorax (two cases) and flanks (two cases). Clinical signs included irregular branching alopecia (resembling 'prehistoric paintings') with erythema (three cases), scaling (one case), central depigmentation (three cases), and central (one case) or peripheral hyperpigmentation (two cases). Central or linear peripheral erosions or ulcerations were observed in three dogs. Pain and increased skin thickness were noted in two cases. Histopathological features from non-ulcerated affected areas revealed a cell-poor interface dermatitis with sub-epidermal bubbling vacuolation, smudging of dermal collagen, and dyskeratotic and apoptotic keratinocytes. Eosinophilic wavy fibrils in the superficial dermis were identified in all cases. Histopathological lesions of ulcerated areas were typical of concurrent thermal burns. A medical

treatment was not given except for levothyroxine in dogs with concurrent hypothyroidism. Heat avoidance was recommended, and cicatricial alopecia developed in three cases.

Funding: This study was self-funded.

Saturday, 15 September, 17:00

Prevalence of serum antibodies against canine papillomaviruses in two geographically distinct regions

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Papillomaviruses (PVs) rank among the main causative agents for the development of certain types of tumours in humans, mainly cervical carcinomas and epidermodysplasia verruciformis (EV). The role of PVs in the development of cancers in the canine species remains controversial, however. We recently described the characterization and sequencing of a new canine PV (CPV3), which had been isolated from a dog suffering of a disease resembling human EV. In this study, we investigated the distribution and possible relevance of CPV3 in two geographically distinct regions. Since ELISA is commonly used in prevalence studies in human medicine, we established a generic capture ELISA to screen 232 sera from Switzerland and 315 sera from South Africa for the presence of antibodies against the major capsid proteins L1 of CPV3 as well as COPV. Our tests revealed a much higher seropositivity in the South African compared to the Swiss population and also a different distribution. In the Swiss population, most positive sera reacted only to COPV. In contrast, in the South African population, we found not only much more CPV3-positive reacting dogs but also a high proportion of sera containing antibodies against both CPV3 and COPV antigens. The results therefore indicate that CPV3 is a common virus among the tested population in South Africa and also, but to a lesser extend, in the tested Swiss population. They furthermore suggest that the rate of canine PV infections as well as the role of certain PVs might depend on the geographical region.

Funding: this study was self-funded.

Saturday, 15 September, 17:15.

Isolation and sequencing of a novel papillomavirus associated with pigmented plaques in four pugs

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Canine pigmented plaque (CEP) is a skin disorder characterized by multiple black papules or plaques on the abdominal area or limbs. This disorder was first described in pugs in which it was initially called lentiginosis profusa. This breed appears to be highly predisposed to developing such lesions but cancerization was never reported. On the other hand, a similar condition was reported in non-pug dogs with a very high rate of cancerization. The association of CEP with papillomavirus infection has been reported in several studies. In the present study, we report four new cases of CEP in pugs. Taking advantage of the circular DNA of papillomaviruses, we carried out rolling circle amplification of the viral genome. Sequencing of the entire genome revealed that the CEP-associated papillomavirus was related to a recently cloned virus isolated from a similar lesion in a Rhodesian ridgeback (CPV3). Using polymerase chain reaction, we demonstrated that all pugs were infected by similar viruses. Interestingly, our sequencing data match those of small sequences of papillomavirus DNA isolated from Japanese pug lesions. It seems likely therefore that pugs affected with CEP are infected with one or several closely related papillomavirus(es). This new virus type is different from CPV3 but belongs to the same genus. Papillomavirus is often detected in lesions of CEP and probably plays a role in the development of such lesions. Further studies are warranted to firmly establish such causality, however.

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Saturday, 15 September, 17:30

The influence of polyunsaturated fatty acids on cytokine gene transcription by peripheral blood mononuclear cells in normal dogs and dogs with atopic dermatitis

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Ten dogs with atopic dermatitis diagnosed based on Willemse's criteria, exclusion of differential diagnoses and positive skin reactivity against *Dermatophagoides farinae* (*Df*) antigens and 10 healthy control dogs were included in this study. Blood was obtained by venipuncture, and peripheral blood mononuclear cells were isolated. Cells were stimulated for 24 h with allergen diluent (negative control) or 50 $\mu g\ mL^{-1}$ of a specific *Df* extract with or without three different polyunsaturated fatty acid (PUFA) combinations: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) and gamma-linoleic acid (GLA)/EPA/DHA. RNA was isolated and quantitative polymerase chain reaction was performed for interferon (IFN)-gamma, interleukin (IL)-4 and transforming growth factor-beta. In atopic and healthy dogs, there was up-regulation of IL-4 and IFN-gamma mRNA transcription after incubation with *Df* allergen. This allergen-induced up-regulation was not reversed by incubation with PUFA from either group. However, in atopic dogs this up-regulation was increased with GLA/EPA/DHA ($P < 0.01$ compared to controls); in healthy dogs it was with ALA ($P < 0.01$).

TGF-beta mRNA transcription was not significantly altered in atopic dogs, but it was significantly decreased in healthy dog cells after culture with EPA/DHA ($P < 0.001$). Based on the results of this study, *Df* extract leads to the up-regulation of IL-4 and IFN-gamma mRNA transcription in peripheral blood mononuclear cells of atopic and healthy dogs but PUFAs do not inhibit either IL-4 or IFN-gamma gene transcription.

Funding: Boehringer Ingelheim Denmark.

Saturday, 15 September, 17:45

Emergence of multiresistant mecA-positive *Staphylococcus intermedius* isolated from canine and feline pyoderma and otitis in Europe

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Resistance to cephalosporins and/or fluoroquinolones in *Staphylococcus intermedius* has until now remained low in Europe, with drugs generally remaining effective for systemic therapy in dogs and cats. This study describes clinical and epidemiological features of skin and ear infection in 11 dogs and one cat with highly resistant *S. intermedius* referred to a veterinary dermatology clinic in Northern Germany. In addition, phenotypic and genotypic characteristics of the isolates are described. Twelve *S. intermedius* resistant to at least five antimicrobial classes were isolated from samples of skin and ear infections submitted to veterinary diagnostic laboratories. They represented 23% of all *S. intermedius* submissions during an 18-month period, and resistance included cephalexin, methicillin and enrofloxacin. Species identification was confirmed by PCR detection of thermonuclease genes (*nuc*). Presence and expression of the gene conferring resistance to all betalactam antibiotics (*mecA*) were demonstrated in all *S. intermedius*. Based on pulsed-field gel electrophoresis, six were indistinguishable and the others closely or possibly related. Diagnoses at referral had been recurrent superficial pyoderma, deep pyoderma, pododermatitis and chronic otitis, all unresponsive to systemic betalactam antibiotics or fluoroquinolones. Eleven animals responded to treatment within 8 weeks. Topical therapy, as the only antimicrobial treatment, was successful in six cases. The emergence of multiresistant, *mecA*-positive *S. intermedius* in Europe is alarming. Zoonotic implications, awareness among veterinary laboratories and strategies for the use of antimicrobials in small animal practice need to be considered.

Funding: This study was self-funded.

Saturday, 15 September, 18:00
– Late Breaking Abstract –

Immunohistochemical detection of filaggrin in the skin of different species

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Filaggrin (FLG) is a protein that facilitates terminal differentiation of epidermal cells and formation of the skin barrier. Recent studies have demonstrated that, in humans, mutations encoding FLG are a major genetic predisposing factor for atopic dermatitis. Furthermore, in immunohistochemical studies, FLG's staining intensity was found to be markedly reduced both in nonlesional and in lesional atopic human skin. The aim of this project was to investigate, using immunohistochemistry, the expression of FLG in the skin of the dog, goat, sheep, cow and pig in preparation for future investigations of FLG expression in canine atopic skin. Formalin-fixed paraffin-embedded sections were stained using an ABC immunohistochemical technique. A polyclonal antibody against human pro-FLG (Zymed Laboratories) was used as primary antibody. Filaggrin staining was consistent in all species. Filaggrin was detected clearly in the epidermal granular layer of all studied animals, except for that of the thorax area of the goat. The outer root sheath of the hair follicles also was stained. The staining in all cases was cytoplasmic and keratohyalin granules strongly stained positive. These results are very similar to those described in human beings and they open the door to further studies regarding the expression of FLG in animals with skin diseases.

Funding: this study was self-funded.