Restructuring effect of phytosphingosine-containing shampoo and mousse on the cutaneous barrier in five atopic dogs: preliminary results of a field study

O. FANTINI*, C. ZEMIRLINE†, M. BELLIARD†, E. LATI§, L. PENO-MAZZARINO§, E. GONTIER¶, E. OLLIVIER† and D. PIN*
*Dermatology Unit, VetAgro Sup Lyon Campus, Marcy L'Etoile, France
†Ceva Santé Animale, Libourne, France
§Laboratoire BIO-EC, Longjumeau, France
¶Bordeaux Imaging Center, Electronic Imaging Department, University of Bordeaux, Bordeaux, France

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

Sources of funding: Ceva Santé Animale.

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

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

M. VARLOUD*, E. HODGKINS† and R. KOCHANOWSKI‡
*Ceva Santé Animale, Libourne, France
†Ceva Animal Health Polska, Warszawa, Poland
‡Ceva Animal Health, Lenexa, KS, USA

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

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

Antimicrobial activity of pomegranate extract against canine skin pathogens

L. RAMIO*, S. CERRATO*, L. AROSEMENA†, M. A. CALVO†, P. BRAZÍS* and A. PUIGDEMONT‡
*UNIVET, Barcelona, Spain
†Department of Animal Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain
‡Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Barcelona, Spain

The prevalence of antibiotic resistance has increased interest in the antimicrobial activity of natural compounds. Punicalagins, the active bioflavonoids present in pome-

granate extract, have been shown to exert antimicrobial, anti-oxidant and anti-inflammatory effects. The aim of this study was to investigate the antimicrobial properties of pomegranate extract against the common canine skin pathogens (*Malassezia spp.* and *Staphylococcus spp.*) by determining the minimum inhibitory concentration (MIC). *Malassezia* and meticillin-resistant and -sensitive *Staphylococcus* isolates were obtained from dogs with yeast overgrowth or superficial pyoderma. Serial dilutions of pomegranate extract in tryptone soy broth medium ranging from 50 to 0.1 mg/mL were incubated with $0.5x10^5$ CFU/mL of each isolate for 30 h at 37°C. The MIC for each isolate was defined as the lowest concentration of the pomegranate extract that inhibited microbial growth. Differences between the mean MICs were analysed by ANOVA and Tukey's multiple comparison *post hoc* tests. The MIC values of the pomegranate extract were 3.2 ± 1.8 mg/mL for meticillin-sensitive *Staphylococcus* strains and 4.8 ± 1.6 mg/mL for meticillin-resistant *Staphylococcus*, and there was no significant difference between the two *Staphylococcus* groups. Inhibition of *Malassezia* was obtained at a MIC of 12.5 ± 0.0 mg/mL. These results suggest that pomegranate extract may represent a new nonantibiotic antimicrobial strategy for managing dogs with skin infections.

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

C. FAVROT*, V. .HAUCK*, P. HÜGLI*, N. FISCHER*, A. ROSTAHER* and M. MELI*
* *Vetsuisse Faculty, University of Zürich, Switzerland*

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases in humans and dogs. Human and canine AD share numerous similarities. Regulatory T cells (T_{regs}) are essential controllers of the immune homeostasis and have been shown to play a key role in human AD. However, the reports of the frequencies of T_{regs} in human atopic patients are conflicting. At this time only two studies have assessed T_{reg} numbers in atopic dogs.

The aim of this work was to explore the role of T_{regs} in the pathogenesis of canine AD by assessing the number of circulating T_{regs} in healthy and atopic dogs and to determine whether this frequency correlates with patient age, gender, disease severity or pre-treatment. Thirty-five atopic and fourteen healthy dogs were involved in the study. Peripheral blood mononuclear cells were stained with monoclonal antibodies (anti-CD4, anti-CD25, anti-FoxP3) and evaluated by flow cytometry. T_{regs} were phenotypically identified as T cells triple positive for CD4, CD25 and FoxP3. The percentage of circulating $CD4^+CD25^+FoxP3^+$ T_{regs} in atopic patients was significantly increased compared to healthy dogs (mean 2.1% versus 1%, $P = 0.002$) and correlated with disease severity [Hill's Pruritus Scale: 48%, $P = 0.003$; Canine Atopic Dermatitis Extent and Severity Index (CADESI)-04 (clinical score): 34%, $P = 0.044$]. Our data suggest that as in humans, $CD4^+CD25^+FoxP3^+$ T_{regs} may play an important role in the pathogenesis of canine AD. Additionally, there is an association between T_{reg} frequency and disease severity. Further investigation is required to improve our understanding of the role of T_{regs} in atopic dogs.

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

A. CORONA*, A. VERCELLI*, N. BRUNI† and L. CORNEGLIANI*
* *Ambulatorio Veterinario Associato, Torino, Italy*
† *Istituto Profilattico e Farmaceutico Candioli & C. S.p.A., Beinasco, Torino, Italy*

Lactoferricin (LC) is a peptide able to inhibit growth and to prevent biofilm formation in some bacteria. The aim of this study was to evaluate the *in vitro* activity of LC against *Staphylococcus intermedius* group (SIG), *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* strains isolated from dogs with pyoderma. The minimum inhibitory concentrations were evaluated using a microtitre plate dilution method. The bacterial inoculums in log-phase growth were prepared in brain heart infusion broth (BHI) with a turbidity of 0.5 McFarland, corresponding to 10^2 to 10^3 cells/mL. The LC solution was diluted in alcohol (prior studies showed that the alcohol exerted no antimicrobial activity) to achieve final concentrations of 7.3%, 5.5%, 3.7% and 2.2% for each bacterial isolate. Appropriate negative and positive controls were included. The plates incubated at 37°C for 24 h, after which 10 μL aliquots from each well were incubated on blood agar at 37°C for a further 24 h. Inhibition was defined as the lowest concentration of LC that prevented growth in the microtitre wells and blood agar. LC showed bactericidal activity against all the bacterial isolates at 7.3% and 5.5% concentrations, but bacterial growth was evident at 3.7% and 2.2%. These results show that LC exhibits *in vitro* bactericidal activity against Gram-positive and Gram-negative bacteria associated with canine pyoderma. Lactoferricin is a potential novel topical antibacterial treatment that could be used instead of conventional antiseptics and/or antibiotics to treat skin infections. However, *in vivo* studies are needed to confirm these results.

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