



SCIENTIFIC DATA : ANTIPARASITIC & ANTISEBORRHEIC SHAMPOO

1) Introduction

Dog skin disorders are among the most common health problems in dogs. Skin disorders in dogs have many causes, and many of the common skin disorders that afflict people have a counterpart in dogs. The condition of dog's skin and coat can also be an important indicator of its general health. Dog skin disorders may be grouped into categories according to the causes. Skin disorders of dogs vary from acute, self-limiting problems to chronic or long-lasting problems requiring life- time treatment. They also need to be differentiated on the basis of being of primary or secondary (due to scratching, itch) in nature, making diagnosis complicated. The health and proper function of the skin is dependent on the health and proper function of the other organs in the dog's body.

The diagnosis and treatment of skin diseases can be difficult and time consuming. Listed below are some common skin diseases and conditions that can affect dogs.

Seborrheic Dermatitis: is a common chronic inflammatory skin condition, characterized by scaling and poorly defined erythematous patches. It may be associated with pruritus, and it primarily affects sebum-rich areas, such as the scalp, face, upper chest, and back. Although its pathogenesis is not completely understood, some postulate that the condition results from colonization of the skin of affected individuals with species of the genus *Malassezia* (formerly, *Pityrosporum*)

Fungal Infections: These include *Malassezia* sp., dermatophytosis (ringworm) and dermal coccidioidomycosis. They are diagnosed by examining skin scrapings, laboratory cultures, and blood tests to identify antibodies. Treatments include antifungal shampoos and rinses in conjunction with topical and systemic antifungal drugs.

Bacterial Infection: Bacterial infection is a common, but usually secondary reaction to an underlying disease such as an allergy. Treatment for bacterial infection is usually a round of oral or topical antibiotics

Acute Moist Dermatitis or Hot Spots: The dog usually causes hot spots himself as he attempts to relieve the pain or itch.

Pyoderma: Pyodermas vary in severity and cover a wide range of infections that result in the formation of pus.

Allergic Inhalant Dermatitis or Atopy: Atopy is an extremely itchy skin disease caused by allergies to microscopic particles in the air. It is diagnosed by both clinical presentation and ruling out other causes such as ectoparasites

Ectoparasites (external parasites): External parasites like mites, fleas and ticks break through the skin and allow bacterial infections to occur. They can also cause allergic reactions. Afflictions are diagnosed by observation and examination of skin scrapings under a microscope. The type of parasite detected will dictate the treatment protocol, which is usually a regimen of anti-parasitic drugs and/or the use of medicated shampoos and rinses.

1) Seborrheic dermatitis:

A common disorder of the skin, seborrheic dermatitis is characterized by the development of erythematous patches with yellow-gray scales that appear most often appear on the face, scalp, upper chest, and back. A milder variant is dandruff, which is manifested by dry, flaking scales on the scalp. The extent of involvement, as well as the severity of symptoms, helps to dictate treatment.



EPIDEMIOLOGY

Estimates of the prevalence of seborrheic dermatitis are limited by the absence of validated diagnostic criteria as well as a grading scale of severity; however, as one of the most common skin disorders,² it affects approximately 11.6% of the general population and up to 70% of infants in the first three months of life may have the condition. Among adults, the peak incidence is in the third and fourth decades of life.³ There appears to be an ethnic predilection, with few cases seen in African-Americans.⁴ Seborrheic dermatitis also occurs more frequently in patients with Parkinson’s disease and in patients treated with certain psychotropic drugs such as haloperidol decanoate (Haldol, Ortho-McNeil), lithium (Eskalith, GlaxoSmithKline, buspirone (BuSpar, Bristol-Myers Squibb), and chlorpromazine (Thorazine, GlaxoSmithKline).

Seborrheic dermatitis is one of the most common dermatoses seen in individuals infected with human immunodeficiency virus (HIV) infection, particularly those who have a CD4 T-cell count of below 400 cells/mm³.⁵ Other medical conditions associated with an increased incidence of seborrheic dermatitis are neuroleptic-induced parkinsonism, familial amyloidosis, and trisomy 21.⁶⁻⁸

Clinical Presentation and Differential Diagnosis

Seborrheic dermatitis is characterized by the development of pruritic, erythematous patches with easily detachable, greasy large scales. Although it may appear in various anatomical locations, it tends to occur in areas that contain numerous sebaceous glands, such as the scalp, face, upper chest, and back (Table 1). Seborrheic dermatitis of the scalp commonly presents as dandruff, a milder eruption, characterized by smaller dry, flaking scales.

Table 1

Manifestations of Seborrheic Dermatitis

Site	Subtype
Scalp	Cradle cap, dandruff
Eyes, face	Seborrheic blepharitis
Chest	Annular
Generalized	Erythrodermic

The diagnosis is generally a clinical one, with a strong emphasis on the patient’s history and clinical examination findings. A number of conditions may be confused with seborrheic dermatitis, such as psoriasis, atopic and contact dermatitis, and erythrasma. In addition, because of the similarities in distribution, seborrheic dermatitis can be easily confused with rosacea.

A skin biopsy is rarely needed to make the diagnosis, but it can be useful if the presentation is atypical. The differential diagnosis is presented in Table 2.

Table 2

Differential Diagnosis Of Seborrheic Dermatitis

Tinea capitis	Erythrasma
Langerhans cell histiocytosis	Wiskott–Aldrich cutaneous lupus
Psoriasis	Dermatomyositis
Atopic dermatitis	Vitamin B deficiency
Contact dermatitis	Zinc deficiency
Rosacea	Drug eruption

PATHOGENESIS

The pathogenesis of seborrheic dermatitis is not completely understood, but there seems to be a strong association with skin colonization with yeasts of the genus *Malassezia*.^{9,10} These yeasts are present on the skin of affected individuals, and antifungal therapy that decreases the number of *Malassezia* organisms present has been shown to be effective in the treatment of seborrheic dermatitis.¹¹

Although no correlation has been made regarding the number of fungal organisms and severity of disease, several hypotheses suggest the exact pathogenic mechanism used by *Malassezia*. The fact that there is a preponderance of disease in sebum-rich areas has led to the idea that fungal metabolites react with triglycerides released from sebaceous glands, producing an inflammatory mediator.¹¹

Another theory is that the lipid layer of the fungus leads to keratinocyte production of proinflammatory cytokines, causing inflammation and the skin eruption.¹² No genetic predisposition has been identified with seborrheic dermatitis.

Adverse Events: The adverse effects associated with topical antifungals (Table 5) are irritant contact dermatitis in a small percentage of patients as well as a burning or itching sensation and dryness in approximately 2% to 3% of patients.²³ Because oral antifungal agents interfere with the CYP 450 system in the fungus, they may also interfere with the host CYP 450 system, limiting their practical use for the treatment of seborrheic dermatitis. Of the antifungals that work via the fungal CYP 450 system, itraconazole and fluconazole (Diflucan, Pfizer) have the weakest binding to human CYP 450 and consequently cause fewer adverse effects. Among the antifungal agents, ciclopirox is better tolerated and better accepted than ketoconazole.²⁴

Table 5

Adverse Effects of Various Therapies

<i>Topical antifungal agents</i> (e.g., azoles, allylamines): irritant contact dermatitis, itching, burning, dryness
<i>Metronidazole</i> : contact sensitization
<i>Selenium</i> : hyperpigmentation (rare)
<i>Tea tree oil</i> : irritant contact dermatitis
<i>Topical corticosteroids</i> : skin atrophy, telangiectasis, folliculitis, hypopigmentation
<i>Immunomodulators</i> : questionable association with lymphoma
<i>Tar</i> : hyperpigmentation, exfoliative dermatitis, skin atrophy, keratoscanthomas, malignancy
<i>Phototherapy</i> : burning, itching, increased risk of malignancy with long-term use



SCIENTIFIC DATA: ANTIPARASITIC & ANTISEBORRHEIC SHAMPOO

Treatments: Tar has historically been the treatment of choice for many dermatological diseases. As early as 1895, Kaposi showed its usefulness for seborrheic dermatitis.⁴⁰ Its method of action likely involves its inherent antifungal properties as well as the ability to decrease the inflammatory response. Studies have also shown the ability of tar to reduce sebum production.⁴¹ Tar has been found to be equivalent to ketoconazole in its fungistatic properties,⁴² but concerns about its safety profile remain.

The use of tar commonly leads to the development of local folliculitis, contact dermatitis of the fingers, exacerbation of psoriasis in affected individuals, local skin atrophy, telangiectases, pigmentation, exfoliative dermatitis, and keratoacanthomas. Kaposi also described tar toxicity, consisting of nausea, vomiting, and tarry black urine when the substance was administered to small children, who commonly are affected by seborrheic dermatitis. There is also a possible association with an increased risk of malignancy, specifically squamous cell carcinoma.⁴³ Therefore, a number of concerns are involved with the use of tar for treating seborrheic dermatitis.

For severe dry flaking, shampoos containing sulfur and salicylic acid are recommended to remove scales. For oily seborrhea, shampoos containing coal tar are effective and retard further scale production. And oatmeal as an ingredient to prevent irritation. Oats contain polysaccharides, which leave a protective film on skin, preventing dryness and itching. Oatmeal just may be one of the best remedies for itchy skin, and its gentle effects are a great alternative to many chemicals and over-the-counter itch treatments. From stopping itching to helping relieve insect bites and stings, from rashes to sunburns. Allantoin/coal tar shampoo is a keratolytic. Also, It works by slowing bacterial growth and loosening and softening scales and crust. Relieving itching, scaling, dryness, and flaking of the skin due to dandruff, eczema, psoriasis, and seborrhea. It may also be used for other conditions as determined by doctor.

II) A new animal model for the purpose of studying superficial infections is presented. In this model an infection is established by disruption of the skin barrier by partial removal of the epidermal layer by tape stripping and subsequent application of the pathogens *Staphylococcus aureus* and *Streptococcus pyogenes*. The infection and the infection route are purely topical, in contrast to those used in previously described animal models in mice, such as the skin suture- wound model, where the infection is introduced into the deeper layers of the skin. Thus, the present model is considered more biologically relevant for the study of superficial skin infections in mice and humans. Established topical antibiotic treatments are shown to be effective. The procedures involved in the model are simple, a feature that increases throughput and reproducibility. This new model should be applicable to the evaluation of novel antimicrobial treatments of superficial infections caused by *S. aureus* and *S. pyogenes*.

An important stage in testing the potential of chemicals as antimicrobial drug candidates is establishment of their effectiveness in an animal model system (12, 30). A useful animal model system should be clinically relevant, experimentally robust, ethically acceptable, and convenient to perform and should provide reliable and reproducible results. We report here on a new model in mice that fulfills these criteria. The tape-stripping model has been developed to test the effectiveness of topical antibiotic treatments of superficial skin infections caused by *Staphylococcus aureus* or *Streptococcus pyogenes*. *S. aureus* and *S. pyogenes* are the most common causative agents of primary skin infections in humans (11, 17).

The existing mouse models for topical treatment of skin infections are the burnt skin model (1, 32, 36) and the skin suture-wound model (5, 14). Either the bacteria are introduced into the skin by injection in a traumatized skin area, as in the burnt skin model, or a bacterium-impregnated nylon suture is implanted into an artificial wound (a scalpel incision through all skin layers), which is then sewn or stapled shut, as in the skin suture-wound model. The area of infection in these models is usually dorsally located to hinder grooming or

cleaning by the animal itself. Antibiotics dissolved in cream or ointment can be applied to the wound during the course of the experiment. At the experimental end point, the animal is killed, the infected area of the skin is cut out, and the number of bacteria in the sample is assayed (1, 5, 14, 32, 36). These mouse models for skin infections have some disadvantages in relation to superficial infections. The burnt skin model has been developed for studying issues related to infections in burn patients and is ethically unacceptable from an animal welfare perspective for the study of localized skin infections, such as impetigo and erysipelas. The skin suture-wound model involves cutting into the deeper layers of the skin and is thus not clinically relevant to purely topical conditions. Here we describe a new mouse model for superficial infections, the tape-stripping model. In this model a way of entry for the infectious agent is created by stripping off the fur and epidermis in a region on the back of the mouse by successive applications of an adhesive bandage.

In the development of this model, we used *S. aureus* and *S. pyogenes* as the infectious agents. Both bacteria are endemic in human populations and are regarded as opportunistic bacteria, usually causing infections in children, immunocompromised patients, or patients suffering from the effects of medical surgery (11, 22, 35). *S. aureus* can be isolated from throat or nasal swab samples from approximately one-third of the population (2, 16, 26, 33, 34) and is also commonly found in the skin flora, together with related species, such as *Staphylococcus epidermidis* (6, 7). Topical infections due to *S. aureus* and *S. pyogenes* are clinically relevant and cause a variety of serious symptoms, including toxic shock syndrome and skin lesions (17, 31), that can progress to sepsis and systemic shock if they are left untreated (3, 10, 24). These bacterial species are also the most common causes of impetigo in humans (13, 17). The antibiotic used to test the model was 2% fusidic acid in ointment (FAO; Fucidin ointment). In vitro FAO shows high levels of activity against several gram-positive organisms and species of staphylococci in particular (19, 20, 23).

MATERIALS AND METHODS

Bacterial strains and growth media.

Staphylococcus aureus FDA486 is a laboratory strain previously used to study wound infections in rats (25). *Streptococcus pyogenes* 301 is a clinical dermal infection isolate obtained from the University Hospital in Uppsala, Sweden. The Dry SPOT Streptococcal Grouping kit (DR0400 M; Oxoid Ltd., Basingstoke, United Kingdom) was used to determine the Lancefield type of the streptococcal strain. The streptococcal bacteria were grown overnight anaerobically at 37°C on defibrinated horse blood agar before they were tested according to the manufacturer's instructions. *S. pyogenes* 301 belongs to Lancefield serological group type A. The in vitro MICs of fusidic acid for these strains, as determined by Etest (AB BIODISK, Solna, Sweden), were as follows: *S. aureus* FDA486, 0.125 µg/ml and *S. pyogenes* 301, 4 µg/ml. *S. aureus* was grown in Luria broth and on Luria agar plates (Oxoid Ltd.). *S. pyogenes* was grown in Todd-Hewitt broth (Sigma Aldrich, Stockholm, Sweden) and on blood agar plates made by mixing 5% (wt/vol) defibrinated horse blood (National Veterinary Institute, Uppsala, Sweden) with Luria agar. *S. aureus* was grown aerobically at 37°C, and *S. pyogenes* was grown anaerobically (10% CO₂) at 37°C.

Tape-stripping infection model.

Animal infection experiments were performed at the Microbiology and Tumor Biology Center, Karolinska Institute (Stockholm, Sweden), in accordance with institutional and national guidelines (ethical permit N154/02). Six- to 8-week-old female BALB/c mice (Taconic M&B, Ry, Denmark) were used for all experiments. The mice were anesthetized by intraperitoneal injection of 10 ml/kg of body weight of a 1:1:2 (vol/vol) mixture of Hypnorm (fentanyl/fluanisone; Janssen-Cilag Ltd., Saunderton, United Kingdom)-Dormicum (midazolam; Hoffman-La Roche AG, Basel, Switzerland)-distilled water. The fur was stripped from the mice with Tensoplast (Smith & Nephew Medical, Hull, United Kingdom), an elastic adhesive bandage. An area of ca. 2 cm² was tape stripped. In order to standardize the degree of barrier disruption elicited by the tape stripping, the transepidermal

water loss (TEWL) was measured by using a DermaLab TEWL probe (Cortex Technology, Hadsund, Denmark). Measurements were made according to the guidelines of the Standardization Group of the European Dermatitis Society (27). TEWL is calculated automatically and is expressed in g/m² h. By tape stripping the back of the mice 7 to 10 times in succession, the TEWL reached approximately 70 g/m² h. Following this procedure, the skin became visibly damaged and was characterized by reddening and glistening but no regular bleeding. Microscopically, this procedure resulted in the controlled removal of most of the epidermal layer, with only a few basal epidermal cells remaining. After stripping of the skin, a bacterial infection was initiated by placing on the skin a 5- μ l droplet containing 10⁷ cells concentrated from an overnight bacterial culture in stationary phase. In each experiment, a group of mice were killed 4 h after infection to control the infectious dose (Table (Table1).1). The mice were treated with FAO (LEO Pharma, Ballerup, Denmark) on a regular basis, as described here. This dose gave a significant reduction in the numbers of CFU in preliminary dose-finding studies (0.5%, 1%, and 2% fusidic acid) and is the dose recommended by the manufacturer for human use. The first application of antibiotic to the stripped skin of the mice was at 4 h postinfection. Thereafter, beginning at 16 h after the first treatment, additional antibiotic applications were made twice daily (in the morning and the evening, with an 8-h interval) for a period of 4 days. For each treatment 25 to 30 mg of ointment was applied (estimated by weighing the pellet of ointment on a spatula). After each day the ointment tube was weighed to determine the average amount of ointment used for each mouse. Two infection control groups were included for each experiment: one consisted of untreated mice and the other consisted of mice treated with placebo ointment. The placebo ointment was identical to FAO except for the lack of the 2% fusidic acid. For all experiments in which untreated or topically treated groups were included, the experiments were terminated 18 h after the last topical treatment in order to avoid carryover effects in vitro. The addition of fucidinase (2.5 units per sample) to the homogenized samples did not influence the numbers of CFU, showing that 18 h is sufficient to avoid a carryover effect. Immediately after the mice were killed, the wounds, approximately 2 cm², were excised and homogenized together with 1 ml of phosphate-buffered saline in stomacher lab system bags by using a Stomacher 80 machine (Seward Ltd., Thetford, United Kingdom) set at 260 strokes per min for 120 s. The homogenates were washed once in phosphate-buffered saline to decrease the concentration of ointment. Suitable dilutions of the homogenates were plated on Luria agar (*S. aureus*) or blood agar (*S. pyogenes*) plates to determine the number of living bacteria (CFU). In order to investigate the reproducibility of the infection with *S. aureus* and *S. pyogenes*, three independent experiments that included untreated and placebo-treated groups were performed. In each experiment the mean CFU was calculated by using log₁₀-transformed data. Based on the averages of these three experiments, the mean, range, and coefficient of variation were calculated.

TABLE 1.
Bacterial counts obtained in the various treatment groups

Group	Mean bacterial count (log ₁₀ CFU/wound) \pm SD (no. of wounds)	
	<i>S. aureus</i> FDAH0 ^a	<i>S. pyogenes</i> 301 ^b
Inoculum size	7.21 \pm 0.08	7.28 \pm 0.11
4 h postinfection	7.03 \pm 0.37 (7)	6.48 \pm 0.29 (16)
Untreated (day 4)	5.89 \pm 0.64 (8)	3.31 \pm 1.62 (16)
Placebo (day 4)	6.03 \pm 0.31 (7)	6.13 \pm 0.37 (16)
FAO (day 4)	4.68 \pm 0.78 (12)	2.04 \pm 1.31 (8)

^aFA = placebo ($P = 0.001$); 4 h = placebo ($P = 0.03$); 4 h = untreated ($P = 0.01$); untreated = placebo ($P = 1.00$).
^bFA = placebo ($P = 0.001$); 4 h = placebo ($P = 1.00$); 4 h = untreated ($P = 0.001$); untreated = placebo ($P = 0.001$).

Bacterial counts obtained in the various treatment groups Suture-wound model.

The established skin suture-wound model was carried out as described previously (14).

Histological examinations: In order to characterize the histopathology of the model, biopsy specimens were taken after the following treatments: immediately after tape stripping, 4 days after tape stripping, and 4 days after inoculation with *S. aureus* and *S. pyogenes* with and without treatment with placebo ointment. Immediately after the animals were killed, 5-mm punch biopsy specimens of excised skin were taken and immediately fixed in phosphate-buffered (pH 7.4) formalin (4%). The formalin-fixed biopsy specimens were



embedded in paraffin and stained with hematoxylin and eosin. For identification of the bacteria, the biopsy specimens were stained with Gram's crystal violet solution (94448; Sigma-Aldrich). The following parameters and semiquantitative scoring system were used to describe the inflammatory response: for scoring of the inflammation (in the dermis, subcutis, muscular tissue, and connective tissues), 0, no inflammation present; 1, little inflammation present; 2, moderate inflammation present; and 3, severe inflammation present; for scoring of the presence of neutrophils, 0, no neutrophils present; 1, a few neutrophils present; 2, moderate occurrence of neutrophils; and 3, abundant occurrence of neutrophils; for scoring of the presence of mononuclear leukocytes, 0, no mononuclear leukocytes present; 1, a few mononuclear leukocytes present; 2, moderate occurrence of mononuclear leukocytes; and 3, abundant occurrence of mononuclear leukocytes; for scoring of presence of bacteria, 0, no bacteria; 1, scattered bacteria; 2, moderate numbers of bacteria; and 3, many large collections of bacteria. The observer was blinded to the treatments for all biopsy specimens.

Behavioral responses of mice.

The mice were observed at least twice each day for signs of fatigue, stress, and aggressiveness. The mice were weighed before and after each experiment.

Statistical analysis.

Statistical analysis of the log₁₀-transformed data was performed to ensure variance homogeneity and normality. Thus, an analysis of variance (generalized linear models [8]) was applied, followed by four predefined pairwise treatment comparisons adjusted for multiplicity by the Bonferroni method (18), yielding a statistical ranking. Furthermore, to ensure robustness in the analysis performed, the nonparametric Kruskal-Wallis approach (21) was used. The method showed no conclusive dissimilarities to the generalized linear models approach. All testing was performed on an overall 5% significance level, meaning that P values less than 0.05 were considered a statistically significant difference. All calculations and analyses were performed with SAS version 8.2 (SAS OnlineDoc; SAS Institute Inc., Cary, NC). Log₁₀-transformed data are presented as the mean and standard deviation (SD) in Table Table1,1, whereas actual values and corresponding median values are presented in the figures.

RESULTS

Establishment of staphylococcal infection.

The number of CFU recoverable from the wound 4 h after application of 10⁷ CFU of *S. aureus* FDA486 was 7.03 ± 0.37 log₁₀ (Table (Table1).1). The different treatment regimens were begun after this initial 4-h period. There was no significant difference (P = 0.05) in the numbers of CFU per wound when 4 h versus 4 days of placebo treatment were compared (6.05 ± 0.31 log₁₀). This is evidence of the successful establishment of a staphylococcal infection in this model. There was a slightly greater reduction in the numbers of CFU per wound that was statistically significant (P = 0.01) when 4 h versus 4 days of no treatment (5.89 ± 0.64 log₁₀) were compared. The differences between the 4-day placebo treatment and 4 days of no treatment were not statistically significant (P = 1.00).

Effect of fusidic acid treatment on staphylococcal infection.

Tape-stripped mice infected with *S. aureus* FDA486 were treated with FAO to test the efficacy of a topical antibiotic treatment in the new model (Fig. (Fig.1).1). Comparison of placebo (6.05 ± 0.31 log₁₀) and FAO (4.68 ± 0.78 log₁₀) treatment of the staphylococcal infection revealed a reduction in the numbers of CFU per wound that was statistically significant (P < 0.001).

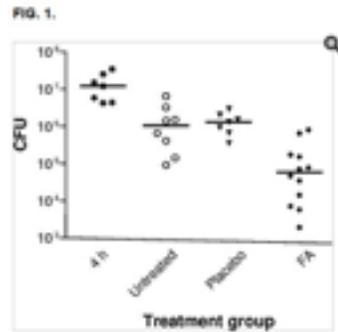


FIG. 1. Tape-stripped mice infected with *S. aureus* FDA338. The number of bacteria (CFU) extracted from each mouse is represented by the symbol for the corresponding experimental group. The median value of the data for each group is shown as a horizontal bar. The number of mice in each experimental group was as follows: 4 h postinfection, n = 7; untreated, n = 5; placebo, n = 5; FAO, n = 12.

Establishment of streptococcal infection. The number of CFU recoverable from the wound 4h after application of approximately 10⁷ CFU of *S. pyogenes* 301 was 6.48 ± 0.29 log₁₀ (Table (Table1).1). Comparison of the numbers of CFU after 4 h and 4 days of placebo treatment (6.15 ± 0.57 log₁₀) revealed no significant difference (P = 1.00). In contrast, comparison of the numbers of CFU at 4 h and 4 days without treatment (3.21 ± 1.62 log₁₀) revealed a significant reduction (P < 0.001). Comparison of the placebo and the untreated groups 4 days after infection confirmed the significance of the reduction in the numbers of CFU in the latter group (P < 0.001).

Effect of fusidic acid treatment on streptococcal infection.

Comparison of placebo and fusidic acid treatments for the streptococcal infection (Fig. (Fig.2;2; Table Table1)1) revealed a very significant effect (P < 0.001) of the antibiotic in clearing the infection. Thus, the mean CFU count after the 4-day FAO treatment (2.04 ± 1.31 log₁₀) was reduced more than 4 log₁₀ compared with that after the 4-day placebo treatment (6.15 ± 0.57 log₁₀). Statistical comparison of the 4-day FAO treatment group and the 4-day untreated group showed that there was no significant difference (P > 0.05)

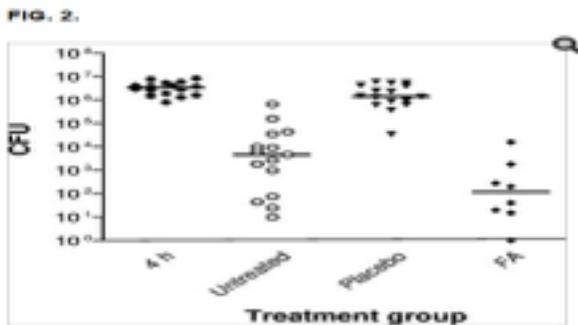
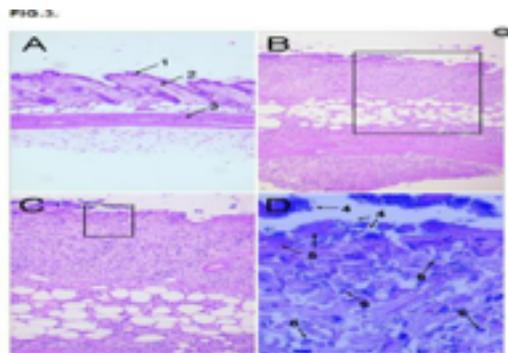


FIG. 2. Tape-stripped mice infected with *S. pyogenes* 301. The number of bacteria (CFU) extracted from each mouse is represented by the symbol for the corresponding experimental group. The median value of the data for each group is shown as a horizontal bar. The number of mice in each experimental group was as follows: 4 h postinfection, n = 16; untreated, n = 16; placebo, n = 16; FAO, n = 8.

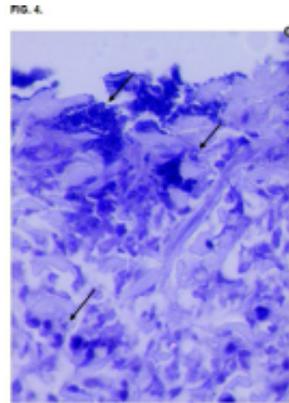
Reproducibility of the model. The reproducibility of the model was assessed in three independent experiments, each of which included both *S. aureus* and *S. pyogenes*, with untreated and placebo-treated mice. The coefficients of variance (CVs) for the untreated mice were 18% (mean, 6.09 log₁₀; SD, 1.10 log₁₀) for those infected with *S. aureus* and 36% (mean, 3.96 log₁₀; SD, 1.41 log₁₀) for those infected with *S. pyogenes*. The CVs for the placebo-treated mice were 30% (mean, 6.21 log₁₀; SD, 1.86) for those infected with *S. aureus* and 12% (mean, 6.62 log₁₀; SD, 0.81 log₁₀) for those infected with *S. pyogenes*.

Histological examination of infected and noninfected mice.

The results for the scores of the inflammatory response, infiltrating neutrophils and mononuclear leukocytes, and the presence of bacteria are presented in Table 2.2. The tape-stripping procedure removed most of the epidermis, although a single layer of epidermal cells or scattered epidermal cells remained. Most of the remaining epidermal cells were necrotic. A minimal inflammatory response was evident immediately after tape stripping, and except for the missing epidermis, the skin appeared normal. Four days after tape stripping an acute subcutaneous phlegmonous inflammation with fibrin deposition and edema was observed with the infiltration of a few neutrophils. The inflammation was most pronounced in the subcutaneous and connective tissues. Inoculation of both *S. aureus* and *S. pyogenes* induced a late stage of subcutaneous fibrinoid necrosis after 4 days, with a few neutrophils mainly seen as nuclear dust deep in the subcutis (Fig. 3). In addition to neutrophils, the inflammatory cell infiltrate consisted of mononuclear cells, including lymphocytes and histiocytes. The inflammatory response was most intense in the subcutaneous tissues. For both mice infected with *S. aureus* and mice infected with *S. pyogenes*, the presence of bacteria (Fig. 4) was less evident in the untreated mice than in placebo-treated mice and the skin appeared dry compared to the skin of the placebo-treated mice. In some of the infected animals, fibrosis and remarkable regenerative changes were observed in the subcutaneous tissue.



Histological appearance of normal dorsal skin of mice (A; magnification, ×100) and *Staphylococcus aureus*-infected skin lesion (B, magnification, ×100; C [boxed area in panel B], magnification, ×200; D [boxed area in panel C], magnification, ×1,000) on day 4. Biopsy specimens were taken immediately after the termination of the experiment, fixed in formalin, and embedded in paraffin. The biopsy specimens were stained with hematoxylin and eosin. The inflammatory cell infiltrate consists of mononuclear cells, including lymphocytes, histiocytes, and, to lesser extent, neutrophils. The inflammatory response is associated with marked fibrosis, edema, and fibrin deposition. Coccoid bacteria are present. The epidermal layer is absent in the infected lesions. Numbered arrows indicate the following: 1, epidermis; 2, dermis; 3, muscular layer; 4, bacteria; 5, fibrin deposition; 6, inflammatory cell infiltrate



Staining of gram-positive bacteria in *Staphylococcus aureus*-infected skin (magnification, $\times 1,000$) on day 4. Biopsy specimens were taken immediately after the termination of the experiment, fixed in formalin, embedded in paraffin, and stained with Gram’s crystal violet solution. Cocci bacteria are present in the superficial layers of the dermis (arrows).

TABLE 2.

Scores for inflammatory response, infiltrating neutrophils and mononuclear leukocytes, and presence of bacteria

Group, day, and parameter	Mean ^a	SD	Untreated		Placebo	
			Mean	SD	Mean	SD
Tape-stripped skin						
Day 0 (n = 5)						
Inflammation score						
Dermal	0.0	0.0				
Subcutaneous	0.1	0.4				
Muscular	0.0	0.0				
Connective tissue	0.0	0.0				
Neutrophils	0.0	0.0				
Mononuclear leukocytes ^b	0.0	0.0				
Presence of bacteria	0.0	0.0				
Day 4 (n = 5)						
Inflammation score						
Dermal	0.8	1.4				
Subcutaneous	2.6	0.5				
Muscular	0.3	0.7				
Connective tissue	1.3	1.3				
Neutrophils	0.3	0.3				
Mononuclear leukocytes ^b	0.0	0.0				
Presence of bacteria	0.0	0.0				
<i>S. aureus</i> , day 4 ^c						
Inflammation score						
Dermal			1.3	1.2	0.0	0.0
Subcutaneous			2.5	0.8	2.4	0.9
Muscular			0.4	0.5	1.6	1.1
Connective tissue			0.4	0.5	1.1	0.5
Neutrophils			0.6	0.6	0.5	0.4
Mononuclear leukocytes ^b			0.4	0.2	1.2	1.7
Presence of bacteria			0.6	0.7	3.0	0.0

S. pyogenes, day 4^d

Inflammation score				
Dermal	0.0	0.0	1.2	1.3
Subcutaneous	2.8	0.5	2.2	1.3
Muscular	0.3	0.5	1.6	1.1
Connective tissue	0.5	1.0	1.1	0.5
Neutrophils	0.3	0.5	0.7	0.3
Mononuclear leukocytes ^b	0.3	0.3	1.7	0.7
Presence of bacteria	0.3	0.3	2.4	1.3

^aScoring quantification is described in Materials and Methods.

^bMononuclear leukocytes include lymphocytes and histiocytes.

^cEight mice were in the untreated group and six mice were in the placebo group.

^dFive mice were in the untreated group and five mice were in the placebo group.

Behavioral responses of the mice. No abnormal behavioral patterns, such as fatigue, stress, or aggressiveness, were observed among the mice at any time during the course of these experiments. The mice gained, on average, 0.5 g of weight during the experiment, with no differences between the various test groups. A small fraction (2 of 48) of the mice displayed signs of deeper infections, with necrotized tissue and softening of the backbone skeletal structure (data not shown)

DISCUSSION

We have established a new model for superficial skin infections caused by *S. aureus* and *S. pyogenes* which we suggest to be a relevant and useful model for localized skin infections in humans. In contrast to previously described models for skin infection (1, 5, 14, 32, 36), the infection route in our model is topical. Partial removal of the epidermal layer of the skin allowed both *S. aureus* and *S. pyogenes* to colonize the skin and to elicit a profound inflammatory response. TEWL, which is a measure of skin barrier integrity, was used in order to ensure that the same degree of barrier disruption was achieved in all the mice and, thereby, to increase the reproducibility of the model.

The infection was maintained throughout the 4-day duration of the experiment (placebo groups). Thus, in the placebo groups, from an initial infection with 10⁷ bacteria of either species, the number of CFU recovered from each wound dropped by less than 1 log₁₀ over the course of the 4-day treatment. Topical treatment with FAO significantly reduced the numbers of *S. aureus* and *S. pyogenes* CFU recoverable after the 4-day treatment (Fig. (Fig.11 and and2;2; Table Table1),1), showing that an established topical treatment is effective in the model. For both *S. aureus* and *S. pyogenes*, the numbers of recoverable CFU were different when the mice were left untreated for 4 days and when the mice were left untreated for 4 h (Table (Table1).1). For *S. aureus* this number was significantly higher (1.2 log₁₀; P < 0.001) than that for the fusidic acid-treated group. For *S. pyogenes* the number of bacteria was also 1.2 log₁₀ higher in the untreated groups than in the fusidic acid-treated animals, although this difference was not significantly different (P > 0.05). A plausible explanation



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for this is that *S. pyogenes* grows best under microanaerobic conditions (17) and that such conditions are more closely approximated by the presence of ointment in this assay. The genetic background of the BALB/c mice may also explain the reduced number of streptococci in the untreated group, as BALB/c mice have been shown to be much more resistant to group A streptococci than C3H/HeN mice (15). However, the use of more susceptible mouse strains may lead to an unacceptably high mortality rate, as observed in C3H/HeN when they are exposed to streptococci. In our model, BALB/c mice were used because the dominating agent in superficial infections, *S. aureus* (11, 17), colonized these mice well. BALB/c mice are also considered relevant because they respond immunologically to the superantigens (staphylococcal enterotoxin B) produced by *S. aureus* (29).

A pronounced and significant reduction in the numbers of CFU was achieved following 4 days of treatment with FAO. However, complete eradication of the staphylococci and streptococci was not reached after 4 days of treatment. This was expected, as superficial infections normally should be treated for 7 to 10 days in order to obtain a successful outcome (13). The fact that the infection was not cleared after this time allows the model to be used to compare various antimicrobial treatments with FAO.

Histologically, inoculation with *S. aureus* and *S. pyogenes* induced a pronounced acute inflammatory response characterized by the presence of neutrophils, lymphocytes, histiocytes, and fibrin deposition. The inflammatory response included most of the layers of the skin. Considering the histology of the infection caused by the staphylococci and streptococci, it most resembles that of human erysipelas, except that our model lacks the epidermal layer. Erysipelas is an acute bacterial infection of the dermis and subcutaneous tissues that is associated with clinical inflammation. Erysipelas is generally caused by group A streptococci (4). The model has less resemblance to the histology of impetigo, which is a contagious superficial pyogenic infection of the skin caused by *S. aureus* and *S. pyogenes*. In impetigo, the epidermis splits just below the stratum granulosum and large subcorneal pustules are formed, and these may also contain bacteria.

The upper dermis contains an inflammatory infiltrate of neutrophils and lymphocytes (9). In our murine model, the formation of subcorneal pustules does not occur. We propose that our model would be a relevant disease model for localized skin structure infections caused by *S. aureus* or *S. pyogenes*, which can occur following skin barrier disruption.

The disruption of the barrier by using tape stripping resulted in a homogeneous removal of the upper epidermal layers in all the biopsy specimens examined immediately after tape stripping. Considering the numbers of CFU, the reproducibility of the model is acceptable, in that it has CVs that ranged from 12% to 36%, depending on the strain and the treatment.

The tape-stripping model presented here is relatively painless and noninvasive for the animal. It is technically quick and simple to perform, with only a few uncomplicated steps involved in preparing the animals for treatment. In the process of validating this new model, we also performed some preliminary experiments using the established skin suture-wound model (14). In our hands, the throughput time per mouse (the total time taken to prepare a wound and inoculate it with bacteria) was approximately 2 min for the tape-stripping model, whereas it was at least 20 min for the skin suture-wound model. The reduction in the time required to process each animal by use of the tape-stripping model relative to that required by use of the skin suture-wound model provides a significant advantage when one is dealing with many animals. Considering the reduction of the numbers of CFU following treatment with FAO, the efficacy of fusidic acid is in agreement with those detected in previous investigations of FAO, e.g., by use of the skin suture-wound model (14, 28). Also, the variability in the model appears to be comparable to that detected in previous studies with the skin suture-wound model (14).



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In conclusion, partial removal of the epidermal layer of BALB/c mouse skin by tape stripping allows *S. aureus* and *S. pyogenes* to colonize the skin, and this colonization is associated with an inflammatory host response, as determined by histology. Infections with both *S. aureus* and *S. pyogenes* can be treated by topical administration of FAO. The model is simple and reproducible and can be used for the evaluation of new antibiotic treatments for superficial skin infections. The model may also be advantageous for studies of the mechanisms involved in superficial skin infections.

III) The Art Of Shampoos In Veterinary Dermatology: Treatment And Prevention Strategies

Topical therapy (locally acting) is extremely important in the management of many dermatological conditions. Several formulations are available for the prescribing veterinary surgeon: shampoo, lotion, spray, ointment, cream, milk and gel. Choice varies according to the case and must take into account the nature and extent of the lesions, animal's temperament and owner time available. Shampoos are nowadays widely used by veterinary dermatologists.

Shampoos: What are they, how do they work, how to use them?

A shampoo is an aqueous solution, with added surfactant(s), cleansing agents and various other therapeutic and/or cosmetic agents. Cleaning agents rid the skin surface of debris and help clear the apical pole of hair follicles.

Washing the skin with a topical cleanser should always precede actual topical therapy. Ideally, a shampoo possessing both cleansing and therapeutical properties should be applied twice.

A shampoo can be used in a limited area (eg chin, feet, dorsolumbar, ventral areas), as in humans for the hairy skin, or more commonly all over the body surface of a dog or a cat for treating generalized conditions.

The mechanical effect (elimination of scales and crusts) of the bath is beneficial in all cases. Water rehydrates the stratum corneum although this effect is temporary in the absence of moisturisers.

At the second application, the shampoo must be left on for several minutes, to allow the active ingredients to be properly absorbed and reach adequate levels in the deep cellular layers. This length of time varies between 5 and 15 minutes according to choice of product, concentration, type of base, and the skin condition. The skin should then be rinsed thoroughly, for at least 5 minutes, to prevent irritation and to enable the skin to become adequately hydrated.

The shampoo may be applied several times a week for 2 weeks. The frequency is then reduced to give the longest interval over which treatment is still effective, usually about 1 to 2 weeks.

Efficacy of shampoo therapy

Clinical improvement is the main criterion to evaluate the efficacy of shampoos (see below for efficacy in specific indications) (2). Their use has increased greatly in North America over the past 25 years, but they have been slow to gain acceptance in Europe (3). However, they are now widely used in the old continent, despite the fact that they were considered as contraindicated and even harmful by many teachers in veterinary schools in the 60's, who recommended "not to wash dogs". This was a mistake and has probably delayed considerably the use of medicated shampoos, which is now considered as being indispensable by the veterinary dermatology community.

The efficacy of shampoos on skin hydration, the surface lipid film and stratum corneum (interesting in case of keratoseborrheic disorders) can be evaluated objectively using a variety of techniques : transepidermal water



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loss (TEWL) measurement, corneocyte counts, measurement of corneal layer thickness, stripping, chemical analysis of lipid film, water content measurement, surface biopsies and corneometry (4-8). In one study (7) corneometry, but not TEWL measurement, was found to give reproducible results. In another study, results from TEWL measurement, corneometry and sebometry were not reproducible and these procedures were therefore deemed to be useless in evaluating effects of topical treatments in the dog (8). Electron microscopy could perhaps be useful (9).

In recent years, there has been considerable progress in improving topical formulations, especially in prolonging the action of active ingredients applied to the skin. Microencapsulation (multilamellar microvesicles, liposomes, spherulites) increases bioavailability of therapeutic agents and promotes immediate and residual moisturising properties. Active agents are released from liposomes by membrane rupture. Spherulite surfactants are amphiphilic (two antagonistic extremities – one hydrophilic, the other hydrophobic). They unit to form lamellar phases and are arranged in concentric layers according to a specific manufacturing process. They are multilamellar, each membrane acting as a diffusion barrier to reduce loss of active ingredients to the external environment. They can act as a vehicle for a great number of active agents, hydrophilic or hydrophobic (lipophilic), released continuously and progressively at the surface of hairs and skin. This surfactant formulation is very useful in dermatology because it allows hydrophilic, active ingredients access to an oily environment and conversely hydrophobic, active ingredients access to an aquatic medium. The type of surfactant varies. In some cases (cationic surfactants), their charge is positive and spherulites attach preferentially to hairs and skin, whilst in other cases (non-ionic surfactants), the charge is neutral, allowing spherulites to penetrate the deeper skin layers. A study has demonstrated that non-ionic spherulites can penetrate the epidermis, hair follicles, sebaceous glands and dermis (10). The presence of chitosanide reinforces the cationic phase and, by creating a film sheath over the hair, promotes excellent moisturising properties.

A new veterinary formulation (micro-emulsion), with excellent solubility of active ingredients, has recently become available.

The use of shampoos in keratoseborrhoeic disorders (11)

1 Keratomodulating agents Keratomodulating agents work in two different ways :

- restoration of normal keratinocyte multiplication and keratinisation. A cytostatic effect is probably exerted on basal cells, thereby reducing their rate of division. Agents working in this way are called keratoplastic (keratoregulating) ;

- elimination of excess corneal layer production, either by increasing desquamation or by reducing intercellular cohesion. Agents that work in this way are called keratolytic.

There are several types of keratomodulating agents. There are also antiseborrhoeic agents which act at the level of the sebaceous gland and its duct (1,12,13).

Salicylic acid is a keratolytic agent. It causes a reduction in skin pH which leads to an increase in:

1) the amount of water that keratin is able to absorb. Stratum corneum hydratation is therefore also increased ;

2) desquamation, via direct effect on intercellular cement.

These actions help soften the corneal layer. Salicylic acid acts synergistically with sulphur, and is often present



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in small quantities in shampoos. Its efficacy varies with concentration.

Coal tar is a keratoplastic (cytostatic) agent. It reduces nuclear synthesis in the epidermal basal layers (13,14). It is also antiseptic and antipruritic. There are many different sources and varieties of this active agent. Tar is a complex mixture of aromatic hydrocarbons, with many constituents (more than 10,000). It is hard to determine which is (are) responsible for therapeutic effects. Standardisation is therefore difficult, and good quality preparations must be used. Smell and consistency of commercial preparations sometimes make it difficult to use, although deodorised veterinary preparations are now available. Side-effects (e.g. skin drying, discolouration of pale coats and irritation) have been reported with high concentrations (over 3 %) (1). Its use is contraindicated in the cat.

Sulphur is keratolytic. It forms hydrogen sulphide in the corneal layer and has numerous other, mainly antiseborrheic, properties (see below). It is also keratoplastic, due to a direct cytostatic effect and possibly because it interacts with epidermal cysteine to form cystine, an important component of the corneal layer (3,12-14). It is gradually being replaced in topical products by other more effective keratomodulating agents with fewer side-effects (e.g. a rebound increase in scaling).

Selenium disulphide is keratolytic and keratoplastic (reduced epidermal turnover and impaired disulphide bridge formation in keratin). It is also antiseborrheic (see below) (3,12,13). It too can cause rebound increase in scaling and sometimes skin irritation.

Ammonium lactate has keratoplastic and keratolytic activity. In the management of human seborrhoea, it has been shown to be effective in removing excessive scale by virtue of its keratoplastic activity (15-18). Its mechanisms of action in seborrheic disorders have not yet been completely elucidated but it seems to stimulate the living epidermis, correcting defects in keratinocyte multiplication and maturation. This facilitates terminal keratinocyte differentiation, leading to more normal desquamation (19,20). Its properties are useful in seborrheic disorders where the ammonium lactate has important moisturising properties (17,18,20). Several clinical studies in man indicate that this substance is very well-tolerated, even when used over prolonged periods (15-19).

2 Antiseborrheic agents Antiseborrheic agents inhibit or reduce sebum production by the sebaceous glands, and help clear the ducts.

Sulphur (see above) is a classic antiseborrheic agent, but is drying and may trigger a rebound effect. It is also antiseptic. It exerts synergistic activity with salicylic acid. This synergism appears optimal when both substances are incorporated into the shampoo in equal concentrations (21).

Selenium disulphide (see above) is antiseborrheic, but also has detergent, irritant and drying effects. It is contraindicated in the cat. Benzoyl peroxide, in addition to being antibacterial, is antiseborrheic, thanks to sebum hydrolysis and reduced sebaceous gland activity. One study showed that 3% benzoyl peroxide shampoos increase transepidermal water loss and decrease skin surface lipid concentration and corneocyte counts (6). Benzoyl peroxide exerts a follicular flushing action which is very useful when treating comedone disorders and/or follicular hyperkeratosis (3,4,13,22). Side effects (irritations, erythematous rash) have been reported especially in concentrations above 5% (4). The skin may also become dry and emollients are therefore always indicated after using this product.

3 Essential fatty acids Various veterinary shampoos have incorporated essential fatty acids for their softening and moisturising properties. One study has demonstrated that in seborrheic dogs, abnormal transepidermal



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water loss could be corrected by applying linoleic acid (23). Some shampoos contain moisturisers : glycerin, lactic acid and fatty acid polyesters. Moisturisers can be stored in multilamellar structures for prolonged release (spherulites), or mono/oligolamellar bodies (liposomes) to ensure hydration levels are maintained.

4 How to use shampoos in keratoseborrhoeic disorders Certain guidelines are suggested : - long- haired dogs with severe seborrhoeic disorders should be clipped. Clipping leads to more effective application and better distribution of the active ingredient ; - shampoos should initially be applied 2 to 3 times weekly. With time, frequency of application can gradually be reduced ; - cases should be monitored frequently. The therapeutic agent often needs to be changed following the development of side-effects, rebound effects or change in clinical presentation (e.g. transition from greasy seborrhoea to dry seborrhoea).

IV The use of shampoos in parasitic diseases

Antiparasitic shampoos, ie containing organochlorines, natural pyrethrins or synthetic pyrethroids, are not considered to be as efficacious as antiparasitic rinses and dips (12) and other formulations (sprays, pump-sprays, powders, spot-ons, line-ons, systemic agents), mainly because they are rinsed and cannot act during a sufficient time (2). Scabies, cheyletiellosis, otodectic mange, tick infestation, trombiculosis and pediculosis are relative indications.

Insecticidal shampoos often contain synthetic pyrethroids chosen for their rapid knock-down effect: these are best used as a convenient one-off treatment to rid an animal of a resident flea infestation. As there is usually little or no residual action once the shampoo is rinsed off, the treated animal is immediately vulnerable to reinfestation by host-seeking fleas. Normally, therefore, shampoos have limited application in the long-term management of flea infestation (pulicosis) and flea allergy dermatitis (24). However, a shampoo containing deltamethrin (0.07%) has been shown recently to maintain a > 90 % antifeeding effect during the hour following challenge for one week (25).

Colloidal oatmeal, an antipruritic agent (see below) is added to bioallethrin , a pyrethroid, in a shampoo to decrease inflammation due to the parasitic infestation.

Benzoyl peroxide shampoos are recommended in the treatment of demodicosis because of their degreasing and follicular flushing effect (3,12).

Many parasitic diseases (e g scabies, cheyletiellosis) and flea allergy dermatitis can cause a keratoseborrhoeic disorder and the affected animals will benefit from application of keratomodulating shampoos (3).

V The use of shampoos in bacterial diseases (pyoderma) Topical therapy is used in canine pyoderma to reduce the cutaneous bacterial population and antibacterial shampoos also remove tissue debris, allowing direct contact of the active ingredient with the organism and promoting drainage (12).

Limited cases of superficial pyoderma can be treated with shampoos alone, particularly if they are used frequently at the beginning (e g every day), then decreasing the frequency of applications depending on the animal's response. However in most cases systemic antibiotics will be administered to ensure a more prompt response, the shampoo playing a supporting role (3). A common indication for long-term use is in the dog that is prone to recurrent folliculitis, either idiopathic or eventually secondary to endocrine or allergic skin disease, even though the pruritus due to the allergic skin disease is controlled. In these situations well tolerated antibacterial shampoos may have a prophylactic effect if used regularly i e every one to two weeks(3,12).

In case of deep pyoderma, clipping is preferable before using shampoos (and soaks). This will prevent the



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formation of a sealing crust and allow the product to contact the lesions (furuncles, ulcers) (12). In such cases, shampoos should be used very frequently at the initiation of treatment.

Agents commonly included in antibacterial shampoos are chlorhexidine, povidone-iodine, benzoyl peroxide and ethyl lactate.

Chlorhexidine (4,26) is a biguanide antiseptic, very effective against most bacteria (Gram + and -), except some *Pseudomonas* and *Serratia* strains. It is bactericidal by action on cytoplasmic membrane which causes leak of intracellular components. Concentrations vary in shampoos from 0.5 to 4% (diacetate or digluconate). It has a prophylactic effect due to its remanence (27,28). It is well tolerated.

Povidone-iodine is a iodophore which slowly releases iodine to tissues (4,12). The titratable iodine is usually of the order of 0.2 to 0.4 per cent. It is bactericidal and acts in a few seconds at 0.005 % (2). It has also a prophylactic effect due to its remanence (28). It is relatively drying which can be compensated by emollients in shampoos. It can be irritant and staining (4).

Benzoyl peroxide (see above) is metabolized in the skin to benzoic acid and much of its microbiocidal activity probably derives from the lowered skin pH (3). This disrupts microbial cell membranes (3,4). It is in fact an oxidizing agent, which releases nascent oxygen into the skin and produces a series of chemical reactions resulting in permeability changes and rupture of bacterial membranes (4). It has an excellent prophylactic effect, the best one in a comparative study with chlorhexidine, complexed iodine and triclosan (28). It is generally used in concentrations of 2 to 3 %, which are well tolerated but irritation can occur at higher concentrations (erythema, pruritus and pain) (4). In a clinical study done in 1983 in 30 dogs with folliculitis, 61 % of dogs responded well to a 2.5 % benzoyl peroxide shampoo, without concurrent therapy (29). In a comparative study on superficial pyoderma, 70 % of 10 dogs responded well to a 2.5 % benzoyl peroxide shampoo (30).

Ethyl lactate is hydrolysed in the skin to ethanol and lactic acid, thus lowering the skin pH and acting similarly to benzoyl peroxide (3). It is used in concentration of 10 %, which is rarely followed by undesirable side effects (irritation, erythema, pruritus) (4). In a comparative study to benzoyl peroxide, 90 % of 30 dogs with superficial pyoderma responded well to a 10 % ethyl lactate shampoo (30). In a recent study comparing two groups of 10 dogs with superficial pyoderma, it was shown that utilization of a 10 % ethyl lactate shampoo twice weekly reduces the length of systemic antibiotic needed in canine superficial pyoderma (31). Other antibacterial agents used in shampoos are hexachlorophene (not much used because of neurotoxicity), hexetidine (only one product) and triclosan (less effective than benzoyl peroxide and chlorhexidine in a comparative study) (28). In a recent shampoo formulated specifically for canine atopy, piroctone olamine (widely used in human shampoos) has been added for its antibacterial and anti-yeast properties (see below).

VI The use of shampoos in fungal diseases Antimycotic shampoos are used as adjunctive therapy for dermatophytosis and Malassezia dermatitis.

They limit contagiousity in case of dermatophytosis but are not effective in treating it when used alone (32). Non antifungal shampoos or shampoos with insufficient antifungal properties can disseminate spores (33). However, keratomodulating shampoos are used before antifungal topical therapy when there is a keratoseborrheic disorder and they are then beneficial in removing infected scales and crusts. In an in vitro study a ketoconazole shampoo was effective to inhibit the growth on Dermatophyte Test Medium of *Microsporum canis* from infected hair, but after more applications than antifungal solutions (enilconazole, lime sulfur, 2% chlorhexidine, povidone iodine) (34). In a review, a miconazole shampoo is considered to be as effective as lime sulfur and enilconazole in treating feline dermatophytosis (33). In a recent study a shampoo containing chlorhexidine (2%) and miconazole (2%) was shown to accelerate the clinical cure but not the mycological cure of cats



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infected with *Microsporum canis* and treated with griseofulvin (35).

Topical therapy is an alternative to systemic treatment in *Malassezia dermatitis*. For extensive lesions antifungal shampoos or lotions are preferable. They can be used with systemic therapy, although there is no formal evidence that the combination is of greater value than systemic treatment alone. Topical therapy alone should not be used as a diagnostic challenge, but it can maintain a remission, thus confirming the diagnosis. Shampoos containing miconazole (2%), chlorhexidine (2 to 4%), a combination of both (2% each), ketoconazole (2%) or a combination of chlorhexidine (2%) and ketoconazole (1%) are the most appropriate (as are rinses such as lime sulfur and enilconazole) (12,36,37). Selenium sulphide shampoos could be less effective (37).

A study has demonstrated the immediate and residual *in vivo* antifungal effect of a shampoo containing piroctone olamine against *Malassezia pachydermatis* (38).

VII The use of shampoos in allergic diseases

All shampoos are likely to remove allergens from the skin, which is supposed to be useful in canine atopic dermatitis. They also help to rehydrate dry skin, which is common in dogs with allergic skin disease. In addition, shampoos with an antipruritic effect can improve the condition of allergic dogs, provided they are used frequently (e.g. twice a week, at least at initiation of therapy). Antipruritic shampoos are considered generally as adjunctive treatments. They are rarely affective as the sole therapy (3,12).

Antipruritic shampoos contain 1% hydrocortisone, 0.01% fluocinolone, 2% diphenhydramine, 1% pramoxine or colloidal oatmeal. A clinical study has demonstrated that shampoos and rinses containing the local anesthetic, pramoxime, are useful (39). The topical fluocinolone shampoos have been shown not to be systematically absorbed in the dog. Controlled studies on efficacy of antipruritic shampoos are lacking (12).

A shampoo specifically designed for canine atopic dermatitis has been recently developed. It contains linoleic acid, mono and oligosaccharides, vitamin E, and piroctone olamine. Linoleic acid can help in restoring the barrier function of the skin (see above) (23), thus limiting the transcutaneous penetration of allergens. In effect, it has been demonstrated that the stratum corneum intercellular lipids are altered in atopic dogs (9). Mono and oligosaccharides are immunomodulator agents, which can inhibit the secretion of proinflammatory cytokines (such as TNF μ) and limit the expression of membrane molecules (such ICAM 1). *In vitro* studies have been done in man (40) and dog (41). Vitamin E is an antioxidant, stabilizes lysosomes, reduces prostaglandin E2 (PGE2) synthesis, and increases interleukin 2 (IL-2) production with resultant anti-inflammatory and immunostimulatory effects (12). Piroctone olamine is an antiseptic agent active on Gram + and Gram - bacteria, dermatophytes and yeast. It is much used in topical formulations in human dermatology against proliferation of *Malassezia furfur*. The concept of this shampoo is promising since its goal is to provide a therapeutic response to defects potentially occurring in canine atopic dermatitis. Controlled trials are needed to document the clinical efficacy of this product in atopic dogs.

VIII Moisturisers

In every skin disorders, and in particular with dry seborrhoea, there is scope for increasing the humidity of the animal's skin, after shampooing, with a moisturiser. It has been demonstrated that skin hydration is less in dogs with scaling than in normal dogs (42).

Moisturisers lubricate, rehydrate and soften the skin. In French, they are all, incorrectly, lumped together as emollients. Moisturisers actually consist of true emollients, emulsifiers/emollients, occlusive dressings and rehydrating agents.



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They restore an artificial superficial skin film. Diluted in water, they can be massaged into the skin or applied as a lotion. Undiluted, they may be sprayed on after a shampoo. They should not be rinsed off. In Europe only emollients and rehydrating agents are found in veterinary products (an emulsifier/emollient combination exists in North America). Occlusive dressings are neither used nor marketed in the veterinary field due to risk of maceration.

Emollients are composed principally of fatty acid polyesters, vegetable oils, mineral oils (no veterinary formulations available) and lanolin.

Lipid emollients, containing lanolin alcohols, liquid paraffin or mineral oils, were borrowed from human dermatology and are now rarely used. Used as an emulsion in tepid water, they do improve coat condition, but also have a greasing effect, a definite disadvantage. One veterinary lipid emollient containing fatty acid polyesters is marketed in France. Local application of essential fatty acids has also been proposed to soften and rehydrate the skin, and reduce transcutaneous water loss (23). No major occlusive effect is involved, and the effects are probably brought about by the incorporation of essential fatty acids (especially linoleic acid) into stratum corneum ceramides.

Non-lipid emollients have rehydrating and softening properties. They reduce smell and improve coat appearance without the greasing effect. The high molecular weight of their active ingredients and their hygroscopic nature make them effective surface-protecting therapeutic agents. Examples include lactic acid, glycerin, propylene glycol, urea and chitosanide.

Active agents can be combined with moisturisers : colloidal oatmeal extracts and aloe vera for antipruritic activity, and coal tar for keratolytic and keratoplastic activity.

Conclusion Treatment and prevention strategies in veterinary dermatology include often the use of medicated shampoos. The therapeutic plan should be defined on short and long term basis to obtain the best results, to cope with owners compliance and to limit potential side effects.

1) - References1. Plewig G, Janssen T. Seborrheic dermatitis. In: Wolff K, Goldsmith LA, Katz SI, et al., editors. *Fitzpatrick's Dermatology in General Medicine*. 7th ed. New York: McGraw-Hill; 2008.2. Gupta AK, Bluhm R. Seborrheic dermatitis J Eur Acad Dermatol Venereol 2004;18(11):26-26; quiz, 19-20.20 [PubMed]3. Johnson MLT. Skin Conditions and Related Need for Medical Care among Persons 1- 74Years, United States, 1971-1974. Series 11, Data from the National Health Survey November, No. 212, DHEW Pub No. (PHS) 79-1660.

Hyattsville, Md: U.S. Department of Health, Education, and Welfare, Public Health Service, National Center for Health Statistics; 1978.4. Mahe A, Simon F, Coulibaly S, et al. Predictive value of seborrheic dermatitis and other common dermatoses for HIV infection in Bamako, Mali. J Am Acad Dermatol. 1996;34(6):1084-1086. [PubMed]5. Mallal SA. The Western Australian HIV Cohort Study, Perth, Australia. J Acquir Immune Defic Syndr Hum Retrovirol. 1998;17(Suppl 1):S23-S27. [PubMed]6. Binder RL, Jonelis FJ. Seborrheic dermatitis in neuroleptic-induced parkinsonism. Arch Dermatol.1983;119(6):473-475. [PubMed]7. Rocha N, Velho G, Horta M, et al. Cutaneous manifestations of familial amyloidotic polyneuropathy. J Eur Acad Dermatol Venereol. 2005;19(5):605-607. [PubMed]8. Ercis M, Balci S, Atakan N. Dermatological manifestations of 71 Down syndrome children admitted to a clinical genetics unit. Clin Genet. 1996;50(5):317-320. [PubMed]9. Gupta AK, Boekhout T, Theelen B, et al. Identification and typing of Malassezia species by amplified fragment length polymorphism and sequence analyses of the internal transcribed spacer and large-subunit regions of ribosomal DNA. J Clin Microbiol. 2004;42(9):4253-4260. [PMC free article] [PubMed]10. Tajima M, Sugita T, Nishikawa A, Tsuboi R. Molecular analysis of Malassezia microflora in seborrheic dermatitis patients: Comparison with other diseases and healthy subjects. J Invest Dermatol.2008;128(2):345-351. [PubMed]11. DeAngelis YM, Gemmer CM, Kaczvinsky JR, et al. Three etiologic facets of dandruff and seborrheic dermatitis: Malassezia fungi, sebaceous lipids, and individual sensitivity. J Invest Dermatol Symp Proc.2005;10(3):295-297. [PubMed]12. Thomas DS, Ingham E, Bojar RA, Holland KT. In vitro modulation of human keratinocyte pro- and anti-inflammatory cytokine production by the capsule of Malassezia species. FEMS Immunol Med Microbiol.2008;54(2):203-214. [PubMed]13. Gupta AK, Einarson TR, Summerbell RC, Shear NH. An overview of topical antifungal therapy in dermatomycoses: A North American perspective. Drugs. 1998;55(5):645-674. [PubMed]14. Pierard-Franchimont C, Pierard GE. A double-blind placebo-controlled study of ketoconazole + desonide gel combination in the treatment of facial seborrheic dermatitis. Dermatology. 2002;204(4):344-347.[PubMed]15. Gupta AK, Nicol K, Batra R. Role of antifungal agents in the treatment of seborrheic dermatitis. Am J Clin Dermatol. 2004;5(6):417-422. [PubMed]16. Peter RU, Richarz-Barthauer U. Successful treatment and prophylaxis of scalp seborrheic dermatitis and dandruff with 2% ketoconazole shampoo: Results of a multicentre, double-



SCIENTIFIC DATA: ANTIPARASITIC & ANTISEBORRHEIC SHAMPOO

blind, placebo-controlled trial. *Br J Dermatol.* 1995;132(3):441-445. [PubMed]17. Caputo R, Barbareschi M. Itraconazole: New horizons. *G Ital Dermatol Venereol.* 2002;137:1-7.18. Nahm WK, Orengo I, Rosen T. The antifungal agent butenafine manifests anti-inflammatory activity in vivo. *J Am Acad Dermatol.* 1999;41(2 Part 1):203-206. [PubMed]19. Abrams BB, Hanel H, Hoehler T. Ciclopirox olamine: A hydroxypyridone antifungal agent. *Clin Dermatol.* 1991;9(4):471-477. [PubMed]20. Gupta AK. Ciclopirox: An overview. *Int J Dermatol.* 2001;40(5):305-310. [PubMed]21. Korting HC, Grundmann-Kollmann M. The hydroxypyridones: A class of antimycotics of its own. *Mycoses.* 1997;40(7-8):243-247. [PubMed]22. Shuster S, Meynadier J, Kerl H, Nolting S. Treatment and prophylaxis of seborrheic dermatitis of the scalp with antipityrosporal 1% ciclopirox shampoo. *Arch Dermatol.* 2005;141(1):47-52. [PubMed]23. de Padua CA, Uter W, Geier J, et al. Contact allergy to topical antifungal agents. *Allergy.* 2008;63(7):946-947. [PubMed]24. Unholzer A, Varigos G, Nicholls D, et al. Ciclopiroxolamine cream for treating seborrheic dermatitis: A double-blind parallel group comparison. *Infection.* 2002;30(6):373-376. [PubMed]25. Parsad D, Pandhi R, Negi KS, Kumar B. Topical metronidazole in seborrheic dermatitis: A double-blind study. *Dermatology.* 2001;202(1):35-37. [PubMed]26. Madsen JT, Lorentzen HF, Paulsen E. Contact sensitization to metronidazole from possible occupational exposure. *Contact Dermatitis.* 2009;60(2):117-118. [PubMed]27. Danby FW, Maddin WS, Margesson LJ, Rosenthal D. A randomized, double-blind, placebo-controlled trial of ketoconazole 2% shampoo versus selenium sulfide 2.5% shampoo in the treatment of moderate to severe dandruff. *J Am Acad Dermatol.* 1993;29(6):1008-1012. [PubMed]28. Gillum RF. Hyperpigmentation associated with selenium sulfide lotion. *J Natl Med Assoc.* 1996;88(9):551. [PMC free article] [PubMed]29. Opdyke DL, Burnett CM, Brauer EW. Anti-seborrheic qualities of zinc pyrithione in a cream vehicle: II. Safety evaluation. *Food Cosmet Toxicol.* 1967;5(3):321-326. [PubMed]

30. Marks R, Pearse AD, Walker AP. The effects of a shampoo containing zinc pyrithione on the control of dandruff. *Br J Dermatol.* 1985;112(4):415-422. [PubMed]31. Pierard-Franchimont C, Goffin V, Decroix J, Pierard GE. A multi-center randomized trial of ketoconazole 2% and zinc pyrithione 1% shampoos in severe dandruff and seborrheic dermatitis. *Skin Pharmacol Appl Skin Physiol.* 2002;15(6):434-441. [PubMed]32. Satchell AC, Saurajen A, Bell C, Barnetson RS. Treatment of dandruff with 5% tea tree oil shampoo. *J Am Acad Dermatol.* 2002;47(6):852-855. [PubMed]33. Henley DV, Lipson N, Korach KS, Bloch CA. Prepubertal gynecomastia linked to lavender and tea tree oils. *N Engl J Med.* 2007;356(5):479-485. [PubMed]34. Hammer KA, Carson CF, Riley TV, Nielsen JB. A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food Chem Toxicol.* 2006;44(5):616-625. [PubMed]35. Josse G, Rouvrais C, Mas A, et al. A multitechnique evaluation of topical corticosteroid treatment. *Skin Res Technol.* 2009;15(1):35-39. [PubMed]36. Wikler JR, Nieboer C, Willemze R. Quantitative skin cultures of *Pityrosporum* yeasts in patients seropositive for the human immunodeficiency virus with and without seborrheic dermatitis. *J Am Acad Dermatol.* 1992;27(1):37-39. [PubMed]37. Nakagawa H, Etoh T, Ishibashi Y, et al. Tacrolimus ointment for atopic dermatitis. *Lancet.* 1994;344(8926):883. [PubMed]38. Meshkinpour A, Sun J, Weinstein G. An open pilot study using tacrolimus ointment in the treatment of seborrheic dermatitis. *J Am Acad Dermatol.* 2003;49(1):145-147. [PubMed]39. Warshaw EM, Wohlhuter RJ, Liu A, et al. Results of a randomized, double-blind, vehicle-controlled efficacy trial of pimecrolimus cream 1% for the treatment of moderate to severe facial seborrheic dermatitis. *J Am Acad Dermatol.* 2007;57(2):257-264. [PubMed]40. Kaposi M. Pathology and treatment of diseases of the skin for practitioners and students [translation, last German edition, under the supervision of Johnston James C., MD, translator and editor.]. New York: William Wood & Co; 1895. Arnold WP. Tar. *Clin Dermatol.* 1997;15(5):739-744. [PubMed]42. Wright MC, Hevert F, Rozman T. In vitro comparison of anti-fungal effects of a coal tar gel and a ketoconazole gel on *Malassezia furfur*. *Mycoses.* 1993;36(5-6):207-210. [PubMed]43. Lin AN, Moses K. Tar revisited. *Int J Dermatol.* 1985;24(4):216-218. [PubMed]44. Lee E, Koo J, Berger T. UVB phototherapy and skin cancer risk: A review of the literature. *Int J Dermatol.* 2005;44(5):355-360. [PubMed]45. Shin H, Kwon OS, Won CH, et al. Clinical efficacies of topical agents for the treatment of seborrheic dermatitis of the scalp: A comparative study. *J Dermatol.* 2009;36(3):131-137. [PubMed] II) - References 1. Akiyama, H., H. Kanzaki, Y. Abe, J. Tada, and J. Arata. 1994. *Staphylococcus aureus* infection on experimental croton oil-inflamed skin in mice. *J. Dermatol. Sci.* 8:1-10. [PubMed]2. Alghaithy, A. A., N. E. Bilal, M. Gedebo, and A. H. Weily. 2000. Nasal carriage and antibiotic resistance of *Staphylococcus aureus* isolates from hospital and non-hospital personnel in Abha, Saudi Arabia. *Trans. R. Soc. Trop. Med. Hyg.* 94:504-507. [PubMed]3. Alouf, J. E., and H. Muller-Alouf. 2003. *Staphylococcal and streptococcal superantigens: molecular, biological and clinical aspects.* *Int. J. Med. Microbiol.* 292:429-440. [PubMed]4. Bonnetblanc, J. M., and C. Bedane. 2003. Erysipelas: recognition and management. *Am. J. Clin. Dermatol.* 4:157-163. [PubMed]5. Boon, R. J., and A. S. Beale. 1987. Response of *Streptococcus pyogenes* to therapy with amoxicillin or amoxicillin-clavulanic acid in a mouse model of mixed infection caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. *Antimicrob. Agents Chemother.* 31:1204-1209. [PMC free article] [PubMed]6. Brook, I. 2002. Microbiology of polymicrobial abscesses and implications for therapy. *J. Antimicrob. Chemother.* 50:805-810. [PubMed]7. Brook, I. 2002. Secondary bacterial infections complicating skin lesions. *J. Med. Microbiol.* 51:808-812. [PubMed]8. Brown, H., and R. Prescott. 1999. *Applied mixed models in medicine.* John Wiley & Sons, Ltd., Chichester, United Kingdom.9. Champion, R. H., J. L. Burton, and F. J. G. Ebling. 1992. *Textbook of dermatology.* Blackwell Scientific Publications, Oxford, United Kingdom.10. Chang, F. Y., J. E. Peacock, Jr., D. M. Musher, P. Triplett, B. B. MacDonald, J. M. Mylotte, A. O'Donnell, M. M. Wagener, and V. L. Yu. 2003. *Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. *Medicine (Baltimore)* 82:333-339. [PubMed]11. Chiller, K., B. A. Selkin, and G. J. Murakawa. 2001. Skin microflora and bacterial infections of the skin. *J. Investig. Dermatol. Symp. Proc.* 6:170-174. [PubMed]12. Craig, W. 1993. Relevance of animal models for clinical treatment. *Eur. J. Clin. Microbiol. Infect. Dis.* 12(Suppl. 1):S55-S57. [PubMed]13. George, A., and G. Rubin. 2003. A systematic review and meta-analysis of

treatments for impetigo. *Br. J. Gen. Pract.* 53:480-487. [PMC free article] [PubMed]14. Gisby, J., and J. Bryant. 2000. Efficacy of a new cream formulation of mupirocin: comparison with oral and topical agents in experimental skin infections. *Antimicrob. Agents Chemother.* 44:255-260. [PMC free article] [PubMed]15. Goldmann, O., G. S. Chhatwal, and E. Medina. 2003. Immune mechanisms underlying host



SCIENTIFIC DATA: ANTIPARASITIC & ANTISEBORRHEIC SHAMPOO

susceptibility to infection with group A streptococci. *J. Infect. Dis.* 187:854-861. [PubMed]16. Grundmann, H., A. Tami, S. Hori, M. Halwani, and R. Slack. 2002. Nottingham Staphylococcus aureus population study: prevalence of MRSA among elderly people in the community. *BMJ* 324:1365-1366. [PMC free article] [PubMed]17. Guay, D. R. 2003. Treatment of bacterial skin and skin structure infections. *Expert Opin. Pharmacother.* 4:1259-1275. [PubMed]18. Hochberg, Y., and A. C. Tanmhane. 1987. Multiple comparison procedures. John Wiley & Sons, Ltd., Chichester, United Kingdom.19. Hurdle, J. G., A. J. O'Neill, and I. Chopra. 2004. Anti-staphylococcal activity of indolmycin, a potential topical agent for control of staphylococcal infections. *J. Antimicrob. Chemother.* 54:549-552.[PubMed]20. Kim, H. B., H.-C. Jang, H. J. Nam, Y. S. Lee, B. S. Kim, W. B. Park, K. D. Lee, Y. J. Choi, S. W. Park, M.-D. Oh, E.-C. Kim, and K. W. Choe. 2004. In vitro activities of 28 antimicrobial agents against Staphylococcus aureus isolates from tertiary-care hospitals in Korea: a nationwide survey. *Antimicrob. Agents Chemother.* 48:1124-1127. [PMC free article] [PubMed]21. Lehmann, E. L. 1975. Nonparametrics. McGraw-Hill International Book Company, New York, N.Y.22. Manders, S. M. 1998. Toxin-mediated streptococcal and staphylococcal disease. *J. Am. Acad. Dermatol.* 39:383-398. [PubMed]23. Mason, B. W., and A. J. Howard. 2004. Fusidic acid resistance in community isolates of methicillin susceptible Staphylococcus aureus and the use of topical fusidic acid: a retrospective case-control study. *Int. J. Antimicrob. Agents* 23:300-303. [PubMed]24. McCormick, J. K., J. M. Yarwood, and P. M. Schlievert. 2001. Toxic shock syndrome and bacterial superantigens: an update. *Annu. Rev. Microbiol.* 55:77-104. [PubMed]25. Nagaev, I., J. Bjorkman, D. I. Andersson, and D. Hughes. 2001. Biological cost and compensatory evolution in fusidic acid-resistant Staphylococcus aureus. *Mol. Microbiol.* 40:433-439. [PubMed]26. Perl, T. M., and J. E. Golub. 1998. New approaches to reduce Staphylococcus aureus nosocomial infection rates: treating S. aureus nasal carriage. *Ann. Pharmacother.* 32:S7-S16. [PubMed]27. Pinnagoda, J., R. A. Tupker, T. Agner, and J. Serup. 1990. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 22:164-178. [PubMed]28. Roholt, I. 1992. Comparative efficacy of sodium fusidate ointment and two formulations of mupirocin ointment against experimentally induced wound infections in mice. *Curr. Ther. Res.* 51:390-395.29. Saloga, J., and J. Knop. 1999. Superantigens in skin diseases. *Eur. J. Dermatol.* 9:586-590. [PubMed]30. Soriano, F., L. Aguilar, and C. Ponte. 1997. In vitro antibiotic sensitivity testing breakpoints and therapeutic activity in induced infections in animal models. *J. Chemother.* 9(Suppl. 1):36-46. [PubMed]31. Spelman, D. 1999. Fusidic acid in skin and soft tissue infections. *Int. J. Antimicrob. Agents* 12(Suppl. 2):S59-S66. [PubMed]32. Stieritz, D. D., A. Bondi, D. McDermott, and E. D. Michaels. 1982. A burned mouse model to evaluate anti-Pseudomonas activity of topical agents. *J. Antimicrob. Chemother.* 9:133-140. [PubMed]33. Uehara, Y., H. Nakama, K. Agematsu, M. Uchida, Y. Kawakami, A. S. Abdul Fattah, and N. Maruchi. 2000. Bacterial interference among nasal inhabitants: eradication of Staphylococcus aureus from nasal cavities by artificial implantation of Corynebacterium sp. *J. Hosp. Infect.* 44:127-133. [PubMed]34. Uemura, E., S. Kakinohana, N. Higa, C. Toma, and N. Nakasone. 2004. Comparative characterization of Staphylococcus aureus isolates from throats and noses of healthy volunteers. *Jpn. J. Infect. Dis.* 57:21-24. [PubMed]35. Wilcox, M. H., J. Hall, H. Pike, P. A. Templeton, W. N. Fawley, P. Parnell, and P. Verity. 2003. Use of perioperative mupirocin to prevent methicillin-resistant Staphylococcus aureus (MRSA) orthopaedic surgical site infections. *J. Hosp. Infect.* 54:196-201. [PubMed]36. Yamakawa, T., J. Mitsuyama, and K. Hayashi. 2002. In vitro and in vivo antibacterial activity of T-3912, a novel non-fluorinated topical quinolone. *J. Antimicrob. Chemother.* 49:455-465. [PubMed] III) - Reference: 1 Legeay Y (1993) Topiques en dermatologie. Encyclopédie Vétérinaire, Paris, 0700, 7p. 2 Curtis CF (1998) Use and abuse of topical dermatological therapy in dogs and cats. Part 1- shampoo therapy. In Practice, 244-251. 3 Halliwell REW (1991) Rational use of shampoos in veterinary dermatology. *J Small Anim Pract* 32 : 401-407. 4 Guaguère S

(1996) Topical treatment of canine and feline pyoderma. *Vet Dermatol* 7 : 145- 151. View pictures View other presentations 5 Campbell KJ, Schaeffer DJ (1993) Effects of four veterinary shampoos on transepidermal water losses, hydration of the stratum corneum, skin surface lipid concentration, skin surface pH and corneocyte count in dogs. In: Proceedings of the ninth annual meeting of the American Academy of Veterinary Dermatology and American College of Veterinary Dermatology, San Diego, 96-97. 6 Campbell KJ et al (1994) Effects of four antiseborrheic shampoos on transepidermal water losses, hydration of the stratum corneum, skin surface lipid concentration, skin surface pH and corneocyte count in dogs. In: Proceedings of the tenth annual meeting of the American Academy of Veterinary Dermatology and American College of Veterinary Dermatology, Charleston, 85. 7 Beco L, Fontaine J (2000) Cornéométrie et perte d'eau transépidermique : validation des techniques chez des chiens sains. *Ann Méd Vét*, 144 : 329-333. 8 Groux D, Bensignor E (2000) Skin characteristics measured by trans-epidermal water loss, corneometry and sebometry are not valuable tools to evaluate the effects of topical treatments in dogs. *Vet Dermatol*, 11(Suppl.1) : 60. 9 Inman AO, Olivry TO, Dunston SM, Monteiro-Rivière NA, Gatto H (2001) Electron microscopic observations of the stratum corneum intercellular lipids in normal and atopic dogs. *Vet Pathol* 38 : 720-723. 10 Barthe N, Jasmin P, Brouillaud B, Guinez C, Coulon P, Gatto H (1999) Assessment of the Bio-Distribution of Non-Ionic Spherulites in Dog Skin. Proceedings of the 16th Annual Congress of the European Society of Veterinary Dermatology and European College of Veterinary Dermatology, Helsinki, 156. 11 Carlotti DN, Bensignor E (2002) Management of keratoseborrheic disorders. *Eur J Comp Anim Pract*, 12 : 123-133. 12 Scott DW, Miller WH, Griffin CE (2001) Muller and Kirk's Small Animal Dermatology, 6th edition, WB Saunders Company, Philadelphia. 13 Kwochka KW (1993) Symptomatic Topical Therapy of Scaling Disorders. In: Current Veterinary Dermatology (Griffin CE, Kwochka KW, Mac Donald JM, Edrs) Mosby Year Book, St Louis, 191-202. 14 Kietzman M et al (1990) Effects of sulphur and coaltar in epidermal metabolism. In: Advances in Veterinary Dermatology I (Von Tschanner C, Halliwell REW Edrs), Bailliere Tindall, London, 460-461. 15 Rogers RS et al (1989) Comparative efficacy of 12% ammonium lactate lotion and 5% lactic acid lotion in the treatment of moderate to severe xerosis. *J Am Acad Dermatol* 21: 714- 716. 16 Klaus MV et al (1990) Evaluation of ammonium lactate in the treatment of seborrheic keratoses. *J Am Acad Dermatol* 22 : 199-203. View pictures View other presentations 17 Jennings MB et al (1998) Comparison of salicylic acid and urea versus ammonium lactate for the treatment of foot xerosis. A randomized, double-blind clinical study. *J Am Podiatr Med Assoc* 88 : 332-336. 18 Uy JJ et al (1999) Ammonium lactate 12% lotion versus a liposome-based moisturizing lotion for plantar xerosis.



SCIENTIFIC DATA: ANTIPARASITIC & ANTISEBORRHOIC SHAMPOO

A double-blind comparison study. *J Am Podiatr Med Assoc* 89 : 502-505. 19 Lavker RM et al (1992) Effects of topical ammonium lactate on cutaneous atrophy from a potent topical corticosteroid. *J Am Acad Dermatol* 26 : 535-544. 20 Vilaplana J et al (1992) Clinical and non-invasive evaluation of 12% ammonium lactate emulsion for the treatment of dry skin in atopic and non-atopic subjects. *Acta Derm Venereol* 72 : 28-33. 21 Leyden JJ (1987) Effects of sulfur and salicylic acid in a shampoo base in the treatment of dandruff: a double-blind study using corneocyte counts and clinical grading. *Acta Derm Venereol*, 39: 557. 22 Ihrke PJ (1980) Topical therapy: specific topical pharmacologic agents - Dermatologic therapy (part II) *Comp Cont Educ Prat Vet* 11 : 156-164. 23 Campbell KJ, Kirkwood AR (1993) Effects of topical oils on TEWL in dogs with seborrhea sicca. In : *Adv. Vet. Derm. II*, Eds Ihrke PJ, Mason IS, White SD, Pergamon Press, Oxford, 157-162. 24 Carlotti DN, Jacobs DE (2000) Therapy, control and prevention of flea allergy dermatitis in dogs and cats. *Vet Dermatol* 11 : 83-98. 25 Franc M, Cadiergues MC (1998) Activité anti-gorgement d'un shampooing à 0.07% de deltaméthrine sur les puces du chien, *Ctenocephalides Felis*. *Rev Méd Vét* 149 : 791-794. 26 Carlotti DN, Maffart P (1996) La chlorhexidine, revue bibliographique. *Prat Méd Chir Anim Comp* 31 : 553-563. 27 Campbell KJ et al (1995) Effects of four antibacterial soaps/shampoos on surface bacteria of the skin of dogs. In: *Proceedings of the eleventh annual meeting of the American Academy of Veterinary Dermatology and American College of Veterinary Dermatology*, Santa Fe, 43- 44. 28 Kwochka AK, Kowalski J (1991) Prophylactic efficacy of four antibacterial shampoos against *Staphylococcus intermedius* in dogs. *Am J Vet Res*, 52 : 115-118. 29 Lloyd DH, Reys-Brion A (1984) Le Peroxyde de benzoyle : efficacité clinique et bactériologique dans le traitement des pyodermites chroniques. *Prat Méd Chir Anim Comp*

19 : 445-449. 30 Ascher F, Maynard L, Laurent J, Goubet B (1990) Controlled trial of ethyl lactate and benzoyl peroxide shampoos in the management of canine surface pyoderma and superficial pyoderma. In : *Advances in Veterinary Dermatology Volume 1*, Eds Von Tscharnner C, View pictures View other presentations Halliwell REW, Baillière Tindall, London, 375-382. 31 De Jaham C (2002) Usefulness of an ethyl lactate shampoo in conjunction with systemic antibiotics in the treatment of canine generalized superficial pyoderma : a controlled clinical study. In : *Proceedings of the 18th annual congress of the European Society of Veterinary Dermatology and European College of Veterinary Dermatology*, Nice, 199. 32 DeBoer DJ, Moriello KA (1995) Inability of two topical treatments to influence the course of experimentally induced dermatophytosis in cats. *J Am Vet Med Assoc* 207 : 52-57. 33 Moriello KA, DeBoer DJ (1995) Feline dermatophytosis. Recent advances and recommendations for therapy. *Vet Clin. North Am Small Anim Pract* 25 : 901-921. 34 White-Weathers N, Medleau L (1995) Evaluation of topical therapies for the treatment of dermatophytosis in dogs and cats. *J Am Anim Hosp Assoc* 31 : 250-253. 35 Paterson S (1999) Miconazole/chlorhexidine shampoo as an adjunct to systemic therapy in controlling dermatophytosis in cats. *J Small Anim Pract* 40 : 163-166. 36 Mason KV (1993) Cutaneous Malassezia. In : *Current Veterinary Dermatology*, Eds Griffin CE, Kwochka KW, Mac Donald JW, Mosby Year Book, Saint-Louis, 44-48. 37 Bond R, Rose JF, Ellis JW, Lloyd DH (1995) Comparison of two shampoos for treatment of *Malassezia pachydermatis*-associated seborrhoeic dermatitis in basset hounds. *J Small Anim Pract* 36 : 99-104. 38 Bourdeau P, Blumstein P, Ibish C, Gardey L., Jasmin P, Gatto H (1999) Antifungal activity of a piroctone shampoo against *Malassezia* populations after a single treatment in the dog. In *Proceedings 16th Annual Congress of the European Society of Veterinary Dermatology and European College of Veterinary Dermatology*, Helsinki, 155. 39 Scott W et al (2000) A clinical study of the efficacy of two commercial veterinary pramoxine cream rinses in the management of pruritus in atopic dogs. *Canine Pract* 25 : 15- 18. 40 Palacio S, Viac J, Vinche A, Huband JC, Gatto H, Schmitt D (1997) Suppressive effects of monosaccharides on ICAM-1/CD54 expression in human keratinocytes. *Arch Dermatol Res* 289 : 234-237. 41 Cadiot C, Ibisch C, Bourdeau P, Gatto H (2000) In vitro assay for detection of canine keratinocyte activation : preliminary results for pharmacological tests of activation/regulation. *Vet Dermatol* 11 (Suppl 1) : 58. 42 Chesney CJ (1998) The intimate envelope: water and the skin. In : *Advances in Veterinary Dermatology 3rd edition*, Eds Kwochka KW, Willemse T, Von Tscharnner C, Butterworth Heinemann, Oxford, 47-56. 43 Koch HJ (1996) Shampoo therapy in veterinary dermatology. *Proceeding of the Third World Congress of Veterinary Dermatology*, Edinburgh, 88-90.