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Innovation for the Management of Ear Infections

EXTRACTED FROM THE ROUND TABLE ON EAR AND SKIN INFECTIONS

published in Clinician's Brief, February 2016


John Angus
DVM, DACVD


Dunbar Gram
DVM, DACVD


Craig Griffin
DVM, DACVD


Domenico Santoro
DVM, PhD, DACVD


Wayne Rosenkrantz
DVM, DACVD


Stephen White
DVM, DACVD

What are the most common treatment regimens in veterinary practice when animals are affected by ear infections?

DG = As a rule, ears are initially treated topically, and systemic antibiotic therapy is reserved for chronic and complicated cases. However, there is a trend towards topical products because of the emerging increase of resistant antimicrobial organisms.

WR = I also think that practitioners are more comfortable using topical therapy for maintenance and prevention of recurrent infections. We are becoming more aware of alternative options to systemic antibiotics especially when dealing with resistant bacteria infections.

DG = Products that also contain ingredients associated with improving epidermal barrier function have become popular in pruritic patients. The development of newer technologies is aiding in increased efficacy of topical products.

What are the most common causes of failure associated with the "classic or current" approaches?

DG = The most common causes of failure are:

- Failure to address or diagnosing the underlying cause
- Inadequate duration of treatment.

We increasingly see super resistant bacterial infections in referral practices. **Actually it is somewhat rare for me to see cases of bacterial ear infections that are not associated with bacterial resistance.**

CG = Same for me. About 60 % of ear infections are resistant!

WR = Another cause of failure we should not neglect is client compliance! We can send home the proper treatment and tell clients what to do- but then is it always getting done properly at home? So any therapies that promote better compliance would help increase the response to treatment. Many of our topical treatment regimens that cut frequency of dosing are moving in the right direction.

What is (are) the definition(s) of "biofilm"?

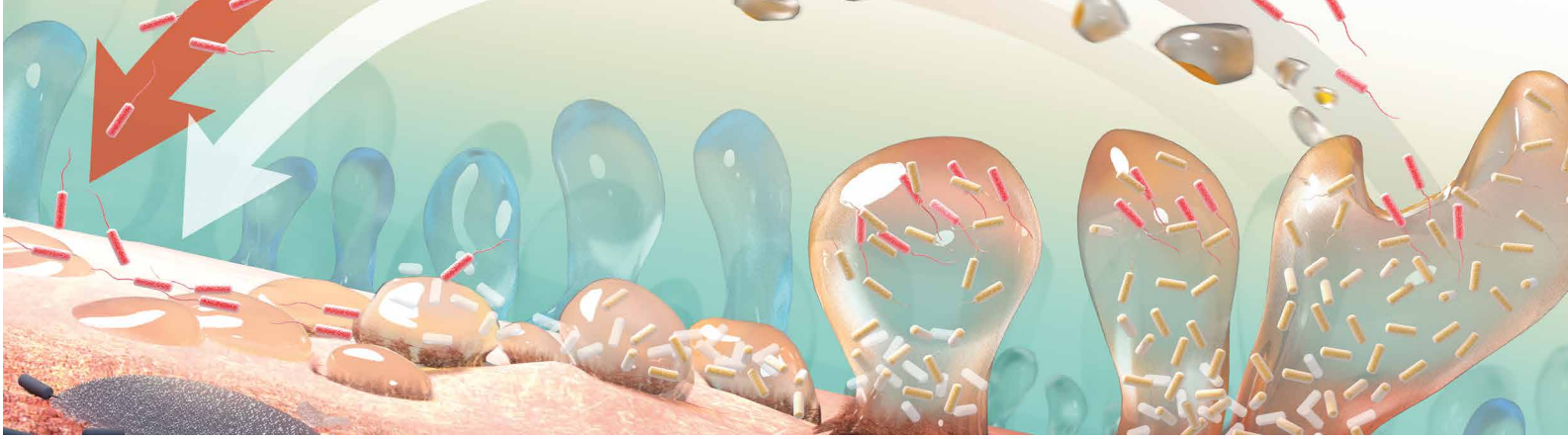
DS = Biofilm is an aggregation of different populations of bacteria. These bacteria live together and create a "community." I like to think of biofilm as a "metropolis" with different species & types of bacteria all living together and all sharing tools to fight whatever aggression is potentially outside. So they secrete material to protect themselves such as polysaccharides; the "slimy material" that we see.

WR = When I think of biofilm, I think of it in a localized site in or on the body, as opposed to it spreading all over the body. It may also be a very important component that can contribute to bacterial "resistance". A perfect localized example is biofilms that may occur in ears and contribute to resistant cases of otitis externa or media.

How would you approach a patient with complications by biofilm formation in ear infections?

SW = In terms of ingredients, on the skin, I would use a product that contains chlorhexidine and miconazole at least to kill bacteria and yeast and just the mechanics of it would also help to get rid of the biofilm. In the ears, I would use a combination of a cleanser and a treatment. The cleanser would ideally be a product that would strip off or break off the biofilm. The treatment would be a product that kills the organisms, recognizing that these days several cleansers also have anti-microbial activities.

WR = I agree entirely with Stephen. In the ear, physically cleaning, flushing and removal of purulent debris, but then also following with the addition of various disinfectants that would penetrate and breakdown the biofilm. There are some recent products currently available that contain MicroSilver that seem to act specifically on biofilm prevention.
We also know Dr Alan Mundell has been a precursor and he has been using MicroSilver for some time with some great results in dogs with chronic resistant ear infections.



Most recently VetBiotek® sponsored a research project documenting that topical products containing various concentrations of MicroSilver (Ag+) were effective at eradicating biofilm formation in an established *in vitro* model for *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*.

This research was conducted by an independent laboratory that utilized an established model for biofilm studies.This study will be presented at the World Dermatology Veterinary Congress next May in Bordeaux, France as it was accepted in the supporting original studies session. We are excited about using Ag+ products as we have several clinical cases showing significant improvement with MicroSilver. These are cases that were not responding to multiple treatment regimens.

CG = What was the lowest concentration of Ag+ that was effective in that study?

WR = It was 0.05 % Ag+, a relatively low concentration as compared to the current treatment products that are now released.

Q MicroSilver has been used for one decade in various human products. What are the benefits from such an ingredient?

WR = It would appear that the MicroSilver has benefits as a preventative agent for biofilm formation. In addition to shampoo therapy, other delivery systems (i.e., mousse, gels, lotions or sprays) may be more effective for localized disease.

DS = So molecules like acetylcysteine, EDTA or MicroSilver are able to disrupt the "shield" and open the door to antimicrobial products.

SW = The whole idea is, for example: You have a dog that comes in and is atopic – rubbing its face, licking its feet – and you don't find much in terms of organisms or just a few. If you have a shampoo with Ag+, you can now tell your client "if you use this product on a routine basis, not only will you be able to take care of any of the infection that takes place now, but you have a good chance to reduce the further incidence of any bacterial infection in relation to atopic dermatitis."

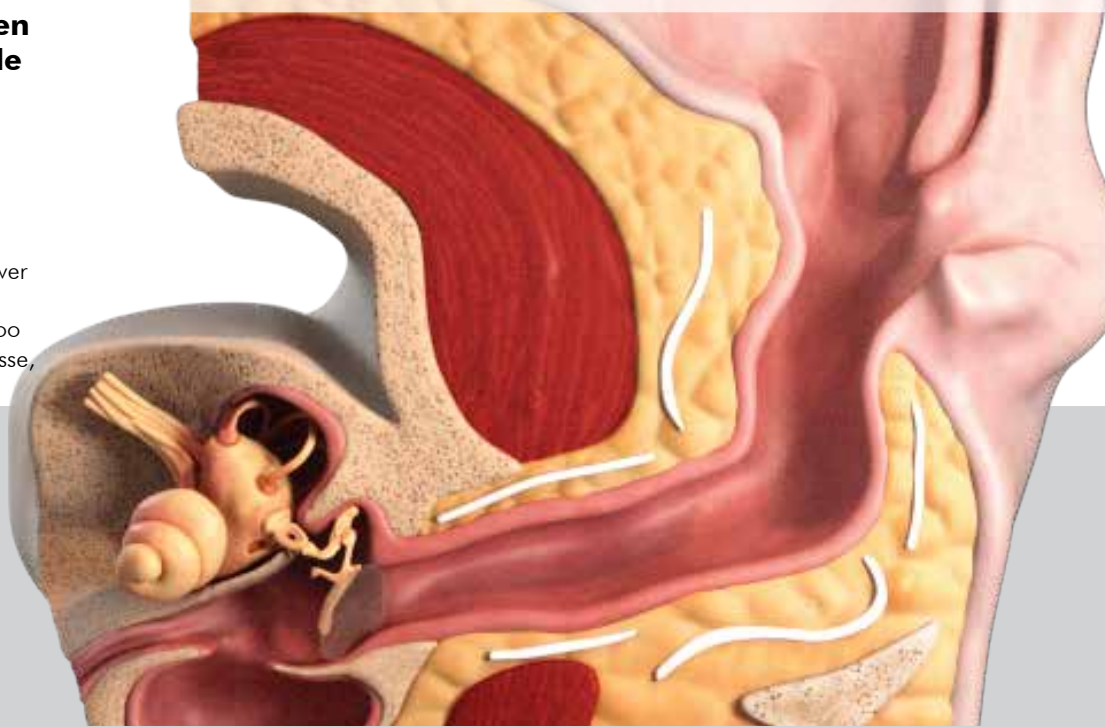
JA = I've treated about 5 total nightmare otitis cases with weekly flush and me applying the micronized silver in clinic. On cytology I see the particles still present on the swab one week later. Owners are clamoring to be able to take it home. Cosmetically – no odor. Looks like grey paint in the canal. **My experience with the MicroSilver is amazing. It is a radical leap forward in ear therapy.**

CG = It is not only how you kill the organisms BUT how you prevent resistance from developing. Multi-modal approaches are needed. An organism must have a genetic mutation that works to become resistant. If an organism needs not one but 2 or 3 genetic mutations, it becomes more difficult for that organism to become resistant.

So having for example, miconazole, chlorhexidine, and MicroSilver should be more effective at preventing resistance.

Main features and benefits of micronized silver:

1. Long lasting antimicrobial effect
2. Kills Bacteria and Yeast including Multidrug-Resistant bacteria (MRSA & MRSP)
3. Broad spectrum (gram +/ gram –)
4. Large particle size means no absorption.
5. It remains on the skin - and it will not cause any detrimental harm to the "good flora."



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Impact of Biofilm in Skin and Ear Infections

EXTRACTED FROM THE ROUND TABLE ON EAR AND SKIN INFECTIONS

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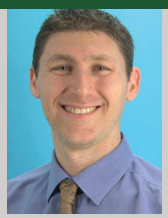
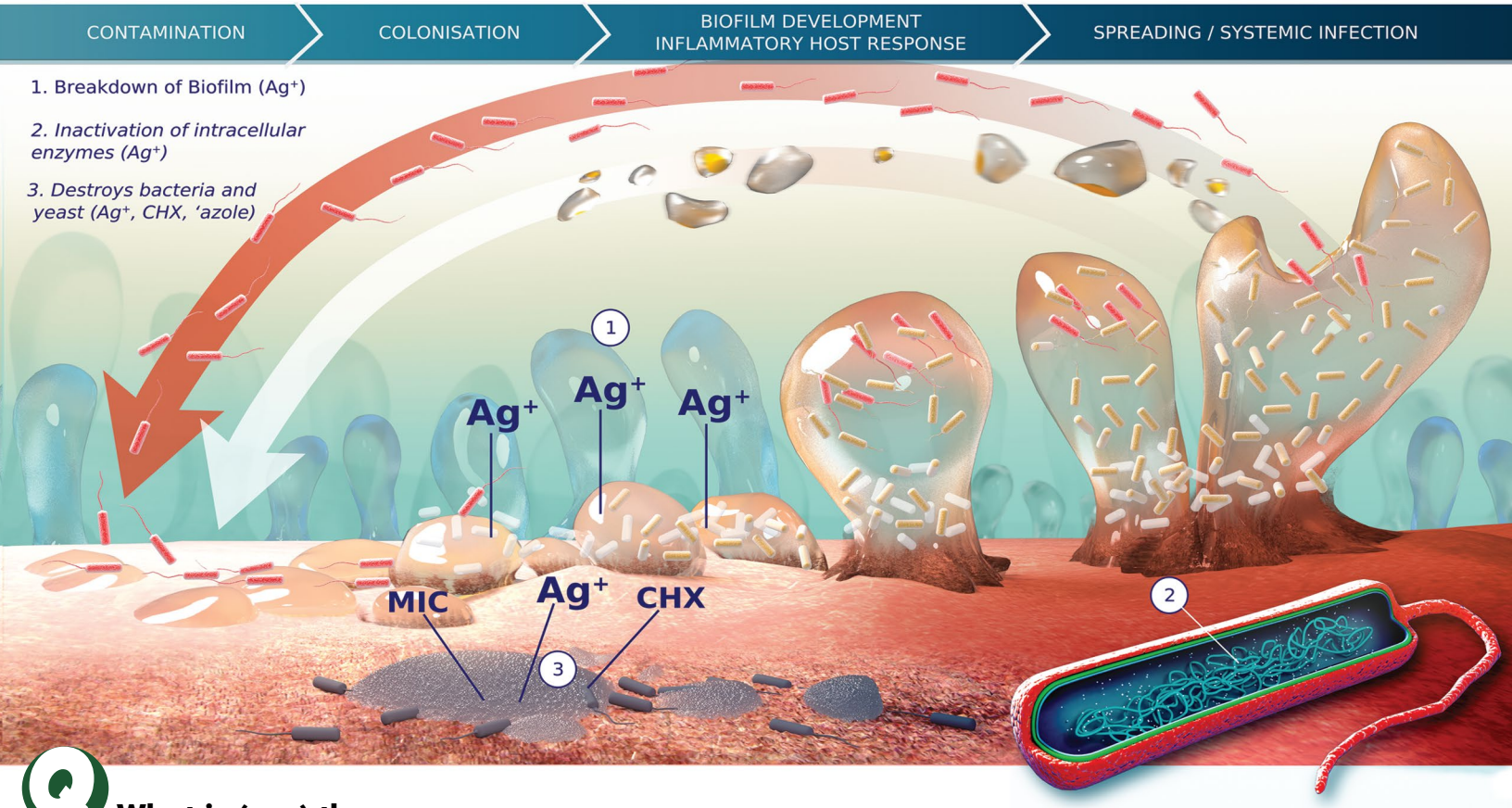


Domenico Santoro
DVM, PhD, DACVD



What is (are) the definition(s) of "biofilm"?

DS = Biofilm has different definitions. The most commonly accepted is an aggregation of different populations of bacteria. These bacteria live together and create a "community." I like to think of biofilm as a metropolis with different species and types of bacteria all living together and all sharing tools to fight whatever aggression is potentially outside. So they secrete material to protect themselves such as polysaccharides; the slimy material that we see. When the metropolis is getting too big, they migrate and split. Paradoxically, the inflammation (the body) helps the metropolis grow.

WR = When I think of biofilm, I think of it in a localized site in or on the body, as opposed to it spreading all over the body. It may also be a very important component that can contribute to the bacterial "resistance." A perfect localized example is a biofilm that may occur in ears and contribute to resistant cases of otitis externa or media.

CG = Some bacteria have the ability to produce a biofilm and some don't. It was shown 40% of canine otic *Pseudomonas* isolates and 30% of the canine otitis Staph strains are able to produce biofilm.[1,2] A study on canine *Malassezia* showed that 95% can produce biofilm.[3]

How would you approach a patient with complications by biofilm formation in ear infections?

SW = For the ears I envision the biofilm as the thick and sticky stuff you find at the bottom of the pot after cooking pasta! If you just use water, it does not wash out as rapidly as if you use soap or some other detergent. …I think this is even more so for the body…and for local lesions such as intertriginous areas, washing with something that has a degree of surfactant or something that would be able to strip away the exudate as well as something that could kill off the organisms…that would be ideal. …In terms of ingredients, on the skin, I would use a product that contains chlorhexidine and miconazole at least to kill bacteria and yeast and just the mechanics of it would also help to get rid of the biofilm. … In the ears, I would use a combination of a cleanser and a treatment. The cleanser would ideally be a product that would strip off or break off the biofilm. The treatment would be a product that kills the organisms, recognizing that these days several cleansers also have antimicrobial activities…

"I think there is sort of an intuitive feeling that the more I can wash things out, the better it will be."

Stephen White, DVM, DACVD

WR = I agree entirely with Stephen. Again thinking of these biofilms as a well protected environment for bacteria with polysaccharide coatings and envelopes—is there a way to disrupt biofilm formation?

In the ear, physically cleaning, flushing, and removal of purulent debris, but then also following with the addition of various disinfectants that would penetrate and break down the biofilm.

CG = This is where some new molecules and a mix of new technologies such as MicroSilver could play a role.

There are some recent products currently available that contain MicroSilver that seem to act specifically on biofilm prevention, and potential elimination of existing biofilms. What is the mechanism of action of micronized silver?

WR = The proposed mechanism of action of the MicroSilver (Ag+) ions against bacteria is related to its ability to inhibit the transmembrane transport of protein. This results in lysis of the bacterial cell wall.

We know that the MicroSilver (Ag+) will prevent bacterial adhesion and, if you recall, adhesion is an important component of the biofilm formation.

There is also some indication that Ag+ will destabilize the binding sites of bacteria to proteins.

MicroSilver has two highly desirable proven properties: Antibacterial and Antibiofilm.

Thanks to its special biologically active surface structure, MicroSilver is highly effective and safe. Products formulated with MicroSilver have an Antibacterial, regulating and stabilizing effect. Harmful microorganisms are neutralized while the damaged skin is gently repaired with the long lasting residual effect of MicroSilver.

Most recently VetBiotek sponsored a research project documenting that topical products containing various concentrations of MicroSilver (Ag+) were effective at eradicating biofilm formation in an established in vitro model for Staphylococcus intermedius and Pseudomonas aeruginosa.

This research was conducted by an independent laboratory that utilized an established model for biofilm studies. This study will be presented at the World Dermatology Veterinary Congress as it was accepted in the supporting original studies session. We are excited about using Ag+ products as we have several clinical cases showing significant improvement with MicroSilver. These are cases that were not responding to multiple treatment regimens.

What are the potential future developments associated with the management of biofilms?

"What I would like to see in terms of ideal product when dealing with biofilms is one that:
- **Dissolves polysaccharides**
- **Kills bacteria."**

Domenico Santoro, DVM, PhD, DACVD

DS = So molecules like acetylcysteine, EDTA, or MicroSilver are able to disrupt the "shield" and open the door to antimicrobial products.

Some of these molecules such as MicroSilver also have an antimicrobial effect.

CG = That is one of the keys. It is not only how you kill the organisms BUT how you prevent resistance from developing. Multimodal approaches are needed. An organism must have a genetic mutation that works to become resistant. If an organism needs not one but 2 or 3 genetic mutations, it becomes more difficult for that organism to become resistant.

So having, for example, miconazole, chlorhexidine, and MicroSilver should be more effective at preventing resistance.

JA = Most of our patients are atopic dogs and these are predisposed to overgrowth by Staphylococcus intermedius. A study in Japan on atopic dermatitis in people showed the correlation between secondary infections and the reduction of natural ceramides in the skin.

So it is not only the combination of the ingredients to kill bacteria that matters, but also the addition of ceramide[3]. This will contribute to a positive reaction in patients with pyoderma, as we know they have altered skin barrier function.

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21st Century Topical Management of Superficial Pyoderma

Galia Sheinberg
DVM, DLACVD

Dr. Sheinberg is a board certified veterinary dermatologist who works in a busy referral practice in Mexico City, Centro Veterinario Mexico. She consults on 240 to 260 dermatology cases per month. She successfully manages over 90% of her superficial pyoderma cases using topical therapy in lieu of antibiotics.

No systemic antibiotics were used on this patient!

Case presentation (summary): English Bulldog mix, 6 years old, weight 40.3Kg. Vet prescribed prednisone 1.3 years ago and the owners continued the medication at a dose of 25mg every day. He had severe polyuria (excess urine) and polydipsia (thirsty). On physical examination, calcinosis cutis (calcium deposits on skin), he had difficulty walking, pyoderma (bacterial infection) and inflammation.

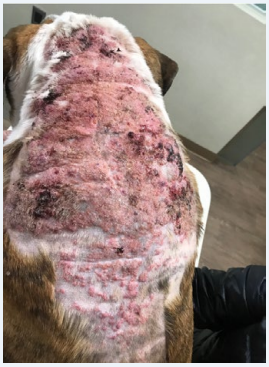
October 30, 2018 First visit - Prednisone was reduced and stopped after 3 weeks. Daily cleaning using BioHex™ Shampoo was prescribed. DMSO roll-on was applied every other day to help reduce calcinosis cutis. Apoquel® (Zoetis USA) 16mg BID was started and tramadol was used for pain management initially. A Hydrolyzed diet ProPlan HA® (Purina USA), was also initiated.

November 15, 2018 Second visit - BioHex Shampoo was reduced to every other day, Rimadyl® 4mg/Kg SID 14 days was started, Apoquel 24mg SID was continued, and DMSO roll-on recommended three times per week.

February 5, 2019 BioHex Shampoo was reduced to 2 times per week, Apoquel® reduced to 16mg SID, DMSO was discontinued.

March 28, 2019 BioHex was reduced to a once a week whole body shampoo. Apoquel was used only when needed. He had lost 10Kg! He was maintained on hydrolyzed diet, as he had no more gastrointestinal problems and minimal itching.

October 30, 2018
First visit



November 15, 2018
Second visit

February 5, 2019

March 28, 2019

Happy Dog!

Acta Derm Venereol 2010; 90: 350–353

INVESTIGATIVE REPORT

Ceramides and Barrier Function in Healthy Skin

Jakob MUTANU JUNGERSTED[1], Lars I. HELLGREN[2], Julie K. HØGH[2], Tue DRACHMANN[2], Gregor B.E. JEMEC[1] and Tove AGNER[3]

[1]Department of Dermatology, University of Copenhagen, Roskilde Hospital, Roskilde, [2]Department of System Biology and Centre for Advanced Food Science, Technical University of Denmark, Lyngby and [3]Department of Dermatology, University of Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark

Lipids in the stratum corneum are key components in the barrier function of the skin. Changes in lipid composition related to eczematous diseases are well known, but limited data are available on variations within healthy skin. The objective of the present study was to compare ceramide subgroups and ceramide/cholesterol ratios in young, old, male and female healthy skin. A total of 55 participants with healthy skin was included in the study. Lipid profiles were correlated with transepidermal water loss and with information on dry skin from a questionnaire including 16 people. No statistically significant differences were found between young and old skin for ceramide subgroups or ceramide/cholesterol ratios, and there was no statistically significant correlation between answers about dry skin and ceramide levels. Interestingly, a statistically significant higher ceramide/cholesterol ratio was found for men than for women ($p = 0.02$).
Key words: age; ceramides; ceramide/cholesterol ratio; gender; stratum corneum; TEWL.

(Accepted March 18, 2010.)

Acta Derm Venereol 2010; 90: 350–353.

Jakob Mutanu Jungersted, Department of Dermatology D92, Bispebjerg Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen NV, Denmark. E-mail: Jungersted@gmail.com

The barrier function of the skin is located in the stratum corneum (SC), where the majority of the very limited transport of substances occurs between the corneocytes in the lipid bilayers (1, 2). The lipid bilayers are composed of ceramides, cholesterol and free fatty acids, and the ceramide profile in particular has been related to the barrier function of the skin. Ceramide 1 and ceramide 3 have been reported to be reduced in the SC of patients with atopic eczema (3–6), and some, but not all, studies have reported a negative correlation between ceramide 3 and transepidermal water loss (TEWL) (3, 7). Limited data are available on variation in skin lipids and ceramide profile due to physiological parameters such as age and gender. Two studies have found overall reduced lipid content in aged human skin (4, 8), but research on healthy skin is very limited (2).

Identification of filaggrin mutations related to atopic eczema, ichthyosis and other eczematous diseases (9–13) has renewed interest in research into skin barrier function, including skin lipids. However, more information about the amount of SC lipids in normal skin is required in order to obtain a better understanding of the diseased skin. The aim of the present study was to evaluate the ceramide profile of healthy volunteers in relation to age and gender, and to correlate ceramide profile with TEWL and clinical perception of dry skin.

MATERIALS AND METHODS

A total of 55 healthy volunteers was included in the study (19 men and 36 women). Thirty-two were < 40 years of age (median age 26 years, age range 18–39 years), 5 were between 40 and 60 years (median 51 years) and 18 were > 60 years of age (median age 76 years, age range 61–88 years). Participants were enrolled after responding to posters at the local library and educational centre, and had no history of any major skin diseases. The study was approved by the local ethics committee (SJ-7, 13986).

Methods

SC was collected from all participants, using cyanoacrylate methods (14). Participants were instructed not to use any moisturizers on the day of the examination. The mid-volar forearm was wiped with acetone to eliminate contamination with surface lipids. A drop of cyanoacrylate tissue-glue (LiquiBand®, Medlogic Global Ltd, Plymouth, UK) was placed on a glass slide and held tightly against the skin for 2 min, and then removed. The slide was stored at –80°C until further analysis by high-performance thin layer chromatography (HPTLC). For HPTLC the skin lipids were separated on silica-coated HPTLC plates, due to the difference in the strength of interaction between the different lipids and the silica gel using a solvent mixture of chloroform:methanol:acetic acid (190:9:1(v:v:v)). The samples were compared with standard curves made from ceramide 5 and cholesterol included on the plate. After separation, the plates were dried, stained with the fluorescent probe primuline and the components were quantified through determination of fluorescence intensity, as described in detail elsewhere (14).

For ceramides we use the simple nomenclature (ceramide 1–9); however, in Fig. 1 for clarity we have included the nomenclature suggested by Motta et al. (15).

TEWL measurements were obtained at the volar forearm from 31 of the volunteers. Measurements were performed on the opposite forearm to where the cyanoacrylate strip was taken, in accordance with guidelines (16).

Data on the clinical perception of dry skin was obtained from 16 healthy participants (median age 32 years, age range 18–51 years; 5 males, 11 females). Individual dryness of the skin, during the last week, expressed on a visual analogue scale (VAS)-score (0 = no dry skin and 10 = severely dry skin), and asked "Do you have dry skin (yes/no)? If yes, do you have dry

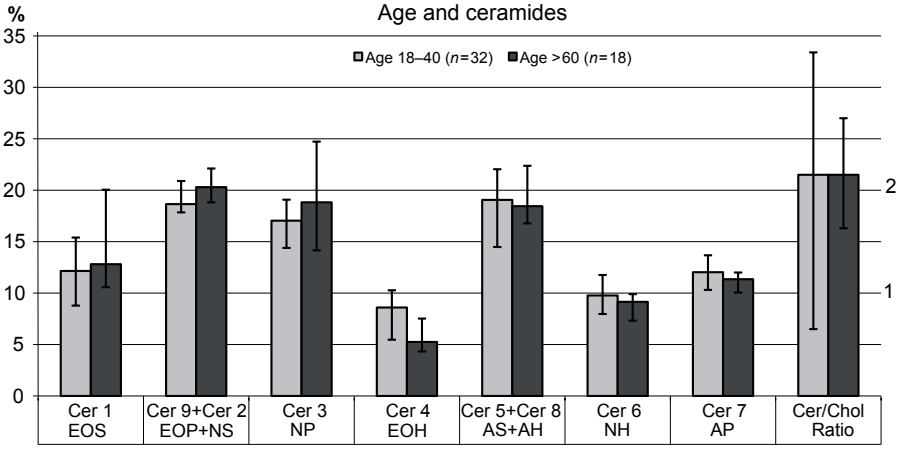


Fig. 1. Comparison of the percentage median stratum corneum ceramide values for healthy young skin and healthy old skin. Percentiles 1 and 3 are shown for each median value. No statistically significant differences were measured. The y-axis represents the median percentage distribution of ceramide 1–9 and is indicated *left*, while the ceramide/cholesterol (Cer/Chol) ratio is indicated *right*. Motta's nomenclature (15) is added for each ceramide: A: α-hydroxy fatty acid; EO: ester-linked ω-hydroxyl acid; N: non-hydroxy fatty acid; P: phytosphingosine; S: sphingosine; H: 6-hydrosphingosine.

skin only in the winter or both summer and winter?" (Questions were asked and samples were obtained in October).

Statistics

For comparison of differences between groups with respect to age and gender, the Mann-Whitney test was used. For correlation studies, the Spearman's rank correlation coefficient was used. p-values < 0.05 were considered statistically significant.

RESULTS

Results of the comparison of the ceramide profile in young and old skin are illustrated in Fig. 1. No statistically significant differences in ceramides or ceramide/cholesterol ratios ($p = 0.57$) were found in SC from young (< 40 years) and old (> 60 years) participants.

Ceramide profiles from the SC of male and female volunteers are shown in Fig. 2. There was a significant difference between the ceramide/cholesterol ratios for men and women (median men 2.0; median women 2.3; $p = 0.02$). No statistically significant differences were found between any of the ceramide subgroups between the age-matched men and women.

The relationship with TEWL for ceramide 1 and 3 is shown in Fig. 3. No significant correlation was found ($p = 0.76$ and $p = 0.57$, respectively). No correlation with TEWL was found for any of the other ceramide classes either, and no correlation between TEWL and the ceramide/cholesterol ratio ($p = 0.60$) was found.

The questionnaire on dry skin showed no significant differences in ceramide classes from volunteers with dry and normal skin, respectively, and no significant correlation between the VAS-score (0–10) and the different ceramides was found. One in 5 males reported dry skin, compared with 7 of the 11 females.

DISCUSSION

Ceramides are thought to play a major role in maintaining the efficient barrier function of the SC. Ceramide 1, in particular, is thought to be of importance in the organization of lipids in the SC (1, 17, 18). However, knowledge of the different ceramides and ceramide/cholesterol ratio in healthy skin is very limited, but is nevertheless important for a better understanding of diseased skin.

No statistically significant difference was found in this study between young and old skin with respect to ceramide profile. Two other groups have previously studied ceramide changes with respect to age. One group examined Japanese volunteers and used roughly the same cyanoacrylate method as ours. They found a decline in the total ceramide content with increasing age in SC, but did not differentiate among the subgroups of ceramides (4), thus the results of this study are difficult to compare with our results, in which the ceramide levels are given as a ratio of cholesterol. The other group used tape-stripping on female Caucasians; they

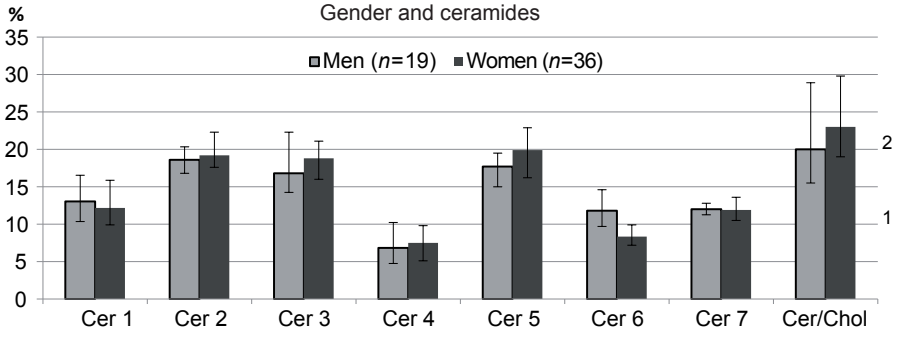


Fig. 2. Comparison of the percentage median stratum corneum ceramide values for healthy men and women and ceramide/cholesterol ratios (Cer/Chol). Percentiles 1 and 3 are shown for each median value. No statistically significant differences were found for the ceramide subgroups, but there was a statistically significant difference in the Cer/Chol ratio between men and women. The y-axis represents the median percentage distribution of ceramide 1–7 and is indicated *left*, while the Cer/Chol ratio is indicated *right*.

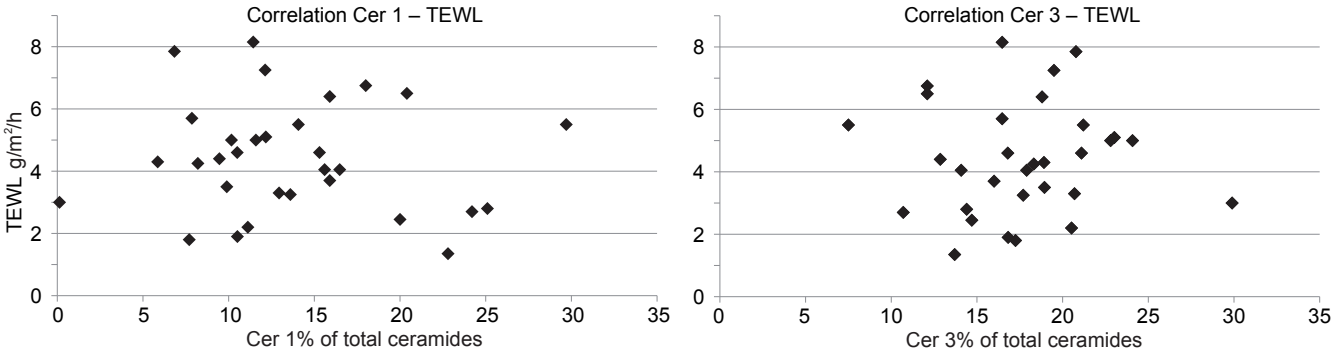


Fig. 3. Lack of correlation of transepidermal water loss (TEWL) and percentage of ceramide 1 and ceramide 3, respectively, $n=31$. p-values are 0.76 and 0.57, respectively.

examined the hands and face, and divided the females into age groups of 21–30, 31–40 and 41–50 years (8). They found a decreased level of all major lipid classes with increasing age, but, like us, they did not find any change in the ratio of either the total ceramide level or in any subgroups of ceramides. An overall reduction in lipids in aged SC has also been reported in a recent study, and was explained by the increasing pH of aged skin (19). However, this explanation is controversial, because conflicting results on pH and skin age exist (20). In conclusion, it appears that there is no correlation between clinically dry skin in elderly people and any ceramide subgroups or ceramide/cholesterol ratio.

It has been demonstrated that the skin response to sodium lauryl sulphate is influenced by the menstrual cycle (21), but whether this correlates with a cyclical change in lipid levels has not been examined; however, since the women in the present study would most probably have been at different stages of the menstrual cycle, the impact of this would be minimal. In addition, there is no difference between genders when evaluated by basal TEWL (16). One group found no statistically significant differences in ceramide subgroups between genders for adult skin (22). They did, however, find differences between the skin of pre-pubertal girls and adults (22). Another group found differences in the abdominal skin, with an increase in total ceramides for men compared with women (23). However, they examined only 3 male subjects, thus caution should be exercised in interpreting gender differences. In the present study no significant differences were found in ceramide profile in relation to gender, but there was a significant difference in the ceramide/cholesterol ratio, whereby men had the lowest ratio, which was closer to the atopic ratio (2). It could be argued that this difference may be due to the use of moisturizers; however, we tried to overcome this by instructing participants not to use moisturizer on the examined area on the day of examination, and a recent study found no difference in SC lipids after one week of two daily applications of a moisturizer compared with untreated control (Jungersted JM 2010, unpublished data).

TEWL has previously been reported to correlate negatively with the amount of ceramide 3 in atopic SC (3). Another group found no correlation between TEWL and total amount of ceramides (7). The present study did not support a correlation between any of the ceramide subgroups and TEWL in a group of healthy volunteers.

Including the above-mentioned observed gender differences with respect to experiences of dry skin, no statistically significant correlations were found between the answers to the questionnaire and the ceramides or TEWL.

This study focused on ceramides in the SC of healthy volunteers, as a basis for better understanding of diseased skin. In future research it would be interesting to determine what happens to SC lipids under different circumstances, such as occlusion, and during different treatment regimes.

ACKNOWLEDGEMENTS

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World Association for Veterinary Dermatology publishes

Revised Guidelines for Treatment of Skin Infections

Proper Identification, treatment and prevention of skin infections have become a major issue in veterinary health in recent years. Recent research has suggested that a majority of superficial skin infections are multidrug resistant. These new guidelines will assist practitioners and staff in diagnosis, treatment and prevention in the clinical setting.

Highlights from ***Recommendations for approaches to methicillin-resistant staphylococcal infections of small animals: diagnosis, therapeutic considerations and preventative measures, Morris, et al. Veterinary Dermatology 2017; 28, 304-e69*** are:

Currently, some degree of antimicrobial resistance has been documented within all Staphylococcus species that infect humans and domestic animals. Even though meticillin is no longer used in clinical practice, the term "meticillin-resistant" has persisted and has been used since the discovery of cephalosporins in the 1970s to indicate strains that are resistant to all beta-lactams except the newest generation of cephalosporins which were specifically developed for treatment of MRSA infections.[1] When a MRS strain expresses co-resistance to at least two additional antimicrobial classes, it may be referred to as multidrug resistant (MDR) and the term extensively drug resistant (XDR) may be used if the strain is nonsusceptible to all but two or fewer antimicrobial classes.[2,6]

Consensus statement 5: Topical therapy, using antibacterial agents with proven anti-staphylococcal efficacy, is the recommended treatment modality for any surface and superficial pyoderma involving MRS, particularly those with localized lesions, and for otitis and superficial wound infection.

The skin is easily accessible by topical treatment and antimicrobial formulations for use in small animals are available in most countries. A systematic review of topical therapy for canine skin infections concluded that evidence from randomized controlled trials was sparse on topical treatments, but that good evidence supported the use of shampoos containing 2–3% chlorhexidine and to a lesser extent of benzoyl peroxide in bacterial skin infections.[3]

Consensus statement 6: Topical therapy should be used as the sole on-animal antibacterial treatment for surface and superficial infections whenever a pet and owner can be expected to be compliant.

Although dermatology texts still recommend systemic antimicrobial therapy for superficial pyoderma with or without added topical medication, this recommendation can be challenged during times of increasing antimicrobial resistance. Newer studies have provided evidence that topical therapy as the sole antibacterial treatment can be effective in superficial pyoderma, providing opportunity to reduce the need for systemic therapy in some cases.[4,5,6]

Consensus statement 8: Empirical drug selection for systemic therapy is always contraindicated when a MRS infection is suspected based on historical factors, due to the high prevalence of multidrug resistance within these strains.

Susceptibility test results should always be available to make treatment decisions once MRS have been identified. However, if MRS is only suspected, for example following previous infections or based on cytological evidence of infection after antimicrobial therapy, a careful, susceptibility test-based approach is indicated to ensure best use of the remaining effective agents.[6]

Further guidelines including proper hospital infection control, hygiene personal protective equipment and disinfection protocols can be found in the full report available at ***www.WAVD.org*** or https://onlinelibrary.wiley.com/doi/epdf/10.1111/vde.12444

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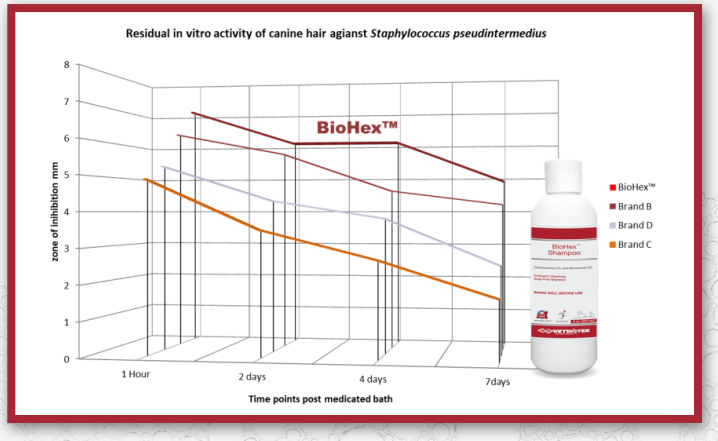
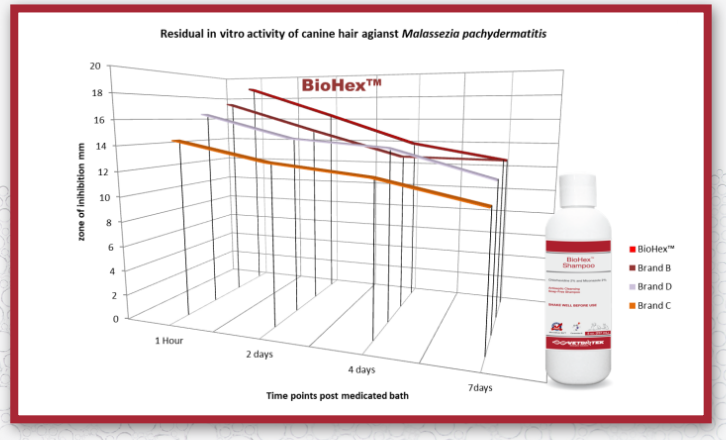
NAVDF technical update

An independent study conducted by Iowa State University entitled, "Residual in vitro activity of canine hair against Staphylococcus pseudintermedius and Malassezia pachydermatis following a single antimicrobial bath," was presented as a poster at the 2018 NAVDF. The study evaluated four market leading antimicrobial shampoos, including BioHex™ from VetBiotek, based on bacterial growth inhibition. While there were similar results in the Malassezia pachydermatis assessment for all test shampoos, there was a statistical significant difference in the performance of the shampoos on the growth inhibition of S. pseudintermedius.



- At day 7, the zone of Inhibition of BioHex Shampoo was 2 times larger than Brand D.
- At day 7, the zone of Inhibition of BioHex Shampoo 2.9 times larger than Brand C.
- BioHex has similar residual antibacterial activity at 7 days post bathing compared to Brand C at 1 hr. post bathing.



- There was no statistical difference in the measured zones of inhibition for hairs against Malassezia pachydermatis between shampoos across all time points, except at 1 hr. post bathing, BioHex was statistically significant versus brand C.

An In-Vivo Study Comparing the Efficacy of BioHex™ Shampoo Versus a 4% Chlorhexidine Gluconate Shampoo, in the Treatment of Canine Superficial Pyoderma.

(Study Abstract - Full Report On-File at VetBiotek)

S. Mannala*, A.R. Rajappa** and M.A. Mathew **

BioHex™ Shampoo

2% Chlorhexidine, 2% Miconazole, MicroSilver BG™ and Ceramide III

INTRODUCTION:

The use of topical antimicrobial therapy has experienced a renewed interest. WAVD Vet Derm 2017; 28: 304-e69. Clinical Consensus Guidelines for the World Association for Veterinary Dermatology***. **Consensus Statement 6:** Topical therapy should be used as the sole on-animal antibacterial treatment for surface and superficial infections whenever a pet and owner can be expected to be compliant. **In Section 7.1 Topical Therapy**, a clinical study using Chlorhexidine is referenced as a topical therapy that resolved or substantially improved clinical signs within 3 weeks in most dogs with susceptible Staphylococci (MSS) superficial pyoderma. The purpose of this study was to determine if differences exist between BioHex Shampoo and a 4% Chlorhexidine Shampoo in efficacy when treating superficial pyoderma and the resolution of respective clinical scores of seborrhea, erythema, lesional spreading and pruritus during the course of treatment.

MATERIALS AND METHODS:

Study Design: This study was a two-group, parallel, multicenter, positive-controlled, blinded, and randomized clinical field effectiveness study in naturally afflicted dogs. A total of sixty-nine (69) dogs, ages 9 weeks to 14 years old, weighing from 2.8 to 199 lbs, were enrolled in this study. This included (34) BioHex-treated cases and (35) Control-treated cases. Bacterial culture and susceptibility testing were performed to confirm enrollment. Failure to confirm a viable isolate during bacterial identification and minimum inhibitory concentration testing resulted in the removal of the dog from the study and was not included in the efficacy evaluation. *Staphylococcus pseudintermedius* was isolated from 69 dogs, including 14 methicillin-resistant strains (MRSP).

Severity of lesions using a four-point scale was used to create a **Primary Clinical Score** (**PCS**) for each dog. After examining a subject, the investigator also assigned a **Secondary Clinical Score** (**SCS**) for seborrhea, erythema, lesional spreading, and pruritus, taking into consideration the extent of coverage, the degree of severity, and the quantity (if applicable) of the clinical sign. Product efficacy was determined based on two criteria. First, there must be a successful resolution of the bacterial infection, which was the basis of the initial diagnosis and study enrollment. Second, the PCS at the conclusion of the study *must be* 0. The secondary clinical scores were used to provide further evidence of treatment effectiveness but were not used in the primary efficacy evaluation.

BioHex Shampoo was applied topically once a week for 2 weeks and 4% Chlorhexidine shampoo (used as a control) was applied topically twice a week for 2 weeks. Initial efficacy evaluations were conducted on Day 17 and again were followed up on Day 24 for any relapses.

REFERENCES:

*** http://onlinelibrary.wiley.com/doi/epdf/10.1111/vde.12444

* Dr. Shajan Mannala, DVM | VetBiotek

** HP Vet Clinic | Indira Nagar, Karnataka, India

VETBIOTEK

RESULTS:

Based upon a reduction of the Primary Clinical Scores to 0 and negative microbiological cultures at the end of treatment, the superiority of BioHex treatment over control was demonstrated by a statistically significant increase in the percentage of dogs that were successfully treated (88% resolution rate in the BioHex treated dogs vs. 40% resolution rate in the control-treated dogs). Furthermore, the evolution of all four of the Secondary Clinical Scores (seborrhea, erythema, lesional spreading, and pruritus) throughout the treatment period were superior in the BioHex-treated group versus the control-treated group. As treatment progressed, skin and coat condition improved more in the Biohex group compared to the control group; there were no other differences noted between the two treatment groups during the physical examinations conducted during the study.

Susceptible Strains of *Staphylococcus pseudintermedius*

Treatment Use Protocol	*Day 1*	*Day 4*	*Day 8*	*Day 12*	*Day 17 Initial Evaluation*	*Day 24 Final Evaluation*
BioHex™ Shampoo	✓		✓		*88% RESOLVED*	*88% RESOLVED (0% RELAPSED)*
4% Chlorhexidine Shampoo	✓	✓	✓	✓	*57% RESOLVED*	*40% RESOLVED (30% RELAPSED)*

- ***BioHex Shampoo was Statistically Superior to 4% Chlorhexidine Shampoo in Treating Superficial Pypoderma (88% Resolved vs 40% Resolved).***
- ***BioHex Shampoo Demonstrated Sustained Antibacterial Effects. One Week Post the Initial Evaluation the BioHex Group had 0% Relapses vs 30% for the 4% Chlorhexidine Group.***
- ***BioHex Shampoo Clinical Scores (Seborrhea, Erythema, Lesional Spreading and Pruritus) Were Statistically Superior to 4% Chlorhexidine Shampoo.***
- ***As Treatments Progressed, the Skin and Coat Condition Improved Greater in the BioHex Shampoo Treatment Group.***

CONCLUSION:

BioHex Shampoo when applied topically once a week for two weeks was effective and superior to a 4% Chlorhexidine Shampoo in the treatment of superficial pyoderma in dogs caused by susceptible strains of *Staphylococcus pseudintermedius* and was well tolerated by the study population with no significant adverse events or other deleterious health effects. The product also demonstrated sustained antibacterial effects lasting over weeks. A use protocol with less treatments will aid in pet owner compliance. BioHex Shampoo is an effective alternative to systemic antibiotics in treating superficial pyoderma.

MicroSilver Video
"The Difference Maker"

* *Dr. Shajan Mannala, DVM | VetBiotek*
** *HP Vet Clinic | Indira Nagar, Karnataka, India*

*** *http://onlinelibrary.wiley.com/doi/epdf/10.1111/vde.12444*

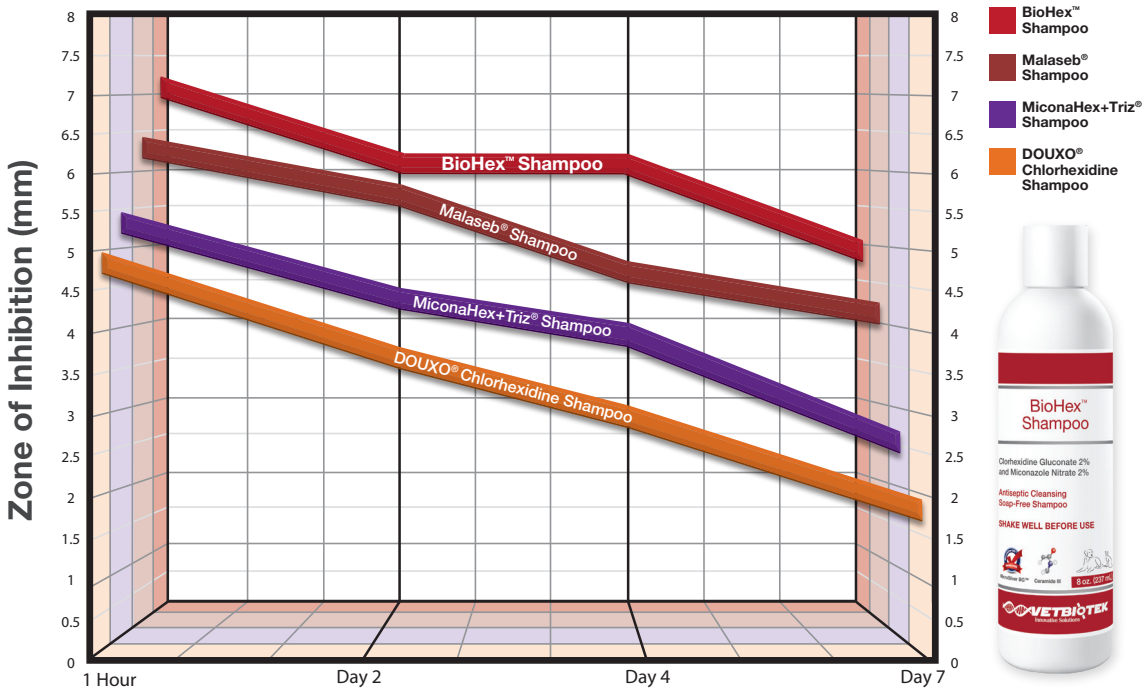
At Day 7, the Zone of Inhibition of BioHex™ Shampoo was:

- 2.9 Times Larger Than CEVA's DOUXO Chlorhexidine Shampoo
- 2.0 Times Larger Than Dechra's MiconaHex+Triz Shampoo
- 1.2 Times Larger Than Bayer's Malaseb Shampoo

Staphylococcus pseudintermedius Time Points Post Bath

Brand	1 Hour	2 days	4 days	7 days
BioHex™	7.1	6.1	6.1	4.9
Malaseb®	6.3	5.7	4.6	4.2
MiconaHex+Triz®	5.3	4.3	3.8	2.5
DOUXO® Chlrx	4.9	3.5	2.7	1.7

Residual *In Vitro* Activity of Canine Hair Against *Staphylococcus pseudintermedius*[1]



(chart 1)

(Full Study Report on file at Nextmune)

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orders@nextmune.com

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