Single blinded, randomized, prospective pilot study to evaluate the effect of L-Mesitran honey-based ointment in the treatment of surface pyoderma in dogs

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Abstract

Pyoderma is a very common skin disease in dogs and should always be considered as a secondary infection to an underlying disease. The diagnosis is based on clinical appearance and the results from cytology examination. Surface pyoderma is the most superficial and mild form of pyoderma where bacteria colonized the stratum corneum, the outer keratinized layer of the skin, and causes an inflammation. Some breeds like boxer, pugs and English bulldogs are predisposed to develop this type of pyoderma, as their anatomy holds several skin folds, where a favourable environment for microorganisms is created.

Surface pyoderma is usually treated topically with antibacterial shampoo and/or topical antibiotics. Use of antibiotics induces a risk of bacteria developing resistance, which is an increasing medical problem. L-Mesitran is a honey based CE-marked wound ointment, which does not contain any antibiotics. Honey has in several studies proved to have a good antibacterial effect and the use of this ointment does not have the risk of inducing resistant microorganisms. The purpose of this study was to evaluate if L-Mesitran was effective for the treatment of surface pyoderma in dogs, in a single blinded, randomized, prospective study.

The study included 40 skin areas with surface pyoderma from a total of 29 privately owned dogs. The dogs were examined and the clinical lesions in the skin were graded (max score of 9) and specimens for cytology were graded with respect to the presence of bacteria and neutrophils at inclusion (day 0) and after 14 days of treatment. The areas were randomly assigned into two treatment groups, 3% chlorhexidine shampoo (Pyoderm) or honey based ointment (L-Mesitran). In four cases the treatment instructions were not followed correctly and thus were excluded from the study. Totally 23 skin areas were treated with Pyoderm and 13 with L-Mesitran. The clinical signs and results of cytology of both treatment groups revealed no significant differences either at inclusion or following the treatment period.

In total 85% of the areas treated with L-Mesitran were considered cured, compared to 78% for the areas treated with Pyoderm. There was no statistically significant difference between the treatment groups with respect to cure rate. The pet owners perceived that the treatment with L-Mesitran was easier and less time-consuming to perform as compared to using shampoo. No side effects were noted with the treatment of L-Mesitran; while two pet owners experienced that their dogs became pruritic (itchy) with Pyoderm treatment.

In summary:

This pilot study shows that L-Mesitran is effective for treatment of surface pyoderma in dogs and that the ointment is safe to use. The results show that L-Mesitran is as effective as shampoo treatment with 3% chlorhexidine. In order to improve statistical power of the study, the study needs to be continued and should include at least 40 skin areas in each treatment group. The pet owners considered the treatment with ointment easier to perform compared to washing with antibacterial shampoo.

INTRODUCTION

The skin’s general structure

The skin is the body’s most important and largest organ. It forms both an anatomical and a physiological barrier between the organism and the external environment (Guaguère & Prélaud 2008). The skin’s function is to prevent loss of water, salts and organic substances, prevent invasion of microorganisms and chemical substances, protect the body from mechanical and chemical damage, regulate humidity and temperature, pressure, touch, pain and itching and to synthesize vitamin D (Sjaastad, Hove & Sand 2003).

The skin consists of three layers, from the inside counting: subcutis, dermis and epidermis.

Subcutis consists of fatty tissue, which has several functions, such as to protect against physical trauma and store fat and fat-soluble substances (Guaguère & Prélaud 2008, Sjaastad et al. 2003).

The dermis forms the next layer. The dermis functions primarily as support tissue to the epidermis (the upper skin). It consists mainly of connective tissue that is both flexible and shock absorbing. Fibroblasts are the predominant cell type and these cells produce; collagen, elastic and reticulin fibers and basic substance. The basic compound is composed of various proteoglycans and glycoproteins. Other cells found in sparse numbers in the normal subcutis are macrophages, lymphocytes, neutrophils, eosinophils, plasma cells, mast cells and melanocytes. In the dermis, which is richly vascular and nerve-fed, there are also the sweat glands, sebaceous glands and hair follicles.

The third and outermost layer of the skin is the epidermis. The epidermis is anchored to the dermis via the dermo-epidermal anchoring struc-
tures in the underlying membrane. This area also has important biological functions, such as for example tissue repair and communication between the different layers of the skin. The epidermis is not supplied by blood vessels, but by diffusion from the dermal blood vessels.

The cells in the epidermis consist of keratinocytes in different degrees of maturity. Keratinocytes are flat epithelial cells and these are structured in several layers, where the most immature keratinocytes at the bottom is called basal cell.

Melanocytes, which synthesize the pigment melanin, and Merkel cells, neuroendocrine cells and is in contact with nerve fibers from the dermis, is also found in the basal layer. The epidermis also contains Langerhans cells, which are antigen-presenting cells.

The basal keratinocytes undergo multiple divisions and reach the next cell layer called the stratum spinosum. This layer can vary in thickness from one to two cells in hairy skin, up to 20 cell layers of pads, mucocutaneous and nose. In this cell layer has cells stopped proliferating and instead started producing the beads (lamellae bodysuits) with content which will then be emptied between the cells and form an essential part of barrier protection.

The final differentiation of keratinocytes occurs in the stratum granulosum. Here the cells harden and become corneocytes, moving towards the top stratum corneum as seemless, quite hard cells arranged as overlapping.

Between the cells of the stratum corneum lipids are produced by keratinocytes in the epidermis. The stratum corneum can be from 40-50 cell layers. It is particularly upper skin that has a thick cell layer, such as nose and pads. The compact layer of cells and lipids between cells, which causes water loss through the skin and minimize the risk of entry of external fluid and microorganisms. (Guaguère & Prélaud 2008, Sjaastad et al. 2003).

The skin's specific structure creates an effective barrier with different components. Its hydrolipida surface, forming an emulsion of fats produced by keratinocytes, sebum and sweat, creating a chemical barrier. This coating also contains transferrin, fatty acids, complement and immunoglobulins, which reduces bacterial proliferation, adherence and colonization. Stratum corneum (compact horn cells) has a fatty layer between the cells which is difficult for microorganisms and water-soluble substances to penetrate if it is intact.

A further advantage is that the stratum corneum is regularly renewed (from the basale cells) cells and the old ones are sloughed off, which means that bacteria that have managed to colonize the skin surface then are removed. If the stratum corneum can still be invaded, the antigen-presenting cells are activated and keratinocytes produce inflammatory mediators. If microorganisms reach down into the dermis they will meet the various cells that will act antibacterial as well as cells involved in the skin's immune system (Guaguère & Prélaud 2008).

Pyoderma is by far the most common cause of dermatitis in the dog (Ettinger & Feldman 2010) and the most common pathogen involved is *Staphylococcus pseudintermedius* (Ettinger & Feldman 2010, Scott et al. 2001). At about 90% of pyoderma are isolated agents of *Staphylococcus pseudintermedius* (Bergvall, lecture 2008).

The bacteria that belong to normal skin flora are found around the mouth and anus even in healthy dogs from puppy stage. They can spread to other parts of the body, to other dogs and people (Scott et al. 2001).

Other bacteria that belong to the normal flora are *Micrococcus spp*, *Staphylococcus epidermidis*, *Clostridium spp*, *Propionibacterium acnes* and coagulase negative staphylococci to name a few (Guaguère & Prélaud 2008, Scott et al. 2001).

When *Staphylococcus pseudintermedius* causes pyoderma, it might also be *Pseudomonas*, *Escherichia coli*, *Proteus mirabilis*, *Corynebacterium spp* or *Staphylococcus aureus*. (Bergvall, lecture 2008, Ettinger & Feldman 2010).

As long as the skin is intact and in good condition, these bacteria usually do not cause infections. It is only when an injury occurs in any
of the skin’s different protective barrier layers that bacteria can be easily absorbed, proliferate and invade underlying tissue and thereby cause an infection. Causes of damage in these protective barrier layers can include traumatic injuries, ectoparasites, seborrhoica diseases (keratosis), allergies, immune-mediated diseases or hormonal imbalance. For this reason, pyoderma is always regarded as a secondary phenomenon to another underlying cause (Ettinger & Feldman 2010, Scott et al. 2001, Öhlén & Bergvall 1999).

Other factors that could cause pyoderma may be external environmental factors or the dog’s anatomy. For example, increased temperature and increased humidity affect the bacterial flora. Similarly, the dog’s own anatomical appearance e.g. skin folds or other clogged areas that are prone to heat and humidity. The altered micro-environment can lead to a proliferation of microorganisms. Risk areas are noses, vulvar folds, ventral fold of the skin and lip creases, to name a few (Guaguère & Prélaud 2008).

Pyoderma can be classified in many ways, the most common and useful classification is based on how deep into the skin the bacterial infection goes. It is then allocated into three types: surface pyoderma, superficial pyoderma and deep pyoderma. Surface pyoderma is a bacterial colonization of the stratum corneum. In superficial pyoderma the infection reaches through the stratum corneum and into the epidermis, the infection can also include upper parts of the hair follicle. In a deep pyoderma the infection reaches into the dermis and the subcutaneous layer can also sometimes be involved (Ettinger & Feldman 2010, Guaguère & Prélaud 2008, Scott et al. 2001, Öhlén & Bergvall 1999).

**Surface pyoderma**

Surface pyoderma is the most shallow and mildest form of pyoderma. Some examples of a surface pyoderma are intertrigo and pyotraumatic dermatitis (hot spots / moist eczema). Intertrigo is an abrasion between the two skin sites which gives a local irritation of the skin. In addition, there are lots of areas of skin resulting in a reduced air circulation, which leads to increased heat and humidity. Cells, sebum, sweat and moisture are not transported away from the skin surface normally. Depending on where the spots are located; urine, saliva or tears can also be collected in the folds. Friction and moisture can macerate the stratum corneum and bacteria can colonize the outer layer of skin. In brachycephala breeds like Pugs and English bulldogs, they occur deep in the nose, while labial fold dermatitis is mainly seen in Spaniel breeds. The dog owner then might think the dog has a very bad breath (halitosis)

The folds can be a problem in obese females or females with an indent-ed vulva. The vulva is a particularly weak and vulnerable area where secretions and urine can cause erosion and the bitch will also lick the site of this irritated area, which further aggravates the damage of the skin. Tail folds dermatitis is seen in breeds with corkscrew tails that are attached to the body, for example in English Bulldog, Pug and Boston Terriers. The other body folds can sometimes be found along the neck and back or between the mammary lines. It is mainly in obese animals that this is a problem (Mason 1991, Scott et al. 2001). Clinically, erythema of the skin and sometimes erosions, and a variable degree of smelly, sticky mucus. The dog often exhibits its itching symptoms in the affected area (Öhlén & Bergvall 1999).

Pyotraumatic dermatitis, a form of surface pyoderma, is also known as moist eczema. Moist eczema occurs when the dog itself traumatizes the skin by scratching, biting or licking because of itching or pain. The most common reasons for this could be ectoparasites, hypersensitivity reactions, otitis, anal sack bursts, foreign bodies in the coat, irritant substances such as dirt, bad shampoos etc. Moist eczema is more common in dogs that have fur with a thick undercoat, such as Golden Retriever, Labrador Retriever, German Shepherd, St. Bernard Dogs and others. The problem is also more common in summer when the weather is hot and humid (Mason 1991, Scott et al. 2001).

Moist eczema occurs rapidly and it shows a clear distinction between the healthy and diseased skin. Clinically erythema is presented as an erosive surface that is covered with pus. The affected area may be either hairless or furry. The dog often has a sharp pain from the eczema and a variable degree of itching (Öhlén & Bergvall 1999).

**Diagnosis of surface pyoderma**

Diagnosis is by anamnesis, the clinical presentation and cytological examination (Antibiotic Policy for dog and cat care in 2009). Cytology samples can be taken in several ways. Either one slide printed directly in the area (imression smear) to collect the material. Material from the skin can also be collected using a scalpel; scratch and then plated out on a slide, or by using a dry or moistened swab. The swab is then rolled out on a microscope slide. The sample is then stained with Diff Quick® for example, or Hema Color. The micro-cabinet can then show bacteria and / or yeast, neutrophils and possibly phagocytic neutrophils under magnification of 1000 (Öhlén & Bergvall 1999).

Moist eczema has a distinctive appearance; making diagnosis of pyoderma possible only using the clinical picture. To assess whether moist eczema is in the form of a surface pyoderma or components of a deeper infection, the remote area of the eczema needs to be inspected carefully. The infection is considered to be deeper when there are findings of papules and pustules in satellite areas around the moist skin and thus requires a different treatment compared with moist eczema in the form of clean surface pyoderma. (Scott et al. 2001, Öhlén & Bergvall 1999).

**Treatment of surface pyoderma**

Surface pyoderma should be treated locally, general treatment with antibiotics is not indicated (Antibiotic Policy for dog and cat care in 2009). In mild cases it is often enough to
use antibacterial wash, which is repeated daily or every other day until the infection is cured. There are several well-functioning antibacterial agents on the market, such as benzoyl peroxide, chlorhexidine, boric acid and ethyl lactate. There are several different brands of chlorhexidine shampoos with varying concentrations of chlorhexidine. Those who have been shown to have better antibacterial effect are the shampoos that have a concentration of 2-4% chlorhexidine. For all antibacterial shampoos, a contact time on the skin of at least ten minutes is needed to achieve an antibacterial effect (Bergvall, K lecture 2008).

Antibacterial wash can be combined with topical treatment with an antibacterial ointment containing 1% hydrogen peroxide, Microcid. In intense cases it may also be required to use topical treatment with antibiotics and glucocorticoids (Antibiotic Policy for dog and cat care in 2009, Bergvall, K lecture 2008).

In Sweden there is only one ingredient registered for animals that contain both an antibiotic and cortisone; Fuciderm™ vet. The gel’s active ingredients are fucidinsyra and betamethasone. Betamethasone is a glucocorticoid which has anti-inflammatory and anti-pruritic (anti-itch) effect. Fucidin is an antibiotic that is particularly active against Staphylococcus spp (Fass 2010).

For the prevention of pyoderma in dogs with folds, the skin folds should be regularly cleaned with antibacterial wash. In extreme cases even plastic surgery should be considered. In cases where the dog has skin folds due to obesity it is of great importance to monitor the dog's diet (Antibiotic Policy for dog and cat care in 2009).

For the topical treatment of moist eczema the infected area must be clean shaven with a margin around. Because moist eczema can be extremely painful for the dog it is recommended that shaving and cleaning are carried out under sedation and analgesia.

Moist eczema can also be treated with antibacterial wash, this requires more mechanical wash than it does with folds dermatitis, it is important that you remove any dried tissue on each treatment. It might also be beneficial to treat moist eczema with a drying medium, such as met-acresol sulphonic acid (Lotagen®), but then the dog should be under sedation / analgesia because this hurts. Even in these patients it is appropriate to combine the washing with an antiseptic ointment and in some cases, even with topical corticosteroid against itch and the inflammatory response. For pet owners to continue treatment at home there should be some form of pain relief given the first few days.

It is essential to prevent any further self-traumatising for example by using a collar. In severe cases, moist eczema needs to be treated with local antibiotics and glucocorticoids (Antibiotic Policy for dog and cat care in 2009).

**Antibiotic resistance**

The National Veterinary Institute (SVA), SVARM 2009 and Antibiotics and animals in Sweden in 2009, reported that both the resistance mode and consumption of antibiotics for veterinary use in Sweden is still good and low in compared to the international situation.

In the media the spread of bacterial resistance in dogs and horses received attention in recent years, which has led to major programs with better procedures for infection control and antibiotic use.

Antibiotic use in animals during 2009 was 15.368 kg, which is the lowest use in 30 years. Since 2006 there has been a downturn in sales of antibiotics in dogs. This is probably related to the first cases of meticillin resistant *Staphylococcus* in dogs that were reported in 2006.

Of the antibiotics prescribed for animals 14% were attributed to pets. The main application in dogs is associated with skin and urinary tract infections where also a prescription of broad spectrum antibiotics dominates, which favor the establishment of multi-resistant bacteria. Multi-resistance means that the bacterium is resistant to three or more antibiotics.

One of the most common bacteria involved in skin infections in dogs is *Staphylococcus pseudintermedius* and around 80-90% of these strains are resistant to penicillin. In 2006 the first case of meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) was discovered and since then the diagnosis of MRSP only increased.

Another 13 cases were diagnosed in 2006, 2007 confirmed 77 cases, 78 cases in 2008 in dogs and four cases in cats. In 2009 as many as 121 confirmed cases in dogs, seven cats and a case of a horse. After molecular biological studies of bacteria it seems that there is a single clone which spread within and between animals hospital in a large part of the country.

The kind that exists in Sweden has also proved common in many European countries, which suggests that it also spread internationally. A diagnosed infection with MRSP should be reported to the Department of Agriculture and county authorities.

Of the MRSP diagnosed cases in the first quarter of 2010, a third was multi-drug resistant. The most common resistance combination of MRSP was to penicillin, clindamycin and erythromycin, a resistance pattern observed in 75% of the cases. Resistance to five or more antibiotics was seen in 9% of the multidrug-resistant MRSP (SVA, 2010).

In Europe multi-resistance is also high for *Staphylococcus (pseud) intermedius*. A German study showed that 23% of *S. (pseud) intermedius* were resistant to at least five antibiotic classes. These samples were collected from the same clinic during an eighteen months period.

The same article also discussed the risk of zoonosis with *S. (pseud) intermedius* from infected animals and their owners, something that was
important to take into consideration, especially given the multi-resistant bacteria. The authors argued that antibiotic resistance will be one of the largest public health problems in the future and veterinarians need to be active in monitoring and controlling resistance and antibiotic use in pets, both nationally and internationally (Loeffler et al. 2007).

In a study in Denmark the transmission of Staphylococcus (pseud) intermedius from dogs with deep pyoderma to their owners was examined. This study found that almost half (46%) of the owners was carrying an identical strain of bacterium which their dog was infected with. Only one person from the control group, who had no dog contact, carried S. (pseud) intermedius. Some of the strains which were examined in both dog and owner were resistant to up to five classes of antibiotics (Guardabassi et al. 2004).

A similar study was done recently in the USA, where they examined the risk of transferring methicillin-resistant S. pseudintermedius between dog and owner. The study revealed that two members out of fifteen had been colonized with the same strain of bacterium their dog was infected with. After one month of treatment of the dogs MRSP could not be isolated from either dog or owner.

Since the study consisted of a small sample, the authors could not determine the amount of zoonosis risk; more samples were needed to clarify this. In one owner in the study they isolated methicillin-resistant coagulase-negative Staphylococci and this strain was only sensitive to chloramphenicol and gentamicin.

Many dogs in the study were bacteria resistant to this particular pattern. However these particular dog owners are not bacteria resistant to this pattern. On the other hand: the owner was working in an animal hospital and it is possible that she picked up the organism from another dog at the animal hospital and not from her own dog (Frank et al. 2009).

Another organism that is regarded as a risk to public health and animal welfare is methicillin-resistant Staphylococcus aureus (MRSA). Previously MRSA was considered exclusively as a hospital infection in humans, but in the 2000s, several reports showed that even animals carry these bacteria.

From the fall of 2006 to April 2010, 29 cases of pets were found, 15 in dogs, 2 in cats and 12 in horses. The isolates from dogs and cats are similar to those found in humans in Sweden. Several countries report that MRSA is increasing in dogs and cats. These are dogs are mainly treated at veterinary hospitals or at home with antibiotics for a long time due to skin infections. The bacterium can also be found in all healthy animals (SVA, 2010).

BACKGROUND

Healing properties of honey

Honey was already used thousands of years ago for medical purposes. Honey was used during ancient Greek times, the Roman Empire and in medieval times as a medical device for wound healing among other things. In fact honey was used until the 1920s before its use declined in the 30- and 40-ies when antibiotics made their entrance. Now that more and more resistant bacteria emerge, honey has begun to re-emerge as a broad spectrum antibacterial product that has no toxic effect on the tissue (Cutting 2007).

Honey has been shown to have many good qualities of wound healing in both humans and animals. It creates a moist wound healing environment, debrides, has a broad spectrum antibacterial action, reduces inflammation, stimulates the immune system, promote the growth of granulation tissue and reduces the risk of scarring (Cutting 2007, Lusby 2002, Molan 2002 o 2006, Simon et al. 2009). It is not just any honey that can or should be used; it must be a honey that is produced for medical use and thus a certain standard. Medical honey is produced in controlled and hygienic conditions with minimal contamination of the handling, antibiotics or other contamination (Cooper 2007, Cutting 2007, Overgaauw 2006).

Most important is to use honey which is sterilized by gamma irradiation (Cutting 2007, Lusby 2002, Molan 2002 o 2006, Simon et al. 2009). The irradiation kills bacteria and spores, but retains honey’s bioactivity (Lusby 2002, Overgaauw 2006). Honey can contain spores of Clostridium botulinum (Overgaauw 2006, Simon et al. 2009) that in deep, anaerobic wounds could begin to proliferate and produce botulinum toxin. This toxin can cause paralysis and cardiac arrhythmias. With the help of radiation the spores are inactivated and the honey is thus safe to use (Simon et al. 2009).

Today there are several different products containing medical honey on the market in Australia, New Zealand, Europe and North America. In Europe, there are several different honey ointments holding the CE Mark (Cooper 2007), which means that the product meets the EU’s basic health, environmental and safety requirements. CE-mark indicates that the product may be sold freely across national borders within the EU (SWEA 2010).

Honey and antibiotics differ in their mode of action. Antibiotics are either bactericidal or bacteriostatic while honey is hygroscopic, which means that it draws out the fluid and therewith dehydrating bacteria. Because honey contains a sufficiently high concentration of sugar it also prevents the growth of microbes.

It is not only its high osmotic effect which gives honey an antibacterial effect, there are many more components that play an important role and many are not even identified (Molan 2002, Simon et al. 2009). An example of another antibacterial component in honey is the enzyme glucose oxidase, which produces gluconic acid and low concentrations of hydrogen peroxide when honey is diluted in the wound bed (Cutting 2007, Lusby 2002, Molan 2002, Simon et al. 2009).
When there is a constant production of hydrogen peroxide it provides a good antibacterial effect, although the concentration is a thousand times less than 3% (v/v) hydrogen peroxide (Molan 2002).

Honey has a very low pH 3.4 to 5.5 (median 4.4) which prevents the growth of most pathogenic bacteria. This low pH also accelerates the healing process by providing more oxygen released from hemoglobin in the capillaries and by proteases disabled. Proteases influence healing by breaking down the growth factors and cell-matrix (Simon et al. 2009).

Several studies have shown that honey has an antibacterial activity against most bacteria such as coagulase negative staphylococci, gram-positive cocci and also antibiotic-resistant strains of MRSA (Cooper et al. 1999 o 2002, French 2005). In these studies clinical isolates from infected wounds were tested in the laboratory for antibacterial efficacy, but there are also many clinical studies published.

Molan (2006) compiled results of 17 randomized controlled trials with a total of 1965 patients, five clinical trials with 97 patients and 16 trials with a total of 533 wounds of experimental animals. The author showed in this article that honey has a good effect on many different types of wounds and thus that there is evidence for a beneficial effect in the use of honey in wound care and prevention.

Honey has been found to have anti-inflammatory effects both in vivo and in animal models, but the mechanism behind is not fully established (Lusby 2002, Molan 2002, Oryan et al. 1998, Simon et al. 2009). The conclusions are that there is an anti-inflammatory component in honey and that it is not just the secondary effect of honey’s antibacterial effect, as studies have shown a good effect on wound healing even in the wounds with almost no or few bacteria (Molan 2002, Oryan et al. 1998).

In animal models, it has also been shown that honey accelerates wound healing by increasing angiogenesis, granulation and epithelisation. It was suggested honey would stimulate an inflammatory response of leukocytes and that this is the reason for faster wound healing (Molan 2006, Simon et al. 2009). Tonks et al., in a study demonstrated that honey stimulates monocytes to produce cytokines, such as TNF-α, IL-1β and IL-6 cytokines that are both pro- and anti-inflammatory.

There are two important adverse effects reported with the use of honey as a medical product. A few patients have complained of a stinging pain right after administration. In addition an allergic reaction could occur. In literature, there are no serious allergic reactions reported after the use of medical honey (Simon et al. 2009).

L-Mesitran

L-Mesitran® is a patented wound care product containing honey from the company Triticum. Their products are available in almost all EU countries and there are also distributors around the world. In Sweden there are two distributors; Rama Medical and Omnidea, where Omnidea is active in the veterinary market. In 2002 the product was CE certified and was thus the first honey-based product in Europe to obtain CE marking. There are six different L-Mesitran products, L-Mesitran Ointment, L-Mesitran Active, L-Mesitran Border, L-Mesitran Hydro, L-Mesitran Net and L-Mesitran Soft. The products are produced for humans, but they have also been used in veterinary medicine, as evidenced by various case reports published in the L-Mesitrans website (L-Mesitran® 2010).

L-Mesitran Ointment contains 48% medical honey. Other components in Medilan® (lanolin), cod liver oil, sunflower oil, calendula, aloe vera, vitamin E and C and zinc oxide.

The ointment is considered safe to use and no side effects or contraindications are registered (L Mesitran 2010). Because L-Mesitran Ointment is a medical product, there is a waiting period for start/exhibition/competition, for horses this is 96 hours and for dogs 14 days (Omnidea u.å).

AIM

The purpose of this study is to evaluate in a single blinded, randomized, prospective study whether the topical treatment with L-Mesitran is effective in the treatment of surface pyoderma in dogs.

MATERIALS AND METHODS

Animals

This clinical pilot study included 40 affected areas of intertrigo or moist eczema in privately owned dogs. Intertrigo in the form of visible inflammatory reaction in the skin with the score for erythema (redness) at least one (0-3) needed to exist. Moreover, the dogs sampled with standardized swabs for cytological examination.

For inclusion in the study a detectable bacterial overgrowth in the form of at least 5-10 bacteria on average per 10 field of view in 1000x magnification (HPF) was required. Nose and vulvar folds, but also lip and tail folds and other areas of the trunk were included. An area of fold dermatitis could be included in the same dog, if these areas were well distanced from each other. Included areas were divided randomly into two treatment groups. The owners took a sealed bag containing any of the treatment options. If several sites were included from the same dog, all areas were treated with the same treatment option. The study was conducted single blinded.
Fold dermatitis in paws (inter-digital) were excluded from the study. Dogs were excluded from the study if treated with antibiotics in some form from two weeks prior to enrollment until study closure. Dogs whose owners did not give written consent for participation in the study, or did not follow the treatment protocol were excluded from the study.

Approval for the study was given by the city’s Animal Review Board and the dispensation was granted as to the requirement that the animals should be bred before the study began.

Collection of cases
Dogs in the study were recruited mainly through telephone contact / direct contact with different Pug and English bulldog breeders from Uppland and Västmanland. Other recruitment methods were advertising via e-mail to veterinary students at SLU and private contacts.

Most of the dogs were examined and sampled in their home environment, while a small number of dogs were examined at University Animal Hospital in Uppsala. Animal owners were asked to give written consent that their dogs were allowed to run in the study, where they also testified that the dog had not been treated with antibiotics during the previous 14 days. Animal owners were informed about the purpose of the study and that they could at any time choose to leave the study. All recruitments of dogs have been made by the undersigned.

Investigation and treatment
All clinical studies and tests have been performed by the undersigned. The dogs were examined and sampled twice, at inclusion day 0 and at study termination day 14. The clinical examination was an assessment of the relevant area for the following clinical signs: erythema, alopecia and the amount of secretions. These symptoms were graded with a four-point scale 0-3, where 0 = absence of, 1 = mild, 2 = moderate, 3 = heavily and was totaled, so that each area of skin could achieve a maximum rating of 9.

When inclusion criteria were first met, at least one of erythema, a cytology sample through a clean cotton swab was rubbed against the affected skin. The swab was then rolled out on a microscope slide. The glasses were marked with name, date and the area where the sample was taken from. Slides were air-dried and stained with Hema Color® (Merck) and analyzed using a microscope at a magnification of 1000 high power field (HPF).

The number microorganisms (coccis, rods and yeasts), neutrophils and phagocytic neutrophils were counted with an average per 10 HPF. The values were recorded on the following scale: 0, <5, 5-10, 10-25 and >25/HPF for each of the investigated microorganisms and cells. This scale was later translated from 0 to 4, where 0 = 0/HPF and 4 => 25/HPF.

The dogs that met the inclusion criteria were randomly divided into one of the two treatment groups and the owner had to carry a sealed, opaque bag containing any of the treatment kits. The bag was not opened in the presence of the undersigned.

The two different treatment regimens consisted of Pyoderma (3% chlorhexidine shampoo, Vibac) and L-Mesitran® Ointment (honey-based ointment, Triticum). Both treatments were performed in a total of 14 days. Animal owners received oral instructions on both methods of treatment by the undersigned and written instructions came also with the treatment bag.

The instructions for the treatment of Pyoderma were as follows: soak the affected area with warm water, massage in 50 cent large clicks the shampoo and leave the shampoo on for 15 minutes. Rinse thoroughly to remove the shampoo and dry thoroughly. The treatment was performed every other day for 14 days.

When treated with L-Mesitran they would wash the affected area with lukewarm water and wipe dry, before a thin layer of ointment was applied on the area. The treatment was performed once per day for 14 days.

Both groups were also instructed to protect the area from the dog licking it, for example by putting on a funnel. Tick and deworming the dog was allowed during the study. The use of another ointment, shampoo or other treatment with antibiotics resulted in exclusion from the study, after registration. The dogs would generally be handled without changing the normal routine (exercise, nutrition).

Results
Return visits with follow-up evaluation was done after 14 days of treatment. Because the experiment was performed blinded to the analyst, the pet owners were asked to clean the treated area before the return visit, so no residue from the treatment would be seen and thus risk revealing which treatment group the dog entered in.

At the return visit a new swab, with cotton swabs, was taken from the treated area, the same method as the initial visit. The sample was rolled out on a microscope slide and stained with Hema Color®. The glasses were labeled with name, date and the sample area. Also a clinical evaluation of erythema, alopecia and amount of secretions in the treated area was performed again, using the same scale as the first visit from 0 (absent) to 3 (severe). Cytology samples were examined again in a microscope with 1000x magnification and micro-organisms and neutrophils were counted in the same way as at the initial visit. The infection would be counted as cured, when the cytological evaluation was at <5 of average /10HPF of coccoid bacteria and rod forming organisms together, and 0 of neutrophils and phagocytic neutrophils.

The animal owners completed an evaluation questionnaire with seven questions at the return visits, which asked their opinion about the treatment they performed. The forms also indicated which treatment they
used and then the form was sealed in an envelope. The envelope was not opened until the final reading of cytology test was performed.

**Statistical analysis**
Clinical and cytological score at inclusion and after treatment were compared between the two treatment groups with frequency analysis. Fisher’s exact test was also used to compare treatment results between the two groups with regard to the cleared and not cleared. The same test was used to compare owners’ evaluation of treatment methods.

**RESULTS**
A total number of samples of 59 areas of skin from 43 privately owned dogs. A total of 40 sites from 29 dogs met the criteria and were enrolled in the study. Of these dogs eight contributed with two fields per dog and one dog with three areas, other dogs contributed with one area per dog.

The dogs were Pug, English Bulldog, Nova Scotia Duck Tolling Retriever, Cavalier King Charles Spaniel, dogue de Bordeaux and Springer Spaniel. 14 of the dogs enrolled in the study were females and 12 were males, with an age from 8 months to 15 years with a mean age of 5 years. The treatment was completed according to protocol on 26 dogs. Overall this resulted in 36 sites that completed the study and 4 were registered as dropouts.

Two of these dropouts, were counted as dropouts because the owners of the animals did not follow the treatment instructions and the other two never started the treatment. All dropouts were in the L-Mesitran treatment group.

23 of the 36 included areas which completed the study were treated with Pyoderma and 13 with L-Mesitran.

**Observations prior to treatment**
No significant differences were detected either clinically or cytological between treatment groups at inclusion day 0 (p >> 0.05). Mean and median scores for clinical symptoms, and cytological results at baseline are presented in Table 1.

**Observations after treatment**
After stopping the treatment at day 14 there was no statistical significance (p >> 0.05) detected between the treatment groups in clinical or cytological respect. No significance (p >> 0.05) could also be seen between the groups in respect of

**Table 1.** Mean (median) of clinical and cytologic results for each treatment group at inclusion.

<table>
<thead>
<tr>
<th>Treatment method</th>
<th>L-Mesitran</th>
<th>Pyoderma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>1,46 (1)</td>
<td>1,48 (1)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1,23 (0)</td>
<td>1,09 (1)</td>
</tr>
<tr>
<td>Secretions</td>
<td>1,69 (2)</td>
<td>1,30 (1)</td>
</tr>
<tr>
<td>Clinical score</td>
<td>4,38 (4)</td>
<td>3,90 (3)</td>
</tr>
<tr>
<td>Cocci</td>
<td>3,15 (3)</td>
<td>3,04 (3)</td>
</tr>
<tr>
<td>Rods</td>
<td>1 (0)</td>
<td>0,96 (0)</td>
</tr>
<tr>
<td>Yeast</td>
<td>1,46 (1)</td>
<td>1,43 (1)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0,38 (0)</td>
<td>0,52 (0)</td>
</tr>
<tr>
<td>Fag. neutrophils</td>
<td>0,15 (0)</td>
<td>0,48 (0)</td>
</tr>
<tr>
<td>Cytological score</td>
<td>6,15 (6)</td>
<td>6,43 (7)</td>
</tr>
<tr>
<td>Total score</td>
<td>10,54 (10)</td>
<td>10,30 (10)</td>
</tr>
</tbody>
</table>
cleared or not cleared. Mean and median duration of clinical symptoms and cytologic results after treatment are reported in Table 2.

Upon completion of the treatment the bacterial count was decreased in all dogs. Clinically speaking all the dogs did a lot better with a lower clinical score than on day 0.

Comparison
Of the total 36 areas, 29 were completely cleared for cytology, according to the criterion that the number of bacteria should be <5/HPF and that there would be no neutrophils.

In the group treated with Pyoderma there were five areas that were not completely cleared and the group treated with L-Mesitran had only 2. All of these seven had clinically improved; the mean clinical score at inclusion was for these 4.14 and 2.29 after treatment.

Cytology was better in all seven; average at inclusion was 6.86 and 4.43 after treatment. Areas treated with L-Mesitran had a 7% better frequency of cure compared to areas treated with Pyoderma (Figure 1), but no statistical significance could be demonstrated between treatment groups.

Animal owners’ evaluation
The study included 15 owners and 14 of these owners completed the evaluation forms. A total of 10 pet owners have dealt with Pyoderma and seven cases were treated with L-Mesitran. Overall the owners were happy with the treatment. Everyone who treated with L-Mesitran would consider using the ointment again and was pleased with the visible result. In the Pyoderma group two out of ten owners would not use the shampoo as a treatment again and two respondents did not think that the treatment gave visibly better results.

A number of pet owners liked the shampoo but they would rather choose a treatment that was less labor intensive to perform when it was available. Three owners tried both methods of treatment and all three perceived L-Mesitran as a simpler treatment to perform in comparison with the Pyoderma shampoo. Two pet owners felt their dogs reacted negatively to such treatment by discomfort or side effects. One of the owners felt that the dog’s skin became more red, itching, increased hairloss and that the tear increased. The second owner felt that two of his dogs had itchy noses during the treatment and a third owner experienced itching after the treatment stopped. Both owners were included in the Pyoderma group.

No side effects or discomfort was observed according to the owners in the L-Mesitran group.

Almost half of the pet owners liked the Pyoderma shampoo although the treatment was difficult to perform. This is in contrast to those who treated with L-Mesitran; there was no perceived treatment difficulty to implement as can be seen from the diagram in Figure 2. However there was no significant difference (p>0.05).

When asked whether the treatment was time-consuming, none of the owners who treated with L-Mesitran thought that the treatment took time to perform. However, more than half of the owners from the Pyoderm group thought that it was a time-consuming treatment as shown in Figure 3. There was also a statistical significance (p <0.03) between the groups.

Other observations
Two owners who treated with L-Mesitran experienced that the first few days of treatment liquids were drained from the treated area. However, they did not think the dogs seemed to perceive it as unpleasant.

DISCUSSION
Shampooing with chlorhexidine shampoo has shown to have good antimicrobial activity in several studies (Kwochka & Kowalski 1991, Lloyd & Lamport 1999 and 2000, Paul 1978, Reme et al. 2005). However it requires a certain concentration of chlorhexidine. A study of Lloyd and Lamport in 1999 showed that a concentration of 3% or more is most desirable.

At inclusion in the study (day 0) there was no significant difference between dogs, clinically and cytology, in the different treatment groups. It was, in other words, an equal distribution between the two groups. After the treatment no significant difference in cured or treated areas was found, as shown in Table 2 and Figure 3.

Table 2. Mean (median) of clinical signs and cytological results for each treatment group after treatment.

<table>
<thead>
<tr>
<th>Treatment method</th>
<th>L-Mesitran</th>
<th>Pyoderma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>0,15 (0)</td>
<td>0,26 (0)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1,15 (0)</td>
<td>0,9 (1)</td>
</tr>
<tr>
<td>Secretions</td>
<td>0,46 (0)</td>
<td>0,30 (0)</td>
</tr>
<tr>
<td>Clinical score</td>
<td>1,77 (1)</td>
<td>1,48 (1)</td>
</tr>
<tr>
<td>Cocci</td>
<td>1 (1)</td>
<td>1,17 (1)</td>
</tr>
<tr>
<td>Rods</td>
<td>0 (0)</td>
<td>0,35 (0)</td>
</tr>
<tr>
<td>Yeast</td>
<td>0,31 (0)</td>
<td>0,17 (0)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0,15 (0)</td>
<td>0,04 (0)</td>
</tr>
<tr>
<td>Fag. neutrophils</td>
<td>0,15 (0)</td>
<td>0,04 (0)</td>
</tr>
<tr>
<td>Cytological score</td>
<td>1,61 (1)</td>
<td>1,78 (1)</td>
</tr>
<tr>
<td>Total score</td>
<td>3,38 (4)</td>
<td>3,26 (2)</td>
</tr>
</tbody>
</table>
It was an uneven distribution regarding the number of dogs between the treatment groups, 23 of them were treated with Pyoderm and only 13 with L-Mesitran. One of the reasons for this skewed distribution was, that some animals contributed to several areas and some owners participated with several dogs. Divisions in each treatment group were made by the owner whom had to pick a sealed bag with treatment. If the same dog had more than one area of affected skin then the same kind of treatment was done on all areas.

The reason for allowing the random division in each treatment group with application on the whole dog, was to minimize the risk of error in following the protocol and thus mal-treatment as this could otherwise be expected to result in increased number of drop-outs. Another reason for the imbalance in numbers between treatment groups was that the cytology samples were taken in the dog’s home environment and then analyzed at the clinic. After sampling and the randomization of treatment we found that some dogs did not meet the inclusion criteria for cytology, in which case they were excluded from the study.

Since the study was blinded for the sampler, whom also studied the cytology samples, the imbalance in the groups could not be detected and the groups could not be equally distributed. With a greater number of affected treatment areas, this issue could have been minimized.

This study suggests a slightly better treatment outcome for the areas treated with L-Mesitran, the group that had fewer areas included. If there had been a more even numerical distribution among the groups, it is possible that the difference would have been even greater in favour of L-Mesitran. As well as larger

**Fig. 1** The proportion of cleared and not cleared compared between each treatment group ($p>>0.05$)

**Fig. 2** Results of the owners’ answer to the question: “was the treatment difficult to perform?” ($p>>0.05$)

**Fig. 3** Results of the owners’ answer to the question: “was the treatment time consuming?” ($p<0.03$)
number of sites in each treatment group there is the need of more total material to show any significance between treatment options. Had the study been double-blind, it would have given more power to the study, but this is something that was not feasible with this study design.

A possible reason for Pyoderma showing a slightly poorer treatment outcomes could be due to lack of contact time. To have an adequate antibacterial effect the shampoo requires a contact time with the skin of at least 10 minutes. The provided margin for this treatment was weak because the owners had been given instructions to leave the shampoo on for 15 minutes. Compliance to the protocol in this regard is not possible to control in this type of study design.

In this study different pet owners’ evaluation of the two treatment methods. By far most owners were happy with their treatment and would choose to use it again if needed. It appeared, however, that there was a clear difference which animal owners experienced the application of an ointment as easier and more convenient compared to a shampoo. The three owners who tried both treatments all agreed that they preferred L-Mesitran over Pyoderm.

Several pet owners who used Pyoderm complained that they would much rather choose a simpler method of treatment if it was available. Almost half of the pet owners who had to shampoo their dogs thought this was a treatment that was difficult to perform, whereas none of the other group perceived their treatment to be difficult to perform.

When asked whether the treatment was time consuming there was even a significant difference (p <0.03) between groups, no one thought L-Mesitran application was time consuming.

A total of four drop-outs were recorded in the study and all were in the L-Mesitran group. Two areas were recorded as drop-outs because the owners never started the treatment and the other two because the owners did not follow proper treatment protocol. The two who never started treatment indicated that it was due to time constraints and one owner stated that she did not think the dog was in need of treatment in this area. The two owners who had not followed the treatment protocol had both failed to treat 3-4 days during the study period. The instructions for the L-Mesitran group was to treat every day. None of the owners who started treatment with L-Mesitran interrupted the course of treatment, which was seen as positive.

Although all four drop-outs occurred in the L-Mesitran group, L-Mesitran, provided better conditions for compliance to treatment instructions in comparison with shampoo, because the owners felt that the treatment of Pyoderm was time consuming and the shampoo treatment, was to some of them, difficult to perform. This might have lead to the fact the shampooing was not performed optimally, which may have contributed to a poorer treatment outcome.

Based on this, it can be assumed to be advantageous to use L-Mesitran, when treatment results are better because the owners have mastered to provide the treatment in a satisfactory manner. It is essential to be responsive to owners’ views. If the animal owner can not perform optimal treatment, it does not help that an agent has been shown to possess good therapeutic properties.

All owners had received an instruction at the start of the study that the treated areas should be protected for example by means of a collar. However, none of the owners thought their dogs had to use a collar and have not complied to this instruction. The dogs were therefore able to access the affected areas and licking or scratching them, despite this L-Mesitran had a good healing rate (85%).

In this study no side effects were noted with the topical treatment with L-Mesitran. Two animal owners experienced that their dogs suffered from itching and also noticed increased redness during the treatment with Pyoderm. This might be caused by residues of the shampoo which were left on skin when it was not dried properly after rinsing. Another option is that one or more components of the chlorhexidine shampoo caused contact hypersensitivity in the patient. These dogs demonstrated an irritation during the treatment with Pyoderm. All, except one whose spots did not heal, were still better clinically and cytological compared to the beginning of the treatment.

In Fass 2010 one can read that products containing chlorhexidine may cause contact dermatitis and urticaria and in very rare cases it can cause anaphylactic shock. No adverse effects of Pyoderm in Sweden are reported by Virbac, who represent the shampoo. In France, however, there is one case reported in 1990 concerning hypersensitivity to Pyoderm in a French dog of unknown breed. However, it is unclear what type of hypersensitive reaction this was (Skarman, E., pp. Msgs, 2010).

Contact Hypersensitivity to chlorhexidine seems to be uncommon, but can not be excluded. Two owners who used L-Mesitran pointed out that the fluid from the areas in the first days of treatment increased. An increased secretion of the skin, is according to the manufacturer completely normal and an expected reaction to treatment with a honey-based product. Because the honey has an osmotic effect, which leads to have a raised secretion on the areas treated with honey (Skarman, O., pp. Msgs, 2010).

One of the dogs in the study had surface pyoderma in the form of moist eczema. According to the animal owner, the moist eczema on the neck of this Pug was almost entirely healed after only two days of treatment. The dog had a combined clinical and cytological total scores of 13 at initial visit, at the return visit it had a total score of 5 and from a cytological view it was completely healed.
CONCLUSION

The results of this pilot study show that topical treatment with L-Mesitran has an equally good effect on surface pyoderma in the dog compared with 3% chlorhexidine shampoo. The treatment method has proven to be safe, no side effects could be observed and the owners experienced the treatment easier to perform compared with shampooing. The results suggest even that L-Mesitran could possibly be an effective treatment option compared with shampooing.

This is a clinical pilot study. In order to arrive at statistically reliable results, the study should continue and should include at least 40 treated areas per treatment group.

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