Papers

Comparison of a chlorhexidine and a benzoyl peroxide shampoo as sole treatment in canine superficial pyoderma

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The clinical and antibacterial efficacy of two shampoos used as a sole antibacterial treatment in dogs with superficial pyoderma were investigated and compared. In a randomised, partially blinded study, a 3 per cent chlorhexidine gluconate shampoo (Chlorhex 3; Leo Animal Health) was compared against a 2.5 per cent benzoyl peroxide shampoo (Paxcutol; Virbac) in 22 dogs with superficial pyoderma. Dogs were washed two to three times weekly with a 10-minute contact time over 21 days. Clinical scores and bacterial counts were assessed on days 1, 8 and 22 and compared within and between treatment groups; overall response was assessed at the end of the study. Twenty dogs completed the study; 15 (68.2 per cent) showed an overall clinical improvement and the clinical signs resolved in three chlorhexidine-treated dogs. In the chlorhexidine-treated group, scores for papules/pustules (P<0.001), investigator-assessed pruritus (P=0.003), total bacterial counts (P=0.003) and counts for coagulase-positive staphylococci (P=0.003) were reduced after three weeks. Scores and bacterial counts did not vary significantly in the benzoyl peroxide-treated group.

CANINE superficial pyoderma has remained a common presentation in small animal practice despite a large number of antibacterial agents being available and licensed for use in dogs in many countries. Pyoderma, irrespective of its depth, was the second most common specific dermatological diagnosis in dogs in a UK survey of over 3700 consultations in small animal general practice (Hill and others 2006).

Staphylococci, particularly those producing coagulase (coagulase-positive staphylococci [CPS]), are the predominant bacteria involved in bacterial skin diseases (pyoderma) in many species. *Staphylococcus pseudintermedius* (formerly *Staphylococcus intermedius*) was isolated in over 90 per cent of cases of canine pyoderma in UK (Lloyd and others 1996) and from more than 80 per cent in Sweden (Holm and others 2002).

Treatment recommendations for canine pyoderma vary with the depth of infection (Ihrke 1976). In superficial pyoderma, bacteria inhabit the skin surface and follicular infundibula without extension into the dermis (Nesbitt 1983). Most standard texts recommend that superficial pyoderma is treated using systemic antibiotic therapy, either alone or in combination with topical therapy (Ihrke 1987, Scott and others 2001). Failure to identify or correct underlying predisposing factors often results in recurrent infections and long-term or repeated antibacterial therapy, with the associated increased risk of drug resistance (Noble and Kent 1992, Holm and others 2002).

In view of the globally increasing antimicrobial resistance in both animal and human pathogens, the use of antimicrobial agents in companion animal practice has come under review as highlighted by a news report in *Veterinary Record* in 2009 (Anon 2009). Although the quantity of antimicrobial agents used for each small animal species and for each disease in the UK remains unknown, canine skin diseases are likely to account for a large part of the total small animal consumption. In the survey by Hill and others (2006), antimicrobials were dispensed to 23 per cent of the 559 dogs diagnosed with skin disease. In addition, the emergence of multiresistant staphylococci such as meticillin-resistant *S pseudintermedius* (MRSP) and meticillin-resistant *Staphylococcus aureus* (MRSA), which are often resistant to all antibacterial products licensed for systemic use in dogs, has highlighted the need for alternatives to systemic antimicrobial therapy (Tomlin and others 1999, Loeffler and others 2007).

The clinical efficacy of antibacterial shampoos in canine superficial pyoderma has been evaluated in only a small number of studies (Lloyd and Reyss-Brion 1984, Ascher and others 1990, Murayama and others 2010a, b, Nagata and others 2006) and these have rarely incorporated quantitative assessments of antibacterial efficacy (Bond and others 1998). The purpose of this study was to investigate and compare, by clinical assessment and quantitative bacteriology, the efficacy of a 3 per cent chlorhexidine gluconate product and a 2.5 per cent benzoyl peroxide shampoo for the treatment of canine superficial pyoderma.

Materials and methods

Ethics

This study was approved by the Royal Veterinary College's Ethics and Welfare Committee and written informed consent for participation was obtained from the owners of each dog.

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Study design

Two shampoos were compared in a randomised, partially blinded study. A 3 per cent chlorhexidine gluconate formulation (Chlorhex 3; Leo Animal Health) was compared with a 2.5 per cent benzoyl peroxide shampoo (Paxcutol; Virbac), which has a UK product license for the treatment of canine superficial pyoderma. Each dog was assigned to either of the treatment groups according to a sequence of computer-generated randomised study numbers. Owners were aware of the treatment that their dog was receiving; they were instructed to discuss details of the treatment only with an assistant investigator who provided the shampoo along with both written and verbal instructions for its use. Dogs were assessed and sampled on days 1, 8 and 22 of the study by one of two clinicians who were blinded to the treatment each dog received and who also carried out the microbiological tests.

Inclusion criteria

Privately owned dogs with a history of pruritus that had been referred to the dermatology service of the Queen Mother Hospital for Animals, Royal Veterinary College, for assessment of skin disease between March 2002 and March 2003, were considered. Superficial pyoderma was diagnosed based on the presence of typical skin lesions such as papules, pustules and epidermal collarettes in commonly affected areas (ventral abdomen, medial thighs, trunk) associated with abnormally high (readily observed) numbers of cocci in multiple oil-immersion microscopic fields (x 1000) on light microscopic examinations of Diff-Quik (DADE) tape-strip or smear samples taken from lesional skin. Dogs were enrolled provided CPS were subsequently isolated on bacteriological examination of samples (see below) and where owners indicated that topical therapy was likely to be practicable for them.

Ectoparasite infestations were ruled out by clinical examination, coat brushings, hair pluckings, skin scrapes or trial acaricidal therapy as appropriate. Flea control with either fipronil (Frontline Spot On Dog/Frontline Spray; Merial) or selamectin (Stronghold; Pfizer), was instituted or continued in all cases on a monthly basis and combined with a permethrin and pyriproxyfen-containing spray for the environment (Indorex; Virbac). *Malassezia* dermatitis and overgrowth were ruled out by cytology as described above. Investigations of other underlying or concurrent diseases were postponed until after the study.

Dogs that required topical or systemic glucocorticoid therapy or antimicrobial therapy other than the study medication were excluded. Before enrolment, withdrawal periods of two weeks for systemic antibacterial agents and four weeks for glucocorticoids and shampoos were specified. None of the owners reported previous known or suspected adverse reactions in themselves or their dogs to either chlorhexidine or benzoyl peroxide when questioned before enrolment.

Treatment groups

The chlorhexidine shampoo was used twice weekly. The benzoyl peroxide shampoo was applied according to the data sheet recommendations, initially on a daily basis for two treatments, then twice weekly. For both shampoos, the owners were instructed to wet the dog's coat thoroughly, apply the shampoo to achieve an overall lather and allow 10 minutes contact time before thoroughly rinsing with water. For the benzoyl peroxide shampoo, a brief shampoo application and rinse were required before the 10-minute wash. Shampoo therapy was not administered during the two days before each re-examination.

Assessment of clinical signs

Owners were asked to score their dog's pruritus (scratching, rubbing, chewing or licking) on a scale of 1 to 5: 1 Absent, 2 Minimal (briefly, occasionally during the day), 3 Mild (frequently during the day), 4 Moderate (more than half of the day) or 5 Severe (day and night), and to state any changes in concurrent medication and to report adverse reactions to the shampoo on either the dogs or themselves. The investigator scored evidence of pruritus (scratching during the consultation, excoriations, intensity of scratch reflexes) on a scale of 1 (absent) to 5 (severe) and assessed the presence and distribution of papules/pustules, collarettes and crust/scale. The lesions were scored as 1 (absent), 2 (one or two lesions present), 3 (involving less than 5 per cent of the skin, 4 (involving 5 to 50 per cent of the skin) and 5 (involving more than 50 per cent of the skin). Coat condition was subjectively assessed as 1 Very good, 2 Good, 3 Fair, 4 Poor or 5 Very poor.

At the end of the study, the clinician assessed the overall response to shampoo treatment of the superficial pyoderma on a scale from 1 to 5: 1 All clinical signs resolved, 2 Much improved, 3 Improved but further treatment required for pyoderma, 4 No change and 5 Worse.

Quantitative bacteriology

Lesional skin, or previously affected sites if lesions had resolved, were sampled using a detergent cup-scrub technique for quantitative assessment of cutaneous bacterial populations (Williamson and Kligman 1965, Lloyd 1984). Briefly, the skin was sampled using sterile polytetrafluoroethylene (PTFE) cylinders (the 'cups') that were applied to the skin and filled with 2 ml of a wash fluid containing sterile PBS with 0.1 per cent Triton X-100. The skin surface within the cup was gently rubbed for one minute with a sterile PTFE rod and the fluid was aseptically transferred into sterile bijou bottles. Aliquots (25 µl) of the wash fluid and of its serial 10-fold dilutions (1:10, 1:1000, 1:100,000, 1:10,000,000) were spread on to paired quadrants on blood agar plates (Oxoid CM271 containing 5 per cent ovine blood) within 40 minutes of collection. Plates were incubated aerobically at 37°C for 48 hours and colony counts of total bacterial (TB) growth and CPS were recorded as $\log_{10}$ (colony-forming units per cm^2 +1). CPS were identified according to colony morphology, size, colour and haemolysis, and bacteriological tests included Gram stain, catalase test, slide coagulation test or tube coagulation test with dog or rabbit plasma and acetoin fermentation (Voges-Proskauer reaction) (Devriese and Hájek 1980).

Statistical analysis

Clinical scores and microbial counts were compared within and between the treatment groups by analyses of variance with subanalyses by Tukey's wholly significant difference test (SAS for Windows version 9.2; SAS Institute). Frequency data relating to overall scores for pyoderma were compared using Fisher's exact test (SPSS version 17.0 for Windows). A P value of less than 0.05 was used to indicate statistical significance.

Results

Animals

Eleven dogs were randomised to each treatment group. The dogs (14 males, eight females) were aged between eight months and 11 years and represented 11 different breeds and four crossbreeds. Coat types included wire-haired (two in the chlorhexidine group, six in the benzoyl peroxide group), shorthaired (seven in the chlorhexidine group, three in the benzoyl peroxide group) and silky to medium-length coats (two in each group). Twenty dogs (91 per cent) completed the study. Adverse reactions were not reported in any of the dogs. One benzoyl peroxide-treated dog was withdrawn because the owner had used a topical glucocorticoid product (Fuciderm gel; Leo Animal Health) on day 5 to reduce self-trauma. One chlorhexidine-treated dog was withdrawn because CPS were not isolated at the first visit, despite the observation of cocci on cytological specimens obtained from representative lesions.

All 20 dogs that completed the study showed signs of pruritus directed towards areas where pyoderma lesions were found. Additional pedal pruritus without lesions of pyoderma on the paws was reported in six dogs. Eighteen dogs (90 per cent) had suffered from recurring skin disease for more than six months before presentation and previous good responses to systemic antibiotic therapy had been observed. Skin lesions of superficial pyoderma observed at the beginning of the study included papules, pustules, focal areas of yellow or haemorrhagic crusts, crusted plaques and epidermal collarettes. In 15 dogs (75 per cent), the lesions were found in the inguinal area and on the medial thighs. Four of those were also affected on the caudal dorsum. Five dogs had lesions involving large areas of the neck, trunk and the proximal limbs.

Clinical scores

Eight of 10 chlorhexidine-treated dogs and seven of 10 benzoyl peroxide-treated dogs improved based on the scores for overall clinical

TABLE 1: Overall clinical response of superficial pyoderma in 22 dogs after 21 days of treatment with either 3 per cent chlorhexidine gluconate or 2.5 per cent benzoyl peroxide shampoo

Treatment group	Number of dogs Resolved	Much improved	Improved	Unchanged	Worse	Withdrawn
Chlorhexidine (n=11)	3	4	1	2	0	1
Benzoyl peroxide (n=11)	0	2	5	2	1	1

TABLE 2: Mean (se) scores for pruritus assessed by investigators and owners in 20 dogs with superficial pyoderma before, during and after treatment with 3 per cent chlorhexidine gluconate (n=10) or 2.5 per cent benzoyl peroxide (n=10) shampoo

	Investigator		Owner	
Day	CH	BP	CH	BP
1	2.9 (0.2)	2.5 (0.3)	3.4 (0.4)	3.6 (0.3)
8	2.4 (0.3)	2.0 (0.3)	3.2 (0.3)	3.3 (0.4)
22	1.6 (0.3)*	2.3 (0.3)	2.4 (0.3)	2.9 (0.4)

Comparison with day 1 value within treatment group, * P=0.003
BP Benzoyl peroxide, CH Chlorhexidine

response of superficial pyoderma (Table 1). Chlorhexidine-treated dogs more frequently showed resolution or marked improvement (7 of 10 dogs) when compared with benzoyl peroxide-treated dogs (2of 10 dogs; $P<0.05$).

Investigator-assessed pruritus reduced after three weeks within the chlorhexidine group ($P=0.003$), whereas the other pruritus scores (investigator-assessed and owner-assessed) did not vary between days or groups (Table 2). Lesion scores for papules/pustules in the chlorhexidine-treated dogs were significantly lower on day 8 and day 22 when compared with day 1 scores (day 8, $P=0.01$; day 22, $P<0.001$). In addition, papule/pustule scores on day 22 in the chlorhexidine-treated group were significantly lower ($P<0.05$) than those of the benzoyl peroxide-treated group (Table 3). Epidermal collarettes and crusts/scales showed no significant difference in scores during and after treatment compared with pretreatment values or between groups. Mean coat condition scores reduced in both treatment groups but the changes were not statistically significant (Table 3). In one wire-haired dachshund, which had received benzoyl peroxide, the hair coat appeared drier and finer at day 22.

Bacterial counts

Within the chlorhexidine-treated group, both TB and CPS counts were significantly reduced on days 8 and 22 when compared with day 1 (Table 4). Within the benzoyl peroxide-treated group, these parameters were not significantly reduced. Counts of TB and CPS did not vary significantly between the treatment groups at each visit.

Further diagnostic investigations

Concurrent allergic skin disease was diagnosed in 14 dogs. Four of those dogs had flea allergic dermatitis, two were diagnosed with adverse food reaction, seven had atopy and one had flea allergic dermatitis with concurrent atopy. In four of the remaining dogs, the owners elected not to pursue further investigations into underlying causes but chose to control superficial pyoderma with topical therapy. In four dogs, an underlying cause could not be identified.

Discussion

The resolution of pyoderma in three chlorhexidine-treated dogs and the overall clinical response of 'much improved' or 'resolved' in nine of the 20 dogs of either group indicate that antibacterial shampoos can be an effective sole treatment in canine superficial pyoderma.

Chlorhexidine is a synthetic cationic biguanide widely used for skin antisepsis in human and veterinary medicine and as a plaque-reducing mouthwash in dentistry. It disrupts cytoplasmic membrane function and coagulates cytoplasmic constituents in many fungi and bacteria (Longworth 1971, Hugo 1999, Odore and others 2000). Chlorhexidine shampoos are active against *S pseudintermedius* in vitro at concentrations of 2 to 4 per cent (Lloyd and Lamport 1999, Lloyd and others 2003), and surgical scrubs are effective on canine skin at concentrations of 1 to 4 per cent (Kwochka and Kowalski 1991, Evans and others 2009). Bond and others (1995) reported a good antibacterial effect of a 2 per cent chlorhexidine gluconate and 2 per cent miconazole shampoo in basset hounds with seborrhoeic dermatitis associated with *Malassezia pachydermatis*, and clinical improvements in dogs with pyoderma were reported in three recent studies using different chlorhexidine formulations (Nagata and others 2006, Murayama and others 2010a, b).

In the present study, the statistically significant reductions in clinical scores of investigator-assessed pruritus and papular/pustular eruptions in chlorhexidine-treated dogs were paralleled by reductions in quantitative counts of total bacteria and CPS, as soon as day 8 but also at day 22. These observations suggest that the papular and pustular eruptions truly reflected superficial pyoderma and support previous cytological evaluations. In addition, investigator-assessed pruritus reduced, which highlights the potential antipruritic effects of antibacterial products in cases of superficial pyoderma, even in the face of frequent concurrent allergic diseases. Although investigator-assessed pruritus remained a subjective outcome measure and relied on evaluation during a short consultation period, repeated assessment of the same dog by the same clinician minimised bias.

Benzoyl peroxide is a broad-spectrum antibacterial agent frequently used in gel or shampoo formulations in human acne at concentrations from 2.5 to 10 per cent (Fulton and others 1974, Kligman and others 1977). Benzoyl peroxide is metabolised in the epidermis to benzoic acid and free oxygen radicals that disrupt bacterial cell membranes (Nacht and others 1981). A prophylactic antistaphylococcal effect of a 3 per cent benzoyl peroxide shampoo was demonstrated on canine skin by Kwochka and Kowalski (1991), and an effect against CPS was shown for a 2.5 per cent benzoyl peroxide shampoo in a semiquantitative study on dogs with pyoderma (Ascher and others 1990).

In the present study, the benzoyl peroxide shampoo was less effective than the chlorhexidine preparation. The only clinical score that significantly improved with benzoyl peroxide was papular/pustular eruption. Although not identified in any of the dogs treated with benzoyl peroxide in the present study, the shampoo has been associated with skin irritation (Lloyd and Reyss-Brion 1984). Such irritation may have affected the clinical scores, but reductions in bacterial counts did not reach statistical significance in the benzoyl peroxide group. The relative merits of these two products in canine superficial pyoderma should be further evaluated in larger studies.

In accordance with previous publications on canine pyoderma, CPS dominated the bacterial flora of lesional skin in these dogs (Lloyd and others 1996, Holm and others 2002), as indicated by the close similarity of counts of TB and CPS. All dogs were enrolled between 2002 and 2003, before the emergence of MRSP in Europe, and although sensitivity testing was not performed, it is likely that these CPS were examples of meticillin-susceptible *S pseudintermedius*. Further studies are needed to determine efficacy of shampoo treatment in canine skin infections caused by MRSP or MRSA. To date, there is very limited evidence of bacterial resistance to chlorhexidine (Fraise 2002, Russell 2004). Preliminary clinical data from dogs are encouraging (Murayama and others 2010a, b, Loeffler and others 2007) and in vitro studies indicated low minimal inhibitory concentrations of chlorhexidine for examples of canine MRSA and MRSP (Baines and others 2008). Although biocide concentrations achievable at the skin surface and in the hair follicle by topical application are likely to be high, it is possible that biocide-resistant strains might be selected for by prolonged use (Strickler and others 1983, Block and Furman 2002).

While shampoo treatment requires compliant patients and is labour-intense for owners, it can reduce the cost and side effects associated with systemic antibiotics, particularly for diseases where long-term maintenance is required (Kunkle and others 1995). It can also improve coat appearance and odour by removing debris, scales and crusts (Curtis 1998). Reducing the use of oral antibacterial agents in dogs might also benefit human health, as frequent prescription of

TABLE 3: Mean (se) scores for papules/pustules, epidermal collarettes, crusts/scales and coat condition in 20 dogs with superficial pyoderma before, during and after treatment with 3 per cent chlorhexidine gluconate (n=10) or 2.5 per cent benzoyl peroxide (n=10) shampoo

	Papules/pustules		Epidermal collarettes		Crusts/scales		Coat condition	
Day	CH	BP	CH	BP	CH	BP	CH	BP
1	3.9 (0.2)	3.9 (0.3)	3.0 (0.3)	2.8 (0.4)	3.2 (0.4)	3.2 (0.4)	2.8 (0.2)	3.4 (0.3)
8	2.7 (0.3)*	3.1 (0.2)	2.6 (0.4)	2.2 (0.4)	2.6 (0.4)	2.7 (0.4)	2.0 (0.2)	2.8 (0.3)
22	1.9 (0.3)†	3.1 (0.3)‡	2.1 (0.4)	2.5 (0.4)	1.9 (0.3)	2.5 (0.4)	1.7 (0.2)	2.5 (0.3)

Comparison with day 1 value within treatment group, * P=0.01, † P<0.001
Comparison of day 22 values between treatment groups, ‡P<0.05
BP Benzoyl peroxide, CH Chlorhexidine

TABLE 4: Mean (se) log10 counts (colony-forming unit/cm² +1) of total bacteria and coagulase-positive staphylococci obtained using a detergent scrub method in 20 dogs before and after therapy with 3 per cent chlorhexidine gluconate (n=10) or 2.5 per cent benzoyl peroxide (n=10) shampoo

	Total bacterial counts		CPS counts	
Day	CH	BP	CH	BP
1	6.21 (0.60)	4.43 (0.46)	6.19 (0.61)	4.43 (0.46)
8	3.41 (0.57)†	4.37 (0.41)	3.30 (0.61)*	4.36 (0.41)
22	3.30 (0.6)†	3.74 (0.61)	3.03 (0.71)†	3.48 (0.71)

Comparison with day one value within treatment group, * P=0.01, † P=0.003
BP Benzoyl peroxide, CH Chlorhexidine, CPS Coagulase-positive staphylococci

antibiotics has recently been identified as a main risk factor for subsequent canine infection with MRSA, an important human pathogen (Soares-Magalhães and others 2010).

While systemic antibiotic therapy is routinely indicated in deep pyoderma and in cases where topical therapy fails or cannot be administered satisfactorily, topical therapy alone should be considered, particularly for cases of superficial pyoderma where long-term management of recurrent infection is required because underlying diseases cannot be identified or corrected.

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Conflict of interest

AL's Senior Clinical Scholarship at the Royal Veterinary College was partially funded by Leo Animal Health between 2001 and 2004. MC was an employee of Leo Animal Health when this study was conducted.

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