Evaluation of aerobic bacteriologic culture of epidermal collarette specimens in dogs with superficial pyoderma

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Objective—To evaluate a method of aerobic bacteriologic culture of epidermal collarette specimens from dogs with superficial pyoderma and compare results with those for aerobic bacteriologic culture of abdominal skin specimens in healthy dogs.

Design—Prospective study.

Animals—22 dogs with epidermal collarettes and 24 healthy dogs.

Procedure—Dry sterile cotton swabs were rolled across epidermal collarettes or hairless areas of abdominal skin in healthy dogs and submitted for aerobic bacteriologic culture. Hemolytic colonies of gram-positive–staining cocci were tested for catalase production, and if results were positive, a coagulase test was performed. Colonies with coagulase activity were tested for the ability to ferment mannitol. Antimicrobial susceptibility testing was performed on all *Staphylococcus* spp that were isolated.

Results—*S intermedius* was isolated from collarettes in 18 of 22 dogs with superficial pyoderma but not from healthy dogs. Estimated sensitivity and specificity of the culture method were 81.8% and 100%, respectively. There were no significant differences in the ability to culture *S intermedius*, the number of *S intermedius* isolates without resistance to antimicrobials, and the number of *S intermedius* isolates resistant to penicillin G when comparing dogs with superficial pyoderma for the first time and dogs with recurrent pyoderma, dogs that did or did not receive concurrent antimicrobials, and dogs with and without underlying allergic disease.

Conclusions and Clinical Relevance—Bacteriologic culture of epidermal collarette specimens was a simple and reliable method for identification of *S intermedius* in dogs with superficial pyoderma, regardless of history of pyoderma or current antimicrobial use. (*J Am Vet Med Assoc* 2005;226:904–908)

Superficial pyodermas are common in dogs[1,2] and often develop secondary to hypersensitivities (atopic dermatitis, food hypersensitivity, or flea-bite hypersensitivity), endocrinopathies (hypothyroidism or hyperadrenocorticisim), or ectoparasite infestations (demodicosis or sarcoptic acariasis).[2-8] *Staphylococcus intermedius* is the most common pathogen isolated from pyodermas in animals, although *Staphylococcus aureus* and *Staphylococcus schleiferi* also have been isolated.[9-13] Intact pustules are reportedly the lesion of choice to culture when bacterial isolation is required.[2,14] However, pustules in superficial pyodermas are often fragile and transient, and clinicians are more likely to observe epidermal collarettes as the sequelae to pustules.[3,14,15]

Epidermal collarettes are circular, often alopecic skin lesions with an exfoliative border.[2,14,15] Although epidermal collarettes are most often found in superficial pyodermas, the methodology of culturing these lesions has not been delineated.[16] The purpose of the study reported here was to evaluate a method of aerobic bacteriologic culture of epidermal collarette specimens in dogs with superficial pyodermas and compare results with those of aerobic bacteriologic culture of abdominal skin specimens in healthy dogs.

Materials and Methods

Dogs—All dogs with superficial pyoderma examined by the dermatology service at the **Veterinary Medical Teaching Hospital, University of California, Davis (VMTH-UCD)** from September 1, 2001, to March 1, 2002, were evaluated for inclusion in the study. Dogs with epidermal collarettes **(Figure 1)** were included in the study. Dogs that had been bathed within 1 week of physical examination or that had been treated with subsequent antimicrobials without resolution of epidermal collarettes were excluded from the study.

Twenty-four healthy dogs were used as controls. These dogs were owned by students, faculty, and staff of the VMTH-UCD and had no clinical signs or history of dermatologic disease, had not received a bath within the week previous to culture, and were not receiving medication other than that used for control of ecto- and endoparasites.

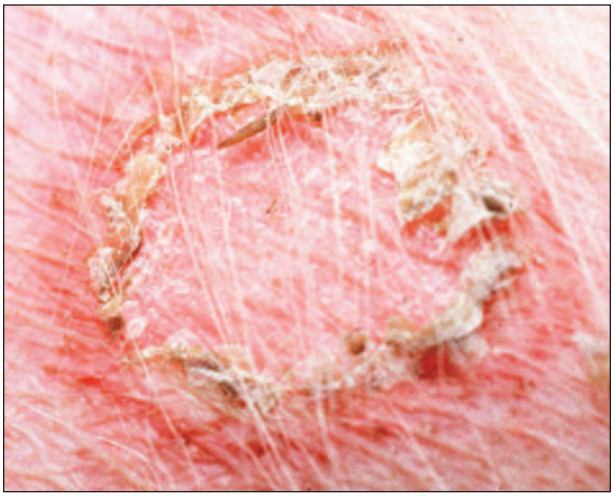

Figure 1—Photograph of an epidermal collarette on the skin of a dog with superficial pyoderma.

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For each dog, age, breed, sex, whether the dog was having its first episode of pyoderma (first-time pyoderma) or had had previous episodes of pyoderma (recurrent pyoderma), concurrent antimicrobial treatment, antimicrobial treatment within the previous year, concurrent corticosteroid treatment, and the area of the body that had the most epidermal collarettes were recorded. All antimicrobial and corticosteroid treatment was recorded, regardless of whether the medication was used specifically to treat superficial pyoderma. The sample for bacteriologic culture was obtained from an epidermal collarette located in the area of the body that had the most collarettes.

Underlying disease was diagnosed by use of standard diagnostic methods. Criteria developed by Willemse[17] and Prélaud et al[18] were used to diagnose atopic dermatitis. A hypoallergenic diet trial was used to diagnose food hypersensitivity.[5] Detection of the organism and response to ectoparasite treatment were used to diagnose flea allergy dermatitis and infestation with *Sarcoptes scabiei* or *Demodex canis*.[19-21] Color dilution alopecia was diagnosed via trichogram,[22] and hypothyroidism was diagnosed via detection of low concentrations of total thyroxine and free thyroxine and a high concentration of thyroxine stimulating hormone,[6] as compared with reference ranges (1.0 to 3.6 µg/dL, 1.0 to 3.5 ng/dL, and 0 to 0.5 ng/mL, respectively) established at the VMTH-UCD. Underlying diseases were defined as diseases that, when treated successfully in combination with antimicrobials, resulted in resolution of the pyoderma or a decrease in the frequency of recurrence of the pyoderma.

Bacteriologic culture of epidermal collarette specimens—In dogs with superficial pyoderma, an epidermal collarette was identified for bacteriologic culture; if necessary, hair was gently clipped with scissors to expose the collarette. A dry, sterile culturette swab[a] was gently rolled across the collarette 3 to 4 times, then placed into transport medium and immediately submitted to the microbiology laboratory at the VMTH-UCD. Standard methods for bacteriologic culture were used.[23] The swab specimen was transferred to culture plates containing blood agar[b] and MacConkey agar[c] and tryptic soy broth.[d] Hemolytic colonies of gram-positive–staining cocci were tested for the production of catalase, and if results were positive, cocci were tested for coagulase activity.[e] If results of the coagulase test were positive, the organism was tested for the ability to ferment mannitol; organisms were identified as *S aureus* if mannitol was fermented or *S intermedius* if mannitol was not fermented or fermentation was delayed for > 24 hours. Specific testing for *S schleiferi* was not performed.

In healthy dogs, bacteriologic culture specimens were obtained from an area on the hairless skin of the caudal aspect of the ventral abdominal midline (approx 1 cm from the midline), as previously described. Bacteria were identified only by results of the coagulase test (positive or negative), and if results were positive, bacteria were further identified as *S aureus* or *S intermedius*. No further tests were performed on bacteria with negative coagulase test results.

Antimicrobials that were tested and their mean inhibitory concentrations included amikacin (16 µg/mL), amoxicillin-clavulanate (8 µg/mL), cefazolin (8 µg/mL), ceftiofur (2 µg/mL), ceftizoxime (8 µg/mL), chloramphenicol (8 µg/mL), enrofloxacin (0.5 µg/mL), erythromycin (0.5 µg/mL), gentamicin (4 µg/mL), oxacillin (2 µg/mL), penicillin G (8 µg/mL), tetracycline (4 µg/mL), ticarcillin-clavulanate (16 µg/mL), and trimethoprim-sulfamethoxazole (2 µg/mL). If a *Staphylococcus* sp was identified on bacteriologic culture, the attending veterinarian chose an appropriate antimicrobial on the basis of susceptibility and determined the duration it was administered (minimum of 3 weeks). Response to the antimicrobial treatment was verified in follow-up examinations (11 dogs), by telephone contact with the owner (7), or both (4). In the latter 4 dogs, follow-up examinations were performed > 6 weeks after the initial examinations. The 15 dogs that had follow-up examinations were evaluated 1 week to 2 years after the initial examinations (median, 5 weeks). Only 1 antimicrobial was administered to each dog. Antimicrobial shampoos were also sent home with the owners of 8 dogs to be used every 2 to 3 weeks.

Statistical analyses—A Fisher exact test[f] was used to compare the proportion of dogs with superficial pyoderma that had *S intermedius* isolated on bacteriologic culture of epidermal collarette specimens to the same proportion in the group of healthy dogs. The sensitivity (proportion of dogs with superficial pyoderma that had *S intermedius* isolated from bacteriologic cultures) and specificity (proportion of healthy dogs in which *S intermedius* was not isolated from bacteriologic cultures) were estimated by use of the observed proportions, and confidence intervals were computed by use of the exact binomial distribution. A Fisher exact test also was used to compare the ability to culture *S intermedius* between dogs with first-time pyodermas and dogs with recurrent pyodermas, dogs that did or did not receive concurrent antimicrobials, and dogs with an underlying allergic disease (flea allergy dermatitis, atopic dermatitis, or food hypersensitivity) and those without (including those with no underlying disease). The number of *S intermedius* isolates with no resistance to the antimicrobials tested and the number of *S intermedius* isolates with resistance to penicillin G were also compared in these same groups. For all analyses, values of $P < 0.05$ were considered significant.

Results

Twenty-five dogs with epidermal collarettes fit the inclusion criteria. Of these, 2 dogs were excluded because a follow-up examination was not performed or the owners were not available by telephone to substantiate a response of the presumed superficial pyoderma to the administered antimicrobials and 1 dog was excluded because of lack of response of the presumed superficial pyoderma to the administered antimicrobial. Erythema multiforme was subsequently diagnosed in this dog via histologic examination of skin biopsy specimens.

The remaining 22 dogs consisted of 3 mixed-breed dogs, 2 Labrador Retrievers, 2 Cocker Spaniels, and 1 each of 15 other breeds. There were 11 spayed females, 9 castrated males, and 2 sexually intact males. Ages of dogs ranged from 0.5 to 14 years (median, 5.5 years). Seven dogs had first-time pyoderma, and 15 dogs had recurrent pyoderma. Underlying diseases diagnosed in dogs included flea allergy dermatitis and atopic dermatitis (n = 4 dogs); flea allergy dermatitis (3); atopic dermatitis (2); atopic dermatitis and food hypersensitivity (2); hypothyroidism (2); atopic dermatitis and hypothyroidism (1); flea allergy dermatitis, atopic dermatitis, and hypothyroidism (1); and *S scabiei* infestation, *D canis* infestation, or color dilution alopecia (1 each). Underlying disease was not detected in 4 dogs. Thus, 13 dogs had evidence of an underlying allergic disease, whereas 9 dogs, including those with ectoparasite infestations, color dilution alopecia, hypothyroidism, or no underlying disease, did not.

Six dogs were receiving antimicrobials for treatment of superficial pyoderma at the time of bacteriologic culture of epidermal collarette specimens; 4 of those dogs were receiving cephalexin (22 mg/kg [10 mg/lb], PO, q 12 h), 1 was receiving lincomycin (20 mg/kg [9.1 mg/lb], PO, q 12 h), and 1 was receiving enrofloxacin (12 mg/kg [5.5 mg/lb], PO, q 48 h). Eighteen dogs had received antimicrobials within the previous 12 months, including the 6 dogs that were receiving antimicrobials at the time of bacteriologic culture; thus, 12 dogs had received

antimicrobials within the previous year but were not receiving antimicrobials at the time of bacteriologic culture. Two of the dogs with first-time pyoderma had received antimicrobials for nondermatologic diseases (dental disease and urinary tract infection), and 2 of the dogs with recurrent pyoderma had not received antimicrobials previously because the pyoderma was somewhat controlled with topical treatments. One dog with recurrent pyoderma was receiving prednisolone (0.05 mg/kg [0.023 mg/lb], PO, q 24 h) but no antimicrobials.

In 15 dogs, most of the epidermal collarettes (and thus the area from which the collarette to be cultured was chosen) were on the ventral portion of the abdomen. Epidermal collarettes were on the dorsal portion of the thorax in 6 dogs and on the muzzle in 1 dog. *Staphylococcus intermedius* was cultured from epidermal collarettes in 18 of 22 (81.8%) dogs with superficial pyoderma. A *Staphylococcus* sp with negative coagulase test results was also cultured from the epidermal collarettes of 2 of those 18 dogs. No other bacteria were identified on bacteriologic culture of epidermal collarette specimens. Of the 4 dogs with negative culture results, 2 had recurrent pyoderma and 2 had first-time pyoderma; 1 dog in each of these pairs was receiving cephalexin at the time of bacteriologic culture. No bacteria with positive coagulase test results were cultured from healthy dogs. The proportion of bacteriologic cultures with positive results for *S intermedius* from dogs with epidermal collarettes was significantly ($P < 0.001$) higher than that of the healthy dogs. Estimated sensitivity and specificity for this culture method were 81.8% (95% confidence interval, 59.7% to 94.8%) and 100% (95% confidence interval, 85.8% to 100%).

Six of 18 *S intermedius* isolates were susceptible to all antimicrobials tested; one of those isolates had intermediate susceptibility to enrofloxacin. Six isolates were resistant only to penicillin G, and 2 isolates were resistant only to tetracycline. The remaining 4 isolates were resistant to multiple antimicrobials including erythromycin and tetracycline (n = 1 isolate); erythromycin, penicillin G, and trimethoprim-sulfamethoxazole (1); erythromycin, gentamycin, penicillin G, and tetracycline, with intermediate susceptibility to ceftizoxime and enrofloxacin (1); and chloramphenicol, enrofloxacin, erythromycin, and tetracycline (1). Thus, the incidence of antimicrobial resistance was highest for penicillin G (8/18 [44%] isolates). Susceptibility results for the *Staphylococcus* spp with negative coagulase test results were identical to that of the concurrently cultured *S intermedius*. Superficial pyoderma was treated with cephalexin in 18 dogs, enrofloxacin in 3 dogs, and lincomycin in 1 dog. There were no significant differences in the ability to culture *S intermedius*, the number of *S intermedius* isolates with no resistance to the antimicrobials tested, or the number of *S intermedius* isolates resistant to penicillin G between dogs with first-time pyodermas and dogs with recurrent pyodermas, between dogs that did or did not receive concurrent antimicrobials, or between dogs with and without an underlying allergic disease.

Discussion

Epidermal collarettes have been described as being a clinical sign suggestive of superficial pyoderma in the dog.[2,14-16] Although these lesions may be representative of any disease causing pustules, superficial pyoderma is the most common of these diseases in dogs, with pemphigus foliaceus arguably the second most common skin disease associated with this lesion.[2,24] In the study reported here, none of the dogs with epidermal collarettes had other clinical signs of pemphigus foliaceus and in all dogs, clinical signs resolved with use of antimicrobials. Various underlying diseases predisposing dogs to the development of pyoderma have been described,[3-8] with various explanations as to pathogenesis. Any pruritic disease may result in self-trauma to the skin and damage the natural barrier to bacteria provided by the intact epidermis.[3] Atopic dermatitis in dogs has been further implicated in superficial pyoderma by the increased ability of *S intermedius* to adhere to corneocytes in atopic dogs; by degranulation of mast cells, making the epidermis more permeable to staphylococcal antigens; or possibly by production of anti-staphylococcal IgG.[3,4,25,26] Endocrinopathies, particularly hypothyroidism and hyperadrenocorticism, have been theorized to compromise a dog's immune system response to bacterial skin infections.[2,6,7,14] The exact mechanism whereby demodicosis contributes to the onset of pyoderma is unclear, as previous associations of immunosuppression in the disease may be more attributable to the pyoderma than the mite infestation.[27,28] Bacterial folliculitis has been recognized as developing in association with color dilution alopecia, presumably as the follicular changes provide a conducive environment for bacterial growth.[29]

In the study reported here, culture of *S intermedius* from 81.8% of the dogs with superficial pyoderma is in agreement with results of other studies,[9-11,30] indicating that this organism is the most common etiologic agent cultured in dogs with superficial pyoderma. In the healthy dogs, the skin of the ventral portion of the abdomen was chosen for bacteriologic culture because it is generally a hairless area and thus precludes clipping the hair coat of healthy pets. In addition, results of other studies[31,32] indicate that *S intermedius* is cultured from the skin of the ventral portion of the abdomen less often in healthy dogs than from dogs with pyoderma. By combining those studies, *S intermedius* was able to be isolated from the abdominal skin in 9 of 29 healthy dogs; however, a moistened cotton swab or a cup-scrub technique with a fluid wash was used.[31-33] The fact that *S intermedius* was not cultured from abdominal skin of healthy dogs in the study reported here supports the specificity of this culture method, which used a dry sterile cotton swab, and the clinical importance of a positive culture result from epidermal collarette specimens. A dry cotton swab (rather than a moistened one) was used because dry sterile cotton swabs are easily accessible to most veterinarians. Eliminating the step of moistening the cotton swab with sterile saline (0.9% NaCl) solution also eliminates a chance for contamination and makes the method used in our study more practical. Specimens for bacteriologic culture of an epidermal collarette may also be obtained by using an iris forceps and lifting the epithelial border.[16] However, we were unable to find data comparing use of that method with the method used in our study. In addition, such a procedure may be more time consuming, and culturing the open lesion inadvertently is possible

because the width of the area under the epithelial border of a collarette is small.

Pyoderma caused by *S schleiferi* has been reported in dogs.[12,13] We did not perform the necessary tests for this organism because at the time the samples were collected, those studies had not been published. Therefore, it is possible that some of the *S intermedius* colonies isolated in this study were *S schleiferi* subsp *coagulans* because this subspecies has coagulase activity and causes delayed or no fermentation of mannitol, similar to *S intermedius*.[13] Similarly, it is possible that the 2 *Staphylococcus* spp that did not have coagulase activity were *S schleiferi* subsp *schleiferi*. As a further confounding effect, *S schleiferi* subsp *schleiferi* may produce a pseudocoagulase, which can result in a false-positive coagulase test result.[13,34] Results of a prospective study[13] of pyoderma in dogs indicate that *S schleiferi* was not isolated from 11 dogs with first-time superficial pyodermas and was isolated from only 8 of 27 dogs with recurrent superficial pyodermas. From a practical standpoint, the importance of not specifically testing for this organism may be minimal because a *Staphylococcus* sp with coagulase activity was cultured in 81% of the dogs in our study, which would have permitted an accurate choice of antimicrobials for treatment of superficial pyoderma. To the authors' knowledge, there are no studies investigating the prevalence of culturing *S schleiferi* from the skin of healthy dogs.

Results of our study are in agreement with results of other studies[11,30,35-40] from various countries indicating a high incidence of antimicrobial resistance to penicillin, near-universal susceptibility to cephalosporins, and rare resistance to enrofloxacin in *S intermedius* isolated from pyoderma in dogs. In our study, the percentage (44%) of penicillin-resistant strains was similar to the percentage (37.9%) detected in a study[35] performed in Norway. Results of a previous study[41] performed at the VMTH-UCD indicated that 7 isolates of *S intermedius* from superficial pyoderma were all susceptible to enrofloxacin at a concentration of 0.5 µg/mL. In our study, there were no significant differences in the resistance profiles between dogs that did and did not receive concurrent antimicrobials, and this suggests that the culture method would be useful in reevaluating the role of *S intermedius* in epidermal collarettes that have not resolved with antimicrobial treatment. However, the number of dogs that had not received any antimicrobials in the year prior to obtaining the culture was too low (n = 4) to compare statistically with those dogs that had received antimicrobials during that period. Resistance patterns of *S intermedius* isolated from pyoderma in dogs are dependent on previous antimicrobial usage.[42,43] However, this may not be apparent in studies with small numbers of dogs.[44]

Most epidermal collarettes are assumed to be attributable to superficial pyoderma in dogs, and the diagnosis is usually confirmed by treatment with antimicrobials chosen by their presumptive efficacy.[2,14,16] In dogs in which collarettes do not resolve with a 3- to 4-week regimen of antimicrobials, reevaluation of the diagnosis and antimicrobial efficacy is indicated.[2,16] In the study reported here, the method of bacteriologic culture can be recommended for evaluation of *S intermedius* and antimicrobial resistance in epidermal collarettes; therefore, pustules on affected skin are not required for bacteriologic culture.

a. HealthLink Transporter, Copan, Brescia, Italy.
b. Blood agar plate, Hardy, Santa Monica, Calif.
c. MacConkey tryptic soy broth agar, Hardy, Santa Monica, Calif.
d. Tryptic soy broth, Hardy, Santa Monica, Calif.
e. Coagustaph, Hardy, Santa Monica, Calif.
f. SAS OnlineDoc, version 8, SAS Institute Inc, Cary, NC.

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