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Brief Communication

Variations in Antibiograms and Plasmid Profiles among Multiple Isolates of *Staphylococcus intermedius* from Pyoderma in Dogs

Furunculosis is defined as an inflammation involving epidermis and corium, usually derived as a pathological continuum of folliculitis and perifolliculitis. Furunculosis is frequently seen in dogs (*Muller et al.* 1989). Canine furunculosis is a complex, multifactorial disorder and various factors have been suggested to be involved in its pathogenesis: bacterial, fungal, and parasitic infections (*Muller et al.* 1989), immunosuppression, impaired neutrofil function (*Latimer et al.* 1982, *Muller et al.* 1989), endocrine abnormalities, seborrhea or allergic conditions (*Muller et al.* 1989). The most common microbiological finding is *Staphylococcus intermedius* (*Muller et al.* 1989), a *Staphylococcus* species which has also been associated with other pathological conditions in dogs, e.g. uterine and urinary tract infections, respiratory tract infections, mastitis, and otitis externa (*Biberstein et al.* 1984). Usually, staphylococcal pyoderma responds to appropriate antibiotic therapy and may completely resolve. However, the disease often recurs subsequent to termination of treatment. Some dogs exhibiting recurrent staphylococcal pyoderma are repeatedly presented to the veterinarians by the progressively frustrated owner. The dogs are usually prescribed topical treatment as well as systemic antibiotics according to the result of bacteriological examination and antibiotic sensitivity test (*Codner* 1988, *Muller et al.* 1989). Antibiotic resistance among *S. intermedius* may complicate the treatment of the disease which may result in fatal bacteriaemia or euthanasia. Autochtonous vaccines, prepared from whole-cells, are frequently used as a supplement in the treatment of long standing canine staphylococcal pyodermas and have been reported to be of some value (*DeBoer et al.* 1990). Usually, autochtonous vaccines and antibiotic sensitivity testings are made from a single *S. intermedius* isolate from the infected dog.

The purpose of the present study was to investigate if it was possible to isolate 2 or more distinct *S. intermedius* strains from the pathological processes of the single dog suffering from furunculosis. Based on the results the relevance of antibiotic sensitivity testings and autovaccines is discussed.

From 11 dogs with a history of furunculosis the laboratory received skin swabs, collected by the practitioner from a typical lesion of the patient. Sufficient data regarding previous antibiotic therapy of the patients were not available. Swabs were submitted to the laboratory in transport medium (Stuart's medium, Difco No. 9340), streaked onto blod agar plates (Volumbia agar, Oxoid CM331, supplemented with 5% sterile bovine blood), and incubated overnight at 37°C aerobically. Predominant growth on primary plates appeared as beta hemolytic porcelain white colonies. Ten colo-

nies producing beta hemolysis and with typical colonial appearance were collected at random from each sample and subsequently identified according to the following scheme: Strains giving a positive reaction for catalase, proteae, lipase, heat stable DNase, and negative reaction for oxidase and hyaluronidase, and showing resistance to bacitracin and sensitivity to furazolidone and novobiocin, were identified as *S. intermedius*. The diagnostic tests were carried out according to *Devriese & Hájek* (1980). Subsequently, strains were subjected to antibiotic sensitivity testing and plasmid profiling. Strains were tested for sensitivity to penicillin, streptomycin, tetracycline, lincomycin, fucidin, sulphonamides, chloramphenicol, amoxycillin + clavulanate. Sensitivity to diagnostic and therapeutic antibiotics was determined on Iso-Sensitest agar (Oxoid CM471) using a tablet diffusion method according to the manufacturer's guidelines (*Casals & Pringler* 1991). Plasmid DNA was isolated by a modification of the method described by *Holmes & Quigley* (1918), using 70 µg/ml lysostaphin (Sigma) for lysis of bacterial cells prior to boiling. Plasmid DNA were subsequently subjected to electrophoresis in 0.8% ag arose gel.

All of 110 isolates identified as *S. intermedius* were sensitive to fucidin, and amoxycillin + clavulanate. Data for the remaining antibiotics are shown in Table 1. From 6 patients all 10 *S. intermedius* isolates possessed identical antibiogram patterns while isolates from each of the other 5 patients could be divided in 2 distinct subgroups. Each antibiogram pattern

Table 1. Resistance to antibiotics and plasmid profiles of 10 isolates of *Staphylococcus intermedius* collected at random from the skin of each of 11 dogs with pyoderma.

Patient No.	No. of isolates	Antibiotic-resistance pattern[a]	Plasmid profile (kb)
889	8	pen-strep-linco-sulfa-chlor	2.3, 4.4, and 7.1
	2	sensitive to all	n.p.[b]
890	10	pen-strep-linco-sulfa	3.0
891	10	pen	n.p.
896	10	sensitive to all	n.p.
903	8	pen-sulfa	n.p.
	2	sensitive to all	n.p.
907	10	sulfa	n.p.
1037	8	pen	3.4
	2	sensitive to all	n.p.
1063	10	sensitive to all	3.0, 19.0, 34.0
1066	6	pen-strep-tet-linco-sulfa-chlor	2.3
	4	sulfa	n.p.
1084	10	sensitive to all	3.0
1125	7	pen-strep-tet-sulfa	2.3, 3.1
	3	pen	2.3, 3.4

a) Determined by a tablet diffusion method using antibiotic tablets containing the following diffusible amounts of antibiotics (abbreviation, break point): 5 µg penicillin (pen, R<23 mm), 100 µg streptomycin (strep, R<23 mm), 80 µg tetracycline (tetra, R<19 mm), 19 µg lincomycin (linco, R<23 mm), 400 µg fucidin (fuci, R<23 mm), 240 µg sulfonamide (sulfa, R<23 mm), 60 µg chloramphenicol (chlor, R<23 mm), 30 µg amoxycillin + 15 µg clavulanate (amc, R<22 mm).

b) n.p. = no plasmids detected.

corresponded to a specific plasmid profile in the isolates form a single patient (Table 1).

Occurrence of *S. intermedius* isolates of varying antibiogram pattern and plasmid profile on individual patients indicate that more than a single strain of *S. intermedius* can be present in the pathological processes of a dog suffering from staphylococcal pyoderma. This is in accordance with infections with *Staphylococcus hyicus* in pigs, the ethiologic agent of exudative epidermitis (*Wegener* 1992).

The presence of several different *S. intermedius* strains on individuals with pyoderma may be of great importance for the correct treatment of the disease. Simultaneous occurrence of strains with different antibiotic resistance or antigenic properties may be crucial for the result of systemic antibiotic treatment or autovaccination. In our laboratory, a tablet diffusion method is used for determination of antibiotic susceptibility. Broth cultures prepared from 10 *S. intermedius* isolates randomly selected from the material are pooled and inoculated on a single agar plate on which antibiotic tablets are subsequently applied. A single resistant strain among 9 susceptible strains can easily be detected by this method. Only antibiotics which are active against all isolates are prescribed for therapy.

Further investigations should clarify the clonal relationship between isolates from each patient with varying antibiograms. This could be performed using a genotypic typing system, e.g. restriction endonuclease analysis of chromosomal DNA. Furthermore, antibiograms of *S. intermedius* isolates before, during, and after antibiotic treatment of dogs with pyoderma should be investigated, and correlated to the loss or uptake of antibiotic-resistance plasmids.

In the light of the present findings, it also seems urgent to investigate the presence of common virulence determinants and major antigens of *S. intermedius,* in order to prepare a common vaccine for the prevention of staphylococcal pyoderma in dogs.

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