Clinical Communication

An outbreak of *Streptococcus canis* mastitis in a dairy herd in Israel

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Abstract

CASE HISTORY: An increase in the bulk somatic cell count (BSCC) of up to 1,000x10^3 cells/ml occurred in a dairy herd in Israel at the end of 2001 and beginning of 2002.

CLINICAL FINDINGS: Bacteriological examination of milk from 69 cows revealed a high prevalence of *Streptococcus* group G bacteria, identified as *S. canis*, affecting 38% of cows and 20% of all quarters. Isolates were sensitive to cephalothin and moderately sensitive to penicillin G. Infected cows were separated from the herd, treated with intramammary antibiotics, milked last, and strict hygiene practices were introduced to the milking routine. The pathogen was cleared from the herd and BSCC decreased to 250–350 x 10$_3$ cells/ml after 6 months.

DIAGNOSIS: *Streptococcus canis* mastitis.

CLINICAL RELEVANCE: Streptococcus canis infection may cause subclinical mastitis and high BMSCC in dairy herds and be resolved by treatment with intramammary antibiotics and the introduction of strict hygiene practices.

KEY WORDS: ***Streptococcus canis, subclinical mastitis, intramammary antibiotics, antibiotic sensitivity test***

Introduction

Streptococcus Lancefield group G bacteria are not common pathogens of the bovine mammary gland, being diagnosed in the udder of only 0.7% of all streptococcal infections (McDonald and McDonald 1976). The ability of these bacteria to cause subclinical mastitis in cattle has been reported in the past, and a good response to penicillin-based preparations was found (Eberhart and Guss 1970; Hamilton and Stark 1970). These bacteria are beta-haemolytic and aesculin-negative, and are part of the natural flora of the respiratory tract of cats and dogs (Cudney and Albers 1982). They have also been incriminated in infections of the genital, urinary and upper respiratory tracts of dogs and humans (Knotek et al 2001; Whatmore et al 2001) and biochemical characterisation has identified isolates as *S. canis*. That bacterium has been isolated from a variety of other animals, including cats, mink (*Mustela vison*), mice (*Mus* spp), rabbits (*Oryctolagus cuniculus*) and foxes (*Vulpes* spp; Devriese et al 1986). Studies of human and animal group G streptococci have confirmed that *S. canis* forms a clear taxonomic group within the pyogenic streptococci. The species appears distinct from the most closely-related species, which include other pyogenic streptococci associated with humans and animals (Efstratiou 1997; DeWinter et al 1999). Infection in humans mainly involves the nasopharynx, but the bacterium is not pathogenic to the host (Lancefield 1940). The pathogenesis in cattle is unclear, and there is controversy about the bacterium's ability to establish itself in the tissues of the udder and the amount of damage caused (Hamilton and Stark 1970; Watts et al 1984).

We describe here dan outbreak and management of subclinical mastitis due to *S. canis* in a dairy herd in Israel.

Materials and methods

Animals and herd description

A closed herd of 69 lactating Holstein-Friesian cows experienced high BSCC from late 2001 to early 2002. The cows were milked twice daily in a double-six herringbone parlour. Nutrition was based on a total mixed ration (Sadeh Eilan Food Centre, Sadeh Eilan, Israel). The cows were housed in an open, free-stall barn.

Management of the herd was considered inadequate, as was the milking routine. No monitoring of the cows for clinical or subclinical mastitis was carried out by the farmer during the year. The farm did not participate in the monthly milk check of the national herd, which included sampling of the milk from each cow in order to record milk yield and somatic cell counts (SCC) and check the composition with respect to fat, protein and lactose. The average daily milk yield per lactating cow, based on bulk milk tank recordings, was 26 kg/day. No data on days in milk or calving numbers of the cows were recorded. Data regarding the BSCC are the result of the average of three bulk milk collections per month taken at irregular intervals. The BSCC was determined using a fossomatic cell counter (AS Foss, Hillerod, Denmark). At the request of the farmer, cows' quarters were routinely examined for evidence of mastitis, and were treated with a combination of 300 mg procaine benzylpenicillin, 100 mg dihydrostreptomycin and 100 mg nafcillin (Nafpenzal DC; Intervet, Boxmeer, Netherlands) after drying off.

Microbiological analysis

Milk samples from each quarter were collected aseptically from all the milking cows in the herd in March and October 2002, immediately chilled and transported to the National Laboratory of

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ATCC	American Type Culture Collection
BSCC	Bulk somatic cell count(s)
cfu	Colony forming units
MIC	Minimal inhibition concentration
NCCLS	National Committee for Clinical Laboratory Standards
SCC	Somatic cell count(s)

Udder Health and Milk Quality in Caesarea. Procedures for collection and transporting milk samples have been described previously (Brown et al 1981; Anonymous 1999). The technician that collected the samples reported there was no evidence of clinical mastitis in any cow on either sampling occasion.

For bacteriological culture, 0.01 ml milk was streaked on to tryptose soy agar (Hylab Laboratories, Rehovot, Israel) containing 7.5% sheep blood, and incubated for 48 h at 37°C. Colonies were tentatively identified to the genus level according to their morphology and haemolytic patterns. Colonies suspected to be streptococci were examined for CAMP factor, aesculin hydrolysis on aesculin-blood agar plates, and the Lancefield serological group using the latex agglutination test (Pathodex, Los Angeles CA 90045, USA); 18 of these were further identified by means of the ID32 STREP (BioMerieux SA, Marcy-l'Étoile, France) and the Apilab Plus 1990 computerised recognition system (Durham, USA).

Antimicrobial drug sensitivity

Standardised methods were applied according to the National Committee for Clinical Laboratory Standards (NCCLS) performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals (Anonymous 2002), to test the sensitivity of *S. canis* strains to antibiotics. Eighteen isolates were tested against penicillin, neomycin, oxacillin, cephalothin and erythromycin (Sigma, St Louis MO, USA). Each isolate and each antibiotic was tested using both antimicrobial drug sensitivity methods.

Briefly, bacteria harvested from blood agar plates were suspended in Mueller-Hinton broth (Hylab Laboratories) to a final concentration of 2×10^6 colony forming units (cfu)/ml of *S. canis*. Two-fold dilution series of the respective antibiotic standards were used for the macrodilution method, and test discs (Beckton Dickinson, Le Pont de Claix, France) for the disc diffusion test. The American Type Culture Collection (ATCC; Rockville MD, USA) strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212, were used as controls. All tests were incubated for 24 h at 37°C, and the minimal inhibition concentration (MIC) was determined as the lowest concentration of antimicrobial agent that prevented visible growth; results are expressed in µg/ml. MIC_{50} and MIC_{90} were defined as the concentration of the antimicrobial agents able to inhibit the growth of 50% and 90% of the isolates, respectively.

Treatment

At the end of January 2002, cows from which *S. canis* was isolated were identified and separated from the rest of the herd. Antibiotic tubes containing a combination of 180 mg penicillin G sodium, 100 mg dihydrostreptomycin and 100 mg nafcillin (Nafpenzal MC; Intervet) were inserted into all four quarters of all infected cows twice at 24-h intervals. Milk from the treated cows was discarded for 5 days after completion of the last intramammary treatment.

In addition, instructions were given for the immediate adoption of an hygienic milking routine for the entire herd, with special attention paid to the infected group. These included the wearing of disposable gloves during milking, that were disinfected with an iodophor-based solution without glycerine, at a concentration of 200 ppm, after every 10 cows. Additionally, post-milking dipping of the teats was performed using an approved, iodophor-based udder disinfectant at the manufacturer's recommended concentration, each udder was wiped separately with a disposable paper towel, and the milking cluster disinfected by dipping it into a chlorine-based solution (with a hypochloride concentration of 400–700 ppm) after milking each cow. The farmer was also instructed to participate in the monthly milk inspections within the framework of the 'Herd Book' organised by the Israel Milk Marketing Board, in order to enable him and the attending veterinarian to diagnose suspected new cases of subclinical mastitis and to participate in a routine udder health programme. This involved regular sampling and submission of samples to the Udder Health Laboratory, for diagnosis of intramammary pathogens derived from clinical cases of mastitis in cows and heifers, 4 days after calving, and was aimed at determining the efficacy of antibiotic treatment during the dry period. Routine sampling was also to aid the diagnosis, isolation and treatment of infected cows or heifers before they infected others in the herd, as well as cows suspected to be infected with subclinical mastitis based on three successive monthly SCC $>200 \times 10^3$ cells/ml. In addition to the bacteriological tests, the efficacy of the iodophor disinfecting solutions was tested by determining their concentration and pH after dilution by the farmer, in order to ensure that reconstitution of the dip was conducted correctly. Infected cows were isolated in separated barns and were milked last.

Ten days after completion of the antibiotic treatment, the cows' quarters were re-sampled, in order to examine the efficacy of therapy and determine the bacteriological recovery rate. At this point, cows from which *S. canis* was subsequently isolated, due to either non-recovery or a recurrent infection during the interval between examinations, received an additional intramammary treatment. These animals were re-sampled upon completion of the additional intramammary treatment. Six months later, quarters from all the cows (n=75) were sampled again. There was no culling of cows as a consequence of their udder disease status during the 6-month period. Data on the monthly BSCC were collected from the beginning of 2001 to December 2002.

Statistical analysis

Data were analysed using Statistix 7.0 (Statistix 2000; Tallahasee FL, USA). Chi-squared analysis was used to ascertain the dependence between the cure rate and number of quarters infected per cow.

Results

Mastitis pathogens grown from milk samples and the numbers and percentages of quarters affected at the first and second samplings are reported in Table 1. *Streptococcus canis* was isolated from 26/69 cows (38%) and 55/274 (20%) quarters sampled; 10 cows were infected in one quarter, six cows in two quarters, seven cows in three quarters, and three cows were infected in all four quarters.

The microorganism isolated was a Gram-positive coccus, which was beta-haemolytic, catalase-negative, CAMP-negative, did not hydrolyse aesculin, and was agglutinated by antibodies of the Lancefield serological group G. The rapid ID32 STREP test resulted in identification of *S. canis*, with 99.9% confidence. The organism was positive for arginine dihydrolase, α- and β-galactosidase, alkaline phosphatase, alanine-phenylalanine-proline arylamidase, and glycyl-tryptophane arylamidase, but negative for β-glucosidase, β-glucoronidase, acetone production, pyroglutamic

Table 1. Numbers and percentages of pathogens isolated from the quarters of 69 cows at the first diagnosis and 75 cows 6 months later, in a dairy herd in Israel.

	Initial diagnosis (n=69)		6 months later (n=75)	
Pathogen	**No. affected**	**% of all**	**No. affected**	**% of all**
Streptococcus canis	55	20	2	0.7
Staphylococcus aureus	16	6	6	2
Streptococcus spp	5	2	10	3
Coagulase-negative staphylococci	23	8	12	4
Corynebacterium bovis	119	43	196	65
Other bacteria	8	3	12	4
No growth	38	14	61	21
Contaminated	10	4	1	0.3
Total	**274[a]**	**100**	**300**	**100**

[a] Two of the 276 quarters were dry

acid arylamidase, n-acetyl beta-glucosaminidase, β-mannosidase, urease production and hippurate hydrolysis. The bacterium fermented ribose, lactose, glycogen, pullulan, maltose, methyl-B-D-glucopyranoside, and saccharose-sucrose, but not mannitol, sorbitol, trehalose, raffinose, L-arabinose, D-arabitol, cyclodextrin, melibiose, melezitose or tagatose. All *S. canis* isolates were phenotypically and biochemically identical.

The MIC_{50} and MIC_{90} values obtained were 0.125 and 0.25 µg/ml, respectively, for cephalothin. For the remaining antibiotics, the MIC_{50} and MIC_{90} values were the same, being 0.0625 µg/ml for oxacillin, 0.0156 µg/ml for penicillin, 16 µg/ml for neomycin, and 0.06 µg/ml for erythromycin. The disc diffusion test disclosed that all of the isolates were sensitive to cephalothin and oxacillin, and 12 were sensitive to penicillin; sensitivity to penicillin was classified as intermediate for the remaining 6/18. All isolates tested were resistant to neomycin and intermediate for erythromycin, according to the NCCLS (Anonymous 2002) inhibition zone specifications.

As no intramammary medication was available that met all the sensitivity test criteria (high sensitivity to cephalothin), treatment with 100 mg nafcillin (Nafpenzal MC) was recommended. In conjunction with the strict hygiene procedures introduced, the bacteriological cure rate for *S. canis* was 20/26 (77%) of infected cows and 49/55 (89%) of infected quarters. The second treatment of six cows that remained infected 10 days after the first treatment resulted in recovery of all the cows and quarters treated.

When the relationship between the success rate of the first intramammary treatment and the number of quarters infected was examined, there was 100% success for cows that had three or four quarters infected, compared with 60% and 67% success in those with infection of one or two quarters, respectively. The difference between the last two figures was not statistically significant. BSCC decreased from an initial $1{,}000 \times 10^3$ cells/ml to 350×10^3 cells/ml about 1 month after completion of treatment, and an average of 250×10^3 cells/ml over the subsequent 6 months.

Discussion

Although *S. canis* does not occur frequently as a mammary pathogen, it may cause a very high incidence of infection within individual herds. In the herd examined in the present study, 38% of cows and 20% of quarters were infected. Since the farm operated as a closed herd, neither the original source of infection nor the length of time it had been in the herd was known. Although cats and dogs are considered possible sources of the bacterium, no evidence of pets near the barn or the milking parlour was found during a visit to the farm or following discussions with the herd owner.

Streptococcus canis is morphologically and epidemiologically similar to *S. agalactiae* (Watts et al 1984). The high infection rate in the herd and the large proportion of infected quarters per cow indicated that there was also a high incidence of transfer of contamination between the cows' teats. The lack of regular checks of individual SCC, and the failure to send samples for bacteriological testing for clinical and subclinical cases of mastitis, prevented early diagnosis and identification of the disease prior to infection of a large number of cows in the herd.

The response to therapy with penicillin, streptomycin and nafcillin was good, and treatment of all infected quarters succeeded in eliminating the organism from nearly all udders (Table 1). The two cows found to still have an infected quarter 6 months after the last treatment were in their dry period during treatment and calved 3 months prior to the last examination. These results suggest that the tissue damage induced by this pathogen may be reversible and that the rehabilitation capability of the tissues is probably dependant on the time elapsed from initial infection of the udder.

It was interesting to note the presence in the herd of two microorganisms, *Corynebacterium bovis* and *Staphylococcus aureus*, that can increase SCC. Despite antibiotic treatment, the proportion of quarters infected with *C. bovis* increased from 43% to 65%. However, it is unlikely that this bacterium was responsible for the dramatic increase in SCC recorded at the end of 2001 and beginning of 2002. According to previous work, the geometric means of SCC in quarters infected with *C. bovis* varied between 40 and 421×10^3/ml of milk, and the bacterium appeared to be a minor pathogen (Djabri et al 2002). The high prevalence of *C. bovis* may be indicative of very poor hygiene and may be seen where teat spraying is practised, and supports the possibility of cow-to-cow transmission of *S. canis*. *Staphylococcus aureus* was found in the first sampling and continued to be present in 2% of quarters in the second sampling, even after treatment. As the SCC data

for each cow were lacking, it is not possible to make a conclusion about the contribution of these bacteria to the BSCC.

In previous studies, *S. canis* was found to be highly sensitive to penicillin G (Barnum and Fuller 1953; Romer 1959). A combination of intramammary antibiotic treatments, prevention of new infections in the herd by immediate isolation of infected cows and milking them last, and imposition of a strict hygienic milking routine achieved positive results in a short period of time, evidenced by the disappearance of the bacterium, and increased yield (data non shown) and improved quality of milk, confirmed by the decrease in the BSCC.

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