Br. vet. J. (1992). **148,** 54

CLINICAL, BACTERIOLOGICAL AND THERAPEUTIC ASPECTS OF BOVINE MASTITIS CAUSED BY AEROBIC AND ANAEROBIC PATHOGENS

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SUMMARY

Twenty cows and three heifers with summer mastitis-like signs were included in the study. Forty aerobic and 38 anaerobic bacterial isolates representing 23 different species were isolated from udder secretions, the mean number of species being 3.4/sample. Pathogens most often were recovered in quantities exceeding 10^5 CFU/ml. Growth of pathogenic bacteria in the blood was found in only one case. Most cases occurred in housed animals and had often been preceded by teat injury. No anaerobic growth was found in milk samples of a control group consisting of 29 cows with ordinary acute mastitis.

Values of indicators of inflammation in milk (NAGase and plasmin activity) were high, mean values being 914.1 and 1.3 units. The animals with aerobic/anaerobic mastitis were treated using various antimicrobial agents. All but three quarters were lost to milk production. Two animals with complete recovery were given a combination of penicillin G and tinidazole.

INTRODUCTION

A severe form of mastitis in which both aerobic and anaerobic bacteria are involved has been recognized (Thomas *et al.*, 1987). It is referred to as summer or heifer mastitis, and is acute, suppurative mastitis characterized by purulent secretion and an unpleasant smell from the affected quarter, often with severe systemic reaction. Non-lactating cattle are affected, heifers predominantly during the summer. There is evidence that cases clinically identical to summer mastitis occur sporadically during winter housing, in non-lactating or even lactating cows, particularly after teat injury (Thomas *et al.*, 1987).

Despite intensive antibiotic therapy, the affected quarter is, as a rule, permanently lost to milk production (Blood & Henderson, 1968; Schalm *et al.*, 1971; Marshall, 1981). Research on summer mastitis has been hampered by its sporadic nature and its variable incidence in different countries, and from year to year.

A proper definition of summer mastitis is still lacking (Thomas *et al.*, 1987). The disease is often diagnosed on clinical grounds as affecting cases exhibiting the

characteristic symptoms. The pathogens most often isolated from milk of affected glands are *Actinomyces pyogenes, Peptostreptococcus indolicus, Streptococcus dysgalactiae* and *Fusobacterium necrophorum.*

The purpose of this study was to investigate typical cases of summer mastitis and cases occurring in winter which resembled summer mastitis. One objective was to determine whether or not the bacteriological aetiologies were different in these forms of mastitis. Quantitative culture methods were used to evaluate their capacity in discriminating potential pathogens from probable contaminants. The aim of the present study was also to study the clinical and inflammatory features of aerobic/anaerobic mastitis and the effects of various therapeutic regimens on the outcome of the disease.

MATERIALS AND METHODS

Animals

The study was conducted from 1986 to 1988 in the Ambulatory Clinic of the College of Veterinary Medicine. The animals were selected on grounds of histories and typical symptoms of summer mastitis (Thomas *et al.*, 1987): the affected quarter was swollen, hard and tender, and the secretion was purulent or serous, with a characteristic bad odour. Three heifers and 20 cows were studied. The details of the animals are shown in Table I. All the animals in the summer group were at pasture when diseased. In 13 animals, systemic signs such as depression, loss of appetite and increase of body temperature were seen.

Milk samples for aerobic and anaerobic culture were also taken from 30 diseased quarters of 24 lactating and five dry cows with ordinary acute mastitis, exhibiting no signs of summer mastitis and from one quarter of 10 clinically healthy cows with low cell counts in their milk (<250 000 cells/ml; estimated using the California Mastitis Test). These control cows were from the same herds as the ones with aerobic/anaerobic mastitis.

Clinical examination and haematological analyses

Each animal was examined clinically before specimen collection and treatment. The examination included assessment of each animal's general conditions, measurement of body temperature, palpation of the udder and lymph nodes and inspection of the appearance of the secretion. A blood sample was taken from the jugular vein of the 13 animals with systemic illness for determination of the haematocrit. Total and differential leucocyte counts were determined for these

Table I
History, clinical features and bacterial isolations of cows and heifers with summer mastitis signs

Cow number	*Season*	*Age (years)*	*Lactation*		*Affected quarter*[1]	*Teat trauma (+/–)*	*Systemic signs*	*Bacterial isolations*[2]
			stage	*(days)*				
1	Summer	6	Dry	(–31)	RH	–	Severe	ACE
2		4	Dry	(–3)	LH	–	Severe	ABCDE
3		2	Heifer	(–90)	RF	–	None	BCDE
4		8	Dry	(–30)	LH	–	Moderate	DE
5		7	Dry	(–5)	RH	+	Moderate	ACE
6		10	Lact.	(+2)	RF	–	None	ABCDE
7		5	Dry	(–95)	LH	–	Moderate	ACD
8	Winter	5	Lact.	(+56)	LF	+	None	ABCDE
9		2	Lact.	(+1)	RH	+	Moderate	ABE
10		3	Dry	(–1)	LF	–	Severe	AE
11		2	Lact.	(+1)	RF	–	Moderate	ABDE
12		4	Lact.	>150	LF	+	None	BD
13		4	Lact.	(+1)	LF	–	Moderate	ABDE
14		5	Lact.	(+3)	LH	–	None	AB
15		2	Lact.	(+62)	RF	+	None	ABDE
16		2	Heifer	(–105)	RH	–	Moderate	B
17		5	Dry	(–8)	LH	–	Severe	ABE
18		6	Lact.	>200	LH	–	None	A
19		2	Lact.	>150	LF	+	None	ACE
20		2	Lact.	(+80)	RH	+	None	C
21		8	Lact.	(+1)	RF	+	Severe	ABE
22		2	Heifer	(–2)	LH	–	None	A
23		12	Dry	(–36)	RH	–	Severe	AE

[1]RF, right fore; RH, right hind; LF, left fore; LH, left hind.
[2]A, *A. pyogenes*; B, *P. indolicus*; C, *F. necrophorum*; D, *Strep. dysgalactiae*; E, others.

samples were collected from animals with systemic symptoms. The site of venepuncture was shaved and carefully disinfected, and 5 ml of blood were transferred into aerobic and anaerobic blood culture bottles (Hemobact™, Orion Diagnostica, Espoo, Finland). The samples were kept at room temperature (blood culture bottles at 37 °C) before transportation (within 24 h) to the laboratory for aerobic and anaerobic culture. Udder secretions were quantitatively inoculated with a 10 μl calibrated loop onto non-selective (blood, chocolate, *Brucella*-blood and egg yolk) agars and selective (kanamycin–vancomycin laked blood (KVLB), neomycin–vancomycin (NV), tryptic soy–serum–bacitracin–vancomycin (TSBV), cadmium–fluoride–acriflavine–tellurite (CFAT) agars and into thioglycollate enrichment broth (Sutter *et al.*, 1985). The plates were incubated in appropriate atmospheric environments for up to 10 days and examined every 48 h. Bacteria isolated were quantified and identified using standard methods (Holdeman *et al.*, 1977; Lennette *et al.*, 1985; Sutter *et al.*, 1985). Sporadic isolates of bacteria in numbers generally less than 10^3/ml of secretion and known to be common skin contaminants were excluded (mainly coagulase negative staphylococci, *Bacillus* spp. and *Propionibacterium acnes*). In-vitro susceptibilities to antimicrobial agents were determined

by the agar dilution method (Sutter *et al.*, 1985). Blood cultures were processed using standard methods.

Indicators of inflammation in the milk

Milk samples from affected quarters were also drawn into a non-sterile plastic tube and frozen for later analysis. This included measurement of milk NAGase and plasmin activity. Free lysosomal enzyme NAGase (*N*-acetyl-β-D-glucosaminidase) activity was analysed using the Milk NAGase Test kit (Eflab, Helsinki, Finland) (Mattila & Sandholm, 1986). Milk plasmin activity was determined using a fluorogenic coumaryl peptide substrate, adapting the method for microtitration plate fluorometry (Mattila & Sandholm, 1986).

Treatment and follow-up

Aerobic/anaerobic mastitis cases were treated with penicillin G (Ethacilin^R, Intervet International B.V., Boxmeer, Holland) 20 000 IU/kg of body weight once a day for 3 days (eight cases), with spiramycin (Spiramycin^R, Aktiebolaget, Leo, Helsingborg, Denmark) 10 mg/kg of body weight once a day for 3 days (four cases) or penicillin G 20 000 IU/kg once a day+tinidazole (Tricanix^R, Orion Pharmaceutica, Espoo, Finland) 6–8 mg/kg twice a day for 3 days (six cases). Five cases were left without antimicrobial therapy. Frequent milking out of the secretion until the quarter was nearly dry was advised and this was also done in most of the cases. The outcome of treatment was monitored within one week after calving or, in lactating animals, 4 weeks after therapy. Follow-up included clinical examination of the quarter and bacteriological culturing of any secretion which could be obtained.

RESULTS

In the winter group, lactating animals were more often affected and visible teat injury preceded infection in the quarter in seven out of 16 animals (Table I). Forty aerobic and 38 anaerobic isolates, representing 23 different bacterial species (9 aerobic, 14 anaerobic), were recovered. Most cases were mixed infections, the mean number of bacterial species/sample being 3.4. The bacterial species isolated are listed in Table II. Nine solitary isolates were found in insignificant quantities ($<10^3$ CFU/ml) and excluded as suspected contaminants (*Streptococcus faecalis, Staphylococcus capitis, Staph. hominis, Staph. hyicus, Staph. simulans, Staph. xylosus, Micrococcus* spp., *Bacillus* spp. and *P. acnes*). Pathogenic bacteria were generally found in secretions in high concentrations; in 65% of isolates at more than 10^5 CFU/ml, in 27% of them at more than 10^6 CFU/ml. The most frequent combination of bacteria was *A. pyogenes* with *P. indolicus*, mostly with one or more other species, found in 10 cases (Table I). There were no marked differences in bacterial findings between cases occurring at pasture during summer and during the winter housing period, nor were any seen between cases with or without teat injury. However, *F. necrophorum* tended to be more common in summer cases (6/7 cases versus 3/16 cases). Bacterial status did not affect the type of clinical symptoms.

Table II
Bacteria isolated from udder secretions from 23 cows and heifers with mastitis caused by aerobic and anaerobic pathogens[1]

Bacteria	*Frequency*	*Percentage of cases*
Aerobes		
Actinomyces pyogenes	18	78
Streptococcus dysgalactiae	10	43
Strep. acidominimus	3	13
Strep. uberis	3	13
Strep. lactis	1	4
Strep. morbillorum	1	4
Staphylococcus aureus	1	4
Proteus mirabilis	1	4
Pasteurella multocida	2	9
Anaerobes		
Peptostreptococcus indolicus	13	57
Fusobacterium necrophorum	9	39
Bacteroides spp.[2]	8	35
B. fragilis	1	4
B. levii	1	4
Peptostreptococcus magnus	2	9
P. micros	1	4
Peptostreptococcus spp.	1	4
Fusobacterium spp.	1	4
Eubacterium spp.	1	4

[1]Presumed contaminants were excluded.
[2]Most closely related to *B. zoogleoformans* (3), *B. capillosus* (1), asaccharolytic indole-negative species (2), saccharolytic indole-positive species (2).

The mean value for milk NAGase activity was 914.1 units (range 492–1324; normal value ≤10 units) and that for milk plasmin 1.32 units (range 0.19–3.23; normal value ≤0.04 units) (Mattila & Sandholm, 1986). Growth of an aerobic or anaerobic bacterial species identical to that in udder secretion sample was found in only one blood sample (*A. pyogenes*). In four samples, coagulase-negative staphylococcal species other than those found in milk were isolated from blood, but they were considered to be skin contaminants. The aerobic bacterial strains except the one *Proteus* sp. were susceptible *in vitro* to penicillin G (minimal inhibitory concentration of 90% of the strains, MIC_{90}, ≤0.06 μg/ml) and spiramycin (MIC_{90}≤1 μg/ml) but resistant to tinidazole. All the anaerobic strains except the one *Bacteroides fragilis* isolate were sensitive to penicillin G (MIC_{90}≤0.06 μg/ml) and all of them to tinidazole (MIC_{90}≤2 μg/ml). Susceptibility to spiramycin could not be evaluated for anaerobic isolates but is effective in theory.

The blood parameters were within normal limits, except for a slight transfer to relative neutrophilia.

Outcomes of therapy were poor. In all cases, systemic signs disappeared rapidly but all except three affected quarters were completely lost to milk production. In most quarters from which post-treatment samples could be obtained the distri-

bution of bacterial species did not change as compared with that prior to treatment. The two totally recovered cases (cow numbers 10 and 16), in which no bacterial growth was found on post-treatment sampling and milk production, milk somatic cell count and NAGase values of the affected quarter were at the same level as in the other quarters, had both been treated with the combination of penicillin G and tinidazole (8 mg/kg).

In the 30 quarters with acute mastitis but no summer mastitis symptoms, no anaerobic growth was found. In the 10 healthy quarters, neither aerobic nor anaerobic growth could be isolated. The bacterial diagnoses in the acute mastitis group were *Staph. aureus* (nine quarters), *Escherichia coli* (seven quarters), *Strep. dysgalactiae* (three quarters), *Strep. uberis* (two quarters), *Klebsiella* sp. (one quarter), *Pasteurella multocida* (one quarter), coagulase-negative staphylococcus (one quarter), mixed infection of aerobes (one quarter), and no bacterial growth (five quarters).

DISCUSSION

Summer mastitis usually refers to severe clinical cases of suppurative mastitis occurring in heifers and dry cows in summer. Bacterial examination of the characteristic foul smelling secretion usually results in the recovery of *A. pyogenes* alone or in combination with other pathogens (Hillerton *et al.*, 1987; Hillerton & Bramley, 1989). In general, the presence of anaerobic bacteria is considered a prerequisite for diagnosis of summer mastitis. A similar condition also occurs in lactating and non-lactating cows in winter, often following teat injuries. Aerobic/anaerobic mastitis could be a broader definition covering all variations of the syndrome.

Bacterial findings in the present study accorded with those in many other sur-

mal were grave but did not differ from those seen in many other cows. Concerning bacteraemia in summer mastitis, Stuart *et al.* (1951) reported 38 experimental cases in which bacterial growth in blood was found in three animals. However, the findings cannot be compared with those in the present study, in which spontaneous cases receiving antimicrobial therapy at an early stage were studied. It has been suggested that pyaemia occurs in acute mastitis, e.g. summer mastitis (Schalm *et al.*, 1971) but evidence for this phenomenon is still lacking. The difficulty of taking sterile blood samples under field conditions was noted in our study, in which apathogenic bacteria from the skin contaminated several blood samples.

Leucopenia was not observed, on the basis of total leucocyte counts for the animals studied. As a rule, bone marrow in cattle is only mildly responsive to infective processes. The relative neutrophilia seen in most cases in this study is typical of the acute phase of disease and has also been seen in experimental summer mastitis (Thomas *et al.*, 1987).

Epidemiological data have been reported in relation to summer mastitis, dealing with its seasonal occurrence and the involvement of the fly vector *Hydrotea irritans*. In contrast to other forms of mastitis, true summer mastitis (heifer mastitis) has been reported to affect principally the front quarters. However, in this study, the hind quarters were more often affected. Hind quarters are known to be more at risk to teat injuries. During the winter housing period, visible or invisible teat trauma probably provokes most cases of this type of mastitis. Most of the numerous bacterial species involved in the syndrome are opportunist pathogens living in the environment of the cow and its different body sites (Sorensen, 1976; Tolle & Reichmuth, 1985). The fly vector is of no significance during winter. On the basis of the present findings, we suggest that summer mastitis-like aerobic/anaerobic mastitis is more frequent than believed in housed animals. The incidence of this form of mastitis seems to be constant. More complete understanding of the role of individual pathogens in the pathogenesis of this syndrome will involve further study.

Suppuration of the affected quarter through tissue destruction rapidly develops in mastitis resulting from a mixed infection of anaerobes and aerobes. Production of copious, foul smelling pus is characteristic. The agent suggested as responsible is *P. indolicus*. Though clinical symptoms in the affected quarter are pronounced, the stage of inflammation can also be monitored by indicators of inflammation in the secretion. Milk NAGase activity reflects tissue damage and milk plasmin activity the stage of proteolysis in the udder. Mean milk NAGase and plasmin values were markedly higher than that reported for acute mastitis caused by aerobes alone (Pyörälä & Syväjärvi, 1987a). A high NAGase level generally indicates a poor prognosis in acute mastitis (Pyörälä & Syväjärvi, 1987b). In this study, however, NAGase values in udder secretion of the recovered cases did not differ from those of cases with no recovery.

Most authors agree that confirmed cases of aerobic/anaerobic mastitis do not respond to therapy (Blood & Henderson, 1968; Schalm *et al.*, 1971; Marshall, 1981). The role of anaerobes may be definitive as regards prognosis. Frequent milking out of the affected quarter was advised in this study. Whether it influenced positively the final outcome is unknown but the authors believe that this procedure relieves acute signs of disease. It is known that *A. pyogenes* alone occurs

sporadically in acute mastitis cases in lactating cows (Hillerton & Bramley, 1989). In such cases, the prognosis is generally quite good. Recovery after therapy, or even spontaneously, may occur.

In the study reported here, a preliminary therapy trial was conducted using a combination of drugs active against aerobes and anaerobes. In addition to penicillin G, which is bactericidal against the aerobic and most anaerobic species isolated, a nitroimidazole derivative, tinidazole, was administered. From the pharmacokinetic point of view, the dosage of 8 mg/kg was quite low (Pyörälä *et al.*, 1990). The tinidazole formulation used was dilute and not very suitable for practical conditions. Because the outcome in aerobic/anaerobic mastitis is extremely poor, even limited therapeutic success is of interest. A therapeutic trial of more cases is needed to confirm the superiority of the combination therapy studied over conventional forms of treatment.

ACKNOWLEDGEMENTS

The authors would like to thank the Walter Ehrström Foundation, Finland, for the financial support for the present study. Orion Pharmaceutica, Espoo, Finland, kindly provided us with tinidazole used in the study.

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(Accepted for publication 26 March 1991)