An Outbreak of Mastitis Caused by Serratia Marcescens

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THERE is a progressively increasing number of microorganisms associated with bovine mastitis. In addition to the organisms commonly associated with mastitis, the list includes species of the genera *Klebsiella, Bacillus, Nocardia,* and *Candida.* The organism, *Serratia marcescens,* which caused the outbreak of mastitis recorded in this article is another addition. Until now *Serratia marcescens* has not been considered significant in mastitis.

S. marcescens (1) also known as *Chromobacterium prodigiosum* or *Bacillus prodigiosus* is an aerobic, motile, gram negative rod that produces a red pigment which is insoluble in water. It and other members of the genus have generally been considered to be saprophytes and not pathogenic for animals or for man. The first isolation was made by Woodward and Clark in 1913 (2) from sputum. Since then human infections have been reported involving septicaemia (3), meningitis (4), pneumonia (5), pseudohaemoptysis (6, 7), skin lesions (8), and urinary tract infections (9).

Wheat et al. (9) who reported the urinary tract infections felt that the organism was introduced after some type of manipulation when multiple antibiotics eliminated all the usual organisms, thus permitting the ready implantation of the resistant Serratia. It has been suggested (10) that the usually saprophytic bacteria may become pathogenic as the result, at least in part, of the increased use of antimicrobial agents.

HERD HISTORY

The herd consisted of twenty-four milking cows housed in a conventional type barn. During the winter of 1952 a mild outbreak of mastitis due to haemolytic *Staphylococcus aureus* occurred. An autogenous bacterin injected subcutaneously into all the cows in the herd at that time appeared to be beneficial. Only an occasional quarter showed signs of mastitis until the cattle were moved to another barn in the fall of 1957 when an increased incidence occurred. Individual quarter samples were collected from all cows in the herd for laboratory examination. The results indicated that milk from twenty-two quarters had an increased cell count and was positive to the California Mastitis Test. Direct culture of the milk samples revealed that sixteen of the quarters were shedding haemolytic staphylocci in large numbers, two yielded *Streptococcus agalactiae,* two yielded *Serratia marcescens* in small numbers and two quarters were negative for bacteria.

As a haemolytic *Staphylococcus aureus* was associated with most of the chronic infection, an autogenous bacterin was again administered. In addition, pencillin was recommended for the treatment of quarters that showed symptoms of mastitis.

Since recurring cases continued to be observed, samples were submitted in February 1958 from ten quarters that had failed to respond satisfactorily to treatment.

Culture of the fresh and incubated milk revealed gram negative organisms from four quarters and no growth from the others. A herd test was conducted on March 20. The milk of the affected quarters was negative on direct culture but from the incubated milk a gram negative organism was isolated which was identified as *Serratia marcescens.* At that time it was isolated from seventeen quarters. The record of the isolation of *Serratia marcescens* in the first two and succeeding tests is given in

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Table 1. The organism was cultured from swabs taken from the teat-cup liners immediately after removal from infected cows but could not be isolated from other parts of the dairy equipment or the environment.

The infection would be classified as mild chronic mastitis. At no time did the animals show systemic reaction. The milk production from the affected animals was lowered. There was evidence of cyclic changes as seen in some mastitis infections namely swelling of the gland, then visibly abnormal milk with clots which was followed in a few days by a normal appearance to the milk. The reaction with the California Mastitis Test (C.M.T.) was three to four plus on all samples drawn.

BACTERIOLOGICAL STUDIES

The organism isolated was a gram negative, short rod occurring singly and exhibiting marked motility when grown at either 25°C or 37°C.

It was found in small numbers by direct culture of the milk in 40 per cent of the total isolations. The remainder were secured from milk that had been incubated for 18 hours at 37°C. The colonies on blood agar after 18 hours resembled colonies of *Escherichia coli*

TABLE I

Laboratory Results on Samples from Quarters Positive for Serratia marcescens

Date of test	Dec. 13/57	Mar. 12/58	Mar. 20/58	Mar. 25/58	Apr. 3/58	May 23/58	June 18/58
Cow number and Quarter	S.m. Cells	S.m. Cells	S.m. Cells	S.m. Cells	S.m. Cells	S.m. Cells	S.m. Cells
1, RH	n ++	P ++	P +		n +	n −	
1, LH	n occ.	n +	P ++		P ++()	n ccc.	
2, RF	n ++	P ++	n ++*	n ++	n +	n occ.	n occ.
2, RH	n −	P ++	P ccc.		P ++()	P +#	n occ.
5, LF	n ++	P +	P ++		P ++()	P +	P +
5, LH	n −	P +	P ++		P ++()	P +	P +
6, RH	n −	P +	P ++*	n +	n −	n −	n occ.
6, LF	n ++	n +	P ++	P ++	P +	n +#	n ++
6, LH	n ccc.	P occ.	P ++	P ++	P +	P ++#	n +
9, RF	n −	n +	n ++		P +	n +	
9, RH	n −	P +	n ccc.		n ccc.	n −	dry
10, RH	n −	P ++	P ++	P ++	P ++	P ++#	n occ.
10, LH	n −	P +	P ++		P ++	P +#	n −
11, RH	P ++	P −	P +		P ++	P +#	n occ.
11, LH	n −	n −	P ++		P +	P occ.#	n −
12, LH	n occ.	P +	P ++.		P ++	P occ.#	dry
16, RF	n −	P occ.	P +		P +	P +#	n occ.
16, RH	n ccc.	P ++	P ++		P ++	P +#	n occ.
16, LF	P +	n −	n −		n −	P +#	n −
18, RH	n −	P ++	P ++		P ++()	dry	
18, LF	n −	P ++	P +	n ++	n occ.		
20, RH	n occ.	n −	P +		P ++()	P +#	n +
20, LH	n −	P +	P ++	P ++	P ++()	P +#	n occ.

S.m.: — Serratia marcescens. P — positive; n — negative

Cells: — less than 500,000/ml, +0.5 — 2 million ++ over 2 million

*Treated on three consecutive days with 2.0, 1.5 and 1.5 gms. neomycin resp. in 20 ml. H_2O.

() Treated on three consecutive days with 1.5, 1.5 and 1.5 gms. neomycin resp. suspended in 20 ml. of Necspan.

#Treated on three consecutive days with 2.0, 1.0 and 1.0 gms. neomycin resp. in 20 ml. H_2O.

since they were white, non-haemolytic, circular, convex with entire edge. The colonies on MacConkey's agar were pink resembling those of lactose positive organisms. When inoculated tryptose agar plates were incubated at room temperature the colonies were white after 18 hours, becoming orange to red after further incubation. They remained colourless when incubated at 37°C. In broth, on about the second day, the organisms produced a red ring with a white sediment when grown at 25°C but no colour at 37°C. The pigment was soluble in alcohol and chloroform but insoluble in water and ether. A clot was produced in litmus milk in 24 hours and slowly digested thereafter. Growth of a stab inoculation in nutrient gelatin produced infundibular liquefaction to bottom of tube in five days with a red ring growth at the surface and a pink floccular deposit. On plain gelatin there was no pellicle and an absence of a brilliant pigment. Other tests showed the organism to be H_2S positive, M.R. and V.P. negative, nitrates reduced, indole negative, catalase positive and Simmon's citrate positive. Acid was produced from dextrose, mannitol, sucrose, sorbitol, galactose, levulose, mannose, maltose and salicin after 24 hours incubation. Dulcitol, inositol, xylose and trehalose were fermented at 48 hours, while lactose, raffinose and inulin were negative.

The organism was classified as *Serratia marcescens* in accordance with the key in Bergey (1) except for its growth at 37°C and its pathogenicity for mice as shown below. The description closely resembles that given for *Chromobacterium prodigiosum* by Wilson and Miles [11].

In vitro tests, using tryptose-agar plates and antibiotic discs, showed resistance to tetracycline (10 mcg), dihydro-streptomycin (10 mcg), chloramphenicol (10 mcg), novobiocin (10 mcg) and penicillin (20 mcg). A zone of inhibition was produced only by neomycin (5 mcg). Other reports indicate that *Serratia marcescens* is resistant to most antibiotics.

The pathogenicity of this organism for mice was determined by the intraperitoneal injection of an 18 hour broth culture grown at 25°C. One tenth of ml. killed mice in 24 hours while mice receiving 0.01 ml. survived. One ml. administered intraperitoneally was lethal to a guina pig and one and a half ml. given intravenously killed a rabbit. The organism was recovered from the heart's blood and visceral organs of the animals that succumbed.

PRODUCTION OF MASTITIS WITH SERRATIA MARCESCENS

An 18 hour broth culture of the organism which had been subcultured five times was diluted in normal saline for intramammary injection. Approximately 500 organisms were introduced into the L.F. quarter in 10 mls. and 25,000 in a similar amount into the L.H. quarter of a normal udder immediately after the evening milking. The next morning the L.H. quarter was swollen and tender and the milk was strongly positive on the C.M. test. The quarter and milk of L.F. appeared normal. *Serratia marcescens* was cultured from the milk of both quarters. By the evening milking the L.F. was positive by the C.M. test, although the quarter did not display as severe a clinical reaction as the L.H. By the third day after injection, the swelling in the L.H. quarter had receded, but clots were present on the strip cup. At this stage the infection resembled the field cases. The L.H. quarter was treated five days after infection with satisfactory results. The L.F. continued to shed *Serratia marcescens* for ten days following infection, after which the organism could not be isolated.

TREATMENT

As the in vitro tests showed the organism to be susceptible to neomycin only, this drug was selected for treatment. The dosage of neomycin given to the L.H. quarter of the experimentally

infected cow consisted of an initial dose of 2 gms. of neomycin in 10 ml. distilled water followed by three daily doses of 1.0 gm. The organism was eliminated by the treatment. The treatment of the infected quarters in the herd was conducted as outlined in Table I.

A recheck of fourteen quarters after treatment with an aqueous solution of neomycin revealed that the organism was eliminated. When the neomycin was suspended in neospan (R) the organism was recovered from five of six quarters treated.

SUMMARY

An outbreak of mastitis, caused by *Serratia marcescens*, which involved seventeen quarters of eleven cows is reported. Experimental inoculation of the organism into animals revealed that it was capable of killing guinea pigs and mice and producing mastitis in cows. Four or five grams of neomycin given by intramammary infusion over a three day period eliminated the organism.

RESUME

Les auteurs rapportent une épidémie de mammite due à *Serratia marcescens* chez onze vaches dont dix-sept quartiers sont affectés. L'inoculation expérimentale entraine la mort du cobaye, de la souris et l'apparition de la mammite chez la vache. Le microbe disparait à la suite de l'administration en infusion mammaire de quatre à cinq grammes de neomycine durant une période de trois jours.

(R) Each c c. contained
penicillin G potassium 25,000 i.u.
Dihydrosteptromycin sulfate 12,500 mcg.
Neomycin sulfate 2,500 mcg.
Polymycin B. sulfate 500 units.

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The Swine Lungworm as a Reservoir and Intermediate Host for Hog Cholera Virus

The author has demonstrated that suspension of adult lungworms procured from apparently healthy swine and having their origin from eggs secured from swine affected with hog cholera were capable in some instances of inducing hog cholera if inoculated intramuscularly into susceptible pigs. The incidence of infection was markedly dependent upon season. It is believed that the evidence points to the lungworm being a host to the virus carrying it in masked form. Under these conditions it must be provoked to pathogenicity by stress.

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