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Serratia marcescens infection associated with early abortion in cows and buffaloes

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SUMMARY

Serratia marcescens was isolated in pure culture from cases of septic abortion in 4 cows on one farm and 10 buffaloes on two other farms. A reddish vaginal discharge was observed after abortion in all animals and in the internal organs of the aborted fetuses. All but two of the isolates produced prodigiosin, and two of the isolates from buffaloes were atypical in that they fermented raffinose. O-serological, bacteriophage and bacteriocin typing revealed four different strains. All cows were infected by the same strain, and this strain was also isolated from the semen of a breeding bull on the same farm. In another farm a strain of serotype O 14 was isolated from 6 of 10 buffaloes, and two other distinct strains were isolated from the remainder. The strain from the cattle was sensitive to gentamicin and so were two of the buffalo isolates. The infected cows were treated with intra-uterine gentamicin and the organism disappeared from cervical mucus after 3 days. Each animal after abortion showed a raised titre of agglutinating antibody to their respective isolate. A survey of 1172 healthy buffaloes and cattle gave an incidence of 1·8% with raised titres towards *S. marcescens*.

INTRODUCTION

Serratia marcescens is an aerobic Gram-negative bacillus of the family Enterobacteriaceae, which is ubiquitous in the natural environment but rarely causes serious sepsis in mammals (von Graevenitz, 1980). However, in the last 20 years attention has focused on the ability of the organism to cause outbreaks of hospital-acquired infections in humans which often prove difficult to control (Christensen *et al.* 1982). There have been few reports of serratia infections in veterinary practice, but they have been isolated from horses (Colahan *et al.* 1984), subcutaneous abscesses in lizards (Boam *et al.* 1970), septicaemia in goats (Wijewanta & Fernando, 1970), exotic birds (Quesenberry & Short, 1983) and systemic infection in domestic pets (Armstrong, 1984).

In bovine species, *S. marcescens* has been associated with an outbreak of mastitis in milking cows (Barnum, Thackeray & Fish, 1958), and was recovered from bull semen (Singleton & Simmons, 1969), but to our knowledge the organism has only once been reported as a cause of bovine abortion (Smith & Reynolds,

1970). In this paper we describe three outbreaks of early abortion in cows and buffaloes due to *S. marcescens* and the antibody response of the herds to the organism.

MATERIALS AND METHODS

Cows. The herd on farm 1 comprised 20 crossbred cows (Gir × Holstein–Friesian), 4 of which were pregnant, and each of these aborted within a period of 10 days.

Buffaloes. Four animals on farm 2 (herd size 46) and six on farm 3 (herd size 37) aborted over a 3-week period. The outbreaks on farms 2 and 3 occurred almost simultaneously, but 6 weeks after the outbreak on farm 1.

Microscopic examination. Impression smears were made of heart, blood, spleen, liver, lung and kidneys of aborted fetuses, and fetal membrane, uterine discharge and cervical material from animals. These were stained by Gram, Ziehl–Neelsen and Leishman methods. Fluid specimens were examined for leptospira by dark-ground microscopy. Specimens for *Trichomonas foetus* were referred to a parasitologist and examined by hanging drop to detect motile trichomonads.

Bacteriological examination. Swabs and tissues for bacteriological studies were inoculated on 5% bovine blood agar, MacConkey agar, campylobacter selective medium (Hi-media, Bombay), Thiol medium (Difco), brucella selective medium (Hi-media), PPLO-medium (Difco), potassium tellurite blood agar and nutrient agar. Cultures for microaerophilic bacteria were incubated in an atmosphere of air containing 10% CO_2 at 37 °C for 72 h, and media for non-fastidious organisms were incubated at 37 °C and 25 °C aerobically for 24–72 h. *Serratia* sp. were identified according to Grimont & Grimont (1978). Atypical strains of serratia were examined further by Dr B. Holmes, National Collection of Type Cultures, Central Public Health Laboratory, London, UK. Serotyping, phage typing and bacteriocine typing of serratia were performed by the methods of Pitt, Erdman & Bucher (1980).

The antibiotic sensitivity of isolates were determined by agar diffusion with commercially available disks (Pasteur Biological Laboratories, India) on Mueller–Hinton agar, and the inhibition zone diameter was expressed in comparison to that of a sensitive control strain (*Escherichia coli* ATCC 25922). The antimicrobial agents contained in the disks were (μg) ampicillin (10), chloramphenicol (30), erythromycin (15), framycetin (50), gentamicin (10), kanamycin (30) and nitrofurantoin (30).

Pathogenicity tests. Seventeen isolates of *S. marcescens* were tested for virulence for mice, as follows. Two mice were used for each strain and each was injected intraperitoneally with 0·2 ml of an 18 h peptone water broth culture, which had a viable count of approximately 5×10^8 c.f.u./ml. A control group of two mice received a similar volume of sterile broth by the same route.

Post-mortem examination was performed on the death of the test animals and on controls which had been killed. Blood and tissue specimens were examined for viable bacteria on blood agar.

An isolate from a cow and a buffalo were each tested for their ability to cause abortion in pregnant guinea-pigs. The dose of bacteria and route of inoculation were as described for the mouse tests. Aborted guinea-pigs and a control were examined, post-mortem, and specimens were collected for culture.

A cow having a pregnancy of 4·5 months was injected i.v. with 5 ml of peptone water broth culture of a strain from an aborted cow. The aborted fetus was examined for internal lesions and the presence of the inoculated organism. Serum of the cow was collected for serology on the day of inoculation and 2 weeks after the abortion.

Antibody tests. Sera from aborted cows and buffaloes were screened for brucella-agglutinins by the Rose Bengal plate test and tube agglutination. Leptospiral antibodies were tested for by an agglutination test with *Leptospira ictero-haemorrhagiae* and *L. canicola* antigens (Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA). Each serum was titrated for antibodies to the isolate of *S. marcescens* made from the animal in tube agglutination tests using organisms killed by heat (100 °C, 1 h) and 0·5% (w/v) phenol. Each titration was performed in duplicate in saline with and without 0·2 M 2-mercaptoethanol to determine total Ig and IgG antibody respectively. Control serum specimens were collected from animals with a normal breeding history in the affected herds and from disease-free farms. The titre of a serum was expressed as the reciprocal of the highest dilution that showed easily visible agglutination.

RESULTS

Three outbreaks of infection occurred first in the cow farm in mid-August 1984 and subsequently in the buffalo farms (2 and 3) in October 1984. All of the cows that subsequently aborted were off their feed prior to abortion, but none showed signs of reduced milk yield. A reddish coloured, apparently bloody, vaginal discharge was observed in cows immediately after loss of the fetus, but this disappeared within 2–3 days. Few red blood cells were found on microscopic examination, but numerous small (3–5 μm) bacilli were seen. One of the cows retained the fetal membrane.

No pre-abortion signs were observed among the buffaloes. The vaginal discharge ceased within a few hours after abortion and the fetal membrane was not retained. An unusual discoloration varying from pink to reddish brown was found in lung, liver, spleen and kidneys of aborted fetuses and in the fluid that had accumulated in the pleural cavity.

The impression smears of specimens from 4 cows and 10 buffaloes revealed Gram-negative, non-acid-fast coccobacillary forms, and *S. marcescens* was isolated from these in pure culture. All but 2 of the 17 isolates produced a magenta red pigment on nutrient agar. Four failed to produce protease enzymes, although all cultures were strongly lipolytic on Tween 80 agar. Nine of the 11 isolates from buffaloes were biochemically atypical since they fermented raffinose.

Epidemiological typing. The isolates from the cow farm were not typable by O-serology (ONT), but were indistinguishable from each other by bacteriophage and bacteriocin typing (Table 1). Two cultures of *S. marcescens* were recovered from the semen of a breeding bull at the same farm, and one of these proved on typing to be identical in phage and bacteriocin type to the index cow strain (strain 1). The other isolate from the bull was weakly pigmented, and was also ONT, but it was not lysed by any of the phages and had a different bacteriocin susceptibility pattern (strain 2).

Table 1. *Type identification of* Serratia marcescens *isolated from cows and buffaloes*

Animal*	Farm	Raffinose	Serotype	Phage type†	Bacteriocin type†	Strain no.
C1	1	–	ONT	6502	1041	1
C2	1	–	ONT	6502	1041	1
C3	1	–	ONT	6502	1041	1
C4	1	–	ONT	6502	1041	1
Bull						
(1)	1	–	ONT	6502	1041	1
(2)	1	–	ONT	NT	0001	2
B1	2	–	ONT	6502	1041	1
B2	2	+	O 14	6300	0160	3
B3	2	+	O 14	6300	0160	3
B4						
(1)	2	+	O 14	6610	5160	4
(2)	2	+	O 14	6300	0160	3
B5	3	+	O 14	6300	0160	3
B6	3	+	O 14	6300	0160	3
B7	3	+	O 14	6300	0160	3
B8	3	+	O 14	6300	0160	3
B9	3	–	ONT	6502	1041	1
B10	3	+	O 14	6610	5160	4

* C, Cow; B, Buffalo. † Mnemonic code (Farmer, 1970).

Typing of the isolates from the buffaloes revealed that 6 of the 10 animals were infected with a single strain of serotype O 14 (strain 3). Cervical swabs from animals B1 and B9 yielded ONT isolates which were indistinguishable from strain 1 of the cow farm (Table 1). Two different strains of *S. marcescens* were isolated from the cervical swabs of buffalo B4; one of these was identical to the isolate from B10 (strain 4) and the other, which was not pigmented, was similar to strain 3.

In order to determine whether carriage of *S. marcescens* was widespread on the buffalo farm 3, faecal samples from animals that had not aborted were examined, and 6 of 43 (14%) yielded serratia. These six isolates were typed, and all proved to be indistinguishable from the index strain (strain 3) of the outbreak on this farm.

Pathogenicity tests. All mice excepting the controls died during the 12–18 h following injection with representative cultures of each strain of *S. marcescens* and a reddish discoloration in all visceral organs was observed post mortem. This pigmentation was not observed in the visceral organs of mice inoculated with the non-pigmented strain B4 (2). The challenge organism was recovered in pure culture from all infected mice. The pregnant guinea-pigs aborted within 5 days and a similar coloration of internal organs was observed in fetal organs from which *S. marcescens* was recovered in pure culture. The control guinea-pig had a full-term pregnancy and a normal delivery. The cow aborted after 3 days. In addition to colour changes in the visceral organs of the fetus, a reddish discoloration of the skin was also observed.

Antibody tests. All serum samples were negative for brucella and leptospiral antibodies. Sera from all the infected animals gave tube agglutination titres of *S. marcescens* antibodies of 40–1280 with the respective isolates in the presence and

Table 2. *Survey of seropositivity towards* Serratia marcescens *among healthy buffaloes and cattle*

Animal	Number tested	No of animals with stated agglutination titre							
		⩽ 10	20	40	80	160	320	640	1280
Buffalo	629	615	0	1	4	3	1	4	1
Cattle	543	535	1	2	0	1	3	1	0
Total	1172	1150	1	3	4	4	4	5	1

Table 3. *Disk-sensitivity tests of* Serratia marcescens *isolated from cows and buffaloes to antibacterial agents*

Isolate from animal	Agent*							
	A	C	E	F	K	G	Ne	Ni
C1, C2, C3, C4, Bull (1) and (2)	S†	S	R	S	S	S	S	R
B1, B9	R	S	R	R	S	S	S	R
B2, B3, B4 (1), B6, B7, B8, B10	R	R	R	R	R	R	R	S
B4 (2) and B5	R	R	S	R	R	R	R	S

* A, Ampicillin; C, chloramphenicol; E, erythromycin; F, framycetin; K, kanamycin; G, gentamicin; Ne, neomycin; Ni, nitrofurantoin.

† S, Sensitive; R, resistant.

absence of 2-mercaptoethanol, but control samples from six non-infected animals in each farm did not agglutinate the outbreak strains of *S. marcescens*. Serum of the experimentally inoculated pregnant cow taken 2 weeks after the abortion had a titre of 320, compared with 10 before inoculation.

A survey of the prevalence of antibody to the index strain of each outbreak among healthy buffaloes and cattle was undertaken. Only 14 of 629 (2·2%) buffaloes and 8 of 543 cattle (1·5%) showed raised serum titres (⩾ 20) towards the test organisms (Table 2).

Antibiotic sensitivity and treatment. Four patterns of antibiotic sensitivity were found among 17 isolates of *S. marcescens*. The isolates from the cow farm, including those from the bull semen, were sensitive to all agents tested except erythromycin and nitrofurantoin (Table 3). The majority of the isolates from the buffaloes were resistant to all seven antibiotics but were sensitive to nitrofurantoin.

The antibiogram of isolates provided some corroboration of the typing results, in that all isolates from the cattle were similarly sensitive to antibiotics. However, the correspondence of type between the cow farm isolates and B4 and B9 (i.e. strain 1, Table 1) was not borne out by their antibiotic susceptibility. The latter isolates differed in sensitivity to ampicillin and framycetin, and these markers served to distinguish them from the cow strain. Nevertheless, some isolates of the distinct strains 3 and 4 gave the same antibiotic sensitivity pattern, and the sensitivity to erythromycin was variable between isolates of strain 3.

Each of the four infected cows was given 160 mg of gentamicin sulphate per day

by the intra-uterine route for 3 consecutive days. Swabs of cervical mucus obtained 2 days after the treatment was implemented did not yield *S. marcescens* on culture. The buffaloes were not treated due to the lack of cooperation of the farm owners.

DISCUSSION

The red pigment prodigiosin produced by *S. marcescens* has in the past been associated with superstitious folklore surrounding the appearance of 'blood' on foodstuffs, in particular starchy foods such as communion host. Although less than 10% of isolates from human infections are pigmented (Acar, 1986), the reddish coloration of the vaginal discharge of the cows and buffaloes that aborted and also of the organs of the fetus was consistent with infection by pigment-producing strains.

The absence of red blood cells in the tissues of aborted fetuses and the discharge of animals suggests that the pigmentation we observed was not due to haemorrhage but to the production of prodigiosin *in vivo*. Smith & Reynolds (1970) also observed a similar discoloration of fetal organs, but they did not examine smears of tissues and fluids to establish whether the discoloration was due to blood or bacterial pigment.

Epidemiological typing showed that the index strains from the cow farm and buffalo farm outbreaks were distinct, but the type characters of the 'cow' strain were similar to two of the buffalo isolates, although their antibiograms were different. The strains from the two buffalo farms may well have been distinct, as the farms were separated by 80 km and there was no obvious link between them. Further typing of these strains was not attempted.

As serotype O 14 was recovered from both buffalo farms this might suggest an endemic distribution for this serotype in the area. However, the results of the seroprevalence survey indicated that past infection with *S. marcescens* was uncommon, as only approximately 2% of healthy buffaloes were seropositive. Serotype O 14 is also very frequent in human infections and accounts for 50–70% of all *S. marcescens* isolated from clinical specimens (Pitt & Erdman, 1984). Rubin (1980) commented that its high incidence in sporadic infection as well as in outbreaks may indicate an enhanced virulence of this serotype, but no evidence for this has been presented.

The source of the infection in the cow farm may have been the breeding bull, whose semen contained two different strains of *S. marcescens*, one of which was associated with the abortion; however, this evidence is only circumstantial. Singleton & Simmons (1969) isolated *Serratia* spp. from bull semen and considered that it was not part of the normal microflora. It is probable that the origin of infection in buffalo farm 3 was faecal material, as isolates from the faeces of non-infected and infected animals were indistinguishable from each other on typing. The role of insect vectors was not investigated. Grimont & Grimont (1978) cited numerous reports of serratia from healthy and diseased insects including crickets, termites, moths and flies. The relative pathogenicity and possible relationships between insect and human or animal strains are worthy of investigation.

The efficacy of antibiotic treatment of these infections is doubtful, as the

infections in the cows would probably have resolved themselves within a few days without the administration of gentamicin. The buffaloes were not treated, and subsequent investigation revealed that four animals developed endometriosis and later proved to be sterile. This may not have been prevented by antibiotic treatment.

In conclusion, we believe that *S. marcescens* was the primary cause of abortion in cows and buffaloes, and this was supported by the pathological evidence. Thus, despite the low virulence of *S. marcescens* for both man and other animals, its role in serious sepsis in animals should not be disregarded.

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