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Salmonella saint-paul infection in calves

By M. M. AITKEN, G. T. H. BROWN, P. W. JONES
AND P. COLLINS
Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 0NN

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SUMMARY

A natural outbreak of *Salmonella saint-paul* infection in two Institute herds was monitored clinically, bacteriologically and immunologically. This paper describes the findings in calves. Morbidity and mortality became apparent 30 days after *S. saint-paul* was first isolated on routine sampling of neonatal calf faeces. All heifer calves were treated with a tetracycline or ampicillin preparation when they showed clinical signs of disease, while the effects of intradermal vaccination with heat-killed *S. saint-paul* were assessed in a proportion of the bull calves.

Antibiotic treatment reduced mortality and the number of persistent excretors; vaccination did not affect mortality but, if given during the first week of life, reduced the duration of faecal excretion. Calves which were untreated and unvaccinated, or vaccinated when older than 16 days, excreted *S. saint-paul* for periods of up to 18 months. Of six 'recovered' calves, which had been negative on faecal culture for 5–8 weeks, four yielded *S. saint-paul* at necropsy. Agglutinating antibody titres were highest in those unvaccinated calves which were persistent excretors.

INTRODUCTION

The effects of *Salmonella dublin* and *S. typhimurium*, the serotypes most frequently isolated from cattle, are well known (Richardson, 1975; Wray & Sojka, 1978) but salmonellosis caused by other serotypes is less well documented. It has been suggested that these 'exotic' salmonellas, like *S. typhimurium* but unlike *S. dublin*, do not establish a permanent carrier state and that they are excreted for only a few weeks or months (Gibson, 1965; Richardson, 1975; Wray & Sojka, 1977; Williams, Bellhouse & Davidson, 1978). Taylor & Davies (1979) reported excretion of *S. saint-paul* for periods of 8 and 21 m, by two out of 23 adult cattle in a herd in Wales; they did not describe disease or infection in calves. An outbreak of *S. saint-paul* infection involving two of our Institute's herds occurred in 1980–1 (Jones *et al.* 1983) and this paper describes the effects on calves and how these were modified by antibiotic treatment or by autogenous vaccination.

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MATERIALS AND METHODS

Animals

The two herds contained 121 and 117 Friesian cows respectively. The cows were loose-housed and calved in a barn on each of two farms. Calving occurred between August and April and calves remained with their dams for 24–30 h. Suckling was encouraged as soon as possible after birth, and calves which failed to suck within 4 h were bucket-fed with their dam's colostrum. Thereafter calves were penned individually, fed pooled colostrum for 2 days and milk substitute (Gold Top Baby Calf Food, BOCM Ltd) from 4 days until 6 weeks of age.

Bacteriology

Isolation, identification and quantitative assay of salmonella organisms was carried out as described previously (Hall & Jones, 1977). An autogenous vaccine was prepared by growing a faecal isolate of *S. saint-paul* on modified brilliant green agar (Oxoid Ltd) containing 120 mg/l sulphadiazine (BGA). This was subcultured on to nutrient agar plates, harvested with saline and killed by steaming for 30 min, as described previously for *S. dublin* (Aitken, Hall & Jones, 1978). The steamed suspension was dispensed in aliquots of 1·0 g (approx. 5×10^{10} organisms) that were freeze-dried to a weight of approximately 50 mg. Reconstitution in 5 ml sterile water was carried out immediately before injection of the material, giving a 10 mg/ml suspension.

Necropsy

Animals that died or were killed *in extremis* by intravenous injection of sodium pentobarbitone (Euthatal – May & Baker Ltd.) were necropsied and the number of salmonellas in bile, liver, mesenteric lymph node and contents of the small intestine was determined. A wider range of tissues and fluids were examined from calves that survived and were subsequently killed. In addition to the above samples the following were also examined bacteriologically; gall-bladder wall, lung, heart, kidney, bladder wall, urine, spleen, adrenal gland, a range of lymph nodes (mandibular, retropharyngeal, bronchial, hepatic, internal iliac, prescapular), contents of the alimentary tract, its walls and associated lymph nodes from a range of sites (rumen, omasum, abomasum, duodenum, caecum, colon, rectum), skeletal muscle, mandibular salivary gland and synovial fluid.

Drugs

Oxytetracycline hydrochloride (Terramycin – Pfizer Ltd), 3 mg/kg intramuscularly (i.m.) or 2 mg/kg intravenously (i.v.), was used on farm 1 and ampicillin trihydrate (Penbritin – Beecham Animal Health) 6 mg/kg on farm 2. On both farms a mixture of light kaolin, pectin, sulphadimidine and dihydrostreptomycin (Streptaquaine sulphate oral suspension – Elanco), approximately 1 ml/kg/day, was given orally. Dehydration was treated with up to 6 ml/kg of an amino acid and electrolyte solution (Duphalyte – Duphar Veterinary Ltd) administered i.v. or intraperitoneally, as required.

Corticosteroid treatment consisted of a combination of prednisolone and dexamethasone (Opticortenol S. – Ciba-Giegy Ltd), 0·2 mg/kg i.v. daily for 4 days, and dexamethasone (Dexafort – Intervet Ltd), 0·06 mg/kg i.m. daily, for 5 days.

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Monitoring and treatment

For 6 weeks before the first isolation of *S. saint-paul*, faecal swabs from all bull calves and their dams were routinely taken as soon after calving as possible (0–4 h). From the time of first recovery of *S. saint-paul* swabbing after calving was extended to include all calves of both sexes and faeces samples were subsequently collected at least once every week.

From day 52 after the first isolation of *S. saint-paul*, 44 infected bull calves from both farms were transferred to a separate unit and 26 were injected intradermally with 8·0 mg (0·8 ml) of heat killed *S. saint-paul;* this inoculation was repeated 9 days later. Initially the calves injected were aged from 4 to 45 days but thereafter alternate calves were vaccinated at 2 and 11 days of age. Unvaccinated calves were housed in the same unit as the vaccinated animals and all received fluid therapy as required but no antibiotics.

The bull calves were examined for clinical signs of salmonellosis twice daily. Faeces were tested three times weekly for the presence and enumeration of salmonellas and serum was sampled weekly for measurement of agglutimating antibody titres to somatic (O) and flagellar (H) antigens by the tube agglutination test.

Heifer calves were kept on their farms of origin, examined daily and treated with antibiotic for 5–7 days from the onset of diarrhoea, pyrexia or anorexia. Faeces samples were taken daily during antibiotic treatment and weekly in other cases for examination as in the case of the bull calves. The antibiotic sensitivity of the organism was checked using Oxoid Multodiscs at the start and after 5 days of treatment.

RESULTS

Infection was first detected when *S. saint-paul* was isolated from a faecal swab taken from a calf 1 h old in herd 1. When faecal swabs were taken from the other calves in this herd, and in herd 2, 5 days later, all 10 in herd 1, but none of the 18 in herd 2, were positive for *S. saint-paul*. At this time there was a lull in calvings in herd 2, but 5 weeks later, when calving frequency increased again, calves in herds 1 and 2 alike were found to have become infected within 72 h of birth (Jones *et al.* 1983).

One calf was killed *in extremis* on day 18 and was found at necropsy to have lesions of enteritis, hepatitis and interstitial pneumonia. These lesions and the numbers of *S. saint-paul* recovered from the tissues (10^3–10^6/g) were compatible with acute salmonellosis. Further calves aged 1–5 weeks died from day 30 onwards after showing diarrhoea and dysentery for 2 days to 1 week. Fig. 1 shows the deaths that occurred up to the time vaccination was introduced.

Initially 11 calves aged 16–45 days, one aged 8 days and three aged 4–6 days, were vaccinated, while nine were kept as unvaccinated controls. One of the 'late' (16–45 days) vaccinated group died after 4 days, as did the 8-day-old calf, but none of the 4- to 6-day-old group. Over this period of time three of the nine unvaccinated calves died aged 16 to 28 days. Subsequently three of 11 calves vaccinated when 2 days old died at 8–15 days of age while two of a further nine unvaccinated calves died aged 13 and 16 days (Table 1).

The duration of excretion of *S. saint-paul* at countable levels (10^2 per g faeces

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Fig. 1. Numbers of calf deaths in 10-day periods from the time of isolation of *S. saint-paul*. ↑, Vaccination introduced.

Table 1. *Numbers and ages of calves which died of salmonellosis after vaccination was introduced*

Age vaccinated (days)	No. deaths/ no. vaccinated	Age at death (days)
16–45	1/11	37
8	1/1	11
4–6	0/3	—
2	3/11	8–15
Unvaccinated	5/18	13–28

Table 2. *Faecal excretion of* S. saint-paul *by vaccinated and unvaccinated calves*

Group	No.	Days excreting 10^2/g or $> 10^2$/g (mean)	
Unvaccinated	13	52	58
Vaccinated at 16–45 days	10	65	
Vaccinated at 4–6 days	3	22	17
Vaccinated at 2 days	8	15	

$t = 2{\cdot}65$. $P = > 0{\cdot}001 > 0{\cdot}01$.

or more) by vaccinated and unvaccinated calves is shown in Table 2. There was no significant difference between the duration of excretion by those vaccinated at 2 compared to those vaccinated at 4–6 days or between those vaccinated at 16–45 days compared to unvaccinated calves. However, vaccination at 2–6 days significantly reduced duration of excretion when compared to the late-vaccinated grouped with unvaccinated calves. Fig. 2 illustrates the difference in duration of excretion when smaller numbers of organisms were detected after enrichment of samples.

High agglutinin titres accompanied prolonged excretion and intradermal vaccination did not appear to enhance O or H agglutinin responses (Fig. 3).

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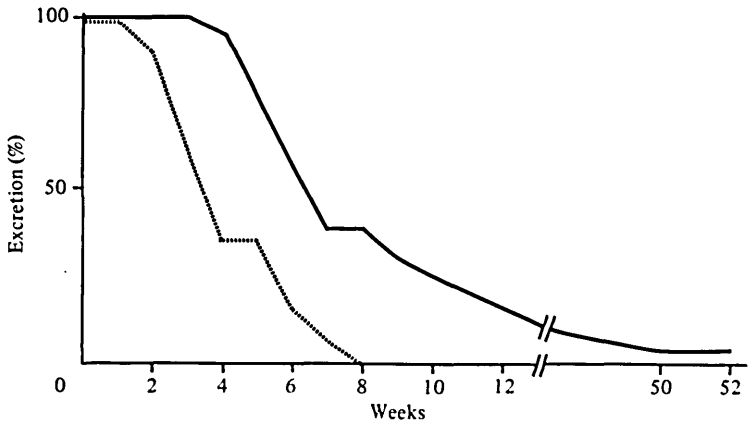

Fig. 2. Duration of excretion of *S. saint-paul* by calves vaccinated at 2–6 days, ▪▪▪▪▪ ($n = 11$), and by those calves not vaccinated or vaccinated at 16–45 days, —— ($n = 23$).

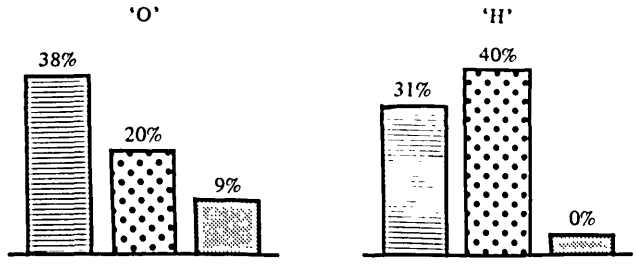

Fig. 3. Percentages of total numbers of calves with agglutinin titres exceeding 1 in 40 in calves unvaccinated, ▤ ($n = 13$), vaccinated late (> 16 days), ▦ ($n = 10$), and vaccinated at 2–6 days of age, ▩ ($n = 11$).

Table 3. *Isolation of* S. saint-paul *from recovered calves at necropsy*

Calf	Age (months)	Time −ve, faecal samples (weeks)		Sites of isolation of *S. saint-paul* at necropsy*
1	6	2	0	
2	3	9	0	
3	3	5	1	ruminal contents
4	8	8	1	omasal contents
5	3	8	1	omasal wall
6	4	8	3	omasal contents, kidney, bladder wall

* 45 sites sampled.

Six animals were killed and necropsied 2–9 weeks after excretion had stopped, when their ages ranged from 3 to 6 months. *S. saint-paul* was isolated from the carcases of four of these animals (Table 3). Three, nos. 3, 4 and 6, had been given corticosteroid treatment after faeces samples had been consistently negative for 3 weeks (no. 4) or 5 weeks (nos. 3 and 6). No. 3 and no. 4 re-excreted *S. saint-paul* for 2 days but in numbers detectable only by enrichment. No salmonellas were recovered from calf no. 6.

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One persistent excretor, with approx. 10^5 organisms/g faeces throughout its life, was killed when 3 months old. Of the 45 tissues examined, 29 were positive (64%); concentrations of 10^5 *S. saint-paul* per ml were recovered from urine and 10^2 per gram from omasal contents, omasal wall and lymph node.

Effect of antibiotic treatment

Oxytetracycline treatment of seven calves in herd 1 failed to prevent death of three of them, but all seven calves treated with ampicillin in herd 2 survived. Nine of the 11 treated survivors excreted *S. saint-paul* for less than 5 weeks and all had ceased to do so by 8 weeks after treatment. Shedding by untreated control heifers lasted for no longer than 9 weeks (Jones *et al.* 1983).

DISCUSSION

Because, in this Institute, calves were being examined routinely, *S. saint-paul* infection was diagnosed before its clinical consequences became apparent. The virulence of the strain involved was similar to that of *S. dublin* when tested experimentally in rats and calves (Aitken & Jones, unpublished). Its clinical course in calves resembled that of *S. typhimurium* in that enteritis invariably preceded death (Wray & Sojka, 1978) but it resembled *S. dublin* in that persistent excretion and infection of tissues occurred.

Fluid therapy was given to all calves as required but appeared to do no more than delay death in the absence of antibiotic treatment. Removal of bull calves to a separate unit permitted a reduction of stocking density, and this measure, together with closer examination and earlier antibiotic treatment of sick calves, reduced the death rate, particularly in herd 2. Most of the deaths in herd 1 occurred before these measures had been taken.

Autogenous vaccination was tried on the grounds that a similar preparation of *S. dublin*, given intradermally, had protected 6-month-old calves against intravenous challenge (Aitken, Jones & Brown, 1982). It was clear that vaccination in this way did not induce protection sufficiently rapidly to prevent deaths and demonstrated the need for earlier protection, possibly by vaccination of pregnant cows. The reduction in the duration of excretion of *S. saint-paul* by calves vaccinated during the first week of life was significant. In the case of bull calves, because of the frequency of sampling, excretion times were accurate to within 1 or 2 days. Data on excretion by heifer calves was less precise.

Calves that had shown disease and were treated with antibiotic did not excrete *S. saint-paul* for longer than did untreated, infected, but apparently healthy calves.

The use of corticosteroids to stimulate re-excretion of pathogens in latently infected animals is now well established (Dennett, Barasa & Johnson, 1976). Although *S. saint-paul* was isolated from the faeces of two of the three treated animals and the organism was recovered from these and another at necropsy, it was surprising that no. 6, which showed infection of three tissues, did not re-excrete. Reinfection of no. 6 during the 3 weeks between completion of corticosteroid treatment and necropsy was unlikely but should be considered. The relative frequency of isolation of *S. saint-paul* from omasal wall or contents has been observed also in the case of *S. dublin* infection (Aitken & Jones, unpublished).

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Agglutinin titres have not been found useful in identifying carriers (Hall *et al.* 1978). Elevated titres accompanied persistent excretion and added to the evidence of tissue infection of individual animals as distinct from passive faecal excretion.

The spread of infection among adult cattle in this outbreak and measures taken to identify the source of infection are described elsewhere (Jones *et al.* 1983). It was likely that calves were infected by their dams or other cows in contact with them at and shortly after calving. Had it been possible to segregate infected animals when they were first detected, to allow cows to calve in boxes free from contamination and to house calves in such a way that contact between them was prevented, the spread of infection might have been considerably reduced. Its effect on calves born in subsequent years will be assessed by continued monitoring of the herds. In the interim, the virulence and persistence of *S. saint-paul* should focus attention on the threat from exotic serotypes and the need for vaccines which will protect against a range of them.

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