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Salmonella saint-paul infection in two dairy herds

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SUMMARY

An outbreak of salmonellosis due to *Salmonella saint-paul* in two dairy herds was first detected during routine examination of calves soon after calving; infection reached 100% amongst calves and up to 60% amongst milking cows. Excretion by cows continued for over 12 months and by calves for up to 18 months.

The most important factor in controlling the spread of infection was reduction of environmental contamination by removal of carriers, prompt antibiotic treatment of sick calves and reduction in stocking densities.

INTRODUCTION

Outbreaks of salmonellosis associated with serotypes other than *Salmonella dublin* and *S. typhimurium* have rarely been described in detail but it is probable that such 'exotic' serotypes resemble the latter rather than the former (Wray & Sojka, 1977). As with *S. typhimurium*, carriers are thought to be rare and excretion that continues after infection is considered to reflect recontamination from the environment. However Richardson (1975) described excretion for 11 months and Taylor (1979) reported continued excretion of *S. saint-paul* for 21 months.

This communication describes an outbreak of infection in cattle which occurred between September 1980 and February 1982.

MATERIALS AND METHODS

Herds. The outbreak occurred on an estate of approximately 800 hectares in Southern England. The estate comprised three dairy farms (farms 1, 2 and 3), a pig unit of 110 sows and gilts and their progeny and an area attached to a Research Institute where animals were housed in isolation units.

The outbreak principally involved cattle at farms 1 and 2, although it spread initially to farm 3 and the isolation units, but was rapidly eliminated from these.

Dairy farms 1 and 2 both housed herds (herds 1 and 2 respectively) of approximately 100 Friesian cows and their calves. Calving took place between August and April in barns. Calves were housed initially in individual pens either within the same building as the collecting yards and parlour (herd 1) or in a separate adjacent building (herd 2). Older calves were housed in groups. Normally heifer calves were retained as replacements and bull calves were sold or removed

to the isolation units attached to the Research Institute. During the initial period of the outbreak this practice was discontinued, bull calves were retained and subsequently the majority were housed in a unit (unit F) approximately 600 yards from farm 2.

At the beginning of the outbreak cows were at pasture but later they were housed in yards until turned out to graze in the following spring. They were fed a 'complete diet' composed of vegetable constituents, minerals and vegetable fat. This was supplemented as necessary during milking with dairy cake. The feed was compounded at a mill, approximately equidistant from the three farms, which was also used to prepare feed for pigs and cattle at farm 3 (herd 3) and the isolation units.

Calves were left with their dams for 24 h after calving and those which did not suck were fed cold-stored colostrum. All calves were weaned onto milk substitute and calf pellets.

Sampling procedures. Prior to the first isolation of *S. saint-paul* in September 1980, rectal swabs were taken from all bull calves within 4 h of birth (these calves were used in experimental infections with *S. dublin*) and examined by enrichment for salmonellas.

Once infection was detected an intensified sampling programme was intiated to monitor the spread of infection and control measures were initiated. To establish the degree of infection all cattle on the estate were examined bacteriologically together with slurry, including pig waste, milk filters, animal feed, human contacts and wild animals.

Regular swabbing of herds 1 and 2 was continued until 6 months after the organism could no longer be isolated. Herd 3 was examined on three occasions, once initially and then 6 months and 14 months later. Milk filters were examined weekly for 2 years and human contacts and wild animals during the first 6 months.

Cows and calves of herds 1 and 2 were swabbed at parturition and calves daily, twice weekly or weekly until infection was no longer detected. Samples were examined by enrichment or plate count.

To detect 'carriers' amongst adult cattle, bacteriological data were compared with serum agglutination tests and skin tests.

During the calving period which commenced in August 1981 all cows and calves of herds 1 and 2 were swabbed as soon after birth as possible. Pre-colostral blood was taken from calves and blood and colostrum samples were collected from cows.

Cattle which showed clinical signs of salmonellosis were examined carefully and those which died or were slaughtered were necropsied.

Isolation of salmonellas from animals. Salmonellas were isolated from cattle by enrichment culture of rectal swabs in 10 ml volumes of Rappaport broth (RAP: Rappaport, Konforti & Navon, 1956) and selenite brilliant green broth (SBG: Difco). The RAP cultures were incubated at 37 °C and the SBG at 43 °C and plated on to modified brilliant green agar (Oxoid CM329) containing 120 mg/l sulphadiazine (BGSD) after 24 and 48 h incubation. Plates incubated at 37 °C were examined after 24 and 48 h and bacteria resembling salmonellas identified biochemically (Buchanan & Gibbons, 1974) and serologically (Kauffmann, 1972). Salmonellas were isolated from cattle at necropsy by enrichment culture of 1 g of tissue or body fluids in RAP and SBG, from human faeces by culture in RAP and SBG, from mice as described by Jones & Twigg (1976) and from birds by

enrichment culture of throat, cloacal swabs and feet in 10 ml volumes of RAP and SBG.

The concentration of *S. saint-paul* in faeces was determined by diluting 1 g in 0·85 % (w/v) saline and spreading appropriate dilutions over the surface of BGSD.

Isolation of salmonellas from feed, slurry and milk filters. Feed samples were examined by enrichment and pre-enrichment (Jones *et al.* 1982) and milk filters and slurry samples by enrichment of half of each filter or 10 g of slurry in 100 ml volumes of RAP and SBG followed by plating on BGSD as above.

Serology. Blood was removed from the jugular vein and separated serum stored at −18 °C. In the serum agglutination test (SAT) doubling dilutions were incubated at 56 °C with an equal volume of *S. saint-paul* 'O' or 'H' antigen. The 'H' titre was read after 4 h and the 'O' titre after 18 h, the end-point being taken as the highest dilution giving 50 % agglutination and the titre expressed as a reciprocal of that dilution. Whey was obtained by incubating colostrum with 0·1 ml rennin (Sigma) at 56 °C for 30 min and filtering the supernatant through a 65 μm filter; SAT titres were obtained as for serum.

Delayed hypersensitivity test. Cattle were tested for delayed-type hypersensitivity to a heat-killed suspension of *S. saint-paul* injected intradermally, as described previously for *S. dublin* (Aitken, Hall & Jones, 1978).

Zinc sulphate turbidity. The concentration of immune globulin in calf serum was estimated by the zinc sulphate turbidity test (McEwan *et al.* 1970).

RESULTS

Extent of initial infection. *S. saint-paul* was first isolated from a bull calf in herd 1 on 27 Sept. 1980. Sixteen bull calves had been examined during the preceding 40 days but were all negative. Two further calves born on the same day – one in herd 1 and one in an isolation unit – were examined on 28 Sept. 1980 and both yielded *S. saint-paul*. Five days later 10/10 calves of herd 1, 9/11 in the isolation unit, and 1/28 of herd 3 were infected; 18 calves in herd 2 were not infected. The infected calf in herd 3, which had been transferred from herd 1, was removed and subsequently no further isolations were made from this herd. Those in the isolation unit were segregated from other stock and infection was contained.

Infection of adult cattle. Milking cows in herd 1 were first sampled 11 days after infection in calves was detected (8 Oct. 1980), and in herd 2 on 9 Oct. 1980; 15/48 and 2/112 respectively were positive. One of the two positives in the latter had been transferred from herd 1 on the morning of 9 Oct. 1980. Fig. 1 shows the percentage of milking cows positive for 8 months after infection was detected. Both herds were examined for up to 595 days and were last positive after 342 days (herd 1) and 382 days (herd 2). There were four clear-cut peaks of excretion from herd 2 and three diminishing ones from herd 1. The last peak of herd 2 coincided with cattle being turned out in the spring. At the start of the outbreak most cows in herd 2 were being milked, compared to half of herd 1. Excretion by dry cows of herd 1 was monitored for 70 days until their number became too few. The percentage excreting was higher than in milking cows, with a maximum of 96 %, (34/35 animals) which was reached later. On two occasions the faeces of dry cows was counted. On the first occasion 13 of 22 animals excreting were shedding more

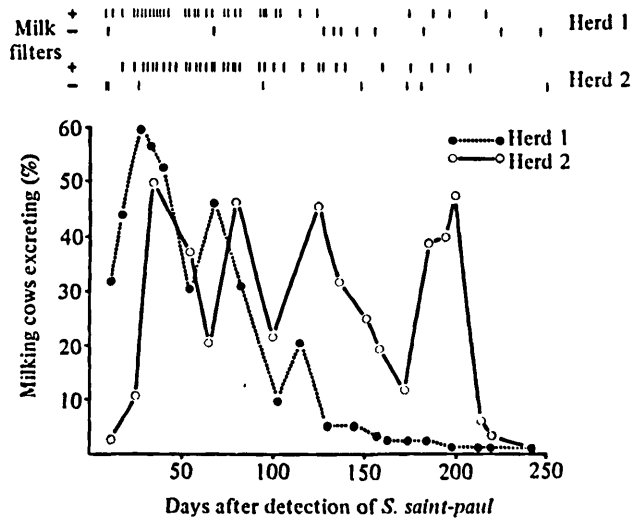

Fig. 1. Excretion of *S. saint-paul* by cows and detection of *S. saint-paul* in milk filters.

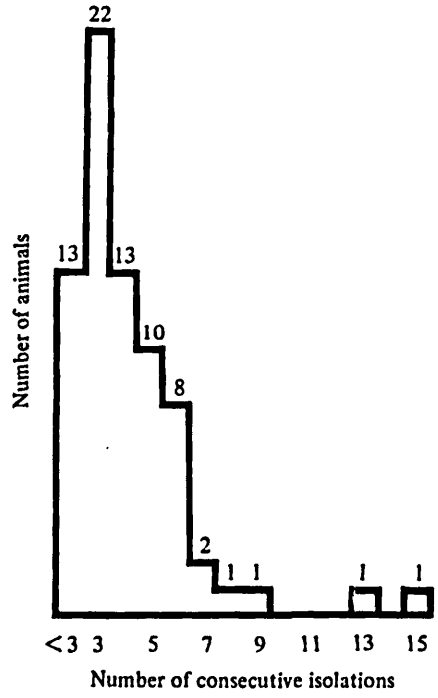

Fig. 2. Detection of *S. saint-paul* from consecutive rectal swabs from cows of herd 1.

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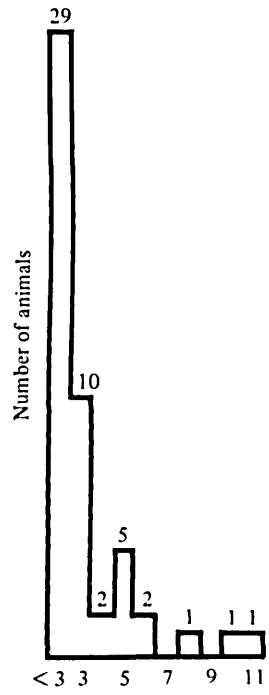

Fig. 3. Detection of *S. saint-paul* from consecutive rectal swabs from cows of herd 2.

than 10^2/g, with a maximum of $8{\cdot}2 \times 10^4$ in one cow that was contributing 75% of all the *S. saint-paul* excreted by the group. When this animal was removed, only 6 of 13 cows excreted more than 10^2/g and the proportion excreting fell from 88 to 68%.

In herd, 1, 23 of 37 (62%) cows sampled shortly after parturition were positive compared with 17 of 25 (68%) from herd 2.

Salmonellas were not recovered from herd 3 or from cattle in the isolation units.

Excretion of S. saint-paul *by 'carriers'*. As more than 200 milking cows were swabbed 25 times it is impractical to present results for individual animals. However, Figs. 2 and 3 show the number of occasions on which cattle were consecutively positive. Cattle in herd 1 excreted more regularly than herd 2, where 48 of 99 animals did not yield positive swabs on three consecutive occasions.

Excretion by cattle which shed salmonellas over an extended period is shown in Tables 1 and 2. In herd 1 there were 14 such animals and the six chosen are examples. Cow 1 excreted almost continually for at least 210 days but organisms could not be counted; it was transferred to herd 2 after 195 days and excreted for another month. Cow 2 scoured shortly after the start of the outbreak (day 20) and excreted for at least 164 days. In an attempt to clear the herd of infection, these two animals, which were the only ones to excrete in three consecutive weeks, were removed. Cow 1 was moved to herd 2 and cow 2 was slaughtered. However, cow

Table 1. *Excretion of* Salmonella saint-paul *by selected cattle of herd* 1

Days after initial isolation	Cow no.					
	1	2	3	4	5	6
11	+	−	−	+	−	+
18	NT	+	NT	+	NT	+
27	+	+	+	+	+	+
30	NT	$5{\cdot}5 \times 10^5$	NT	NT	NT	NT
32	+	$9{\cdot}0 \times 10^4$	+	+	+	+
39	+	+	+	+	+	+
53	+	+	+	−	+	+
61	NT	NT	NT	NT	NT	NT
67	+	+	+	−	+	+
81	+	+	+	+	+	+
102	+	+	−	+	+	−
116	$< 10^2$	$< 10^2$	−	$< 10^2$	$< 10^2$	−
130	+	+	−	+	−	−
144	$< 10^2$	$1{\cdot}5 \times 10^3$	−	$< 10^2$	$< 10^2$	−
158	+	+	−	−	−	−
163	−	+	−	−	−	−
172	+	+	−	−	−	−
185	+	+	−	−	−	−
192	−	$1{\cdot}3 \times 10^4$	NT	NT	NT	NT
194	$< 10^2$	$3{\cdot}2 \times 10^4$	NT	NT	NT	NT
199	NT	NT	+	−	−	−
213	NT	NT	+	−	−	−
214	+	NT	NT	NT	NT	NT
221	+	NT	+	NT	NT	NT
242	NT	NT	+	−	−	−
243	−	NT	NT	NT	NT	NT
263	−	NT	NT	NT	NT	NT
270	NT	NT	−	−	−	−
290	−	NT	NT	NT	NT	NT
311	NT	NT	+	−	−	−
339	NT	NT	−	−	−	−

− = *S. saint-paul* not isolated.
+ = *S. saint-paul* isolated by enrichment.
$< 10^2$ = *S. saint-paul* isolated by enrichment but could not be counted on BGSD.
$5{\cdot}5 \times 10^5$, etc. = no. of *S. saint paul.* per gram of faeces counted on BGSD.
NT = not tested.

3, which was excreting from days 27 to 81, recommenced shedding by the time of the following sampling and was positive for another 112 days.

The six animals in herd 2 (Table 2) all had similar patterns of excretion extending over approximately 160 days. They were confirmed as 'persistent excretors' by two faecal counts at days 191 and 193, when, although 23 and 18 animals were positive, only 10 and 5 respectively were shedding organisms in sufficient numbers to be counted. Between days 151 and 159 and days 191 and 193 the number of organisms excreted by five of the six animals had increased. All six animals were negative by day 213, shortly after they had been turned out in the spring; the number of positive animals declined rapidly at that time (Fig. 1).

'Carriers' could not be identified by skin tests or serology. Herd 1 was skin tested and bled for serology between days 51 and 65. Thirteen of 84 animals gave positive

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Table 2. *Excretion of* Salmonella saint-paul *by selected cattle of herd* 2

Days after initial isolation	Cow no.					
	7	8	9	10	11	12
12	−	−	−	−	−	−
25	NT	−	−	+	−	−
37	+	+	−	+	+	+
52	+	+	+	+	+	+
65	NT	+	−	+	−	+
79	+	+	+	+	−	−
100	+	+	+	+	−	+
124	+	+	+	−	+	+
137	+	+	+	−	−	+
151	$3{\cdot}0 \times 10^4$	$1{\cdot}3 \times 10^2$	$2{\cdot}8 \times 10^5$	$9{\cdot}2 \times 10^4$	$< 10^2$	$5{\cdot}0 \times 10^2$
159	$1{\cdot}1 \times 10^3$	$1{\cdot}3 \times 10^3$	$5{\cdot}0 \times 10^5$	$1{\cdot}5 \times 10^4$	$< 10^2$	+
172	+	+	+	+	+	−
187	+	+	+	+	+	−
191	$1{\cdot}7 \times 10^5$	$6{\cdot}0 \times 10^3$	$6{\cdot}0 \times 10^3$	$1{\cdot}4 \times 10^5$	$5{\cdot}4 \times 10^5$	NT
193	$1{\cdot}4 \times 10^5$	$2{\cdot}7 \times 10^3$	$2{\cdot}7 \times 10^3$	$1{\cdot}2 \times 10^5$	$5{\cdot}4 \times 10^4$	$1{\cdot}7 \times 10^3$
213	−	−	−	−	−	−
219	−	−	−	−	−	−
242	−	−	−	−	−	−
263	−	−	−	−	−	−

− = *S. saint-paul* not isolated.
+ = *S. saint-paul* isolated by enrichment.
$< 10^2$ = *S. saint-paul* isolated by enrichment but could not be counted on BGSD.
$3{\cdot}0 \times 10^4$, etc. = no. of *S. saint-paul* per gram of faeces counted on BGSD.
NT = Not tested.

skin reactions but only one of these (cow 6, Table 1) was identified bacteriologically as a 'persistent excretor'. There was no correlation between skin test and SAT (Fig. 4) nor did the SAT identify animals positive bacteriologically. The animal with the highest titres (O, 1/1280; H, 1/80) was not excreting at the time of sampling although it had done so on three previous occasions.

Positive skin reactions were observed in seven animals of herd 2 tested at day 79 but there were no correlations between skin tests and agglutination titres (Fig. 5) or bacteriological results. None of the animals identified bacteriologically as 'persistent excretors' gave positive skin reactions.

Infection in calves. The calves of herd 1 were all infected after 7 days (Fig. 6) but infection declined slowly over the next 160 days as newly born calves became infected and older calves became clear. The mean duration of excretion by 42 calves for which complete data are available was 39 days with a range of 7–162 days. Fifty-two days after the start of the outbreak most bull calves were removed to unit F (Aitken *et al.* 1983). The infection rate in herd 2 also quickly reached 100%; the negative results up to day 42 were associated with a later calving season. The mean duration of excretion by 22 calves was 19 days with a range of 1–64 days. As in herd 1, excretion was shortened by removal of bull calves to unit F and persistently excreting calves to the isolation units. Only 11 bull calves remained in the herds after others were removed to unit F but they excreted for a mean of 48 days compared to 28 days for heifer calves.

Salmonellas excreted by calves exceeded those voided by adult cattle. Thus both

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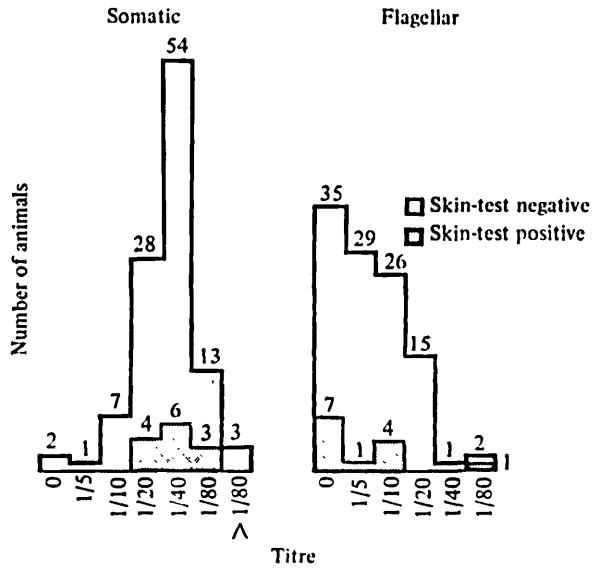

Fig. 4. Serum agglutination titres of cows of herd 1.

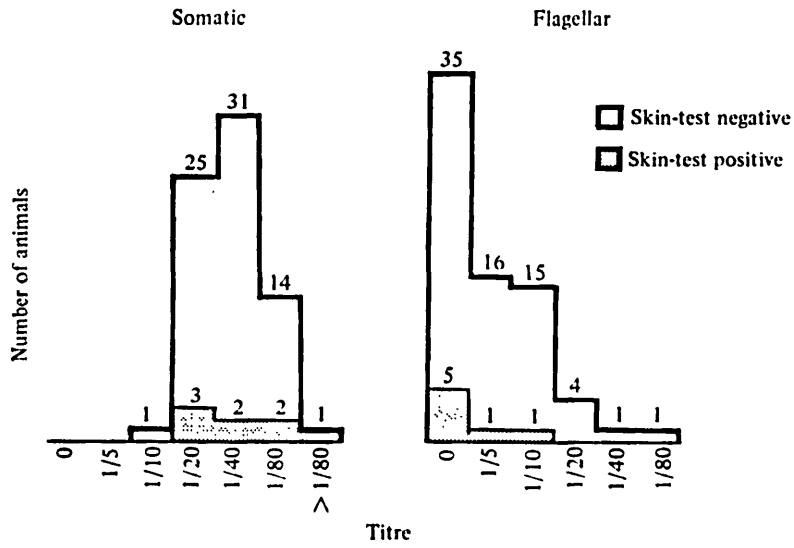

Fig. 5. Serum agglutination titres of cows of herd 2.

sick and healthy calves usually had counts between 10^6 and 10^8/g within 4 days of birth and continued to excrete up to 10^4/g for more than 30 days. The level was considerably reduced by treatment with antibiotics, falling within 24 h from greater than 10^7/g to numbers only detected by enrichment.

A total of 144 calves were born into herds 1 and 2 during the 1980–1 season and of those 122 became infected: 80 were positive when first swabbed, 40 more at the second swabbing and the other two at the third. Ninety-eight were swabbed within 12 h of birth and 54 were positive. Those not sampled within 12 h were all positive

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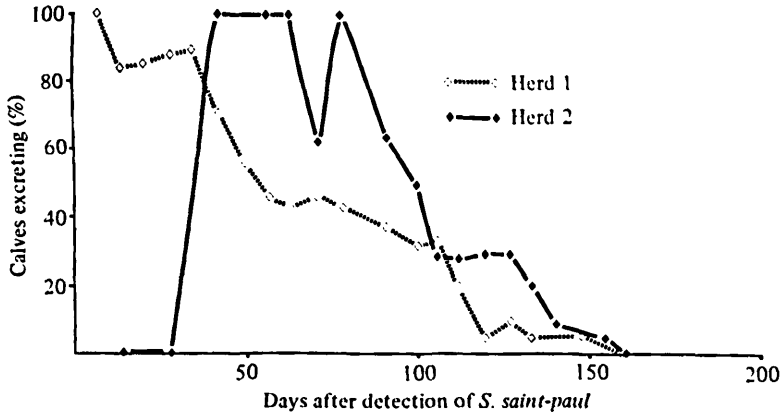

Fig. 6. Excretion of *S. saint-paul* by calves.

at first examination. Thus with the exception of eight calves which were not swabbed in the first week of life all of the calves which became infected were excreting within 7 days and 79% within 72 h of birth. These figures would be higher if swabs had been taken from all animals soon after birth.

Isolations from human contacts. Faecal samples were examined from 16 'close contacts' (people regularly in contact with the infected cattle or who had drunk raw milk) and 27 'distant contacts' (in occasional contact with infected cattle). Two of the 'close contacts' excreted. One was excreting when first examined and remained positive for a minimum of 54 days. The other became infected after 25 days and remained positive for 43 days.

Isolations from feed, slurry and milk filters. Salmonellas were not isolated from feed or feed ingredients but slurry from herds 1 and 2 was positive when sampled 9 days after detection in calves. Samples from herd 3 and pigs were negative. A milk filter from herd 1 was positive when first tested (day 9) and from herd 2 at the third attempt on day 18 (Fig. 1). Although usually detectable on filters it was occasionally absent, even when isolations from cattle exceeded 30%. On other occasions filters were positive when only one animal in a herd was excreting.

Isolations from wild animals. With the exception of one isolation from a house-mouse at farm 1, isolations were made only from starlings, rooks and one crow (Table 3). All isolates came from the area of the infected buildings, even though many of the animals were derived from areas to which grazing cattle had access (Table 4). Most isolations were made from feet (18/26); isolations from mouth (9/26) and cloaca (12/26) were less common.

Observations during the 1981–2 *calving season.* During the 1981–2 calving season infection was not detected in herd 1. The first cow calved on 11 Aug. 1981 and the last on 21 Jan. 1982; 113 calves were sampled. Calving in herd 2 lasted from 17 Sept. 1981 to 31 Dec. 1981 and the tenth of 89 calves sampled within an hour of parturition was positive. Rectal swabs from all cows were negative. Although the infected calf was segregated from calves born previously, infection spread to 23 of 33 clean calves born subsequently and housed in the same unit, and also to one born the day before and initially housed in the same loose box. In an attempt to break the cycle of infection the forty-fourth and all subsequent calves were

Table 3. *Wild animals examined for the presence of* Salmonella saint-paul

Animal	No. examined	No. positive
House mouse (*Mus musculus*)	30	1
Brown hare (*Lepus europaeus*)	54	0
Rabbit (*Oryctolagus cuniculus*)	3	0
Pheasant (*Phasianus colchicus*)	95	0
Partridge (*Perdix perdix* and *Alectoris rufa*)	43	0
Wood pigeon (*Columba palumbus*)	11	0
Starling (*Sternus vulgaris*)	83	20
Rook (*Corvus frugilegus*)	15	4
Crow (*C. brachyrhynchos*)	7	1
Total	341	26

Table 4. *Distribution of starlings and rooks infected with* Salmonella saint-paul

	No. positive	
	Starling	Rook
Farm 1	14/27	4/9
Farm 2	6/27	NT
Farm 3	0/27	NT
Estate	0/2	0/6
Total	20/83	4/15

removed from the herd to an isolation unit within 12 h of birth and none became infected.

The spread of infection in 1981–2 was slower than during the previous year (Fig. 7). Of the 24 calves which acquired infection only 16 were positive when first swabbed and nine did not become positive until more than 7 days old. The mean duration of excretion was 11 days, with a range of 1–35 days. The maximum number of organisms in faeces was $2{\cdot}0 \times 10^6$/g; none of the animals were sick. Twenty-nine days after the start of this second outbreak, when excretion was at a maximum, all calves were treated with oxytetracycline (250 mg/day for 3 days), after which excretion declined.

Serum antibody in cattle at parturition exceeded that in whey (Table 5), and whilst 'H' titres were comparable with those recorded earlier (Figs. 4, 5), 'O' titres tended to be slightly higher. Specific antibody was not detected in the pre-colostral serum of the majority of calves and most of those with low titres also had high zinc sulphate turbidity levels; those with high turbidity and no antibody against *S. saint-paul* were born to cows with no activity in whey.

Clinical observations. Fourteen bull calves and four heifer calves of herd 1 died or were killed *in extremis* between 33 and 65 days after infection was detected. No calves in herd 2 died. Calves that were treated with antibiotics following periods of anorexia and scouring or which died in unit F have been reported upon by Aitken *et al.* (1983). *S. saint-paul* was also the cause of or a contributory factor to disease in eight cows equally divided between the two herds. These included one animal that aborted, two that died (one from acute salmonellosis and one from milk fever and salmonellosis), four that were slaughtered after periods of scouring and two that scoured but recovered. The organism was recovered from either the faeces of these animals or their tissues at necropsy.

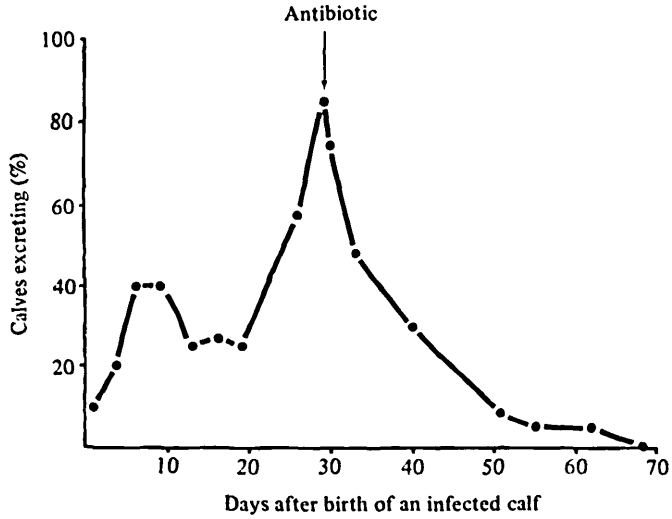

Fig. 7. Excretion of *S. saint-paul* by calves of herd 2 during the 1981–2 calving season.

Table 5. *Agglutination tests on the serum and whey of cattle sampled at parturition during the* 1981–1982 *calving season*

	No. of animals with titres of:							
	0	1/5	1/10	1/20	1/40	1/80	1/160	1/320
				Herd no. 1				
Cows								
Serum								
O	7	2	6	5	35	18	6	1
H	38	18	15	7	2	0	0	0
Whey								
O	30	17	19	11	9	6	0	0
H	68	9	7	3	4	1	0	0
Calves								
Serum								
O	56	5	6	7	6	3	1	0
H	78	1	4	0	0	1	0	0
				Herd no. 2				
Cows								
Serum								
O	2	2	4	10	22	13	3	1
H	22	19	7	6	3	0	0	0
Whey								
O	17	18	22	7	3	3	1	0
H	55	6	6	2	2	0	0	0
Calves								
Serum								
O	41	7	8	3	4	2	0	0
H	60	2	2	0	1	0	0	0

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DISCUSSION

The problem with investigating outbreaks of salmonellosis is that the organism is usually well established and the environment heavily contaminated before its presence is suspected. Fortunately in this case calves were being swabbed at birth as a preliminary to using them in experiments with *S. dublin*, otherwise salmonellosis may not have been suspected for at least 20 days, when the first calf became anorexic and scoured. By this time the organism had become widely distributed. The outbreak started in herd 1 and spread to herd 3 and the isolation units by movement of infected calves, and to herd 2 by transfer of cattle that had been brought into herd 1 prior to calving and returned afterwards. The organism did not establish in herd 3 or the isolation units because infected calves were removed or isolated and further introductions prevented.

Source of infection. The origin of the outbreak was not determined. Animal feed was not contaminated, although one of these was the source of a later outbreak (Jones *et al.* 1982). A human was positive when first sampled but he could easily have become infected from the cattle. Eight heifers had been introduced to herd 1 at 3 weeks prior to the discovery of the organism, and since 50% of these were positive when first examined compared to 32% of the herd, it is possible that the organism was acquired in this manner. However, infection had not been reported on the farm of origin. Other possible sources such as gulls (Williams *et al.* 1977) were not examined.

Infection in adult cattle. Once established the organism spread rapidly, particularly amongst pregnant cows close to parturition. This may have been due to increased susceptibility or close association, especially in the calving barns. Several peaks of excretion occurred amongst cows in both herds. This may have been related to nutrition and particularly to episodes of ketosis. The last peak in herd 2 was closely associated with animals being turned out to graze and probably did not occur with herd 1 because fewer 'persistent excretors' were present.

Several shedders were detected in both herds, indicating that 'exotic' serotypes may be just as likely to produce such animals as is *S. dublin*. This is in contrast to the observations of Richardson (1973), Wray & Sojka (1977) and Williams, Bellhouse & Davidson (1978). A large number of animals may become actively infected and continue to excrete for several months. Environmental contamination by such animals probably accounts for the large number of animals that occasionally excrete passively. Herd 1 was cleared of infection in 1980–1 by removal of 'persistent excretors' and this prevented a second outbreak amongst the calves.

Carriers were detected only by bacteriological examination and, unfortunately, this would not be feasible for most outbreaks, although counts on faecal samples could occasionally be useful to distinguish 'persistent excretors' (which usually excrete more than 10^5/g) from 'passive excretors'.

Bacteriological examination of cattle at calving has been advocated to identify 'latent carriers' (Richardson, 1973, 1975; Osborne *et al.* 1977; Counter & Gibson, 1980). Cattle in this study were more frequently infected at parturition but this was also associated with excretion for several weeks preceding calving. 'Persistent excretors' identified in 1980–1 did not re-excrete at calving in 1981–2.

There was little disease in adult animals and, apart from one that aborted and one that died of acute salmonellosis, was often associated with concurrent disease such as milk fever or ketosis.

Infection in calves. Calves were infected at birth or acquired infection soon after birth. They were born in barns on deep litter and remained in this heavily contaminated environment for several hours before transfer to single pens. The use of open-sided (herd 1) or solid-sided (herd 2) pens did not appear to limit infection, which spread to all but two calves born during the outbreak, but the rate of spread was reduced by solid-sided pens. Hygienic measures to prevent cross-infection similarly were not successful, possibly due to infected starlings that perched on the partitions between pens. At farm 1, particularly, starlings were often infected, especially in the first few months of the outbreak. They may have been important in the spread of infection within herds, rather than between herds. Other animals not closely associated with infected premises did not become infected.

Infected calves usually excreted large numbers of organisms (up to 10^8/g of faeces), even without apparent signs of disease, and the environment of the calf houses was heavily contaminated. This was reduced by treating with antibiotic at the first sign of disease, thus dramatically reducing the number of organisms excreted.

Although a few calves excreted for many months, infection declined rapidly once the influx of susceptible calves ceased at the end of the first calving season. In herd 2, periods of excretion were shorter and no calves died, compared to 20 % mortality in herd 1, probably because the density of calves was less. The reason why bull calves excreted the organism for longer and why infections in males were more often fatal is not clear. Most bull calves were removed from both herds for experimental vaccination (Aitken *et al.* 1983) and this reduction in stocking density eased problems of congestion caused by reluctance to sell calves. On farms with less-enlightened management this might not have happened.

Infection during the 1981–2 *calving season.* The infection in herd 2 a year after the original outbreak was initiated by one calf from which an infected meconium swab was obtained at birth, even though the dam was not infected. Although infection spread from this animal to other calves the rate and extent of spread was not as great as in the preceding year. This may have been due to acquired resistance or to a reduction of environmental contamination, by treatment with antibiotic and early removal of susceptibles. Although the calf unit was infected there was no spread to adults due either to the isolation measures introduced or a lack of susceptibles.

Serological results at calving revealed only one animal with a titre considered to be indicative of recent infection (Hall *et al.* 1978). 'In utero' infections of calves could not be demonstrated by serology.

Control measures. During the course of this outbreak many methods to control infections and reduce mortality were attempted. The use of an autogenous vaccine and antibiotics have been described elsewhere (Aitken *et al.* 1983). Other measures included restriction of movement of stock and personnel, the use of impermeable clothing and disinfectant sprays, removal of predisposing factors such as dietary imbalance, reduction of stocking densities, segregation of susceptible animals,

prompt treatment of sick animals and removal of 'persistent excretors'. It is difficult to speculate on the effect of individual methods, but they were all designed to reduce the degree of contamination of the environment. The latter is caused primarily by the faeces of 'persistent excretors' from which calves may be infected either at birth, particularly when communal calving facilities are used, or when housed in the same buildings as adults. Once infected, calves excrete such large numbers of organisms that less than 1 g of faeces may contain a lethal dose (Aitken & Jones, unpublished observations). Thus any measure that reduces environmental contamination may help to break the cycle of infection.

Methods which appeared particularly successful were prompt antibiotic treatment of sick calves, removal of adult carriers and reduction in stocking densities or segregation of susceptible animals.

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