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Experimental *Salmonella* infection in calves. 2. Virulence and the spread of infection

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SUMMARY

Calves with a serum 'H' titre of 1/160 developed a chronic form of salmonellosis after infection with *S. dublin*. Their growth rate was severely retarded by this illness. An aerogenic strain of *S. dublin* was found to be much more virulent than an anaerogenic strain. Faecal samples were superior to mouth swabs in detecting animals carrying *S. dublin*.

During transport two uninoculated calves became infected and after transport stress, latent carriers of *S. dublin* with faecal samples negative for *Salmonella* for 5 weeks, restarted excretion. At slaughter *Salmonella* were isolated from viscera and organs and from the surface of five of six carcasses.

INTRODUCTION

It is generally accepted that *Salmonella* infections are commonly transmitted via the mouth and the alimentary system (Gibson, 1965). Infected droplets may also play a part in the spread of animal salmonellosis (Taylor, 1965). Tannock & Smith (1971*a*, *b*) induced a carrier state in mice and sheep by intranasal inoculations. This fact, and the report of possible airborne transmission of *S. abony* in a hospital ward (Stankovic, Barac, Rkman & Lupi, 1971), support the possibility of airborne spread of infection.

The virulence of the bacteria is also important in the spread of salmonellosis. Walton & Lewis (1971) observed that anaerogenic strains of *S. dublin* are less virulent for mice than aerogenic strains.

Experimental salmonellosis was studied in four calves penned together, two inoculated with an aerogenic strain of *S. dublin* and two with an anaerogenic strain. The effects of transport stress on latent carriers was also studied, together with cross-contamination of uninoculated controls.

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MATERIALS AND METHODS

Animals

Four Guernsey calves, 1 week old, were purchased from two different farms. None of them had been vaccinated against *Salmonella*. Faecal samples were examined daily for 1 week to exclude the possibility of a natural infection. Although the farms had no history of *Salmonella* infections, all four calves had a serum 'H' agglutinin titre of 1/160 against *S. dublin*. In spite of this, it was decided to use the calves and study the effect of this possible protection on an experimental infection. All four calves were penned together and maintained at a temperature of 14–16° C. After inoculation they were kept until faecal samples had been negative for *Salmonella* for at least 5 weeks. Towards the end of the experiment two new calves of the same breed were introduced to study the effect of cross-contamination.

Bacteriology and serology

The four calves were inoculated orally with *S. dublin* at an age of 2 weeks. Animals 410 and 411 were inoculated with $1{\cdot}2 \times 10^8$ viable cells of an anaerogenic strain (W) of *S. dublin* (WM 0023, of bovine origin, kindly provided by Dr J. Walton, University of Liverpool) resistant to nalidixic acid. Calves 412 and 561 were inoculated with $1{\cdot}2 \times 10^8$ viable cells of an aerogenic strain (L) of *S. dublin* (06/8 B2, kindly provided by Dr A. H. Linton, University of Bristol), isolated from an outbreak of salmonellosis in calves and resistant to neomycin, tetracycline and ampicillin. Faecal samples taken as described in the previous paper and, from the 5th day, mouth swabs, were taken daily and examined bacteriologically by the methods described by Grønstøl, Osborne & Pethiyagoda (1974), except that tetrathionate broth (Muller-Kaufmann modification) (TB) was used in parallel with selenite broth (SB) for enrichment. When blood samples were taken for serology, the blood clot was examined for *Salmonella* by incubation in brain, heart infusion broth and subculturing on bismuth sulphite agar and brilliant green agar.

Because of the high 'H' agglutinin titres in the sera of the calves faecal samples from the dams were examined bacteriologically for evidence of *Salmonella* infection, but they were all negative.

Post-mortem samples and sera were examined using the materials and methods described by Grønstøl *et al.* (1974).

RESULTS AND DISCUSSION

After inoculation, all four calves developed symptoms of the chronic form of salmonellosis. Temperatures did not rise above 103° F. except in calf 410, where the temperature reached 105° F. the third day after inoculation. They all excreted small numbers of *Salmonella* in their faeces, rarely exceeding 10^3 bacteria/g. Diarrhoea persisted for more than 2 weeks in a more or less severe form and, despite feeding reasonably well, the calves put on little weight and were severely under weight at slaughter.

Table 1. *Changes in the 'H'-agglutinin titres in the sera of calves following oral infection with* $1{\cdot}2 \times 10^8$ *cells of* S. dublin

Days after inoculation	Inoculated calves				Control calves	
	410	411	412	561	1261	1262
0	1/160	1/160	1/160	1/160	.	.
2	1/160	1/80	1/320	1/160	.	.
13	1/320	1/160	1/320	1/160	.	.
19	1/160	1/160	1/160	1/320	.	.
26	1/320	1/80	1/160	1/40	.	.
35	1/160	1/80	1/160	1/80	.	.
40	1/20	0	−*	.	.	1/80
63	1/20	0	0	1/160	0	0

* Not examined.

Table 2. *'H'-agglutinin titres in the dams of experimental calves*

Mother of calf no.					
Inoculated calves				Control calves	
410	411	412	561	1261	1262
1/80	1/160	1/160	1/80	0	1/160

The development of chronic salmonellosis rather than an acute form was probably caused by the calves being protected to some degree by humoral antibodies. The 'H' titres throughout the experiment are shown in Table 1. Because of the high 'H' titres in the calves at the start of the experiment, sera from their dams were examined approximately $2\frac{1}{2}$ months after parturition (Table 2).

Rankin & Taylor (1970) found a fourfold increase of antibodies in the udder compared with serum, and the antibodies could readily be transferred to calves through colostrum during the first hours after birth. They also found that calves having a serum 'H' titre of 1/128 or more did not develop a bacteraemia when challenged with 10^7 cells of *S. typhimurium*, although they developed severe diarrhoea. This agrees with our findings for *S. dublin* and implies that if calves with passive immunity become infected, they are more likely to contract chronic salmonellosis, which will considerably retard their growth rate.

For 3 days after inoculation, both the anaerogenic (W) and the aerogenic (L) strains were isolated from faeces of all four animals on one or more occasions, and on the third day strain L was isolated from the blood of calf 410, which had been inoculated with strain W. After the third day, only strain L was isolated from all four calves. More faecal samples than mouth swabs were found to be positive for *Salmonella* (Table 3). Faecal samples from calves 410 and 411, infected with strain W, were negative for *Salmonella* after 1 week, but the other two continued to show the presence of the organism for 18 and 29 days respectively. Calves 410 and 411 did, however, show positive mouth swabs on days 18 (410) and 25, 28 and 29 (411) respectively, suggesting they had been licking material contaminated by calves 412 and 561.

Table 3. *Recovery of* S. dublin *from faecal samples and mouth swabs of calves after oral infection by* $1{\cdot}2 \times 10^8$ *cells of* S. dublin

	Calves inoculated with anaerogenic strain W				Calves inoculated with aerogenic strain L			
	410		411		412		561	
Days after inoculation	Mouth swab	Faeces	Mouth swab	Faeces	Mouth swab	Faeces	Mouth swab	Faeces
1	.	W & L	.	W & L	.	–	.	W & L
2	.	–	.	–	.	W & L	–	W & L
3	.	W & L	.	W & L	.	W & L	.	–
4	.	–	.	L	.	L	.	–
5	–	L	–	–	L	L	–	–
6	–	L	–	L	L	L	–	–
7	–	–	–	–	L	–	–	–
8	–	–	–	–	L	L	L	L
9	–	–	–	–	L	L	L	L
10	–	–	–	–	L	L	–	L
11	–	–	–	–	L	L	–	L
12	–	–	–	–	L	L	L	L
13	–	–	–	–	L	L	–	L
14	–	–	–	–	–	L	–	–
15	–	–	–	–	L	L	–	L
16	–	–	–	–	L	L	–	–
17	–	–	–	–	–	L	–	–
18	L	–	–	–	–	L	–	L
19	–	–	–	–	–	L	–	–
20	–	–	–	–	–	L	–	–
21	–	–	–	–	–	L	–	–
22	–	–	–	–	–	L	–	–
23	–	–	–	–	–	L	–	–
24	–	–	–	–	–	L	–	–
25	–	–	L	–	–	L	–	–
26	–	–	–	–	–	L	–	–
27	–	–	–	–	–	L	–	–
28	–	–	L	–	–	L	–	–
29	–	–	L	–	–	L	–	–

There was a great difference in the virulence of the two strains of *S. dublin*, i.e. in their ability to invade, multiply and persist within an animal. The only blood sample positive for *S. dublin* came from calf 410 on the third day after inoculation, when its temperature rose to 105° F., and strain L was isolated in spite of the calf being inoculated with strain W.

Because the calves excreted a mixture of the two strains on the day after inoculation, it is assumed that cross-contamination occurred, although the possibility of contamination of the anus of one calf by the faeces of another cannot be completely ruled out. Strain L persisted much longer than strain W in all four calves; beyond the third day after inoculation, only strain L was isolated from faecal samples, mouth swabs and post-mortem samples. Considering only strain L, calves 412 and 561 were infected with $1{\cdot}2 \times 10^8$ cells, whereas calves 410 and 411 were infected by cross-contamination. Although it is impossible to assess the

Table 4. *Isolations of* S. dublin *from post-mortem samples of calves infected orally with* $1{\cdot}2 \times 10^8$ *cells* S. dublin

	Inoculated calves				Control calves	
Sample	410	411	412	561	1261	1262
Swab of outside of carcass	+	−	+	+	+	+
Swab of inside of carcass	−	−	−	−	−	−
Muscle from forelimb	−	−	−	−	−	−
Muscle from hindlimb	−	−	−	−	−	−
Pool of head lymph nodes	+	−	−	−	−	−
Mesenteric lymph nodes	−	−	−	−	+	+
Hepatic lymph nodes	−	−	−	−	−	−
Parotid salivary gland	+	−	−	−	−	−
Liver	−	−	−	−	−	−
Spleen	−	−	−	−	−	−
Lung	−	−	−	−	−	−
Caecum	−	+	+	+	+	+
Small intestine	+	−	−	+	−	+
Gall bladder	−	−	+	+	−	−

cross-infective dose, it is likely to have been smaller than the inoculum of 10^8 cells. The difference in the duration of excretion in the two sets of calves (Table 3) may reflect the difference in size of infective doses, as reported by de Jong & Ekdahl (1965).

Samples of feed, water and flies from the pen were examined bacteriologically during the experiment, but all were negative for *Salmonella*.

To test the effect of stress during transport, 5 weeks after the last occasion on which *Salmonella* had been demonstrated in faecal samples the calves were transported for 7 hr. together with two uninoculated calves, but separated from them by a double partition. The following day, three of four inoculated calves and both uninoculated control calves were positive for *Salmonella* from faecal samples.

Thirty-six hours after transportation all six calves were slaughtered and examined bacteriologically (Table 4). All calves except 411 became contaminated on the surface of their carcasses during the slaughter operations. Salmonellas were also found in the intestines, the mesenteric lymph nodes, or the gall bladder of one or more animals; caecum samples from all but calf 410 were positive. In calf 410 salmonellas were isolated from the parotid salivary gland and from a pool of lymph nodes from the head. This calf had been eager to lick the other calves and possibly for that reason carried *Salmonella* in these organs, although mouth swabs were positive on only one occasion. After refrigerated storage of the carcasses at 4° C. for 3 days, none was positive for *Salmonella* on the surface.

The finding of *Salmonella* in viscera and organs of all the calves and in the faeces of three out of four indicated that at least one, but probably more than one, of the four inoculated calves were latent carriers of strain L, and the stress of transport restarted its excretion. In contrast, in an earlier experiment with inoculated calves, all but one of which had cleared the infection before being moved (Grønstøl *et al.* 1974), transport did not restart excretion. In that experiment, the

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calves produced an acute response to infection, whereas in the current one they developed chronic salmonellosis. Though agglutination tests failed to confirm the possibility, the first group of calves may have developed a higher titre of immunizing antibody.

In general, the results clearly confirm the greater virulence of aerogenic strains in meat animals as well as mice (Walton & Lewis, 1971); a fact explaining the failure of up to 10^{10} cells of Walton's anaerogenic strain (W) to kill sheep or cause extensive cross-infection (Kitchell & Leach, 1973).

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