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Salmonella dublin infection of calves: use of small doses to simulate natural infection on the farm

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SUMMARY

Small numbers of *Salmonella dublin* were used to infect calves in an attempt to simulate natural infection on the farm.

Twenty calves were exposed to *S. dublin* by one or more of the following methods:

Sucking cows which were excreting *S. dublin* in their faeces ($\leqslant 10^2$–10^5 organisms/g).

Housing on *S. dublin* contaminated bedding.

Drinking *S. dublin* contaminated water (10^2–10^4 organisms/ml). During this experiment some calves were given therapeutic doses of oxytetracycline.

After exposure the calves were examined for faecal excretion of *S. dublin* (in some instances mouth swabs and blood samples were also examined) and for clinical signs of illness. Most of the calves became infected with *S. dublin* but excretion was usually sporadic and the numbers of salmonellas excreted were small. No clinical signs of salmonellosis were observed by *S. dublin* was isolated from one calf at *post-mortem*.

Another six calves, dosed orally with either 10^6 or 10^8 *S. dublin*, showed signs of mild illness and although three calves had diarrhoea excretion of salmonellas was intermittent. *S. dublin* was isolated from one of these calves at *post-mortem*.

INTRODUCTION

Experimental reproduction of salmonellosis in calves is usually achieved by the oral inoculation of large doses of salmonellas which are unlikely to bear relationship to the smaller doses which presumably cause disease under farm conditions. To consistently produce *Salmonella dublin* infection by the oral route, it has been necessary to use doses of 10^7–10^{10} organisms (Smith & Jones, 1967; Nazer & Osborne, 1977) and 10^6–10^{10} organisms in the case of *S. typhimurium* (Rankin & Taylor, 1966; DeJong & Ekdahl, 1965; Wray & Sojka, 1978).

In attempts to produce the disease with smaller doses of salmonella, routes other than the oral route have been used. Nazer & Osborne (1977) found that 10^4 organisms administered into the lumen of the duodenum produced severe disease, whereas larger doses were necessary when the nasal and intratracheal routes were used. Similarly, Forbes, Oakley & MacKenzie (1977) used intravenous inoculation to produce more consistent results because oral inoculation had given variable results.

The purpose of the present experiments was to investigate *S. dublin* infection by methods which may be considered more likely to occur on the farm. In some cases, young calves were suckled by cows which were active *S. dublin* excreters and in other cases calves were placed in a contaminated environment. The calves were then examined daily both bacteriologically and clinically for evidence of salmonella infection.

MATERIALS AND METHODS

Experimental animals

Calves with S. dublin *infected cows*

Three Friesian cows, which were naturally infected with *S. dublin* and excreting the organisms daily in their faeces were allowed to suckle calves less than one week of age.

Cow A arrived at the laboratory 2 weeks after a normal calving and suckled three colostrum-fed calves (1, 2 and 3) for a period of 98 days.

Cow B calved a normal calf (4) which was allowed to suck and in addition two colostrum-deprived calves (5 and 6) were set on 2 and 3 days after parturition. The calves were suckled for 60 days.

Cow C had recovered from dysentery caused by *S. dublin* and excreted the organism for 30 days after arrival at the laboratory. She suckled two colostrum-fed calves (7 and 8) for 45 days during which *S. dublin* was isolated from her faeces for 24 days.

Calves housed in buildings contaminated with S. dublin

Faeces and bedding from the above cows A and B were allowed to accumulate for two weeks in loose boxes. A group of four Friesian calves (9, 10, 11 and 12), 1–2 weeks old, was housed in a contaminated box for 50 days and another group of three yearling Friesian calves (13, 14 and 15) was similarly housed for 22 days. *S. dublin* was isolated from the bedding for 3 weeks.

Calves given water contaminated with S. dublin

Three groups of Friesian calves were given drinking water contaminated daily with *S. dublin* infected faeces so that the number of salmonellas ranged from 10^2 to 10^3/ml. The groups, which consisted of two 7-week-old calves (7 and 8), five 3- to 4-month-old calves (1, 2, 3, 7 and 8) and three calves (13, 14 and 15) older than a year were exposed for 70, 18 and 75 days respectively.

In later experiments, three 6-month-old calves (16, 17 and 19) were treated with therapeutic doses of oxytetracycline (Pfizer Ltd) after access to *S. dublin* contaminated water. Two calves (18 and 20) remained untreated. The antibiotic was given intramuscularly and then orally 3 and 15 days respectively after access to the contaminated water. A month later these calves were given oral oxytetracycline 1 and 7 days after access to water to which a peptone broth culture of *S. dublin* was added to obtain a concentration of approximately 10^4 organisms/ml.

Experimental oral infection

After overnight starvation, six calves, 1–2 weeks old, were dosed orally with 50 ml peptone broth that contained the required number of *S. dublin*. The culture of *S. dublin* had been isolated from the faeces of cow B. Three calves (21, 22 and 23) received a dose of 10^8 organisms and the other three calves (24, 25 and 26) received 10^6 organisms.

All the young calves in the above experiments received 4 pints of sterilized milk twice a day and were weaned at 7–8 weeks onto a diet of hay, concentrates and water.

Examination of the calves

All calves were examined clinically and body temperatures were recorded before the experiment started. Freedom from salmonella infection was confirmed by bacteriological examination of faeces on arrival at the laboratory and before beginning the experiment.

In all experiments, clinical examination of calves and faeces collection were carried out on average 4–5 times a week. In three experiments, saliva swabs and heparinized blood samples were collected at frequent intervals. All calves were examined *post-mortem* for the presence of pathological lesions.

Bacteriological examination of specimens for salmonella

Faecal samples and contents from the gastro-intestinal tract (rumen, abomasum, small and large intestine and bile) were cultured on modified brilliant-green agar (Oxoid CM329) by direct plating and after enrichment in selenite broth for 24 h at 37 °C. The plates were incubated for 24 h at 37 °C and then examined for the presence of salmonellas.

At post-mortem examination 1–2 gm portions of the following organs (representative number of mesenteric and hepatic lymph nodes, liver, spleen, kidney, lungs, tonsils and parotid gland) were macerated in nutrient broth with a Colworth Stomacher and 1 ml samples treated as above. One ml of heparinized blood was inoculated into nutrient broth, incubated for 24 h at 37 °C and plated on brilliant-green agar.

The *S. dublin* serum agglutination test (SAT) using the somatic (O '9, 12') and flagellar (H 'gp') antigens was performed as described by Wray, Morris & Sojka (1975). In one experiment, the faeces of calves 4, 5 and 6 sucking cow B were examined during episodes of diarrhoea for Rotavirus by electron-microscopy (Wray *et al.* 1981) and campylobacters by the methods described by Lander & Gill (1980).

RESULTS

Calves with S. dublin *infected cows*

Of the eight calves in the three groups studied, *S. dublin* infection occurred in the three calves with cow A and one (4) of the calves with cow B (Table 1). *S. dublin* was isolated from these calves only by enrichment techniques.

Infection was detected in calves 1 and 2 at 30 and 20 days respectively, all three calves suckled by cow A were excreting *S. dublin* around days 40–47 and days 62–69. With the exception of a three day period observed in calf 1, salmonella excretion

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Table 1. *Isolation of* S. dublin *from calves in contact with* S. dublin *infected cows*

Cow	No. of *S. dublin* /1 g faeces (range)	Duration of experiment (days)	Calf	Colostral antibody status	Day after exposure to infection on which *S. dublin* isolated from: Faeces	Saliva	Blood	SAT titre at end of experiment (O '9, 12' H 'gp')	
A	10^3–10^5	98	1	+++	30, 43, 46, 48, 62, 67, 68, 69, 75, 77	—	—	—	1/10
			2	+++	20, 40, 69, 77, 88	—	—	—	—
			3	+++	47, 48, 62, 68, 85	—	—	—	—
			No. of samples		60	40	15		
B	10^3–10^4	60	4*	+++	1, 2, 4, 5	—	ND	1/10	1/640
			5	—	—	—	ND	—	1/10
			6	—	—	—	ND	—	—
			No. of samples		30	30			
C	$\leqslant 10^2$	45†	7	+++	—	—	—	—	1/20
			8	+++	—	—	—	—	—
			No. of samples		27	20	12		

* Cow B's calf; † *S. dublin* isolated from cow's faeces for 24 days; ND denotes not done.

was intermittent. Calf 4 was infected with *S. dublin* during its first week of life but the two colostrum-deprived calves, 5 and 6, which were also in contact were not infected. Cow C excreted small numbers of *S. dublin* and ceased to excrete salmonellas 24 days after the experiment commenced but neither of the calves being suckled became infected.

Salmonellas were not isolated from the oral swabs and blood samples and none of the calves had clinical signs suggestive of salmonellosis. Rotavirus was detected in calf 5 and *Campylobacter jejuni* in calf 6 during episodes of diarrhoea.

At the end of the experiments one calf (4) had an elevated *S. dublin* flagellar titre, the titres in the other calves remained low.

Calves housed in S. dublin *contaminated buildings*

In the first experiment of 50 days duration, three (9, 10 and 11) of the four calves became infected with *S. dublin* (Table 2) which was isolated from the faeces of two calves (9 and 11) on two occasions and once from the faeces of the other calf (10). With the exception of a sample from calf 9, which contained 10^2 salmonellas/g faeces, salmonellas were detected in the other faeces samples only by enrichment methods. Calf 9 was infected early in the experiment excreting *S. dublin* on days 1 and 4 (Table 2), whereas the other two calves were infected later.

S. dublin was isolated once from the blood of calf 11 before being isolated from the faeces. Enrichment cultures of the mouth swabs from calves 9 and 10 yielded *S. dublin* on five and three occasions respectively. At *post-mortem* examination, *S. dublin* was isolated from the hepatic lymph nodes of calf 12, although the organism had not been isolated during life. Calf 9 showed an elevated *S. dublin* 'H' titre, whereas agglutinins were not detected in the other 3 calves.

In the second experiment of 22 days duration, salmonellas were not isolated from any of the yearling calves. An elevated *S. dublin* SAT titre was detected in calf 13, which had been vaccinated. At *post-mortem* examination of calf 10, a rumen abscess causing adhesions to the pylorus of the abomasum was found but lesions were not detected in the other calves.

Calves given water contaminated with S. dublin

Of the eight calves in the three groups which were given contaminated drinking water, six calves, 7, 8, 3, 13, 14 and 15 became infected with *S. dublin* (Table 3). Four calves (7, 8, 3 and 13) excreted *S. dublin* on only one occasion. Calf 14 excreted *S. dublin* on 6 days, four of which were consecutive (Table 3). At *post-mortem* examination *S. dublin* was not isolated from any of the calves. In calf 14 lesions of interstitial nephritis were found. SAT titres in all the calves with the exception of 13 which had been vaccinated, were low.

Effect of oxytetracycline on calves drinking water contaminated with S. dublin

In the first experiment when the water was contaminated with *S. dublin* infected faeces, neither the treated nor untreated calves became infected with *S. dublin*.

In the second experiment, the water was contaminated with a culture of *S. dublin* grown in the laboratory and the three treated calves became infected with *S. dublin* as did an untreated calf. *S. dublin* was isolated from the mesenteric lymph nodes and lower small intestine of one of the treated calves at *post-mortem*

Table 2. *Isolation of* S. dublin *from calves placed in* S. dublin *contaminated buildings*

Experiment duration	Age of calves	Calf	Days after exposure to infection on which *S. dublin* isolated from: Faeces	Blood	Saliva	*S. dublin* isolated at PM	SAT titre at end of experiment (O '9, 12' H 'gp')	
50 days	2 weeks	9	1, 4	—	2, 4, 9, 10, 17	—	—	1/80
		10	14	—	4, 17, 30	—	—	—
		11	13, 22	10	—	—	—	—
		12	—	—	—	+	—	—
		No. of samples	45	10	40			
22 days	1 year	13*	—	—	—	ND	1/20	1/640
		14	—	—	—	ND	—	1/10
		15	—	—	—	ND	—	—
		No. of samples	20	10	20			

* Vaccinated with *S. dublin* vaccine (Mellavax, Burroughs Wellcome). ND denotes not done.

Table 3. *Isolation of* S. dublin *from calves given water contaminated with* S. dublin

Group	No. of *S. dublin*/1 ml water* (range)	Duration of experiment in days. In parenthesis No. of samples taken	Calf	Age in weeks	Days after exposure to infection on which *S. dublin* isolated from faeces	*S. dublin* isolation at PM	SAT titre at end of experiment (O '9, 12' H 'gp')	
A	10^2–10^3	70 (57)	7	7	19	ND	—	1/20
			8	7	43	ND	—	—
B	10^2–10^3	18 (15)	1	14	—	—	—	1/20
			2	14	—	—	—	—
			3	14	1	—	—	—
			7	17	—	—	—	1/20
			8	17	—	—	—	—
C	10^2–10^3	75 (43)	13†	55	25	—	1/20	1/160
			14	55	33, 39, 66, 67, 68, 69	—	1/10	1/10
			15	55	25, 74	—	1/20	—

* Contaminated with infected faeces; † Vaccinated *S. dublin* vaccine (Mellavax, Burroughs Wellcome).
ND denotes not done.

Table 4. *Isolation of* S. dublin *from calves infected experimentally*

Calf	Dose *S. dublin*	Days after infection on which *S. dublin* isolated from: Faeces (40)*	Saliva (40)	Blood (20)	*Post-mortem* findings: *S. dublin* isolated	SAT titre at end of experiment (O '9, 12' H 'gp')
21	10^8	4, 8, 9, 10, 14, 15	—	—	—	—
22	10^8	1, 2, 3, 6, 8	2, 4	—	Tonsil Liver Intestine	1/320
23	10^8	7, 8	—	—	—	—
24	10^6	2, 3, 5, 7, 8, 9, 10, 11	7	—	—	1/320
25	10^6	8, 9, 10, 11, 19, 22, 24, 25, 26, 27	10, 17, 21, 24	3, 6, 7, 10	—	1/1280
26	10^6	6	—	—	—	—

* Figure in parenthesis indicates number of samples taken.

examination. Lesions were not detected at *post-mortem* examination and SAT titres were low.

Experimental oral S. dublin *infection*

The calves showed elevated rectal temperatures, 1–3 days after infection and some refused their food. Three calves 22, 23 and 26 had diarrhoea of 3–5 days duration but the clinical signs were mild and did not persist for more than 6 days. All the calves excreted *S. dublin* in their faeces within 1–8 days of infection (Table 4). The number of occasions on which *S. dublin* was isolated varied from once in calf 26, to ten in calf 25. In four samples, 10^4 *S. dublin*/g faeces were detected, although salmonellas were isolated mostly by enrichment. Salmonellas were not detected more than 9 days after infection in three calves (22, 23 and 26) and in the three other calves (21, 24 and 25) salmonellas were isolated 11–27 days after infection.

Saliva swabs from calves 22, 24 and 25 yielded salmonellas on a number of occasions and salmonellas were isolated from blood samples from calf 25 before being isolated from the faeces.

At *post-mortem* examination, *S. dublin* was isolated from calf 22. Three calves (22, 24 and 25) had *S. dublin* flagellar titres of 1/320 and above. A number of small infarcts was found in the kidneys of calves 22 and 24, and in calf 23 extensive interstitial nephritis was found.

DISCUSSION

The object of the present experiments was to simulate the methods by which *S. dublin* infection of cattle might occur under field conditions on a self-contained farm. Some calves were used in more than one experiment but this was considered

valid because under farm conditions animals are likely to be exposed continually to infection and cattle of all ages may be infected. Indeed some calves not infected in the first experiment became infected subsequently.

Three methods were used to simulate natural infection and many calves became infected during the various experiments but the number of salmonellas in their faeces was small and excretion was intermittent and for short periods. None of the calves showed clinical signs of salmonellosis.

Taylor & Burrows (1971) found that calves which grazed pasture to which slurry containing 10^6 *S. dublin*/ml had been applied became infected, but no infection resulted when the contamination rate was reduced to 10^3/ml. Hall & Jones (1978) fed cattle salmonella contaminated sewage sludge, but did not isolate salmonellas from the faeces or carcasses at *post-mortem*. In the present experiments the calves were exposed to numbers of salmonellas not dissimilar to those recorded by Sojka, Thomson & Hudson (1974) during a study of faecal excretion by two *S. dublin* infected cows. Higher counts may occur during clinical disease in adult cattle and counts of 10^6/g faeces were observed by Aitken *et al.* (1978) during experimental *S. dublin* and liver fluke (*Fasciola hepatica*) infection, and in calves Smith & Jones (1967) found faecal counts of 10^7/g faeces. To produce clinical signs of disease in the present experiments, it was necessary to use salmonella doses of 10^6 and 10^8 orally. However, during the initial stages of an outbreak, animals are unlikely to be exposed to such a weight of infection and there seems to be little evidence that salmonella multiply to any extent in the farm environment (Rankin & Taylor, 1969; Wray, 1975; Jones, 1980). Consequently the question arises as to the possible role of other factors in the pathogenesis of bovine salmonellosis, especially factors which will allow the organism to multiply in the intestine.

Although animals of all ages may be infected with *S. dublin*, young animals are more susceptible. However, when eight young calves were placed in contact with *S. dublin* excreters, only one became infected during the first week even though two calves were colostrum-deprived which might have been expected to increase their susceptibility to infection. In another experiment the treatment of some calves with oxytetracycline did not appear to either cure or aggravate *S. dublin* infection. Smith & Jones (1967) noted that experimental disease was more severe in calves on a milk diet than in calves on a diet of concentrates; but in the present experiment suckling calves did not appear to differ from weaned calves with ruminal digestion in their susceptibility to infection.

Experimental oral infection with broth cultures of *S. dublin* were more consistent in producing clinical disease and excretion of salmonella, although it must be pointed out that the dose given was probably higher than the calf could acquire from the contaminated environment. In these calves, salmonella excretion was often intermittent, which is a feature of the natural disease (Gibson, 1965). *S. dublin* was isolated from oral swabs from two calves on days when faecal samples were negative which suggests that mouth swabs may be a useful adjunct for the diagnosis of salmonella infection. Whether the mouth was contaminated from the environment of the calf or indicated infection of the salivary glands could not be determined, although in one calf *S. dublin* was isolated from the tonsils at *post-mortem*.

S. dublin was isolated from the tissues of some calves at autopsy and while calves

are generally considered to recover completely from infection, it is possible that later activation of latent persistent infection may be important in the pathogenesis of salmonellosis in adult animals. The difficulties encountered in the present experiments in producing clinical disease with small numbers of *S. dublin* in naturally contaminated material suggest that other factors may be involved regarding both the organism and the animal.

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