J. Hyg., Camb. (1986), 97, 237–246
Printed in Great Britain,

Salmonellosis in two dairy herds associated with a sewage farm and water reclamation plant

By F. G. CLEGG

Ministry of Agriculture, Fisheries and Foods, Veterinary Investigation Centre, The Elms, College Road, Sutton Bonington, Loughborough, Leicestershire

C. WRAY

Central Veterinary Laboratory, New Haw, Weybridge, Surrey

A. L. DUNCAN and W. T. APPLEYARD

Veterinary Investigation Centre, Sutton Bonington, Loughborough, Leicestershire

(*Received 20 February 1986; accepted 19 March 1986*)

SUMMARY

Two dairy herds, situated on a sewage farm, were monitored for the presence of salmonellas following outbreaks of *Salmonella dublin* infection. In addition an *S. dublin* control scheme, which involved examination of adult animals and calf vaccination, was instigated.

During the period 1975–84, 12 salmonella serotypes and 10 phage types of *S. typhimurium* were isolated from the cattle and their environment although their presence was seldom associated with disease. Two adult *S. dublin* excreters were detected but it was concluded that none of the tests employed to examine the adult animals was sensitive enough. The prevalence of disease in the calves was low and although vaccination may have been beneficial it did not eradicate *S. dublin* infection. Thus *S. dublin* persisted in adults and calves during the 8-year period but its presence was seldom associated with disease. The results are discussed with regards the disease risk to animals from the agricultural use of sewage sludge and the public health aspects.

INTRODUCTION

Salmonella investigations are usually confined to the duration of a specific outbreak and seldom exceed 1–2 years. Adult animals which become infected with *Salmonella dublin* may become persistently infected, whereas the development of the carrier state with other serotypes is rare (Wray & Sojka, 1977). However during a survey of slurry samples from 187 dairy farms, Jones & Matthews (1975) isolated salmonellas from 11% of samples in the absence of clinical disease. Thus it is desirable not only from the veterinary aspects but also from the public health aspects that prolonged studies are undertaken on infected premises to study the relationship between the presence of salmonella and disease.

Following an outbreak of *S. dublin* infection in two dairy herds situated on

sewage farm (site of a water reclamation plant) it was decided to carry out further investigations to study: (*a*) The possible presence of other salmonella serotypes, their relationship to disease and to public health. (*b*) The persistence and diagnosis of *S. dublin* infection on the farm. (*c*) The control of *S. dublin* infection in calves using a live vaccine.

MATERIALS AND METHODS

Farm histories

The two farms, A and B are owned by a Water Authority and lie immediately outside the boundary of a large city. Figure 1 shows that the farms are isolated from other agricultural enterprises as they lie between a main road and a large river.

Following outbreaks of cholera during the 1860s several Acts were made relating to sewage utilization and prevention of pollution of rivers. One such Act, passed in 1876, made it an offence to discharge solids or liquid sewage into an inland water course without first making it inoffensive. Because the river running to the south of the city was a matter of concern, the corporation took possession of the two farms in 1878 and the first sewage was received on Farm B in 1880.

Sewage treatment plant

The soil in this flat valley consists of 30–45 cm of loam over considerable deposits of gravel. It was considered suitable for treatment with sewage as the water percolated through the soil and gravel and after a period in use the land was allowed to dry and the residual solids were ploughed in. Crops were grown including cereals, kale, potatoes and sugar beet, some of which was used for cattle. It was decided to cease potato cultivation, and sugar beet and grass were extended. This was successful but as the volume of sewage increased in the 1920s consideration was given to the construction of a treatment plant which came into operation in 1936. Sludge was dried out and sold for agricultural use. Following post-war development, other conurbations were connected to the city's sewage system and there was a need for expansion. A sludge digestion plant was completed in 1954 and the sludge was used on agricultural land. The modern aeration plant was completed in 1960 and instead of irrigating 800 acres of farm land, a fully treated sewage effluent was discharged. It is calculated that at present 30000 tonnes of air-dried sludge are put on to the land annually. The human population served by the plant is 465000.

Farm A

This farm covers 48 ha and the herd was established in the 1930s. In 1972, *S. dublin* infection was diagnosed in calves and the use of *S. dublin* vaccine (Mellavax, Wellcome) on heifer calves was commenced; bull calves being sold within a few days of birth. As both farms are closed except for the purchase of bulls, the route of infection may have been indirect. Sewage effluent was used for the irrigation of the grass fields by a system of graded pipes. Some of these were broken with the formation of puddles around the cracked or broken pipes.

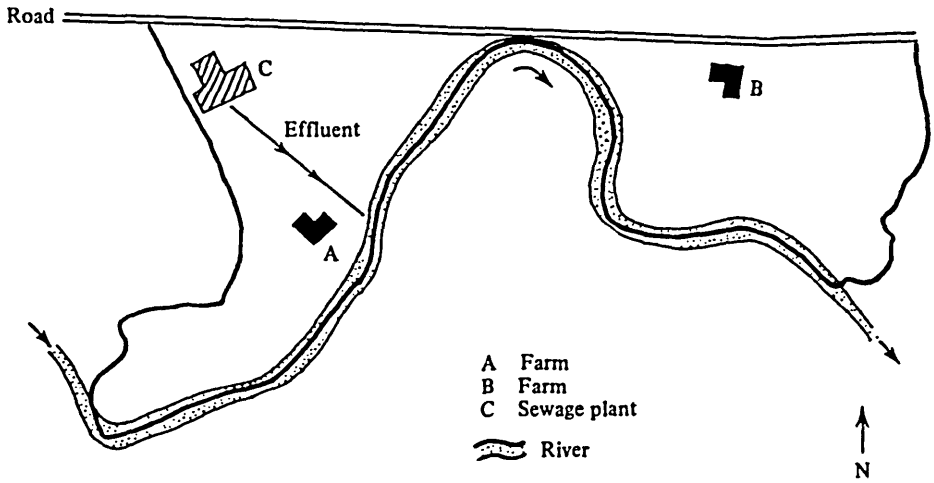

Fig. 1. Diagram of the location of the farms and sewage plant.

Farm B

This farm of 105 ha is adjacent and lies to the east (Fig. 1) and it is run as a separate unit. Effluents have not been used for irrigation and there is no current sludge disposal on this farm. In 1971, *S. dublin* was introduced following the purchase of cattle and an outbreak of *S. dublin* associated abortion occurred in a group of heifers. The use of a live *S. dublin* vaccine was as on Farm A. A pig unit exists on this farm.

Both farms have extensive and adequate cattle buildings with many boxes for the isolation of individual animals. Sparrows around the farm buildings are controlled every 6 months.

The cattle population of both farms is as follows:

	Farm A			Farm B		
	Cows	Heifers	Calves	Cows	Heifers	Calves
1971	215	130	70	110	60	40
1976	220	125	80	200	110	60
1984	150	250	100	275	40	60

Milk from both farms is sold to a local dairy and pasteurized before re-sale.

Sampling

In November 1976 regular sampling commenced in an attempt to control the *S. dublin* infection and to monitor the occurrence of other salmonella serotypes. The procedures which continued until the end of 1984 were as follows: (*a*) Initially the cows were examined by rectal swab sampling. (*b*) Any animal found to be excreting salmonella was placed in isolation and swabbed every 3–4 days. If the animal was found to be excreting *S. dublin* rather than other serotypes a decision would be made about slaughter or disposal. (*c*) As each animal calved rectal and vaginal swabs and milk samples were collected together with a rectal swab from the calf. (*d*) Serological examination of the herd for the estimation of agglutination titres to *S. dublin* and *S. typhimurium* was to be undertaken for 2 years. (*e*) Swabs

https://doi.org/10.1017/S0022172400065323 Published online by Cambridge University Press

were to be laid weekly for 2 days in the drain of the collecting yards of the farms and placed in sterile containers for transport to the laboratory. (*f*) In-line milk filters used on both Sunday evening and Monday morning milkings were collected in sterile containers and transported to the laboratory. (*g*) Examination of the effluent from the sewage plant was to be carried out.

Bacteriological procedures

Rectal and vaginal swabs were incubated in 15 ml selenite F broth (Oxoid CM 395) at 37 °C for 24 h and sub-cultured onto desoxycholate citrate agar (Oxoid CM 35). Non-lactose fermenting colonies were confirmed as salmonella by slide agglutination tests and submitted to the Central Veterinary Laboratory for confirmation and identification. In the case of *S. dublin* isolates these were checked for aggultination in acriflavin and if positive by phages and biochemical tests (Walton & Lewis, 1971) to confirm whether they resembled vaccine strains.

The drain swabs and milk filters were pre-enriched by incubation at 37 °C for 18 h in 250 ml buffered peptone water (CM 509: Oxoid) and 100 ml transferred to double strength selenite broth which was treated as above.

Serum samples

Serum samples were obtained from adult animals within 1–3 days of parturition and a total of 584 animals in the herd were sampled during the first 2 years of the survey. The samples were examined for the presence of agglutinins to *S. dublin* and *S. typhimurium* by the methods described by Wray, Sojka & Callow (1977). The tests were incubated at 50 °C. Flagellar titres were read after 4 h incubation and somatic titres after overnight incubation.

RESULTS

Salmonella isolations

Farm A

The salmonella isolations on Farm A during the period 1976–84 are shown in Table 1.

In August 1976, *S. dublin* was isolated from a calf and further investigations in September revealed the presence of *S. hadar*, *S. typhimurium* (DT204) and *S. dublin* in the calves. At this time a cow died of *S. typhimurium* (DT193) septicaemia and another phage type 204 was also isolated from a cow. The community physician was informed and it was discovered that a cowman was excreting *S. typhimurium* (DT204) and his child *S. hadar*. Both of these organisms were cultured from drain swabs and the same phage type of *S. typhimurium* was also isolated from sparrows on the premises.

Subsequently the only serotypes isolated from cattle were *S. dublin* and *S. typhimurium* (three different phage types); the last serotype was isolated only from the adults. Three different salmonella serotypes which included *S. dublin*, were isolated from the milk filters and six different serotypes from the drains. Of the different *S. typhimurium* phage types detected in the drains, two were isolated concurrently from the cattle.

Table 1. *Farm A – isolation of salmonella from animals and their environment*

Date	Salmonella serotype isolated from: Cows	Calves (no. of calves)	Milk filters	Drains	Other species
1976 Aug.	—	*S. dublin* (×1)	—	—	
Sept.	*S. typhimurium* (193)*	*S. dublin* (×2)	—	*S. hadar*	*S. hadar* (human)
Oct.–Dec.	*S. typhimurium* (204)	*S. hadar* (×3) (×1)	—	*S. typhimurium* (204)	*S. typhimurium* (204)
	S. dublin	*S. typhimurium* (204) (×4)	—	—	(sparrows) (human)
1977 June	*S. dublin*	—	—	—	
Aug.–Nov.	—	*S. dublin* (×5)	*S. typhimurium* (RDNC)†	—	
				—	
1979 Jan.–March	*S. typhimurium* (20)	—	—	*S. typhimurium* (20)	
Nov.	—	—	—	*S. typhimurium* (49)	
1980 Sept.–Dec.	—	*S. dublin* (×5)	—	*S. hadar*	
1981 Jan.–March	*S. dublin*	—	—	—	
	Cow 167–73	*S. dublin* (×14)	*S. typhimurium*	*S. typhimurium* (40)	
	Cow 55–80 (×2)‡	—	—	*S. typhimurium* (44)	
	Cow 477–78	—	—	—	
1982 Oct.	—	*S. dublin* (×1)	—	—	
1983 March. *S. dublin*	—	*S. dublin*	*S. virchow*		
	Cow 431/79	—	—	—	
	Cow 62/78	—	—	—	
	Cow 454/80	—	—	—	
May	*S. typhimurium* (204)	—	—	*S. typhimurium* (204a)	
July	*S. dublin*	*S. dublin* (×3)	—	*S. agona*	
	Cow 432–79	—	—	—	
	Cow 42–78	—	—	—	
	Cow 419–78	—	—	—	
Aug.–Nov.	*S. typhimurium* (49a)	*S. dublin* (×6)	—	—	
1984 May	*S. dublin*	—	—	Group E	
July	Rough *Salmonella*	—	—	*S. typhimurium* (12)	
Sept.–Nov.	—	—	*S. give*	*S. give*	

* Phage type in parentheses.

† Does not conform to a recognizable phage pattern.

‡ Number of isolations.

Table 2. *Farm B – isolation of salmonella from animals and their environment*

		Salmonella dublin isolated from		Serotype of salmonella isolated from		
Date		Identification number of cow (no. of isolations)	Numbers of infected calves (No. clinically ill)	Milk filter	Drains	Other species
1975	Dec.	—	8 (×8)	—	—	
1976	Oct.	—	2	—	—	*S. derby* (pig)
1977	March	—	2†	—	—	
	July	—	—	—	*S. typhimurium* (40)*	
	Aug.–Nov.	29–73 (×6)	5	—	—	
		342–74 (×1)	—	—	—	
		347–74 (×1)	—	—	—	
		316–74 (×2)	—	—	—	
1978	July	—	—	—	—	
	Aug.–Oct.	S325–74 (×1)	5 (×4)	*S. senftenberg*	*S. dublin*	
		61–75 (×5)	—	—	—	
		357–74 (×2)	—	—	—	
		347–74 (×1)	—	—	—	
		85–75 (×1)	—	—	—	
1979	Jan.	—	—	—	*S. typhimurium*	
	Nov.	—	—	—	*S. dublin*	
1980	Jan.	—	2†	—	—	
	Dec.	—	5 (×5)	*S. dublin*	—	
1981	Jan.	—	2 (×2)	—	*S. virchow*	
	Oct.	—	—	—	—	
1982	July	—	—	—	*S. hadar*	
	Nov.	—	—	—	*S. typhimurium*	
1983	March	—	1	—	—	
	June–July	62–78 (×1)	—	*S. typhimurium* (204)	*S. typhimurium* (204)	
		—	—	—	*S. saint-paul*	
		—	—	—	*S. panama*	
		—	—	—	*S. virchow*	
	Sept.	—	1 (×1)	—	—	
	Dec.	—	—	—	*S. bredeney*	
1984	Jan.–Feb.	—	—	—	S. virchow	
	April	—	1†	—	—	
	July	—	—	—	*S. mbandaka*	
	Oct.–Nov.	—	—	—	*S. typhimurium* (110)	

* Phage type.
† Vaccine-like strain of *S. dublin*.

Table 3. *Serological findings in the cattle*

	Number of cows with serum agglutination titre in the range of								
	Somatic			Flagellar phase one			Flagellar phase two		
S. dublin ...	0–20	40 'O' 9 antigen	80	0–40	80–160 H 'gp' antigen	320	0–40	80–160	320
Cows	576	22	8	578	26	2			
Aborting heifers 1973	20	6	5	29	2	—			
Cows from which *S. dublin* isolated	7	3	1	9	2	–			
S. typhimurium		'O' 4, 5 antigen			'H' 'i' antigen			'H' 1, 2 antigen	
	528	—	—	501	26	1	517	11	—

Farm B

The salmonella isolations on Farm B during the period 1976–84 are shown in Table 2. *S. dublin* was the only serotype isolated from cattle and two cows, 61–75 and 29–73 (Table 2) were persistent excreters and culled. Three different salmonella serotypes, which included *S. dublin*, were isolated from the milk filters and eight different salmonella serotypes, which included three different *S. typhimurium* phage types were isolated from drain swabs.

Serological findings

The results of the serological investigation are shown in Table 3. Five of the original group of heifers, in which the initial *S. dublin* infection occurred had somatic *S. dublin* titres of 80 and above. In contrast, only one of the *S. dublin* infected cows and eight animals, from which *S. dublin* was not isolated had somatic titres of 80 and above. Flagellar ('gp') titres of greater than 160 were found only in two *S. dublin* free cows.

None of the animals had *S. typhimurium* somatic agglutinin titres greater than 20 and only one had a flagellar titre of 320.

Clinical salmonellosis

Farm A

A cow died of *S. typhimurium* septicaemia in 1976 and later in the year *S. dublin* was isolated from an aborted fetus. During 1980 and 1983 clinical *S. dublin* infection was diagnosed in 4 and 9 calves respectively; disease during the latter period occurred when unvaccinated male calves were mixed with vaccinated females.

Farm B

Clinical *S. dublin* infection of calves occurred during 1978, 1980 and 1983 when 4, 7 and 1 calf respectively were ill. Four of the calves died.

Samonellae in the effluent from the water reclamation plant (sewage plant)

Sampling was carried out twice, in February and March 1978 and the following serotypes were isolated:

February 1978 – *S. agona, brandenberg, bredeney, oranienburg, panama, paratyphi B, typhimurium, heidelberg.*

March 1978 – *S. agona, brandenberg, hadar, livingstone, newport, oranienburg, panama, paratyphi B, senftenberg, virchow.*

DISCUSSION

On both farms, *S. dublin* persisted over the 9-year period of the survey although the disease incidence was small considering the size of the herds. The adult carrier is believed to play a key role in the perpetuation of infection (Field, 1948), but only two cows were diagnosed as persistent excretors and their removal from the herd did not reduce the incidence of infection. *S. dublin* was isolated from a number of cows at parturition but only one of these animals yielded *S. dublin* the

following year. In this situation it is difficult to decide whether these animals were latent carriers or passive carriers, which merely reflect an infected environment. Frik (1969) suggested that the persistent carrier state in adult cattle follows concurrent infection of *S. dublin* and *Fasciola hepatica* and the absence of persistent excretors may reflect the parasites absence on these farms.

Throughout the period of the investigation, *S. dublin* was isolated from calves every year except 1979. Some isolations coincided chronologically with isolations from adults but on a number of occasions it was isolated only from calves. The question therefore arose as to the likely source of calf infection; on some occasions the calves were probably infected from the cows but on other occasions the calves were probably infected from their environment. Gibson (1961) has shown that *S. dublin* may survive for 10 months in faeces splashed on walls and it is possible that cycles of infection in calves may contribute to the persistence of *S. dublin* on farms.

There was little *S. dublin* disease in the calves but it is not known to what extent the use of vaccination suppressed illness. However, it should be pointed out that one disease outbreak occurred when unvaccinated bull calves were mixed with vaccinated heifer calves. It is suggested earlier that cycles of *S. dublin* infection in the calves may have contributed to its persistence. If this is the case vaccination is more likely to be beneficial than when direct spread from the dam occurs because a longer period for the development of immunity occurs. The investigation did show, however, that vaccination did not eliminate infection and vaccine like strains of *S. dublin* were isolated on a number of occasions.

The diagnosis of *S. dublin* infection in the adult animals presented many problems. Serological tests were of little value in these herds where infection is endemic, and few animals had titres indicative of infection. In herds where recent infection has occurred many animals have high levels of agglutinins to *S. dublin* (Lawson *et al* 1974; Wray, Morris & Sojka, 1975) and it is possible that the low titres observed reflect the decline in titre that occurs with time. Rectal swabs were also of debatable use and the best time to sample animals would appear to be at parturition when *S. dublin* may be excreted by a number of routes. Milk filters and drain swabs were also of value in confirming the presence of the salmonella on the farm. Thus 12 serotypes of salmonella and 10 phage types of *S. typhimurium* were detected during the survey. However, it should be pointed out that *S. dublin* was isolated from milk filters and drain swabs on two occasions each although it persisted on the farms during the 9-year period of the survey. This would suggest that although milk filters and drain swabs are a simple and cheap method of monitoring farms, there are limitations as to their use.

Despite the presence of many different salmonella serotypes on the two farms they were seldom associated with disease or even infection. Indeed on Farm B although a wider range of serotypes was detected in the environment than on Farm A they were never detected in the cattle. This was unexpected because unlike Farm A neither effluent nor sludge was applied to pasture and the question arises as to their likely source. Examination of the effluent on two occasions resulted in the isolation of a number of different salmonella serotypes. Similarly, Harbourne, Thomas & Luery (1978) found a wide variety of salmonella serotypes in sewage effluent which contaminated the water courses and it is likely that Farm B was contaminated from the river. However, one cannot rule out other

possible sources such as spray, birds or vermin. Linklater, Graham & Sharp (1985) monitored sewage sludge for the presence of salmonellas and found little evidence of risk to animals when known contaminated sludge was spread on pasture; provided that the recommended guidelines were followed. Our findings over a period of 7 years add further support to their conclusions; they also confirm and extend those of Jones & Matthews (1975) who demonstrated the presence of salmonellas on a number of dairy farms in the absence of disease.

Although salmonellas were demonstrated in the milk filters from both farms on a number of occasions; the only associated human infection occurred during 1976 when farm personnel consumed raw milk. Such farm outbreaks are not infrequent and difficult to prevent; however it is possible that pasteurization may have prevented community outbreaks associated with the resale of milk from this farm during a period when the national incidence of milk-borne salmonellosis was increasing (Sharp, Paterson & Barrett, 1985).

We wish to acknowledge the help of Mr D. R. Jones, MRCVS who brought the case to our attention, the management and staff of Severn Trent Water Authority farms for their co-operation, the scientific staff of the Veterinary Investigation Centre and Mr J. Coles for maintaining the records.

REFERENCES

FIELD, H. I. (1948). A survey of bovine salmonellosis in mid and west Wales. *Veterinary Journal* **104**, 251–266.

FRIK, J. F. (1969). *Salmonella dublin* – infecties bij runderen in Nederland: epizootiologie en pathogense in verb and met fascioliasis. Doctorate in animal health thesis. Utrecht University.

GIBSON, E. A. (1961). Salmonellosis in calves. *Veterinary Record* **73**, 1284–1295.

HARBOURNE, J. F., THOMAS, G. W. & LUERY, K. W. (1978). Salmonellae in effluent waters and river sites in North Yorkshire. *British Veterinary Journal* **134**, 350–357.

JONES, P. W. & MATTHEWS, P. R. J. (1975). Examination of slurry from cattle for pathogenic bacteria. *Journal of Hygiene* **74**, 57–64.

LAWSON, G. H. K., MCPHERSON, E. A., LAING, A. H. & WOODING, P. (1974). The epidemiology of *Salmonella dublin* infection in a dairy herd. I. Excretion and persistence of the organism. *Journal of Hygiene* **72**, 311–329.

LINKLATER, K. A., GRAHAM, M. M. & SHARP, J. C. M (1985). Salmonellae in sewage sludge and abattoir effluent in south-east Scotland. *Journal of Hygiene* **94**, 301–307.

SHARP, J. C. M., PATERSON, G. M. & BARRETT, NICOLA, J., (1985). Pasteurisation and the control of milkborne infection in Britain. *British Medical Journal* **291**, 463–464.

WALTON, J. R. & LEWIS, L. E. (1971). Preliminary studies of some biochemical properties of enteric and genital isolates of *Salmonella dublin* and a possible correlation with reduced virulence. *Veterinary Record* **89**, 112–113.

WRAY, C. MORRIS, J. A. & SOJKA, W. J. (1975). A comparison of indirect haemagglutination tests and serum agglutination tests for the serological diagnosis of *Salmonella dublin* infection in cattle. *British Veterinary Journal* **131**, 727–737.

WRAY, C. & SOJKA, W. J. (1977). Reviews of the progress of dairy science: bovine salmonellosis. *Journal of Dairy Research* **44**, 383–425.

WRAY, C., SOJKA, W. J. & CALLOW, R. J. (1977). The serological response in cattle to salmonella infection. *British Veterinary Journal* **133**, 25–32.