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# Persistence of *S. typhimurium* in a large dairy herd

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## SUMMARY

*Salmonella typhimurium* 49a infection in a large dairy herd persisted for 3·5 years. Illness initially occurred in cows and calves but latterly although there were fewer clinical cases milk filters were culturally positive on 26 out of 73 samplings. Three associated human disease incidents occurred. Individual milk samples identified one cow as an excreter and the organism was recovered from the mammary gland of this animal at slaughter. Correlation between calving pattern, the times of calving and the occurrence of positive milk filters suggest that the cow may have been excreting the organism intermittently from the udder for 2·5 years.

## INTRODUCTION

In adult cattle, the development of persistent infection following clinical disease is usually associated with particular salmonella serotypes. Persistent *Salmonella dublin* infection is not uncommon, whereas the development of the carrier state with other serotypes is rare (Wray & Sojka, 1977) and investigations of outbreaks associated with these serotypes are confined to the duration of the outbreaks which rarely exceeds 2 years (Clegg *et al.* 1986). These workers also reported that a number of different serotypes may be recovered from milk filters and drain swabs in the absence of disease. Milk is more usually contaminated by infected faeces and salmonella excretion from the udder is uncommon (Wray & Sojka, 1977). Udder excretion of salmonella was reported by Giles & King (1987) and Morisse *et al.* (1984*a*) found salmonellas in 10–60% of aseptically drawn milk samples in herds which had experienced acute outbreaks. Davis (personal communication) reported continuing *S. saint paul* infection by a dairy cow whose mammary lymph node was infected. In tracing the source of cheese contaminated with *S. typhimurium* phage type 10, Ogilvie (1986) cultured the organism from one quarter of a cow which shed salmonellas for 36 days.

This report of herd infection with *S. typhimurium* DT 49a is of interest because of the duration of the infection and the prolonged mammary excretion.

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## FARM HISTORY

The herd numbered 220 in 1983 but declined in numbers to 190 in 1987 and was one of nine dairy herds under the same management where heifer calves were reared centrally and calving heifers were then distributed to the herds. Movement of machinery and staff took place between the farms. Solid manure was spread on arable land adjacent to the dairy herd and slurry was spread on nearby pasture.

There were 109 hectares of grazing and cows were turned out onto leys or permanent pastures in spring and brought into two strawed yards in the autumn. It was difficult to keep the straw yards dry because of dense stocking, poorly sited water bowls and the effluent from the silage clamp situated between the two yards. The calves were housed in converted cowsheds and penned individually before being mixed in small groups. Pooled colostrum was often stored for 1–2 days before being fed to the calves. Milk was pasteurized before sale but originally farm staff had taken raw milk for their own consumption.

## SAMPLING

Following the initial isolation of *S. typhimurium* in 1983, in-contact animals were sampled by rectal swabs followed by herd screening. For 3 months washings from nylon milk filters were cultured and then milk socks (Alfa Laval) were substituted for sampling purposes and these were cultured on a regular basis until March 1987.

## BACTERIOLOGICAL METHODS

The milk socks were placed in 250 ml Muller–Kauffman tetrathionate broth (CM 343 Oxoid, Wade Rd, Basingstoke) and incubated at 43 °C. Subcultures were made at 24 and 48 h onto modified brilliant green agar (CM 329 Oxoid). Individual milk samples were taken from the recording jars and cultured for salmonella on the farm by adding approximately 10 ml of milk to an equal volume of double strength selenite F broth (CM 395 Oxoid) and after 24 h incubation at 37 °C. subculturing the broths onto modified brilliant green agar. Rectal swabs were cultured in single strength selenite broth and subcultured as previously described. Milk samples taken on three occasions from the suspect cow were cultured onto 5% sheep blood agar (CM 271 Oxoid) and McConkey medium (CM 109 Oxoid) as well as the enrichment broth technique described above. Direct cultures were prepared from mammary tissue and lymph nodes. Cultures were also prepared after the tissues had been macerated. One-centimetre cubes of tissue were flamed in methanol and then macerated (Stomacher; Seward Medical, London) in 15 ml sterile buffered peptone water.

Ten millilitres of this homogenate were added to 100 ml of tetrathionate broth which was processed as previously described. Colonies were confirmed as salmonella at the Central Veterinary Laboratory, Weybridge. Phage typing was carried out at the Department of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London.

## INVESTIGATIONS

The sequence of events is summarized in Table 1. At the time of the initial isolation in 1983, two calves and four cows were affected. One of the cows subsequently died. Three children on the farm were ill with abdominal pains and diarrhoea, all had drunk raw milk and stools were culturally positive for *S. typhimurium* (Table 1). In April 1984 positive milk filters and a chronically affected cow prompted a further investigation. The cows were grazing two fields, one field was separated by a single line electric fence from a pond and a ditch which took slurry and effluent from the farm. The pond may have received overflow from the domestic cesspit and was stagnant and overgrown with algae. The ditch flowed with a thick effluent. No slurry had been spread on the fields. Swabs taken from the pond and effluent were negative for salmonella. Rectal swabs taken from the whole herd, however, detected three cows excreting salmonella. Human illness occurred again in September and one child and one adult were found to be excreting the organism.

A further visit in March 1985 followed an outbreak of salmonellosis in calves. There were reported cases of illness in the household and two further human excreters were detected. Another visit in March 1986 followed an outbreak of salmonellosis in calves that had started in October 1985. Sampling showed that salmonella was widespread in the calf environment, including the pooled waste milk stored prior to feeding to calves. Corrective building work had been carried out and although there was no seepage from the soakaway chambers the surrounding pasture was wet and a recommendation was made to fence this off. It was again emphasized that raw milk should not be used for human consumption.

Following further positive milk filters a visit was made in August 1986 to take individual milk samples from all 131 cows in milk. Although one positive milk sample was obtained further samples from the same cow (837) were negative. Individual milk sampling was repeated and another cow (832) was identified, which was also positive on three further occasions. This animal was slaughtered and the udder and mammary lymph nodes were cultured. In August 1986 two rinses of the milking equipment were cultured for salmonella.

## RESULTS

The isolations of *S. typhimurium* 49a made from rectal swabs taken from cows and calves on this farm during the period 1983–87 are shown in Table 2. During the same period 73 milk filters were cultured of which 26 were positive for *S. typhimurium 49a* (Table 3). The 26 isolations were made from filters cultured during the lactations of cow 832. During these lactations 35 filters were cultured with negative results as were 12 filters collected when cow 832 was not in lactation.

Individual milk samples were cultured on 12 occasions during 1985 and 1986. *S. typhimurium 49a* was recovered from one cow each time the whole herd was sampled and on two further occasions from cow 832 (Table 1). The correlation with the lactations of cow 832 is shown in Table 3. Salmonellas were not recovered from the milking equipment rinses.

*S. typhimurium* was recovered from one of the four areas of udder tissue

Table 1. *Sequence of events: isolations of* S. typhimurium *49a from various sources*

| | 1983 | | | | 1984 | | | | | | 1985 | | | | | | | 1986 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month... | 4 | 5 | 6 | 7 | 4 | 5 | 6 | 7 | 8 | 9 | 2 | 3 | 5 | 6 | 8 | 11 | 12 | 2 | 7 | 8 | 9 | 10 |
| | Source | | | | | | | | | | | | | | | | | | | | | |
| Milk filters | — | — | — | — | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | — | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 |
| Cow rectal swabs | 3 | 27 | 7 | 1 | 1 | 4 | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — |
| Calf rectal swabs | 1 | 1 | 4 | — | 4 | — | — | — | — | — | — | 4 | 1 | — | — | — | — | — | — | — | — | — |
| Human | 3 | — | — | — | — | — | — | — | — | 2 | — | 2 | — | — | — | — | — | — | — | — | — | — |
| Individual milk samples | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | 3 |

Table 2. *Results of rectal swab culture (number cultured (number positive) for* S. typhimurium *49a)*

| | 1983 | | | | 1984 | | | 1985 | | | 1986 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month... | 4 | 5 | 6 | 7 | 3 | 4 | 5 | 3 | 4 | 5 | 3 | 4 | 8 | 9 | 10 |
| Rectal swabs | 3 | 445 | 1201 | 38 | 3 | 10 | 454 | 32 | 1 | 0 | 38 | 0 | 268 | 43 | 2 |
| cows | (3) | (27) | (7) | (1) | — | (1) | (4) | — | — | — | — | — | (1) | — | — |
| Rectal swabs | 1 | 1 | 54 | 0 | 0 | 0 | 0 | 10 | 0 | 5 | 2 | 12 | 0 | 0 | 0 |
| calves | (1) | (1) | (4) | — | — | — | — | (4) | — | (1) | — | — | — | — | — |

Table 3. *Milk filters examined (and culture positive) for* S. typhimurium *49a and the duration of the lactations of cow 832* (⊢---⊣)

| Month | 1983 | 1984 | 1985 | 1986 | 1987 |
|---|---|---|---|---|---|
| 1 | — | — | 2 | 1 | — |
| 2 | — | 1 | 1 (1) | 2 (1) | 1 |
| 3 | — | 2 | 2 (1) | 1 | 1 |
| 4 | — | 2 (2) | 1 | 2 | — |
| 5 | 2 | 2 (1) | 2 | 2 | — |
| 6 | 6 | 2 (2) | 1 (1) | 2 | — |
| 7 | 3 | 2 (2) | 1 | 1 (1) | — |
| 8 | — | 1 (1) | 2 (1) | 3 (3) | — |
| 9 | — | 2 (1) | 1 | 4 (3) | — |
| 10 | 1 | — | 2 | 4 (3) | — |
| 11 | 1 | 1 | 1 (1) | 1 | — |
| 12 | — | 1 | 2 (1) | 1 | — |

cultured and, on direct culture, from the lymph nodes but only after the latter had been macerated. Histopathology showed a chronic interstitial mastitis.

## DISCUSSION

Mammary excretion of salmonella by cattle is uncommon and most likely to occur following *S. dublin* infection; Osborne and others (1977) isolated the organism from the milk of three of ten *S. dublin* infected cows following caesarian section. Milk may also be contaminated by farm personnel (Galbraith & Pusey, 1984) and consequently farm staff were issued with disposable gloves to prevent contamination of milk filters during their attachment or removal. It was also considered that the milk filters could have been contaminated by inadequately cleaned and sterilized milking equipment as occurred recently in an outbreak of milk-borne *S. braenderup* food poisoning in 1986 (J. C. Bell, personal communication). In that incident salmonellas could still be recovered from the milking equipment when it was no longer possible to isolate the organism from the cows.

The initial outbreak of salmonellosis in the spring of 1983 was unremarkable and typical of many outbreaks encountered where positive milk filters arise from faecal contamination of the milk. Since milk may also be contaminated indirectly from infected water courses and ponds (Harbourne, Thomas & Luery, 1978; Smith, Jones & Watson, 1978, Williams, 1975, Morisse *et al.* 1984*b*) those on the farm were fenced off as a precautionary measure. Salmonellas were not widespread in the environment because no other serotypes or phage types were recovered from the filters; unlike the study of Clegg *et al.* 1986, who isolated four different salmonella serotypes from milk filters during a 6-year survey.

Attempts were made to disinfect the teats before collection of milk samples because the udders were soiled but this proved ineffectual and samples were subsequently obtained from the recording jars. Since salmonellas were not recovered from rectal swabs or from other milk samples, faecal excretion of salmonella did not appear to be widespread and it was considered to be more

efficient to screen the herd rapidly in this way and risk the possibility of false positives from subsequent samples from the same jar. In the event false positives did not occur and salmonellas were not recovered from the subsequent milk samples obtained through the same cluster and jar.

The close correlation between the positive milk filters and the duration of the lactation of cow 832 suggested that this cow or another cow with the same calving pattern may have been the source of infection from April 1984 until October 1986. Positive milk filters were obtained in April 1984 after cow 832 had calved but before clinical cases were seen. Milk filters became positive after this animal calved in 1985 and this was followed by an outbreak of salmonellosis in the calves. During the later stages of the investigation, the herd was divided into a fresh calved group and a late lactation group, milk filters from these groups were cultured separately and the late lactation group which contained cow 832 was positive; milk filters have remained negative since cow 832 was removed from the herd.

The evidence suggests that from April 1984 until October 1986 one cow (832) was responsible for the continuation of the outbreak for this 2·5 year period. Excretion of *S. typhimurium* over such a long period of time is most unusual but the recognition of such an occurrence has important consequences for animal and human health. It is interesting that cow 832 was not one of the cows detected as a rectal excreter in 1983. It is tempting to conclude that the udder became infected during this period when several cows were excreting *S. typhimurium* in their faeces.

After the slaughter of cow 832 salmonellas were recovered from its udder and supramammary lymph node by homogenizing excised cubes of tissue for culture. Using conventional techniques, Ogilvie (1986) failed to isolate salmonella from the mammary tissue or from the lymph nodes of a cow which was producing *S. typhimurium* infected milk.

The associated human infection followed the consumption of raw milk. Advice has previously been given that milk should not be taken for human consumption until at least three consecutive negative milk filters have been obtained. In this outbreak, however, such a recommendation would have re-exposed the consumers to infection on a number of occasions. Salmonellas may survive in colostrum stored at 5–11 °C for up to 32 days (Wray & Callow, 1974) and because pooled colostrum, milk from cows with mastitis and other spoilt milk was fed to calves, large number of calves could have been exposed to infection for long periods.

Since 1983, milk and cream in Scotland has been compulsory pasteurized and no general outbreaks of milk-borne salmonellosis have occurred in humans. A significant number of human cases have, however, continued to occur in farm workers and their families from drinking raw milk, and the Agricultural Wages Board have agreed that if milk is supplied in part payment of wages it must be pasteurized.

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