Epidemiol. Infect. (1990), **105**, 295–305
Printed in Great Britain

The epidemiology of salmonella infection of calves: the role of dealers

C. WRAY[1], N. TODD[2], I. MCLAREN[1], Y. BEEDELL[1] AND B. ROWE[3]

[1] *Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey KT15 3NB*

[2] *MAFF, Veterinary Investigation Centre, Langford, Bristol BS18 7DX*

[3] *Division of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT*

(*Accepted 24 April 1990*)

SUMMARY

Salmonellas were detected in the environment of 10 of the 12 calf dealers' premises studied. The cleaning and disinfection routines were often ineffective and salmonellas were isolated from 7·6% and 5·3% of the wall and floor samples before disinfection and 6·8% and 7·6% afterwards. Eight different salmonella serotypes were detected, of which the commonest were *Salmonella typhimurium*, predominantly phage type DT204C, and *S. dublin*. Plasmid profiles were used to fingerprint *S. typhimurium* DT204C and the results indicated that with the exception of one of the premises, prolonged salmonella-persistence in the environment was not occurring.

Three separate epidemics of salmonellosis in calves were studied by use of plasmid profile analysis. The results illustrated the role of dealers, and their subcontractors, in the dissemination of salmonellas. The study concludes with suggestions for methods to reduce the spread of salmonellas in the calf marketing chain.

INTRODUCTION

To understand fully the epidemiology of salmonellosis in calves it is necessary to investigate each link in the calf marketing chain. At its simplest the chain consists of the production farm, which is usually a dairy farm supplying surplus calves to the market, and a dealer who purchases and sells these calves to a rearing farm. In reality the network is far more complicated (J. Lund, pers. comm.; [1]) and calves may appear in more than one market and pass through a number of dealers. This mixing of young susceptible calves and their subsequent transportation proves to be an efficient mechanism for the rapid dissemination of salmonellas [2]. Although many studies have been undertaken on rearing farms (see [3]), we are unaware of any published reports of investigations on dealers' premises.

On rearing farms, the number of calves infected with salmonella on arrival is usually 1% or less [4, 5] and it is therefore unlikely that the examination of the calves alone on dealers' premises would provide much information about the

spread of salmonella infection. It has been shown, however, that *Salmonella typhimurium* can persist for long periods in calf houses, even after cleaning and disinfection [5, 6] and monitoring salmonella infection in the environment could therefore be an important part of investigating dealers' premises.

Another possible strategy to determine the factors common to different disease outbreaks caused by particular strains of salmonella is by retrospective tracing of the movement of salmonella-infected calves. At the time of writing, *S. typhimurium* DT204C is the commonest strain isolated from calves during the last few years (Wray, unpublished data). The study of its epidemiology requires a suitable method for finger-printing the organism. Using a combination of biotyping and plasmid profile analysis it has been possible to divide this phage type into a number of different sub-types [7]. This paper describes the extent and persistence of salmonella contamination in 12 dealers' premises and further results from the use of the finger-printing scheme to study the spread of salmonella infection through the dealer network.

MATERIALS AND METHODS

Dealers' premises

Twelve dealers' premises situated in different parts of the country were sampled twice a week, once before cleansing and disinfection (usually Friday) and once prior to restocking (usually Monday). The premises were sampled for 5 weeks during the Autumn of 1986 and for 4 weeks during the Spring of 1987. Table 1 gives details of the different dealers; their activities and a brief description of their premises.

Sampling procedures

Walls of the calf pens

Five sites on the pen(s) walls were sampled before and after cleaning. If salmonellas were isolated, the sites were re-examined on subsequent visits until negative results were obtained. Different sites were chosen when salmonellas were not detected. The swabs consisted of fist-sized pieces of absorbent cotton-wool which had been autoclaved in labelled jars containing 250 ml buffered peptone water. Using disposable gloves the cotton-wool was squeezed out and then wiped vigorously over a wall area of approximately 36 square feet, starting from floor level and working upwards, to include corners and overhead ledges as appropriate. The soiled swab was replaced in the jar and the gloves discarded.

Floors of the calf pens

Fist-sized pieces of absorbent cotton-wool, which had been previously wrapped and sterilized were moistened in jars containing 250 ml selenite broth. After drawing back the bedding the swab was rubbed vigorously over an area of approximately 20 square feet and then placed in the jar containing selenite broth. Five sites were chosen in the pen(s) and sampled before and after cleaning. On subsequent visits the sampling routine was as for the walls; positive sites were re-sampled and different sites examined if salmonellas had not been detected.

'Dirty' samples were cultured directly into selenite broth because it was

Table 1. *Details of the dealers' premises*

Dealer	Weekly purchase	Weekly throughput of calves	Days on premises	Trade	No. of markets used	Sold to other dealers	Cleaning methods employed	Construction of premises		
								Walls	Pen divisions	No. of pens
1	500	500	1	Direct* export	> 10	Few	Weekly pressure hosing/ steam clean and disinfectant	Rendered brick	Tubular steel	18
2	1000	900	1	Direct export	> 50	10–20%	Weekly pressure hosing/ steam clean and disinfectant +lime wash	Limewashed stone	Wooden hurdles	7
3	200	200	1	Direct export	> 10	Few	Weekly pressure hosing and disinfectant	Breeze-block	Metal	250
4	40	40	Not known	Direct	< 10	None	Weekly pressure hosing	Rendered brick	Metal	4
5	100	100	1–3	Direct	5	None	Weekly pressure hosing and disinfectant	Rendered brick	Concrete	7
6	200	200	1–2	Direct export	15	50%	Weekly pressure hosing and disinfectant	Marine ply	Marine ply	5
7	1–300	1–300	0–5	Direct	3	None	Weekly pressure hosing	Breeze-block	Tubular steel	10
8	1000	1000	1–3	Direct export	> 20	None	Weekly steam cleaning	Cement finish	Wood	14
9	100	80	1–3	Direct	5	50%	Weekly pressure hosing	Rendered brick	—	2
10	1500–4000	All	< 1–2	Direct export	All	None	Sweep out twice weekly + small amount of disinfectant Monthly pressure hosing and lime wash	Rendered brick	Concrete	7
11	200	200	< 12 hrs	Direct	None	None	Weekly pressure hose	Rendered brick	Tubular steel	8
12	100	100	3	Direct export	5	None	Swept out fortnightly and disinfectant applied	Hardboard	Wood	28

* Supplied direct to rearer.

considered that contaminating organisms might have overgrown any salmonellas had a pre-enrichment medium been used. After cleaning, it was considered that the number of salmonellas would be less and possibly sub-lethally damaged and that pre-enrichment in buffered peptone water would increase the sensitivity of the technique.

Drains

The main drain was sampled weekly, the swab being placed in the drain on the day of the clean visit and collected at the next visit, when the swab was placed in a jar containing 100 ml selenite broth. The swabs usually remained in the drain for 3–4 days.

Bacteriological procedures

Wall and clean-floor swabs

The buffered peptone water containing the swab was incubated for 18 h at 37 °C and 1 ml was then transferred into 100 ml of Rappaports broth (Oxoid CM669). After incubation at 43 °C for 48 h, subcultures were made onto brilliant green agar (Oxoid CM329) which was incubated at 37 °C for 18 h and then examined for the presence of salmonellas.

Dirty-floor and drain swabs

The selenite broths containing the swabs were incubated for 18 h at 37 °C and subcultured onto brilliant green agar plates. After incubation at 37 °C for 18 h the plates were examined for the presence of salmonellas.

Identification of salmonellas

All suspect salmonella cultures were submitted to the Central Veterinary Laboratory for serological identification. Cultures of *S. typhimurium* were phage-typed at the Division of Enteric Pathogens, Central Public Health Laboratory and multiply resistant strains of *S. typhimurium* were examined for plasmid content [7, 8]. Lysis in alkaline sodium dodecyl sulphate was followed by purification in phenol-chloroform and electrophoresis at 140 V for 3·5 h in 0·7 per cent agarose. The gels were stained with ethidium bromide and photographed over ultra-violet light. The molecular weights of the plasmids were determined approximately by comparison with standards of known molecular weight.

Initially each new profile is given a Non-Recognized-Type (NRT) number and checked for reproducibility and stability. If the new plasmid profile is recognized in subsequent disease incidents, then the NRT is added to the typing scheme. Thirty-three distinct plasmid profile types have been recognized and each has been ascribed either a letter (A–Z) or a number (1–7).

RESULTS

Salmonella isolations from dealers' premises

Salmonellas were isolated from 10 of the 12 dealers' premises (Table 2). Eight different salmonella serotypes which included eight different *S. typhimurium* phage types were isolated. Plasmid profile analysis detected eight different

Table 2. *Isolation and salmonellas from calf dealer's premises*

Dealer	Wall		Floor		Drain	Serotype isolated (phagetype and plasmid profile type)
	Pre-cleaning	Post-cleaning	Pre-cleaning	Post-cleaning		
1	11/50*	12/50	8/54	13/49	3/6	Stm, ++ 204C, (E, P)
2	0/25	0/25	0/25	0/25	0/5	
	1/25	2/25	0/25	0/25	0/9	Stm, 107
3	0/50	0/25	0/30	5/30	0/10	Stm, 204 *S. binza*
	1/36	0/36	0/36	1/36	0/12	Stm, 204C (N)
4	0/25	0/15	0/25	0/15	0/3	
5	0/42	0/35	1/42	0/35	1/11	Stm, RDNC *S. virchow*
6	0/25	0/25	0/25	0/25	0/8	
	5/20	3/20	0/20	0/20	0/6	*S. dublin* *S. havana*
7	0/25	0/25	0/25	0/25	ND	
	0/15	0/20	1/20	0/15	ND	*S. binza*
8	5/27	0/28	ND	ND	1/6	Stm, 110, 204C (0, E) *S. agama*
	1/20	0/20	0/20	0/20	0/8	Stm.
9	1/18	0/20	0/1	ND	ND	*S. dublin*
10	6/30	7/25	5/30	7/25	2/5	Stm 204C, (B, E, X) *S. dublin*
	1/20	2/20	3/20	3/20	1/4	Stm 193, 204C, *S. dublin* *S. heidelberg*
11	0/25	0/25	0/25	0/25	0/5	
	0/20	0/20	0/20	0/20	0/4	
12	2/45	1/15	2/45	0/15	ND	Stm 49, 204C (E)
	10/36	6/12	8/36	4/12	ND	Stm 204, 204a, 193, 204C, (E, F, 2) *S. derby*
Total	44/579 (7·6)	33/486 (6·8)	28/524 (5·3%)	33/437 (7·6%)	8/102 (7·8%)	

* Numerator, no. of salmonella isolations; Denominator, no. of samples; Stm, *S. typhimurium*, phagetype (plasmid profile DT 204C) RDNC, does not conform to recognizable pattern; ND, not done

patterns in *S. typhimurium* DT204C. The percentage of salmonella isolations from the different sampling sites was similar and it is of interest that after cleaning and disinfection salmonellas could still be isolated on many premises. However, the survival of salmonella did not appear to relate to the disfection programme. The different serotypes and phage types, however, did not persist except on the premises of dealer 1 (Table 1 & 2, Fig. 1). Preliminary studies had been undertaken on the premises of this dealer and following the isolation of salmonellas, disinfectant was sprayed on the walls and floors at the end of the third week of the study using a pressure sprayer. This removed all the long standing dust and cobwebs but further isolations of the same plasmid profile type were made from

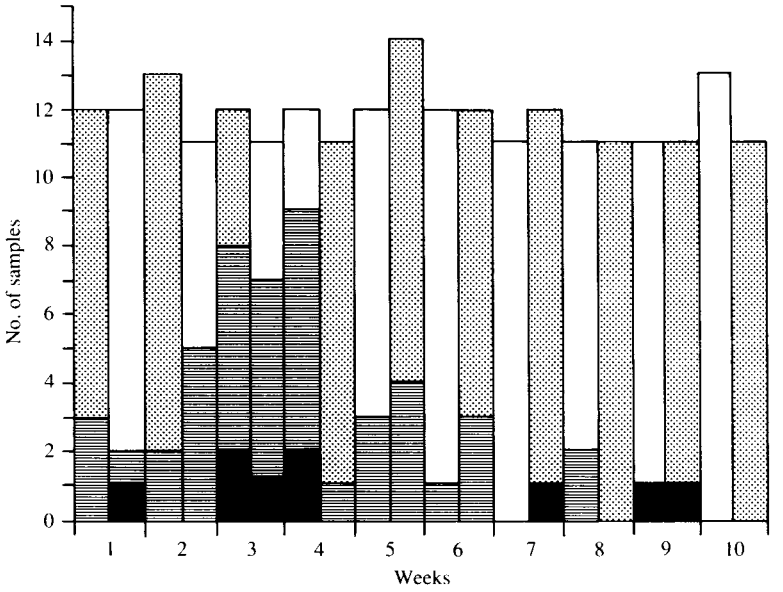

Fig. 1. Persistence of *Salmonella typhimurium* DT 204C in the premises of Dealer 1. The arrow indicates extensive cleansing and disinfection. ■, salmonellas isolated from drains; ▤, salmonellas isolated from walls or floor; ▨, samples collected before cleaning; □, samples collected after cleaning.

the cleaned premises on a number of occasions (Fig. 1) and the buildings were not salmonella-free for 10 weeks.

Salmonellas were isolated from both the walls and floor of four premises, from only the walls of four premises and from only the floors of two premises. On the premises where drain swabs were used salmonellas were isolated from 7·8% of the swabs. On four premises, drain swabs were negative throughout the period of the survey although salmonellas were isolated from the other sampling sites. The design and construction of the various premises differed markedly and salmonella contamination did not appear to relate to the different types of building material. Likewise the degree of salmonella contamination did not appear to be related to the throughput of calves; thus the number of isolations from a small dealer's premises [12] were similar to those on the premises of the largest dealer [10]. Dealer 2 was also a large dealer and salmonellas were not isolated during the survey in the Autumn but during the Spring the standard of hygiene declined for a period and salmonellas were isolated. Premises 11 and 7 were farmers' co-operatives and the only salmonella isolation, *S. binza* was from the floor of 7. Dealer 12 used a converted poultry house which was inadequately cleaned on the many occasions when some calves remained on the premises.

Use of plasmid profile analysis for tracing the spread of S. typhimurium *DT 204C Salmonella typhimurium DT204C*

This is usually resistant to sulphonamides, streptomycin, chloramphenicol, ampicillin, tetracyclines, neomycin and trimethoprim. Previous studies [7] showed that isolates of this phage type could be divided on the basis of their ability to

Table 3. *The epidemiological survey of* S. typhimurium *DT 204 C 'E' type plasmid profile (neomycin sensitive, failure to ferment m-inositol at 25 °C)*

Rearer	County in which affected farm(s) situated	Date of arrival on farm in 1985	Suggested relationship between early incidents from tracing calf movements through dealers and markets
1	Sussex	9 Jan.	? A
2	Lincolnshire	19 May	B
3	Cambridge	1 June	C
4	Cornwall	2 June	C
5	Norfolk 1	5 June	D
6	Norfolk 2	6 June	D
7	Somerset	12 June	D
8	Norfolk 3	18 June	C
9	Norfolk 4	20 June	D
10	Worcester	24 June	1 E
11	Northumberland	24 June	2
12	Lincoln	26 June	F 3
13	Cheshire	29 June	

Letters indicate dealers. Numbers indicate markets.

ferment m-inositol at 25 °C, and further sub-division could be achieved by plasmid profile analysis. To date, 33 distinct stable plasmid profiles have been recognized and while the frequency of the different sub-types has varied, the technique has been of value in our epidemiological studies. Three examples of its use for retrospective tracing of the spread of *S. typhimurium* DT204C in calves are as follows.

S. typhimurium *DT 204C, Type E*

Strains of this type fail to ferment m-inositol at 25 °C, are neomycin sensitive and possess three plasmids of approximately 120, 79 and 6·5 Md.

First isolated from calves supplied by a dealer to a farm in Sussex, further isolations of this subtype are listed chronologically in Table 3. In May 1985 it was isolated from calves in Lincolnshire, and 12 isolations were made from calves in areas as part apart as Cornwall and Northumberland in June 1985. During the period from the first week in July to the first week in October, when monitoring ceased, a further 16 outbreaks of disease were associated with this type of *S. typhimurium* DT 204C.

Table 3 shows the suggested relationship between 12 of the outbreaks caused by this subtype. Two dealers (C and E) were associated by supplying the calves to the rearing farms either directly, or indirectly through other dealers and markets.

S. typhimurium *DT 204C, Type O*

Strains of this type ferment m-inositol at 25 °C, are resistant to both neomycin and apramycin and possess six plasmids of 120, 90, 81, 42 and 4·3 & 3·9 Md. Five

https://doi.org/10.1017/S0950268800047890 Published online by Cambridge University Press

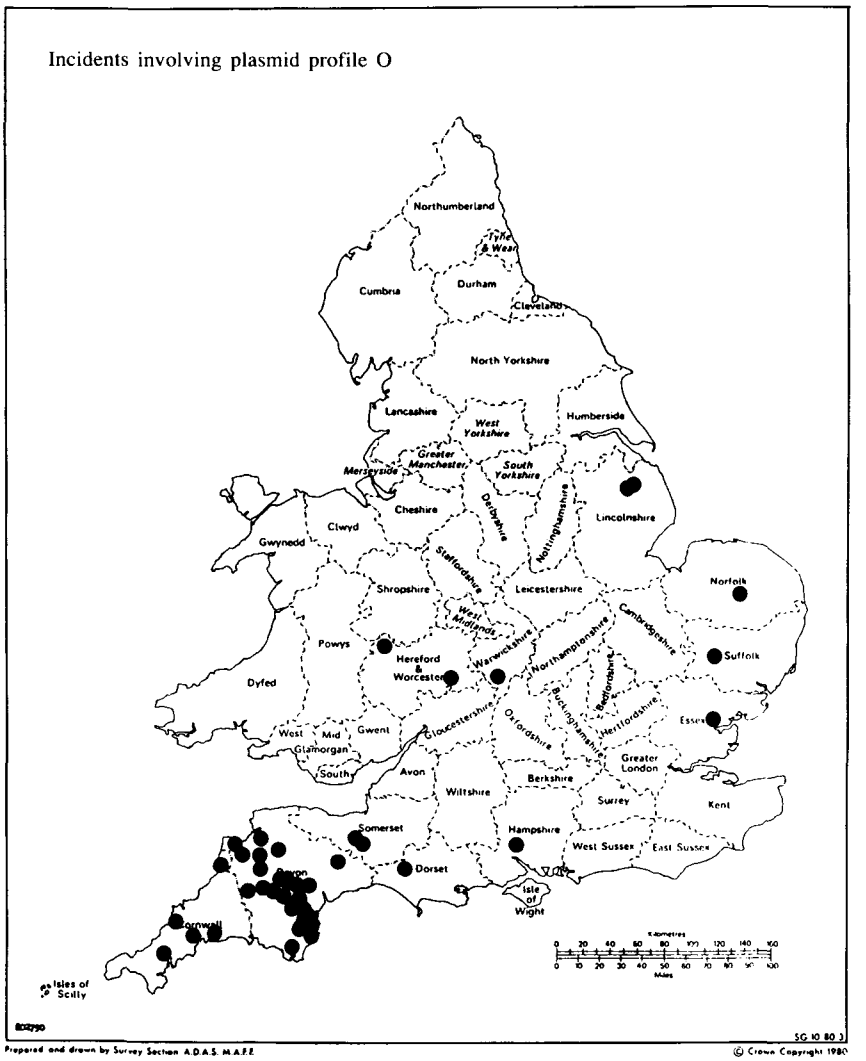

Fig. 2. Incidents of *Salmonella typhimurium* DT 204C, O plasmid profile. Maps prepared and drawn by Survey Section ADAS MAFF, Crown Copyright 1980.

disease incidents associated with this type were detected before 1986; of these three occurred in the West Midlands and two in the South-West Region. Between August 1986 and February 1987, 43 disease incidents were caused by this sub-type (Fig. 2), of which 24 were in Devon, 5 in Cornwall, and 14 from other counties in England. One dealer was associated with six of the earlier outbreaks in Devon and Cornwall, and he also supplied calves to the dealer associated with the Norfolk incident. Five of the later incidents in a localized area of the South-West, which included salmonella infection on self-contained dairy farms, indicated the likelihood of a common vector.

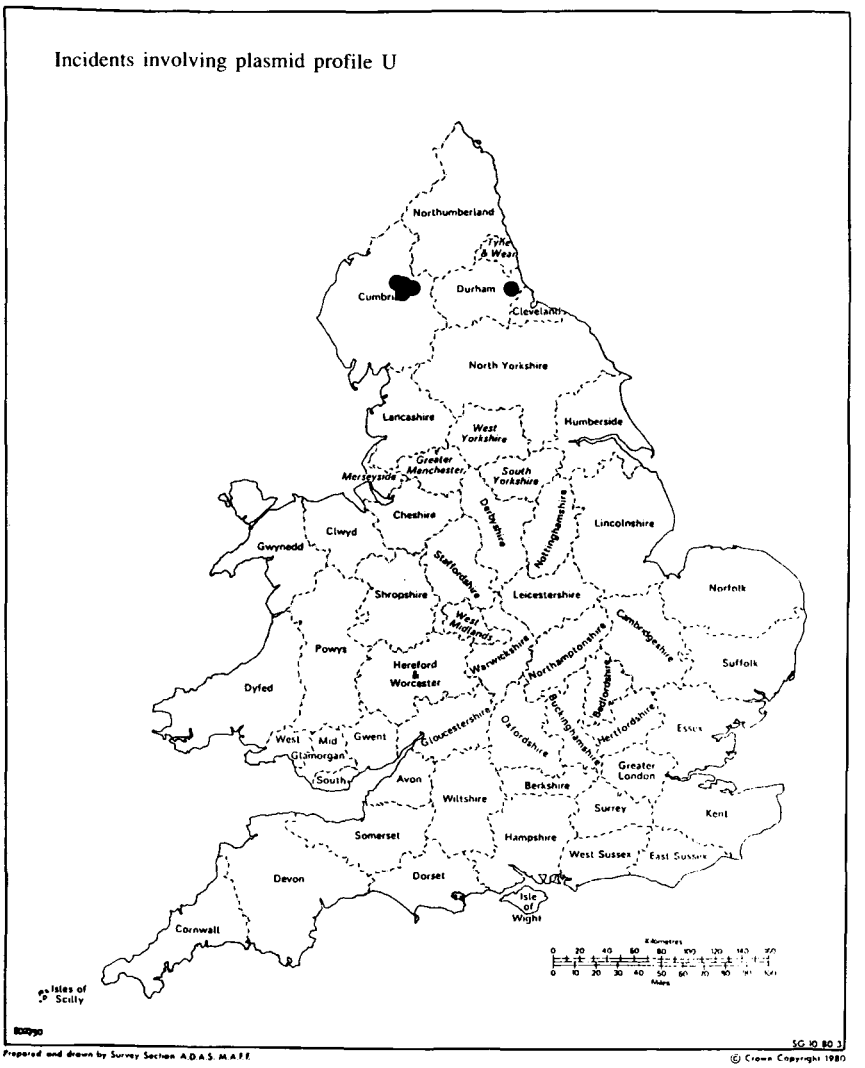

Fig. 3. Incidents of *Salmonella typhimurium* DT 204C, U plasmid profile. Maps prepared and drawn by Survey Section ADAS MAFF, Crown Copyright 1980.

S. typhimurium *DT 204C, Type U*

Strains of this profile are resistant to neomycin, sensitive to apramycin, ferment m-inositol at 25 °C and possess five plasmids of 130, 100, 35, 30 & 4·5 Md. Five incidents occurred with this strain during a 9-week period from December 1986 to January 1987 (Fig. 3). One incident was associated with market purchased calves, and three with a dealer; the remaining incident involved possible contact with one of the earlier incidents.

https://doi.org/10.1017/S0950268800047890 Published online by Cambridge University Press

DISCUSSION

A wide variety of salmonella serotypes and phage types were isolated from the dealers' premises sampled in this study and with the exception of those of Dealer 1, salmonella persistence was for short periods. The commonest *S. typhimurium* phage type (DT204C) was isolated on a number of occasions on the same premises but the isolation of different plasmid profile types suggested that prolonged persistence was not occurring. In contrast, on calf rearing premises a smaller range of salmonella serotypes and phage types was encountered, and the repeated isolation of the same plasmid profile type indicated persistence in the environment [5–7]. This difference between the results on dealers' and rearers' premises may relate to the degree of contamination. On the former, it is likely that only small numbers of infected animals are passing through, whereas on the latter infection may spread rapidly to all animals in a group which then remain on the premises for several weeks [5]. Epidemiologically the presence of salmonellas on dealer's premises, even for short periods, is important, because the throughput of large numbers of calves destined for many different rearing units may result in their widespread dissemination of infection. The commonest salmonellas in calves are *S. dublin* and *S. typhimurium* DT 204C and it is not surprising that they were detected on 9 of the 12 premises. However, incubation of Rappaport's broth at 43 °C has been shown to inhibit the growth of some salmonella serotypes, especially *S. dublin* [10] and it is possible that the prevalence of this serotype has been underestimated. A number of other serotypes and phage types were also detected but as they are seldom associated with disease in calves [9] it is likely that they may be of reduced virulence for calves.

One important finding was the inadequacy of the cleaning and disinfection routines on many premises. Indeed on one of the premises (Fig. 1) the salmonella isolation rates increased after cleaning and disinfection and it has been suggested that the use of pressure hoses may cause aerosols and spread salmonellas to other parts of the building [11]. It is also possible that vigorous cleansing and disinfection may have washed salmonellas out from previously untouched areas.

Overall the isolation rate of salmonellas was not high on most premises. However, lapses of hygiene or the keeping of calves on the premises for more than a day may result in salmonella contamination. It is clear however, that the disinfection routines were often failing to achieve their objective and further studies on cleansing and disinfection routines and their relationship to the design of buildings are desirable.

Plasmid profile analysis enabled retrospective tracing of the spread of salmonellas during three unrelated salmonella epidemics and clearly demonstrated the role of the dealer in salmonella dissemination. Of interest was the further spread through selling calves to other dealers. However, because of the high incidence of sub-clinical infection in market purchased calves [5, 10] it is inevitable that gaps will occur in the identifiable chain of transmission. Since some of the outbreaks occurred on self-contained farms, which did not purchase calves, it is possible that a common vector was responsible for the spread of salmonella between farms during some of the localized outbreaks. Whilst it is not possible to identify any vector with certainty, the possibilities would include farm visitors

and farm personnel whose work may take them onto different farms in the same area. Another possibility is wild-life, and although salmonellas have been isolated from many species of animals [12] such infection generally appears to indicate a contaminated environment [13].

The investigation has shown that: 1, Dealers' premises may act as reservoirs of infection but this is usually a short term phenomenon, 2, Cleaning and disinfection routines were not always satisfactory and often failed to achieve their objective, 3, Purchase of calves by as direct route as possible should reduce salmonella dissemination.

ACKNOWLEDGEMENTS

We should like to thank our colleagues Messrs N. Allsup, R. Bicknell, D. Counter, D. Gibbons, J. Lund, G. Pritchard and G. Purvis of the Veterinary Investigation Services and the staff of their respective laboratories for their help and assistance. We should also like to thank Mr E. Boden, for permission to include Table 3 previously published in the Veterinary Record.

REFERENCES

1. Farm Animal Welfare Council report on the welfare of livestock at markets. London: HMSO, Ref. Book 265.
2. Anderson, ES. Drug resistance in *Salmonella typhimurium* and its implications. Br Med J 1968, 3; 333–9.
3. Wray C, Sojka WJ. Reviews of the progress of dairy science: bovine salmonellosis. J Dairy Res 1977; **44**: 383–425.
4. Rankin JD, Taylor RJ, Burrows MR. Salmonella infection in young calves. Vet Rec 1969; **85**: 582.
5. Wray C, Todd N, Hinton MH. The epidemiology of *Salmonella typhimurium* infection in calves: excretion of *S. typhimurium* in the faeces of calves in different management systems. Vet Rec 1987; **121**: 293–6.
6. Twiddy N, Hopper DW, Wray C, McLaren I. Persistence of *S. typhimurium* in calf rearing premises. Vet Rec 1988; **122**: 399.
7. Wray C, McLaren I, Parkinson NM, Beedell Y. Differentiation of *Salmonella typhimurium* DT 204C by plasmid profile and biotyping. Vet Rec 1987; **121**; 514–6.
8. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; **145**: 1365–73.
9. Animal Salmonellosis; Central Veterinary Laboratory, New Haw, Weybridge, UK, KT15 3NB MAFF 1987.
10. Peterz M, Wiberg C, Norberg P. The effect of incubation temperature and magnesium chloride concentration on growth of salmonella in home-made and in commercially available dehydrated Rappaport–Vassiliadis broths. J Appl Bact 1989; **66**: 523–8.
11. Hinton MH, Ali EA, Allen V, Linton AH. The excretion of *Salmonella typhimurium* in the faeces of calves fed milk substitute. J Hyg 1983; **91**: 33–45.
12. McDiarmid, A. Diseases of free-living wild animals. FAO Agricultural Studies no. 57: 1962: Rome.
13. Jones PW, Twigg G. Salmonellosis in wild mammals. J Hyg 1967; **77**: 51–4.