The excretion of *Salmonella typhimurium* in the faeces of calves fed milk substitute

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SUMMARY

A total of 495 calves in 16 batches were examined (117 calves in 4 batches in 1979 and 378 in 12 batches in 1982). They were purchased in markets, transported by road to a farm in Somerset and reared on a milk substitute diet for a period of up to five weeks. *Salmonella typhimurium* phage type DT 193 was endemic in 1979 and phage type DT 204c in 1982. The mortality rates in the two years were 9·4% and 1·9% respectively. The causes of death were not investigated although the majority were probably due to salmonellosis.

The rate of isolation of *S. typhimurium* from the rectal faeces of calves in all groups was either zero or relatively low on arrival. It rose to a peak (which was higher in 1979) in the second or third weeks before declining to low levels by the end of the fourth week of residence on the farm.

Data from 162 calves, examined twice weekly for four weeks in 1982, indicated that the distribution of infected calves, based on the number of times that *S. typhimurium* was isolated from each, was not random. The calves could be assigned to two main categories; those from which the organism was never isolated and those from which it was isolated at least twice. This suggested that salmonella infected calves actively excreted the organism.

The association between salmonella excretion and medication of sick animals with antibacterial drugs was strongest during the second week. Over the four-week period nearly 40% of the calves found to be excreting *S. typhimurium* were not treated, indicating a high incidence of subclinical infection.

Salmonella excretion by the calves followed a regular pattern and infection was self-limiting within five weeks. The peak in the salmonella excretion rate and the mortality rate were higher in 1979 when phage type DT 193 was the endemic strain. However, in 1982 the calves received 100 p.p.m. furazolidone in their milk ration during the first week of their stay on the farm, and this may have contributed to the differences noted between the two years.

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INTRODUCTION

Chloramphenicol resistance amongst strains of *Salmonella typhimurium* isolated from farm animals in the U.K. was uncommon before 1976 (Sojka & Wray, 1980; Linton, 1981). Since that time the incidence has risen considerably, and this has been due principally to an epidemic, which commenced in 1977 in calves of phage types DT 193 and DT 204, resistant to a range of antibacterial drugs including chloramphenicol. The early features were described by Anon. (1978), Davies *et al.* (1978) and Threlfall, Ward & Rowe (1978*a, b*) together with additional comments in the correspondence columns of the *Veterinary Record* (e.g. Appleyard & Clegg, 1978; Threlfall *et al.* 1979; Davies, 1979). A detailed study of the genetical and molecular characteristics of both phage types DT 193 and 204 has also been published (Willshaw *et al.* 1980).

The epidemic of these phage types has proved extremely serious because they became widely disseminated across the counties of England, Wales and Scotland and have been responsible for a large number of incidents of infection in calves and, to a lesser extent, in adult cattle and other farm animal species (MAFF, 1982). Infection has also been reported in man (Threlfall *et al.* 1978*b*). Yet, despite this, there is surprisingly little published data on the epidemiology of these *S. typhimurium* infections on farms which specialize in rearing young calves. In 1979 a single batch of 40 calves reared on a farm in Somerset was studied. It was found that *S. typhimurium* phage type DT 193 was isolated from relatively few calves on their arrival on the farm. The excretion rate then rose to a peak on the 14th day before declining to low levels by the fifth week (Linton, Timoney & Hinton, 1981). Since that time an additional 455 calves reared in 15 batches (three in 1979 and 12 in 1982) have been monitored and the data obtained from these, together with that from the previous batch, are presented in this paper.

MATERIAL AND METHODS

A total of 495 calves were examined in four batches between May and November 1979 and in 12 batches between May and July 1982 (Table 1).

There were some differences in the methods employed on each occasion and these are indicated, where appropriate, below.

The calves and their management

The calves, principally Friesians, were purchased at markets in various parts of the United Kingdom and transported by road to the rearing farm which is situated in the county of Somerset.

The farm building was modified between 1979 and 1982 when the roof was raised and the system of ventilation improved, otherwise the general policy of management was similar in both years. The calves were housed in one of a number of rooms and an all-in, all-out policy was operated for each of these. The calves were kept individually in wooden pens linked together. The partitions between the pens were solid while the pen floors, which were raised above the concrete floor, consisted of slats. Calves in adjacent pens could contact each other at both ends of the pen.

The calves were fed twice daily (once daily on Sundays in 1982) with a milk substitute diet which contained the growth-promoting antibiotics flavomycin and

zinc bacitracin. In 1982 furazolidone was incorporated in the milk (100 p.p.m.) during the first week that the calves were resident on the farm.

A variety of parenteral and oral antibacterial drugs, obtained from a veterinary surgeon, was used to treat animals showing signs of clinical illness, e.g. diarrhoea. In 1982 the records of treatment for each calf were retained for analysis although details of the drug(s) were not always recorded by the farm staff.

In addition, in 1982 the ear tag of each calf was noted and the probable county of the farm of origin determined from the MAFF register of farm code numbers.

In three batches in 1979 some calves left the farm before the end of the period of observation since they had grown sufficiently to be exported to the continent (Table 1).

Bacteriological techniques

Swabs of rectal faeces (alginate in 1979 and cotton-wool in 1982) were collected at intervals during their stay on the farm and placed in selenite broth, usually on the day of collection but, failing that, on the next day after storage at 4 °C. The broth, made up from basic ingredients in 1979 (Hobbs & Allison, 1945) and from selenite broth base and sodium biselenite (Oxoid CM 395 and L 121) in 1982, was incubated in air at 37 °C (1979) or 42 °C (1982) before subculture at 18 h and 42 h onto Brilliant Green Phenol Red (BGPR) agar. The BGPR was made up according to the formula of Edel & Kampelmacher (1969) in 1979 and from purchased materials in 1982 (Oxoid CM 329). The BGPR plates were incubated overnight at 37 °C in air, and salmonella-like colonies were investigated using routine biochemical and serological techniques (Gillies, 1956; Edwards & Ewing, 1972). In 1979 the *in vitro* resistance to antibacterial drugs was determined using a disk diffusion method (Linton, Howe & Osborne, 1975). The disks used included ampicillin (A) (25 µg), chloramphenicol (C) (50 µg), kanamycin (K) (30 µg), streptomycin (S) (25 µg), sulphafurazole (Sf) (500 µg) and tetracycline (Te) (50 µg). In 1982 the drugs were incorporated into plates of isosensitest agar (Oxoid CM 471) with 1.2 % lysed horse blood. These were inoculated from a master plate, which had been incubated for 4 h, with a multipoint inoculator. The final concentration of drugs in the agar was A (50 µg/ml), C (50 µg/ml), K (30 µg/ml), S (10 µg/ml), sulphadiazine (Sd) (250 µg/ml), Te (50 µg/ml), trimethoprim (Tr) (1.5 µg/ml) and furazolidone (F) (35 µg/ml).

Analysis of the results

The data from all 16 batches is presented in the first part of the results. More detailed analysis was restricted to the data obtained from 162 calves in five batches examined in 1982 and in which the sampling regimen was strictly comparable (Nos. 7-9, 15 and 16; Table 1). The calves were sampled twice each week during four weeks. Four of the batches consisted of 36 calves, and these were housed in 18 adjoining pens situated on each side of the room. In one batch (No. 9) only one side of the room was filled with 18 calves.

The statistical analysis was undertaken using 'Minitab' (Ryan, Joiner & Ryan, 1981), which is available on the University of Bristol's Honeywell Computer. When dependent values (*y*) were proportions (%) they were subjected into inverse-sine transformation before the calculation of the regression equation (Steel & Torrie, 1980).

Table 1. *The rate of isolation of Salmonella typhimurium from calves fed a milk substitute diet*

Batch no.	Month of arrival on farm	The time in days that the calves had been resident on the farm															
		1	2*	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	May '79	5/40†	—	—	—	—	—	—	—	—	—	—	—	—	28/39	—	—
2	June '79	—	—	3/37	—	3/37	—	—	—	—	14/37	—	—	13/36	—	—	—
3	Nov. '79	—	0/19	—	—	5/19	—	—	—	—	12/19	—	9/19	—	—	—	13/19
4	Nov. '79	—	—	1/21	—	—	3/21	—	—	—	10/21	—	15/21	—	6/24	—	13/20
5	May '82	2/24	—	—	—	—	—	5/24	—	—	—	—	—	—	—	—	—
6	May '82	—	0/12	—	—	—	1/12	—	—	2/12	—	—	—	2/12	—	—	4/12
7	June '82	—	2/36	—	—	—	7/36	—	—	14/36	—	—	—	14/36	—	—	11/36
8	June '82	—	0/36	—	—	—	5/36	—	—	13/36	—	—	—	16/36	—	—	19/36
9	June '82	—	0/18	—	—	—	8/18	—	—	—	5/18	—	—	8/18	—	—	—
10	June '82	2/36	—	—	8/36	—	—	10/36	—	—	—	15/36	—	—	10/36	—	—
11	June '82	0/36	—	—	5/36	—	—	4/36	—	—	—	10/36	—	15/36	—	—	—
12	June '82	—	2/36	—	—	—	—	6/36	—	—	—	13/36	—	7/36	—	14/36	—
13	July '82	—	2/36	—	—	—	4/36	—	—	—	—	—	—	14/36	—	—	—
14	July '82	3/36	—	—	—	—	4/36	—	—	12/36	—	—	—	18/36	—	—	11/36
15	July '82	—	—	—	—	6/36	—	—	—	—	—	—	—	—	—	—	—
16	July '82	—	2/36	—	—	4/36	—	—	—	12/36	—	—	—	16/36	—	—	10/36

Batch no.	Month of arrival on farm	The time in days that the calves had been resident on the farm																No. calves which died	No. calves sold
		17	18	19	20	21	22	23	24	25	26	27	28	29	32	33			
1	May '79	—	—	—	—	—	12/37	—	—	—	—	9/36	—	—	—	2/35	5	0	
2	June '79	13/36	—	—	12/36	—	—	—	9/34	—	—	5/27	—	—	—	—	1	9	
3	Nov. '79	—	—	12/19	—	—	—	9/18	—	7/16	—	—	—	—	—	—	1	2	
4	Nov. '79	—	—	13/20	—	—	—	6/16	—	—	4/15	—	—	—	—	—	4	2	
5	May '82	11/24	—	—	—	5/23	—	—	—	6/23	—	1/12	3/23	—	4/23	—	1	0	
6	May '82	—	—	—	2/12	—	—	2/12	—	—	—	—	—	—	—	—	0	0	
7	June '82	—	—	—	9/36	—	—	5/35	—	—	5/35	—	—	—	—	—	1	0	
8	June '82	—	—	—	15/36	—	—	11/36	—	—	8/36	—	—	—	—	—	0	0	
9	June '82	3/18	—	—	4/17	—	—	1/17	—	—	2/17	—	—	—	—	—	1	0	
10	June '82	—	5/36	—	—	3/36	—	—	—	1/36	—	—	—	—	—	—	0	0	
11	June '82	—	2/35	—	—	0/35	—	—	—	0/35	—	—	—	—	—	—	1	0	
12	June '82	—	8/36	—	—	—	—	—	4/34	—	—	4/34	—	—	—	—	2	0	
13	July '82	—	—	—	5/36	—	6/36	—	—	—	4/36	—	—	3/36	2/36	—	0	0	
14	July '82	—	—	—	10/36	—	5/35	—	—	—	4/35	—	—	1/35	2/35	—	1	0	
15	July '82	—	—	—	10/36	—	—	6/36	—	—	—	4/36	—	2/36	—	—	0	0	
16	July '82	—	—	—	10/36	—	—	6/36	—	—	—	5/36	—	4/36	—	—	0	0	

* Calves examined on the second day were examined within 24 hours of arrival.
† The numerator is the number of calves excreting *S. typhimurium* in their faeces. The denominator is the number of calves examined.

Table 2. *The distribution of 162 calves examined in 1982 according to the number of times S. typhimurium was isolated from eight swabs of rectal faeces collected twice weekly over four weeks*

Batch no.*	Calf nos.	No. of times that <i>S. typhimurium</i> was isolated from individual calves								Average isolation rate	Total excretors	
		0	1	2	3	4	5	6	7			8
7	1-18	5	1	3	6	2	—	1†	—	—	2.17	13
	19-36	7	4	2	2	1	2	—	—	—	1.56	11
8	1-18	7	1	1	3	5	1	—	—	—	2.06	11
	19-36	3	0	5	3	4	3	—	—	—	2.78	15
9	1-18	8	1	2	3‡	3	1	—	—	—	1.72	10
15	1-18	6	1	7	1	3	—	—	—	—	1.67	12
	19-36	4	2	2	7	3	—	—	—	—	2.17	14
16	1-18	5	0	3	7	3	—	—	—	—	2.17	13
	19-36	7	0	7	4	—	—	—	—	—	1.44	11
No. calves		52	10	32	36	24	7	1	—	—	1.97	110

* See Table 1.

† This calf died after the sixth swab was collected.

‡ One calf died after excreting at three successive samplings.

RESULTS

Excretion of salmonella in 1979 and 1982 considered together

Salmonella typhimurium was the only serotype isolated in this survey. Phage type DT 193, resistant to ACKSSfTe, was isolated in 1979 and phage type DT 204c, resistant to ACKSSdTeTr, was the endemic strain in 1982. The isolation rate of *S. typhimurium* from the calves in all 16 batches followed a similar pattern (Table 1). The rate was zero or relatively low on arrival on the farm. It rose to a peak during the second or third week of residence, before declining thereafter. Regression analysis revealed that the excretion rate could be predicted by two significant quadratic equations ($P < 0.001$). These were $\sin^{-1}\sqrt{y} = 5.79x - 0.172x^2$ for 1979 and $\sin^{-1}\sqrt{y} = 4.17 - 0.128x^2$ for 1982 when y equals the proportional rate (%) for salmonella excretion and x the length of time, in days, of residency on the farm. In both years the predicted peak in the isolation rate occurred between the 15th and 17th day, being higher in 1979. The values were 56 % and 30 % in 1979 and 1982 respectively.

Excretion of salmonella by selected batches of calves in 1982

S. typhimurium was isolated from 110 (68 %) of the 162 calves in batches 7-9, 15 and 16 (Table 2). The average isolation rate/swab varied between 1.44 and 2.17 for each group of 18 calves. The difference between means was not significant. On the other hand the distribution of calves according to the number of times that the organism was isolated differed from Poisson expectations ($\chi^2_{(5)} = 41.3$, $P = < 0.001$) (Table 2). The principal reason for this was the fact that the number of calves which excreted only once was lower than would have been expected from a random distribution. The average number of new excretors, at each of the eight

Table 3. *The distribution of the samplings of nine groups of 18 calves sampled eight times in 1982, according to the number of new excretors recorded at each sampling*

Batch no.*	Calf nos.	No. of new excretors recorded at each of eight samplings										Average no. new excretors/ sampling	Total excretors
		0	1	2	3	4	5	6	7	8			
7	1-18	2	3	1	0	2	—	—	—	—	1.63	13	
	19-36	2	4	0	1	1	—	—	—	—	1.38	11	
8	1-18	3	1	2	2	—	—	—	—	—	1.38	11	
	19-36	3	1	2	1	0	0	0	1	—	1.88	15	
9	1-18	6	0	1	0	0	0	0	0	1	1.25	10	
15	1-18	1	5	0	1	1	—	—	—	—	1.50	12	
	19-36	1	3	2	1	1	—	—	—	—	1.75	14	
16	1-18	3	1	1	2	1	—	—	—	—	1.63	13	
	19-36	5	0	2	0	0	0	0	1	0	1.38	11	
No. of samplings		26	18	11	8	6	0	0	2	1	1.53	110	

* See Table 1.

samplings of the groups of 18 calves, varied between 1.25 and 1.88 calves sampled (Table 3). The difference between these means was not significant. The distribution in the number of samplings according to the number of new excretors identified (bottom line of Table 3) did not differ from Poisson expectations ($\chi^2_{(5)} = 6.4$) although the average number of new excretors identified in the groups of 18 calves at each of the eight samplings differed significantly with time ($P = < 0.001$; Table 4). The number of new excretors/sampling could be predicted from the equation $y = 1.34 - 0.179x^2$ ($P = < 0.001$), when y is the number of new excretors in a group of 18 calves and x is the sample number.

A total of 60 patterns of excretion were recorded amongst the 162 calves, 51 of which occurred in only one or two calves (39 and 12 patterns respectively). The nine most common patterns involved 47 (29%) of the calves (Table 5).

It was not possible to determine the spread of *S. typhimurium* within each room, since the calves were examined only twice weekly. Nevertheless, the calves could be assigned to one of five categories depending on whether the calf's neighbours excreted salmonella or not (column headings in Table 6). The distribution of calves which either did not excrete *S. typhimurium* in the eight samples, or which excreted the organism once or twice or more in these five categories was not significant ($\chi^2_{(4)} = 2.4$). This suggests that non-excretors and excretors were similarly distributed and were not grouped together in defined areas within each room.

Excretion of salmonella and drug administration

A total of 93 (57%) of the 162 calves examined in batches 7-9, 15 and 16 were medicated with antibacterial drugs by the farm staff during the first month of their stay on the farm. In all 43 (39%) of the 111 calves that excreted *S. typhimurium* were not treated while 30 (59%) of the 51 calves not identified as excretors were treated. The distribution of calves according to the number of times that they were treated was close to Poisson expectations ($\chi^2_{(3)} = 4.1$) (Table 7).

Table 4. *The distribution of the nine groups of 18 calves examined in 1982, according to the number of new salmonella excretors that were identified in each group on each of the eight sampling occasions*

Sample no.	No. of new excretors identified in each of nine groups of 18 calves*									No. of new excretors/ sampling	Average no. new excretors/ sampling	Accumulative total of excretors identified
	0	1	2	3	4	5	6	7	8			
1	4	3	2	—	—	—	—	—	—	7	0.78	7
2	0	3	2	2	1	—	—	—	1	25	2.78	32
3	0	0	1	1	5	—	—	2	—	39	4.33	71
4	1	1	4	3	—	—	—	—	—	18	2.00	89
5	3	3	1	2	—	—	—	—	—	11	1.22	100
6	4	4	1	—	—	—	—	—	—	6	0.67	106
7	7	2	—	—	—	—	—	—	—	2	0.22	108
8	7	2	—	—	—	—	—	—	—	2	0.22	110
Total samplings	26	18	11	8	6	0	0	2	1			

* Two groups of 18 calves from batches 7, 8, 15 and 16 and one group of nine in batch 9 (Table 1).

Table 5. *The patterns of excretion of S. typhimurium exhibited by at least four of 162 calves examined in 1982*

Sampling occasion								No. calves
1st week		2nd week		3rd week		4th week		
1	2	3	4	5	6	7	8	
—	+	+	+	—	—	—	—	4
—	+	—	+	—	—	—	—	4
—	—	+	+	+	+	—	—	9
—	—	+	+	+	—	—	—	5
—	—	+	+	—	—	—	—	9
—	—	+	—	—	—	—	—	4
—	—	—	+	+	—	—	—	4
—	—	—	—	+	+	—	—	4
—	—	—	—	—	+	+	+	4

+, *S. typhimurium* isolated; —, no salmonella isolated from a rectal swab.

Table 6. *The distribution of each of 162 calves, examined eight times in 1982, according to whether they, and each other of their neighbours, excreted salmonella or not*

No. of times salmonella was isolated from eight swabs	salmonella excretion status of neighbouring calves*					Total
	++	+-	+w	--	-w	
0	19	20	6	5	2	52
1	5	4	1	0	0	10
> 2	45	36	7	10	2	100
Total calves	69	60	14	15	4	162

+, salmonella isolated from one swab or more; —, salmonella not isolated from any swab; w, one side of the calf was next to a wall.

The calves could be placed in one of four categories depending on whether or not they either excreted salmonella or received drugs during any one week (Table 8). The overall difference between the observed and expected distributions was significant ($\chi^2_{(9)} = 86$, $P < 0.001$). The principal differences which contributed to this χ^2 value were recorded in weeks 1 and 2 and were as follows: S = salmonella excretion, M = medicated, (n) = contribution to overall χ^2 value of 86.

Week 1. M+S—more calves than expected (12.2)

M—S+less calves than expected (7.5)

M+S+less calves than expected (7.6)

Week 2. M+S+more calves than expected (20.9)

M—S—less calves than expected (9.1)

Calf mortality

A total of 18 (3.6%) of the 495 calves died (Table 1). The mortality rate was 9.4% and 1.9% in 1979 and 1982 respectively. This difference is significant ($\chi^2_{(1)} = 14.5$, $P = < 0.001$). The cause of death was not determined although the majority of calves probably died as a consequence of salmonellosis.

Table 7. *The distribution of 162 calves reared in 1982 according to the number of times that they were medicated with antibacterial drugs during their four-week stay on the farm*

Batch no.*	No. of times the calves were treated†				Average no. of treatments/calf	No. of calves examined initially
	0	1	2	3		
7	18	14	4	—	0·61	36
8	12	21	3	—	0·75	36
9	8	9	1	—	0·61	18
15	17	11	8	—	0·75	36
16	14	11	9	2	0·97	36
Total calves	69	66	25	2	0·75	162

* See Table 1.

† At least three days separated individual treatments of calves treated twice or three times.

Table 8. *The association each week between medication with antibacterial drugs and the excretion of S. typhimurium by 162 calves reared in 1982*

<i>Salmonella</i> excretion	Drug treatment	Week resident on the farm			
		1	2	3	4
—	—	109	64	83	115
+	—	20	47	50	29
—	+	26	12	9	6
+	+	7	39	20	10
Total no. calves		162	162	162	160*

* Two calves died during the third week.

County of origin of the calves

The probable county of origin was determined for 344 of the 378 calves examined during 1982. A total of 37 'pre 1974' counties were represented, with 8–13 of these being identified in all but the smallest batch (No. 6) of calves examined (Table 9).

DISCUSSION

Salmonella typhimurium has been endemic on this farm for many years and during that time many hundreds of calves have been reared on the premises. salmonella excretion followed a regular pattern in all batches of calves examined in 1979 and 1982 and in the majority of animals it appeared to be an active infectious process (Table 2).

Factors affecting the increase in salmonella carriage

The incidence of infection was low on arrival on the farm but then rose to reach a peak in the second or third week before declining to low levels by the fifth week. There are a number of factors which may influence the increase in salmonella carriage by the calves, and these will be considered separately.

Table 9. *The distribution of calves, reared in 12 batches in 1982, according to the county of the farm of origin*

	Batch no.												Total
	5	6	7	8	9	10	11	12	13	14	15	16	
England													
Northern Region													
Cumberland	2	—	—	2	2	2	6	14	12	7	6	13	66
Durham/Yorks.	1	—	1	—	—	1	—	1	—	—	—	—	4
Westmorland	1	—	1	1	1	2	1	5	7	3	—	5	27
Midland Region													
Hereford	—	—	—	5	—	1	—	—	—	6	—	—	12
Lancs.	—	—	—	—	—	—	—	—	1	6	5	7	19
Other counties*	—	—	—	—	2	3	—	—	—	3	—	—	8
E and S.E. Region†	1	—	—	1	2	—	—	—	—	1	6	1	12
S.W. Region													
Devon/Dorset/Wilts.	2	—	—	—	—	—	1	—	1	1	—	2	7
Somerset	1	5	2	—	2	4	2	2	7	—	1	5	31
Scotland													
Ayr/Dumfries/Wigtown	—	—	3	2	1	—	3	1	4	2	2	1	19
Lanark	3	—	1	6	—	1	7	3	—	4	3	2	30
Other counties‡	1	—	3	1	1	—	1	—	3	3	—	—	13
Wales													
Cardigan	—	—	15	—	—	4	—	—	—	—	—	—	19
Cardarthen	1	3	8	3	—	16	9	6	—	—	6	—	52
Pembs.	—	1	1	7	—	2	1	1	—	—	—	—	13
Other counties§	1	1	—	4	1	—	3	1	—	—	1	—	12
Not known	10	2	1	4	6	—	2	2	1	—	6	—	34
Total calves	24	12	36	36	18	36	36	36	36	36	36	36	378
No. counties represented	10	4	10	13	9	10	12	9	10	13	9	8	37

* Shropshire, Staffordshire, Warwickshire, Worcestershire.

† Lincolnshire, Berkshire, Buckinghamshire, Hampshire.

‡ Aberdeen, Bute, Caithness, Dumbarton, Fife, Perth, Stirling.

§ Brecon, Caernarvon, Glamorgan, Monmouth, Radnor.

Infection rates on arrival on the farm

It is generally accepted that the dissemination of *S. typhimurium* phage types DT 193 and 204 in the United Kingdom was entirely due to the large-scale movement of calves through dealers' premises and markets (Anon., 1978). Excreting calves were detected in two-thirds of the 14 batches swabbed within 24 h of their arrival on the farm. This indicates that infection was being brought onto the premises regularly, although the initial absence of excretors was not necessarily associated with a low peak in the maximum excretion rate recorded later in a batch.

Residual infection in the environment

There is a tendency for *S. typhimurium* to persist on farms where there is rapid movement of calves on and off the premises and where there is minimal disinfection between batches (Anon., 1978). The farm studied operated an all-in, all-out policy for each room and these were thoroughly cleaned and disinfected between batches. Inevitably, small traces of faeces could be detected in the nooks and crannies of the woodwork of a proportion of the pens, although the wood in direct contact with the calf was invariably free of visible faecal material and swabbing of these 'clean' surfaces on several occasions has never yielded *S. typhimurium* (unpublished observations). The role of the small and generally inaccessible foci of potential infection has yet to be assessed, although it is probable that the number of viable salmonella persisting in any room is likely to be relatively small.

Cross-infection between calves

In this study the calves were swabbed at approximately twice-weekly intervals and consequently it was not possible to assess the importance of cross-infection between calves in each batch. However, since excreting and non-excreting calves were similarly distributed amongst the pens (Table 6) it is possible that cross-infection alone was not a primary factor in the spread of infection. In addition, the pens themselves had solid sides, and this feature of construction has been recommended since it assists in limiting the lateral spread of infection (Hughes *et al.* 1971; Linton *et al.* 1974).

It is possible that the salmonella may have been spread on the equipment used at feeding time, although we have never been able to isolate *S. typhimurium* from either the machine used for the bulk mixing of the milk substitute or the feeding buckets either before or after they had been used to feed an individual calf (unpublished observations).

The concrete floor of each room was cleaned each day with a pressure hose. This process inevitably caused aerosols, and these may have been responsible for spreading infection amongst the calves.

Salmonella serotype and prophylactic drug therapy

It is possible that either the serotype, or the phage type within a serotype, is of importance in determining the maximum incidence of salmonella excretion. Excretion rates were much lower between 1969 and 1972 when *S. dublin* was the endemic strain in the cattle population (Linton *et al.* 1974) while, in the present

study, the excretions and the mortality rates were higher in 1979 than in 1982, when *S. typhimurium* phage type DT 193 was endemic.

The differences between 1979 and 1982 may have been due in part to the prophylactic use of furazolidone in 1982. The use of this drug in salmonella control was found to be ineffective in eliminating *S. dublin* from calves infected on arrival at the rearing farm (Heard, Jennett & Linton, 1972), although the possibility remains that its use reduced the severity of infection within the batches of calves monitored in this investigation.

Factors affecting the decrease in salmonella carriage

The reasons for the decline in salmonella carriage after the third week has yet to be explained although the self-limiting nature of *S. dublin* and *S. typhimurium* infections in young calves by the fifth or the sixth week has been reported previously (Linton *et al.* 1974; Anon., 1978). It is possible that the decline in excretion rates is associated either with the development of the calves' immune systems or with the evolution of an intestinal microflora which leads to the competitive exclusion of salmonella from the intestinal tract. In any event it would appear that neither the serotype nor the maximum rate of salmonella excretion influenced the time at which the infection rates declined to low levels.

Conclusions

It is clear from these studies that the epidemiology of salmonella infections in intensively reared calves is still poorly understood, although the predictable nature of the infection would suggest that common factors probably operate for each batch of calves.

It is possible that the management of the calves may be important, since the level of excretion of *S. dublin* has been shown to be influenced, to some extent, by the system of husbandry practised on the farm (Linton *et al.* 1974).

The role of cross-infection will only be resolved by the daily swabbing of calves, while a more thorough evaluation of environmental factors is also required. These include the potential role of aerosols in the spread of infection between calves and the importance of small foci of residual infection on the pens in the maintenance of infection from batch to batch.

Thorough cleaning and disinfection, together with an all-in all-out policy, has been recommended as a means of breaking the cycle of salmonella infection between batches of calves (Hughes *et al.* 1971; Anon., 1978). We would not wish to question this suggestion since poor husbandry encourages the persistence of *S. dublin* on calf units (Heard *et al.* 1972) and *S. typhimurium* has been shown to persist on farms which practise minimal disinfection (Anon., 1978). Nevertheless, the apparent success of disinfection may be due in part to the natural tendency for salmonella infections in calves to be self-limiting after a few weeks, and consequently the importance of disinfection routines in salmonella control needs further critical evaluation.

Finally, the calf itself has also been neglected by research workers, and further study is required to define how the development of both its immune system and its intestinal microflora may contribute to the decline in the rate of salmonella carriage that occurs by the time the animals are six to eight weeks of age.

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REFERENCES

- ANON. (1978). Salmonella strains resistant to chloramphenicol. *Animal Disease Report* **2**, No. 2, 4-8.
- APPLEYARD, W. T. & CLEGG, F. G. (1978). Chloramphenicol resistant *S. typhimurium*. *Veterinary Record* **103**, 542.
- DAVIES, G. (1979). Chloramphenicol-resistant *S. typhimurium*. *Veterinary Record* **104**, 128.
- DAVIES, G., SOJKA, W. J., WRAY, C. & WILLIAMS, B. M. (1978). Chloramphenicol-resistant *Salmonella typhimurium*. *Veterinary Record* **102**, 67.
- EDEL, W. & KAMPFELMACHER, E. H. (1969). *Salmonella* isolation in nine European laboratories using a standardized technique. *Bulletin of the World Health Organisation* **41**, 297-306.
- EDWARDS, P. R. & EWING, W. H. (1972). *Identification of Enterobacteriaceae*, 3rd ed. Minneapolis: Burgess.
- GILLIES, P. R. (1956). An evaluation of two composite media for preliminary identification of shigella and salmonella. *Journal of Clinical Pathology* **9**, 368-371.
- HEARD, T. W., JENNETT, N. E. & LINTON, A. H. (1972). Changing patterns of salmonella excretion in various cattle populations. *Veterinary Record* **90**, 359-364.
- HUGHES, L. E., GIBSON, E. A., ROBERTS, H. E., DAVIES, E. T., DAVIES, G. & SOJKA, W. J. (1971). Bovine salmonellosis in England and Wales. *British Veterinary Journal* **127**, 225-238.
- HOBBS, B. C. & ALLISON, V. D. (1945). Studies on the isolation of *Bacterium typhosum* and *Bacterium paratyphosum* B. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **4**, 12-19.
- LINTON, A. H. (1981). Has Swann failed? *Veterinary Record* **108**, 328-331.
- LINTON, A. H., HOWE, K. & OSBORNE, A. D. (1975). The effects of feeding tetracycline, nitrovin and quindoxin on the drug-resistance of coli-aerogenes bacteria from calves and pigs. *Journal of Applied Bacteriology* **38**, 255-275.
- LINTON, A. H., HOWE, K., PETHIYAGODA, S. & OSBORNE, A. D. (1974). Epidemiology of salmonella infection in calves (1): its relation to their husbandry and management. *Veterinary Record* **94**, 581-585.
- LINTON, A. H., TIMONEY, J. F. & HINTON, M. (1981). The ecology of chloramphenicol-resistance in *Salmonella typhimurium* and *Escherichia coli* in calves with endemic salmonella infection. *Journal of Applied Bacteriology* **50**, 115-129.
- M.A.F.F. (1982). Animal Salmonellosis 1980/81. Annual Summaries.
- RYAN, T. A., JOINER, B. L. & RYAN, B. F. (1981). *Minitab Reference Manual*.
- SOJKA, W. J. & WRAY, C. (1980). A survey of drug resistance in salmonellae isolated from animals in England and Wales from 1975 to 1978. *British Veterinary Journal* **136**, 463-477.
- STEEL, R. G. D. & TORRIE, J. M. (1980). *Principles and Procedures of Statistics*. Tokyo: McGraw-Hill Kogakusha.
- THRELFALL, E. J., WARD, L. R. & ROWE, B. (1978a). Spread of multiresistant strains of *Salmonella typhimurium* phage type 204 and 193 in Britain. *British Medical Journal* **ii**, 997.
- THRELFALL, E. J., WARD, L. R. & ROWE, B. (1978b). Epidemic spread of a chloramphenicol-resistant strain of *Salmonella typhimurium* phage type 204 in bovine animals in Britain. *Veterinary Record* **103**, 438-440.
- THRELFALL, E. J., WARD, L. R. & ROWE, B. (1979). Chloramphenicol-resistant *S. typhimurium*. *Veterinary Record* **104**, 60-61.
- WILLSHAW, G. A., THRELFALL, E. J., WARD, L. R., ASHLEY, A. S. & ROWE, B. (1980). Plasmid studies of drug-resistant epidemic strains of *Salmonella typhimurium* belonging to phage types 204 and 193. *Journal of Antimicrobial Chemotherapy* **6**, 763-773.