

The epidemiology of *Salmonella dublin* infection in a dairy herd

I. Excretion and persistence of the organism

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SUMMARY

This paper describes the epidemiologically relevant events that took place in a dairy herd infected by *Salmonella dublin*. The evidence presented indicates that it may be possible to eliminate infection from the farm and that residual infection or persistent excretion are uncommon. In two animals infection persisted, in one instance in the tonsil and in the other in the gall bladder. In this latter case the infection remained from the neonatal period until adulthood. It is possible that both these animals are relevant in a more general context and are indicative of the source of infection in outbreaks in which the origin of infection cannot be determined by more routine examinations.

INTRODUCTION

Clinical salmonellosis of cattle is most commonly caused by *Salmonella dublin* or *Salmonella typhimurium*. Whilst the sources of the latter organism are many and varied *S. dublin* is more restricted in its distribution and may be considered as relatively host specific (Report, 1965).

During the investigation of outbreaks of *S. dublin* infection many authors have been unable to detect a possible source of infection. Thus Meissner & Koebe (1931), Craig, Davies & Massey (1941), Field (1948) and Gibson (1961) failed to detect carrier animals in outbreaks of calf salmonellosis, while Bythell (1946), Field (1948), Grunsell & Osborne (1948), Barron & Scott (1949) and Smith & Rutherford (1965) were sometimes unsuccessful in their attempts to determine the likely source of infection in salmonellosis of adult cattle. These observations indicate deficiencies in our knowledge of the epidemiology of *S. dublin* infections in cattle.

Since December 1969 we have had the opportunity to study in some detail a closed herd of dairy cattle, in which *S. dublin* infection was present. The following account is a description of the steps taken in attempting to eliminate the infection from the herd and the relevant epidemiological observations made during this period. The serological tests carried out during this investigation are the subject of a separate paper (Lawson, Wooding & McPherson, 1974).

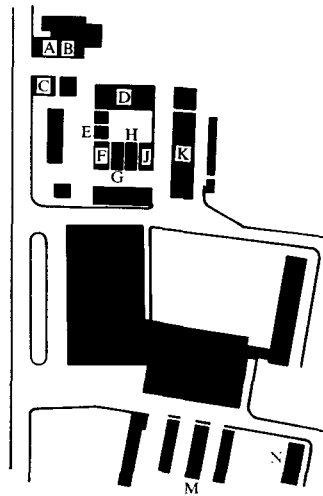


Fig. 1. Plan of infected farm and adjoining premises. *A*, emergency calf house; *B*, isolation byre for cows; *C* and *E*, calf houses; *D*, byre; *F*, *G*, *H* and *J*, court accommodation; *K*, piggery; *M* and *N*, detached calf accommodation.

The farm and stock

The farm steading is a mixture of traditional farm buildings and a newer but largely standard byre, court and calf house.

The principal livestock maintained on the farm are a dairy herd of Ayrshire cattle, flocks of Cheviot and Suffolk sheep, a hysterectomy-derived pig unit and a flock of laying hens. The Ayrshire herd consists of between forty and fifty milch cows and their replacement stock. Male animals have been introduced infrequently, the last introduction being an adult bull in January 1968. In addition to the largely self-contained dairy herd, a number of home-bred Ayrshire and cross Shorthorn calves and occasional batches of purchased calves, have been fattened for beef. The purchased calves which originated from various sources were assembled and then maintained at a rearer's premises until at 6 weeks of age they were moved to the farm.

The farm is similar in character to other dairy farms in the Southern Uplands of Scotland, but, for various reasons, the movement of stock and passage of personnel within the farm is greater than in most herds. There is additional animal accommodation (*M* and *N*) housing calves occasionally, close to the farm but separated from it by buildings not used to accommodate livestock (Fig. 1). The farm and the premises *M* and *N* are largely self-contained areas, but there is always some traffic, mainly of persons and vehicles, between the two.

The farm had a previous history of salmonellosis dating back to 1963. In October/November of that year 62 six-week-old cross Friesian calves were brought into the farm for beef production. Shortly after their arrival mortality occurred associated with *S. dublin* and following rectal sampling three calves were identified as being infected with *S. dublin* and one calf with *S. typhi-murium*: this latter organism has not been encountered on the farm since that isolation. Excretion in this group of calves appeared to have ceased by January 1964 and three clear

samples were obtained from all these calves between January and March 1964. In January and February of the same year, infection with *S. dublin* had spread into the home-bred calves, two of which died and four others excreted the organism. These latter calves were removed to other premises and eventually slaughtered in June of that year. Limited sampling after this time appeared to indicate that excretion had ceased and at no time from 1964 until the outbreak in 1969 was *S. dublin* encountered either in the routine necropsy of calf deaths or in bacteriological samples taken for diagnostic or other purposes from the farm. During the period in question all cattle, sheep or pigs dying on the farm were subjected to post-mortem examination and ancillary bacteriological examination.

Endemic fascioliasis, which may influence the carrier state in salmonellosis (Frik, 1969), is not present on the farm.

MATERIALS AND METHODS

Bovine faecal samples

Calves less than 4 months old were sampled by rectal swabbing using cotton wool swabs. Other young stock and adult cattle were sampled from the rectum using individual polythene gloves. Faecal swabs were placed in 10 ml. volumes of Selenite F broth (Cruickshank, 1965). Faecal samples from older cattle were handled in a variety of ways: (1) an estimated 2 g. sample was inoculated into 10 ml. of Selenite F broth or (2) an estimated 20 g. sample was placed in 100 ml. of Selenite F broth, or (3) two to five samples of approximately 5 g. each from different animals were introduced into 100 ml. of Selenite F broth. In many cases samples were examined by methods (1) and (2) simultaneously. Selenite broths were inoculated as soon as possible after taking the sample, and generally within 3 hr. of sampling. Method (3) was employed for herd tests and where positive results were obtained further individual samples were taken and examined. 100 ml. volumes of Selenite F broth were dispensed in 300 ml. wide-mouth jars with 'twist off' caps.*

Drain swabs

Gauze drain swabs (Moore, 1948) were used and left in position for 24 or 48 hr. before being transferred to 100 ml. volumes of Selenite F broth or Selenite F broth containing 1% lauryl sulphate (Jameson, 1961).

Placentae

A large portion of placental tissue was introduced into 100 ml. Selenite F broth.

Milk samples

Aseptically taken quarter samples from groups of 5 cows were bulked and 100 ml. of milk inoculated into 100 ml. of double strength Selenite F broth.

Bovine tissues

All cattle that have left the farm since infection was first detected have been examined bacteriologically at slaughter. Healthy animals were transported to the Edinburgh Corporation Slaughterhouse direct from the farm and slaughtered

* 'Vapour Vacuum', 66 Whitecap International Ltd., Poole, England.

within 2 hr. of their arrival (with the exception of two animals) at the abattoir. The apparently normal animals were killed and dressed either in booths or in the line system; the others were killed in the slaughterhouse isolation block for a variety of reasons including potential infection.

The following tissues were removed as soon after slaughter as possible and conveyed to the laboratory in individual polythene bags; both tonsils, all the hepatic lymph glands, all the mesenteric lymph glands, the superficial nodes of the colic lymph glands (Sisson, 1963), the last 46 cm. (approx.) of ileum and the gall bladder. The portion of intestine and the gall bladder were ligated before removal from the remainder of the viscera.

At the laboratory the lymph nodes were dissected free of fat, flamed twice to extinction after immersion in methyl alcohol for periods of 30 sec., ground with sand in a mortar and inoculated into an appropriate volume of Selenite F broth. Hepatic and colic lymph nodes were inoculated into one or more 10 ml. amounts of broth and tonsils and mesenteric lymph nodes were inoculated into one or more 100 ml. volumes of Selenite F; approximately 100 ml. of bile were added to 100 ml. of double strength Selenite F broth. The portion of small intestine was opened longitudinally, washed gently in running water and the mucosal surface dried with a paper towel. After flaming the whole sample was ground and inoculated into 100 ml. Selenite F in a similar manner to the other tissues.

Meal samples

Approximately 30 g. samples were shaken with 100 ml. distilled water for 15 min. The meal was allowed to sediment and the supernatant decanted into a pot containing 100 ml. double strength Selenite F broth.

Rodents and sparrows

The cadavers of rodents and house sparrows were flamed externally and the abdomen opened with sterile instruments. The entire abdominal and thoracic contents were removed, ground with sand and transferred to appropriate amounts of Selenite F broth.

Cultural procedures

The 10 ml. Selenite F broths were incubated for 18–24 hr. at 37° C. whereas the 100 ml. and larger volumes were normally incubated for a full 24 hr. before sub-culturing to deoxycholate citrate agar (Oxoid CM 35) or to 0.5% sucrose deoxycholate citrate agar (modified Oxoid CM 227). Plates were incubated for 18 hr. and representative non-lactose fermenting colonies were sub-cultured to MacConkey agar (Oxoid CM 7). Colonies on MacConkey agar were subjected to slide agglutination with Salmonella 'O' and 'H' antisera* and to such other standard identification procedures as were necessary. Colonies giving the typical agglutination reaction (O 9+; H gp+, H mt-) were tested in Kohn two tube media (Oxoid CM 179 and CM 181) and those giving the correct biochemical reactions were accepted as *S. dublin*. Representative strains were all confirmed as *S. dublin* by the Central Public Health Laboratory.

* Wellcome Reagents Ltd.

Macro-colony examination

Cultures for macro-colony examination were stored in screw-capped nutrient agar slopes in the dark, at room temperature. Macro-colonies were cultured using modification of the methods suggested by Jameson (1966). Inocula taken from 18 to 24 hr. peptone water cultures were placed on the agar surface by means of a 2 mm. diameter loop. The loop was fashioned from two turns of 24 SWG Nichrome wire one upon the other, with the plane of the loop at right angles to the stem of the inoculating wire. Nineteen colonies were equidistantly inoculated on to each 85 mm. diameter plate, the site of inoculation being determined by a standard placed underneath the agar plate. Macro-colonies were grown on a number of different media incubated at various temperatures; differentiation appeared most satisfactory on two of the media incubated at 32° C. for 3–4 days. These media contained proteose peptone (Oxoid L 46) 2·4 g.; triphenyl tetrazolium chloride 0·004 g.; Teepol 610 (B.D.H.) 0·2 ml.; de-ionized distilled water 200 ml. and either Noble agar (Difco) or Ion agar No. 2 (Oxoid L12) 2·4 g.

When two strains were being compared this was always done on the same plate.

Biotypes of S. dublin

The biochemical reactions of the strains isolated during the investigations were examined in arabinose, dulcitol, xylose and rhamnose and in Stern's glycerol medium (Hall & Taylor, 1970). Single colonies of the isolate to be examined were inoculated into peptone water and incubated for 8 hr. at 37° C.; one drop of this culture was then inoculated into the sugar media. Stern's glycerol media was inoculated from 18 to 24 hr. cultures on solid media.

*Control measures**Disinfection*

Buildings. Potentially infected faeces and bedding material from the courts, calf-houses, and isolation byres were, with appropriate precautions, removed prior to cleansing and disinfection. This material was ploughed into arable land either immediately or after stacking in the centre of a midden of uninfected manure.

After removal of the bedding the potentially contaminated areas of the accommodation were soaked overnight with a cresol-type approved disinfectant solution. The area was then scrubbed with detergent solution* and washed. Finally, formalin fumigation wherever possible or alternatively a re-spraying with the disinfectant solution completed the operation.

The cleansing and disinfection was carried out by the farm staff as circumstances demanded while excretors were still being detected. In May 1970, when all cattle went out to grass, all cattle buildings were cleansed and disinfected. Throughout the period of observation foot baths containing cresol-type disinfectants were used at the entrances to the various sections of the animal housing by the farm staff and visitors.

Animals. When they had ceased excreting, and immediately before the animals

* By-prox (British Petroleum Ltd).

rejoined their appropriate group within the herd, the hind quarters and tails of infected calves and cows were washed with detergent solution and rinsed with chloroxylenol solution.*

Movements of stock

Non-essential movements of stock were eliminated. Two weeks after infection was first detected the movement of cows between court *J* and the byre *D* ceased: only dry cows thereafter occupied court *J* (Fig. 1).

Recording

Written daily records of all events were maintained throughout the investigation.

RESULTS

The epidemiologically relevant events immediately preceding the onset of the 1969 outbreak are confused and we are still in some doubt as to their relative significance.

Events seemed to be initiated by a cow (N21) calving in court *J* (Fig. 1) on 20 November 1969. The calf was taken to the calf house *E* and on 22 November became ill showing pyrexia and respiratory symptoms. The following day in court *G* a 2-month-old calf (U24) which had come from the calf house on 21 November, was found to be pyrexia and exhibiting symptoms compatible with a mechanical injury to the locomotor system. This animal was immediately segregated from the remainder of the group. On 28 November it developed pneumonic symptoms and diarrhoea, and was found to be excreting *S. dublin*. Shortly after this most of the young calves in calf house *E* showed symptoms and on 1 December, 7 of the 13 calves were found to be excreting the organism. A possible human contact existed between the immediately postparturient cow, N21, and a known *S. dublin* outbreak in calves on an unrelated premises (*T*) some two miles distant; this contact will be mentioned later.

The disease in young calves

The disease in calves less than 3 months of age was characterized by pyrexia, respiratory symptoms, and diarrhoea. In older calves infection may have been associated with transient anorexia. These symptoms were similar to those described by Craig *et al.* (1941).

At the commencement of the investigation calf-house *E* contained 13 calves, ranging from a few days of age to about 3 months, housed in double pens. All calves were treated with chloramphenicol at 150 mg./lb. body weight intramuscularly for 3 days, and 4 days later moved to clean accommodation in a converted byre *C*. This was to remove the known excreting calves from the proximity of the majority of the herd, and to enable the calf-house to be washed and disinfected to receive new-born calves. Fifteen days after moving the calves, clinical symptoms occurred in 5 of them which were then treated with ampicillin

* Dettol (Reckitt and Colman).

(100 mg./30 lb. body weight); temperature and faecal samples taken previously suggested that 3 of these calves had new infections and were not relapsed cases.

Two young calves were destroyed because of their clinical condition, one (N21's calf) on 2 December 1969 and the other on 8 January 1970. Ultimately all 13 calves were demonstrated to have been infected at some time, and faecal excretion by the group persisted for 53 days after the initial discovery of infection in the calf-house.

The drain from the calf-house *C* was sampled at a trap immediately outside the building during the period when it held infected calves. Calf effluent soaked into the straw bedding and percolated into the drains undiluted by water. Positive drain swabs were obtained during the period of faecal excretion and also on the day following the last known excretion by a calf. The subsequent drain swab taken 8 days later proved negative. The calf pens were then cleaned and disinfected: thereafter all faecal and drain swabs proved negative, including a group sample taken two days after castration and de-horning on 13 April 1970. These results are summarized in Table 1.

Faecal excretion in older calves

At the start of the outbreak, courts *F*, *G* and *H* (Fig. 1) held 13 (5–11 months old), 11 (3–5 months old) and 13 (12–18 months old) calves and young stock respectively. Examination of faecal samples from court *F* revealed two animals U10 and U16 excreting between 10 and 29 December. One further group sample (containing animals U4 to U9) proved positive on one occasion (15 December) but an individual excretor could not be identified. Six samples taken between January and April failed to demonstrate further excretion by animals in court *F*.

Court *G* contained the animal U24 which had been isolated because of possible injury at the time when it was found to be infected. This animal was destroyed on 2 December. We were unable to detect any further faecal excretors in either courts *G* or *H* when each group of animals was sampled on six occasions.

Faecal excretion by adult cows

The whole herd was sampled on ten occasions between 3 December and 5 February; thereafter group faecal samples were examined monthly and additional individual samples taken when necessary.

When infection was first detected the majority of the cows were tied up in their own stalls in the byre, except for two periods in the week when they were put out in small groups into an exercising yard immediately outside the byre. As there were more cows than the byre could accommodate, 20 cows shared accommodation, alternating between 10 stalls in the byre and court *J*. This arrangement was terminated on 12 December, from which date only dry cows were housed in court *J*.

During the 12 months following the discovery of infection 8 excreting cows were identified. Five of the 8 cows belonged to the group which shared housing in the byre and court *J* (Fig. 1). Faecal excretion was not associated with observable clinical symptoms in any of the adult cows.

Table 1. Faecal excretion of Salmonella dublin by calves, and related events

Calf or sample	December											January							February		
	1	2	†	15	18	22	23	29	5	8	12	19	22	23	26	27	28*	29	4	19	23
Calf U17	0	.	.	0	.	0	.	0	0	D
U21	0	D
U28	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U29	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U30	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U33	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U34	0	.	.	0	.	0	.	0	0	.	0	.	0	0	.	.	.	0	0	.	0 ¹
U35	0	.	.	0	.	0	.	0	0	.	0	.	0	0	.	.	.	0	0	.	0 ¹
U36	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U37	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U38	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U39	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
U40	0	.	.	0	.	0	.	0	0	.	0	.	.	0	.	.	.	0	0	.	0 ¹
Drain swab	0	0	0
Sparrows	0
Sparrow droppings	0
D Killed.	0

* Pen C cleaned and disinfected.
 † All calves treated Chloramphenicol 2-4 December; moved to calf house C on 8 December.
 0 Sampled, positive for *S. dublin*. 0, Sampled, negative for *S. dublin*. 0¹, Also negative on 20 March, 15 April, 4 May and 21 October.

When infection was detected in a faecal sample the individual cow was placed in isolation in the small byre *B* until at least 5 clear samples were obtained after which the animal was returned to the herd. Despite the removal of excreting animals from the herd, new cases continued to be encountered and, in an effort to control the spread of infection, the bi-weekly exercising period was stopped from 12 December. Isolation of individual adult excretors was discontinued at this time.

An additional cow (S18) excreted between 17 and 29 December followed by a period when no further excretion was detected, until cow R28 was found to be infected on 26 January. This animal had been introduced into the byre on 12 January after having given 6 previous negative samples. She calved on 24 January and 2 days later a group sample indicated infection which was confirmed by an individual sample 4 days later. A further animal (S34) introduced into the byre on 26 January, gave a negative sample on 5 February, calved on 7 February and excreted on 9 and 11 February.

Since we were unable to account for a source of infection for either of these animals (the last known previous excretion being detected in calves on 22 January) an extensive examination of the cow stalls was made on 16 February. *S. dublin* was isolated from scrapings taken from two of the stalls at the bottom of the byre which had been used by the court cows, but currently was occupied by cows G17 and S34. The organism was not recovered from R28's stall and neither of the cows previously standing in R28 or S34's stalls could be incriminated as infected either on serological grounds (Lawson *et al.* 1974), or by the detection of faecal excretion. Following this, all the byre stalls were thoroughly cleaned and disinfected. The detection of excretion in S34 and R28 suggested a possible relation between calving and excretion, and from this time all calving animals were sampled daily for at least 7 days following the calving. No further excretion was detected until the cow G17 proved positive on 2 June. This cow had been sampled 13 times between 3 December and 21 April, being negative on each occasion except for a positive group sample taken on 3 December which included faeces from this animal. The excretor was not detected by individual samples taken two days later. Excretion by G17 was detected intermittently between 2 June, the day she calved, and 6 July; 10 of the 25 samples taken during this period proved positive. Twenty-four further daily 20 g. samples were negative prior to her slaughter on 30 July.

During previous sampling of infected cows we had been of the opinion that 2 g. faecal samples were sometimes insufficient to detect excretion. However, in no case had excretion continued long enough to allow a quantitative estimation to be made. From the cow G17 we were able to examine 17 faecal samples, using 20 g. and 2 g. portions from the same sample. *S. dublin* was not identified in any of the 2 g. inoculations but was isolated from 5 of the 20 g. portions of faeces. Faecal excretion among these adult cows is summarized in Table 2.

Drain swabs were taken on a limited number of occasions and at least 3 of these were in appropriate positions when some cows were known to be excreting. None of these or any other swabs from drains containing effluent from adult cows proved positive.

Faecal sampling of all the adult milking animals in the herd continued on a

Table 3. *Salmonella dublin* status of yearlings and calves (during December 1969 to February 1970) and the time of their slaughter or first calving

Month	Year of slaughter or first calving		
	1970	1971	1972
January	—	—	—
February	—	0 0 0	0° 0°
March	—	0 0 0	0 0 0° 0°
April	—	0 0 0 0	—
May	0 0 0 0 0 0 0 0	0* 0 0 0	—
June	—	0 0	—
July	—	—	—
August	0 0 0	—	—
September	—	0° 0° 0° 0° 0° 0°	—
October	0 0 0 0	0°	—
November	0	0	—
December	0	—	0

Animals slaughtered	Animals sampled at first calving	Status (December 1969–February 1970)
0	0°	Known <i>S. dublin</i> excretors
0	0°	Possible serological evidence of infection
0	0°	No known excretion or serological evidence of infection
0*		Animal U38 – positive culture <i>S. dublin</i> from bile at slaughter

monthly basis until January of 1971, a year after the discovery of infection. Parturient cows continued to be sampled as described previously until March 1971, by which time all had calved once since the appearance of herd infection. In addition the heifers which had been infected as neonates were similarly examined at the time of their first calving between September 1971 and February 1972. (Table 3).

Miscellaneous investigations

Milk

Examination of group milk samples from the herd taken on 5 December 1969 failed to demonstrate the presence of *S. dublin* and no further milk samples were taken.

Placentas

Placentas from 17 animals were obtained during the period June 1970 to March 1971. In 10 instances the placentas came from potentially infected animals at their first parturition following herd infection; these included 5 known faecal excretors and 5 with possible serological evidence of infection. In no case was *S. dublin* isolated from a placenta.

Other domestic livestock

Faeces from all the housed sheep, farm dogs and representative samples from poultry were examined at the outset and proved negative. The hysterectomy-derived piggery (K) contained the only other livestock in close proximity to the main cattle herd and these were not thought to be at risk owing to the isolation

procedures in operation. On the 9 February 1970 an 8-month-old pig died with gross lesions of lymphosarcoma, and a fibrinous enteritis. *S. dublin* was isolated from the small intestine of this pig and from a further litter-mate also affected with lymphosarcoma in which the organism was recovered from pneumonic lesions at necropsy on 21 May. Initially, the origin of these infections was inexplicable until further investigation revealed a possible explanation. The drains from the byre, calf-house and piggery join a common main drain which is liable to occasional blockage. When the main drain becomes blocked, effluent flows back into the piggery drains which are on a lower level than the other buildings; the pen first affected by this back-flooding is the pen which housed the infected pigs and the drain was known to have become blocked during the late autumn of 1969. No further evidence of infection within the piggery was discovered and no other possible methods of infection could be substantiated.

Rodents and sparrows

Though rats (*R. norvegicus*) and mice (*Mus musculus*) were not particularly common on the farm it was possible to examine 16 specimens caught in the area adjoining calf-house *E*. *S. dublin* was not recovered from any of these rodents. Large numbers of house sparrows (*Passer domesticus*) used the infected calf-house *C* for roosting, and their presence constituted a possible method of spread of infection. Sparrow droppings collected from this house on 8 and 12 January yielded *S. dublin*; thereafter samples taken from this site were negative. The viscera of 15 sparrows collected in this house also failed to yield the organism. In addition, large bulked samples of all recognizable faecal droppings collected on 12 January from 5 roosts and feeding perches elsewhere on the farm failed to yield *S. dublin*.

Animal feeds

Representative samples of fish meal, protein concentrate (2 samples), prepared calf feed and milk substitute (2 samples), in use at the commencement of the outbreak were examined for the presence of *S. dublin* with negative results.

Calf accommodation M and N

On 17 January a 4-week-old Friesian calf was introduced to accommodation *M*. A routine faecal sample taken on admission yielded *S. dublin* as did the viscera at necropsy on 19 January. Subsequent to these isolations, mortality due to *S. dublin* took place in young dairy calves in block *N*. We were satisfied however, that there was no connexion between the infections in premises *M* and *N* and the farm outbreak because the macrocolony type of the isolates from the calves in *M* and *N* were identical, but distinct from any of the types derived from the dairy farm (see below).

Examination of tissues from slaughtered animals

This work has not been completed because it is dependent on the culling of animals from the milking herd. To date 30 of the original 55 adult cows present in the herd at the time of infection, together with 21 of the 32 calves and all the 13 yearlings have been examined.

Table 4. *Salmonella dublin* status of adult cows (during December 1969 to February 1970) and the time of their slaughter

Month	Year of slaughter		
	1970	1971	1972
January	—	00	0
February	0	00	00
March	—	—	—
April	0	00	—
May	000	—	00
June	—	—	—
July	000 ¹	—	—
August	—	00 ²	—
September	00	00	—
October	—	—	—
November	—	—	0
December	00	00	—

Infected status at December 1969/February 1970:

0 Known faecal excretors *S. dublin*.

0 Possible serological evidence of infection ('O' > $\frac{1}{80}$ or 'H' > $\frac{1}{80}$ or CFT (P) > $\frac{1}{4}$).

0 Not thought to have been infected.

¹ Cow G17 positive *S. dublin* at slaughter (see text).

² Cow N21 (see text) and all others negative at slaughter.

Adult cows

Ten cows known to have been faecal excretors or to have had raised serological titres (Lawson *et al.* 1974), have been examined at slaughter. The details of these adults are given in Table 4. *S. dublin* was recovered from the tonsils, and from a 20 g. sample of the intestinal contents of animal G17. This latter material, portions of the spleen (20 g.) and the liver (20 g.), the supramammary lymph nodes and seven portions of the small intestinal wall were examined in addition to the tissues routinely examined. The macrocolony type produced by the isolates obtained at slaughter was identical with those produced by isolates recovered from faecal samples between 2 June and 6 July.

One animal, N21, had shown a more persistent serological titre than any of the other animals and for this reason the following additional tissues were examined: supramammary lymph nodes, mediastinal lymph nodes, gall bladder wall, and small intestinal contents. *S. dublin* was not isolated from any of these tissues. The remaining 19 cows believed to be non-infected failed to yield the organism at slaughter.

Yearlings (court H)

Faecal excretion had not been demonstrated in this group although serological tests indicated that at least 2 animals had been exposed to infection. These animals were slaughtered 5–10 months after possible exposure and proved culturally negative.

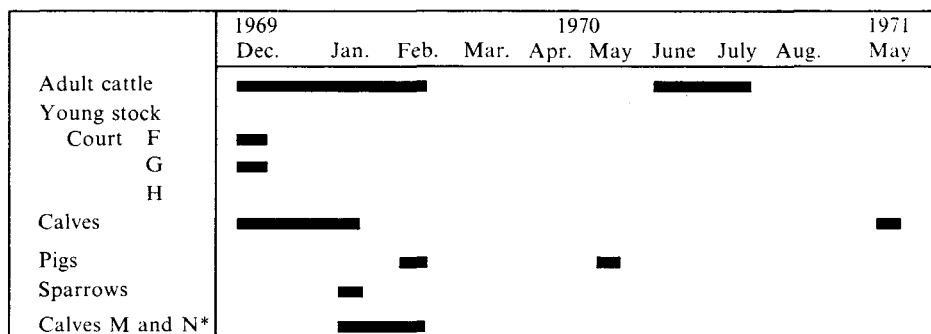


Fig. 2. Summary of known infection in outbreak. * Infection originated from different source.

Calves (courts F and G)

Both courts contained animals shown to have been faecal excretors and serological examination indicated the presence of additional infected animals. Thirteen of the 23 animals were slaughtered 10–22 months after exposure and all proved negative.

Calves (calf-house E)

This group were all known faecal excretors as neonates. Eight of the 10 animals were slaughtered 12–24 months after infection and *S. dublin* was isolated from one (U38). Following neonatal infection, 10 negative samples had been obtained from this animal before the recovery of the organism from the gall bladder at slaughter. Three animals (W5, 8 and 9) thought to be susceptible and not previously exposed to infection, had been introduced into the court occupied by U38 3 days before her slaughter. Faecal examination and culture of the tissues of these animals at slaughter 42 days later failed to yield *S. dublin*, although serological examination of paired serum samples yielded some evidence that they may have been exposed to infection. These results are recorded in Table 3 and the known infections of the whole outbreak are summarized in Fig. 2.

Identity of strains isolated

Macro-colony examination

Macro-colonies grown from cultures isolated from a number of the infected cattle were compared. The macrocolonies proved to have a range of forms and if the whole range of isolates had not been available those at the extremities of the range might have been considered to be dissimilar. We were unable to link any particular colony type with the calves, young stock or adults, each group seeming to contain some of the variations observed. The macrocolony type of *S. dublin* present on the premises T which had been a possible source of infection for the outbreak (see Results: History) differed slightly but consistently in appearance from any of the farm isolates. This difference caused us to critically re-examine the events leading up to the discovery of infection and helped us to conclude that it was extremely unlikely the two outbreaks were related.

The majority of macrocolony examinations were carried out on single isolates from individual animals but in the case of cow G17 a series of 10 cultures isolated at different times were examined. All isolates produced identical macrocolonies.

The macrocolonies produced by the two isolates obtained from pigs were similar to each other and did not differ from some of the bovine isolates. The isolate obtained from the bile of animal U38 at slaughter 17 months after neonatal infection differed by being anaerogenic and of different macrocolony type to the isolates from the initial herd infection. In view of the long period between calf-hood infection and slaughter we do not feel that these colonial differences preclude the possibility that the isolates had a common origin.

Biotype

Seven out of the 12 isolates examined from the farm outbreak could be classified as belonging to Biotype D. However, altogether 6 biotypes were represented in these isolates; A, B, D, G, T and L. The variability of these results was confirmed by repeated examinations of fermentation reactions in arabinose and Stern's glycerol media using replicate and different batches of media. Acid was produced from arabinose within 14 days by 12 out of the 33 isolates from the farm with a range of from 2 to 19 days (mean 15 days).

DISCUSSION

Investigations into the epidemiology of *S. dublin* infection in cattle have largely concentrated on studies of clinical infection or of localization in the gall bladder in known excretor animals (Field, 1948; Gibson, 1958). The majority of these studies have taken place on cattle from areas where fascioliasis is endemic. This inter-current disease which affects the localization of the organism, in the tissues of the bovine (Frik, 1969) does not occur in the herd we investigated. As Watson, Wood & Richardson (1971) point out, no attempt has been made to examine the tissues of non-excreting animals in known infected herds. In the herd studied by these authors the results obtained from the slaughtered animals suggested that in some cases at least the animals were undergoing recent infection, and their results resembled those found in animals slaughtered after some days of travel (O'Conner, Murphy & Timoney, 1967). For this reason their results give little information on the possible persistence of the organism in the tissues of recovered animals. One difficulty in examining bovine tissues is the volume of material which may potentially harbour the organism and the obvious impracticability of examining more than a small proportion of this tissue. We are aware of this shortcoming in our own study and it was with this in mind that the lymphoid tissues examined were chosen.

The herd studied in this report was exceptional in the facilities for repeated sampling and for the handling of animals prior to slaughter.

The investigation described gives some indication of the problems which might be encountered in trying to eliminate *S. dublin* from a cattle herd and also provides a rational explanation for the appearance of clinical infection in young animals

in the absence of the introduction of infection to the herd or the apparent absence of known carrier animals. Two carrier animals were detected and one of these (G17) was a young animal in the herd at the time of the previous clinical infection in 1963 and may have been infected since that time. At slaughter *S. dublin* was isolated from the tonsils which may have been shedding organisms into the alimentary canal at a level which had not been detectable by faecal sampling during the previous 24 days. Persistent tonsillar infection has been demonstrated in sheep (Gitter & Sojka, 1970) although in the instance cited it was not associated with persistent faecal excretion. The demonstration of *S. dublin* in the gall bladder of U38, 17 months after infection, indicates a further potential hazard in any attempt to eliminate *S. dublin* from a herd. Although we have no estimate of the number of organisms present in the bile, the repeated negative faecal samples from this animal (U38) following calthood infection may indicate that only small numbers of organisms were present in the gall bladder. This finding is contrary to the observations of others who have regularly associated gall bladder infection with detectable faecal excretion (Gibson, 1958; Frik, 1969).

In three instances excretion by adult cows was closely associated with parturition, and in cow G17 the evidence suggests that this may have been a quantitative increase rather than an absolute change in excretor status. In 1 of the other 2 cows (R28) the serum titres indicated that infection had taken place some 2 months prior to excretion and parturition. These observations demonstrate a mechanism for the previously postulated link between calving and the appearance of clinical herd infection (Report, 1965). This parturition-associated excretion may be restricted to a period following infection because the examination of the remainder of the herd at parturition over the next year failed to disclose further excretors.

There was a significant difference in the incidence of infection between varying ages of calves. All the neonate calves rapidly became infected following exposure whilst the older calves in court A varied in response. These latter animals were all 'loose-housed' together but despite the presence of at least 3 excretor animals other members of the group did not become infected as judged by the absence of serological responses and failure to detect faecal excretion. In other circumstances without this evidence it might have been assumed that all the group had been infected.

Rats and mice have been incriminated as potential carriers of infection (Gibson, 1958). However, house sparrows probably constitute at least as great a potential threat in the spread of infection. In the autumn, flocks of this species disperse locally over a radius of a few miles (Summers-Smith, 1963). Our evidence suggests that this species is unlikely to be commonly involved in the spread of infection either between or within infected farms.

Simple and easily applied methods for detecting infected herds or premises are of great practical value. Robinson (1966) suggested that drain swabs were useful for detecting *S. typhimurium* infection among adult cattle. In our investigations drain swabs provided an accurate assessment of the *S. dublin* excretor status among calves but we have no confidence in their use for detecting

low levels of excretion by adult cattle infected with this serotype when the animals are housed in byres.

Macrocolonies, like most epidemiological markers, did not provide an absolute guide to the identity of different isolates thought to originate from the same source. However, they were most useful in the demonstration of identity between the isolates obtained from G17 before and at slaughter. In the absence of this information the isolates from the tonsil and intestine might have been considered to have originated from infection in the lairage. Our experience in biotyping the isolates obtained in this outbreak would indicate that, where carbohydrate fermentation is mutative (Kauffman, 1954), the mutation rate is likely to be so variable that some of the biotypes of Hall & Taylor (1970) do not have epidemiological significance.

The control of spread of *S. dublin* infection amongst housed adult cattle can be achieved by simply restricting the movement of animals, whilst infection in calves can be controlled by isolation and disinfection. The detection of the carrier animal still remains a problem and failure to identify these animals may result in the re-appearance of infection at a later date.

This study would not have been possible without the whole-hearted co-operation of a large number of people; in particular, we would like to acknowledge the valuable assistance of the Veterinary Staff of Edinburgh Corporation abattoir and the Staff of the involved farm.

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