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

II. Serology

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SUMMARY

A number of serological tests were evaluated in a study of *Salmonella dublin* infection in a dairy herd. None of the tests used detected either of the two carrier animals from which *Salmonella dublin* was isolated at slaughter 7 and 17 months after the herd infection. The complement fixation tests used proved to be a better guide to the presence of recent herd infection than the conventional 'O' or 'H' agglutination tests.

INTRODUCTION

Serological tests have been employed during studies of bovine salmonellosis both in the field disease (Field, 1948) and in experimentally infected cattle (Frik, 1969). These workers employed conventional salmonella 'O' and 'H' antigens. The passive haemagglutination test has been utilized in studies on salmonellosis of other species (Seiburth, 1957) and although this test differs in sensitivity it appears to measure a similar antibody to that determined by conventional 'O' agglutination tests. The serological responses of cattle to infection by *Brucella abortus* as measured by the complement fixation test (Jones, Hendricks & Berman, 1963) or the Coombs test (Cunningham, 1968) sometimes differ from the response measured by the agglutination titre (Wisniowski, 1964), and are therefore additionally a better indication of the time of the infection.

The following is an account of the serological responses encountered in a herd of cattle infected with *Salmonella dublin* and the relationship between these results and the recovery of the organism during life and at slaughter. The details of the detection of infected cattle and the circumstances of the outbreak have been discussed in a previous paper (Lawson, McPherson, Laing & Wooding, 1974).

MATERIALS AND METHODS

Sera

Blood samples from the jugular vein of calves or the coccygeal vein of adult cattle were taken into vacutainer tubes.* Serum was obtained from these samples after clotting, using centrifugation when necessary, and stored at −20° C.

Serological tests

'H' antigen. Nutrient agar slopes were inoculated with an eighteen hour peptone water culture of *S. dublin* (1420/69). After incubation for 18 hr. at 37° C the growth

* Becton Dickinson.

was washed off with 0·2% formol saline. This concentrated antigen suspension was stored at 8° C. and diluted with normal saline before use. Batches of antigen were standardized by titration with a positive bovine *S. dublin* serum in a similar manner to the procedure employed for *Br. abortus* antigen (Alton & Jones, 1967), the density of the diluted antigen corresponding approximately to Brown's opacity tube 3. *S. dublin* antisera used for standardization were kept in small aliquots at −70° C.

'H' titres were read by recording the amount of deposited agglutinated antigen. Some difficulty was experienced in accurately estimating the end-point as there was a tendency for some sera to produce slight agglutination over two and occasionally more dilutions. For this reason the test result was recorded as the last dilution giving 50% or more agglutination. In later tests a quantitative control of this end point was included for comparison; this utilized a known positive serum to which a half unit of antigen had been added.

'O' antigen. A non-motile strain of *S. dublin* (322/68) was grown on nutrient agar slopes using the method employed for the production of 'H' antigen. The growth was washed off the slopes with the minimum amount of normal saline and 20 volumes of absolute alcohol added to one volume of antigen suspension. The mixture was held at 50° C. in a water bath for half an hour after which the antigen was washed twice in normal saline by centrifugation and re-suspended in 0·25% formol saline. Dilution and standardization were as for the 'H' antigen.

Complement fixation test antigens. The two antigens employed were the alcohol treated 'O' antigen (CFT(A)) suitably diluted in veronal buffer and a phenolized suspension (CFT(P)) prepared from the non-motile 322/68 strain in a similar manner to that employed in the production of *Br. abortus* agglutinable suspension (Alton & Jones, 1967).

Agglutination tests

Doubling dilutions of antisera were prepared in 75 × 10 mm tubes and equal volumes of antigen added to the diluted sera to make a final volume of 1·0 ml. 'H' agglutination tests were incubated at 37° C. for 2 hr., allowed to stand overnight at room temperature and then recorded as the amount of agglutinated antigen deposited at the bottom of the tube. 'O' agglutination tests were incubated overnight at 37° C. and the density of the supernatant antigen compared with similarly incubated standards containing 1, $\frac{3}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ units of antigen suspended in saline.

Coombs tests

'O' agglutination tubes showing no agglutination were centrifuged to deposit the antigen which was then washed three times in 1 ml. volumes of normal saline and finally resuspended in 0·4 ml. rabbit anti-bovine serum optimally diluted with normal saline. The tubes were re-incubated overnight at 37° C. and the test read as the amount of deposited antigen. The rabbit anti-bovine serum did not agglutinate *S. dublin* 'O' antigen in tube agglutination tests at a dilution of 1/2, and in the Coombs test it was used at a dilution of 1/80.

Complement fixation tests

Sera to be tested were heated at 56° C. for ½ hr. All tests were carried out in WHO Perspex trays using veronal buffer (Oxoid BR16) as diluent. Defibrinated washed sheep cells at 2 % and equal volumes of suitably diluted haemolytic serum* in buffer (containing 2 units haemolysin) comprised the haemolytic system.

After preliminary complement titration, antigens were titrated in the presence of excess complement using the serum from an animal known to have been infected by *S. dublin*. The quantity of antigen chosen as a result of this titration was that amount which gave the highest titre with the positive serum. In the test itself sera were serially diluted in 0·2 ml. volumes of buffer and to these were added one volume (0·2 ml.) of diluted antigen and one volume of diluted guinea-pig complement (containing 2 MHD_{100} complement). After overnight fixation at 5° C. the test trays were warmed at 37° C. for five min. and one volume of haemolytic system added. The trays were agitated to mix and then incubated at 37° C. for 1 hr. A range of control dilutions prepared without antigen was always included for each serum, and sera showing anti-complementary activity in the range of a positive result in the test itself were discounted. A positive control bovine serum, stored in small volumes at −70° C. was titrated with each series of tests.

RESULTS

It is known that sera from cattle thought not to have been exposed to infection with *S. dublin* show titres against both the 'O' and 'H' antigens of this organism (Field, 1948). Presumably these antibodies are induced by exposure of the animals to other members of the enterobacteriaceae or other bacteria which possess related antigens.

For comparative purposes the serum titres found in a herd of cattle on a neighbouring farm from which routine necropsies and cultural examination of clinical material over a period of many years failed to demonstrate any evidence of infection with *S. dublin* are listed in Table 1.

Adult cattle

The serum titres found in the herd at the time of active infection are given in Table 2. Where faecal excretion of *S. dublin* was detected from individual animals the titres obtained subsequent to the discovery of infection are given. The later results obtained after the period in which infection was demonstrated in adult animals are given in Tables 3*a* and 3*b*. In all the tests carried out during the period of active infection, a high proportion of known infected animals showed titres in the upper range. In every test, however, a varying number of animals showed raised titres which did not correlate with known infection. The distribution of serum titres found in the 'O', 'H', Coombs and CFT(A) tests approximated to a normal distribution about the mean. However, in the case of the CFT(P) the test tended to divide the animals into two distinct groups although the lower group still contained known infected animals. The CFT employing alcohol antigen

* Haemolytic serum (horse), Wellcome Reagents.

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Table 1. *Distribution of serum titres (reciprocal) against* Salmonella dublin *in 29 adult cattle in an uninfected herd*

Agglutination test			CF test (phenol antigen)	
	'O' antigen	'H' antigen		
Titre	No. of animals	No. of animals	Titre	No. of animals
10	0	7	< 4	28
20	0	13	4	1
40	18	9	8	0
80	11	0	16	0
Mean titre	55·0	24·0	0·138	

(CFT(A)) proved more sensitive and yielded higher titres with the sera from known infected animals than the test carried out with the phenol antigen (CFT(P)). This increased sensitivity avoided some of the problems associated with the interpretation of result from sera which were anticomplementary. Later experience, however, indicates possible disadvantages in the use of the test employing an alcohol antigen (see below). In each of the four tests ('O', 'H', Coombs and CFT(A)) many titres fell within the range where some doubt might exist as to the interpretation of the result.

Some six months after the period of active infection the mean 'O' and 'H' titres had varied little from the previous tests (Table 3*a*) although in the case of the 'O' agglutination tests the number of animals with titres of 1/320 and above had fallen considerably. The most dramatic reduction in the mean herd titre occurred with the CFT(P), where of eleven animals previously showing high titres all but one (a known infected animal) had returned to 1/8 or less. By this time the titres in the CFT(A) had fallen more markedly than in the 'O' and 'H' tests but less than the titres in the CFT(P).

Fifteen months after infection the whole herd showed an increase in the level of 'O' and 'H' titres, not associated with known infection, and the majority of sera gave 'O' titres of more than 1/80. The CFT(A) showed a number of animals with newly acquired titres of 1/32 or more which might have been considered as indicative of infection but these titres could not be related to known past or present infection. The CFT(P) on these animals with raised CFT(A) titres gave levels of less than 1/8 in every case except animal N21 which retained her raised titres until slaughter (CFT(A) 1/32; CFT(P) 1/16).

Of the adult animals exposed to infection and subsequently slaughtered, only one animal (G17) yielded *S. dublin* and a comparison between this animal and those negative at slaughter is shown in Table 4. All these animals, except two not thought to have been infected were slaughtered more than 4 months after the period of active infection.

Table 2. *Serum titres in adult cattle, infected farm December 1969–January 1970 (infected phase)*

'O'		'H'		Coombs		(CFT(P)		CFT(A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.	Titre	No.
10	1	< 10	7	40	5	< 4	27	< 8	5
20	8	10	15	80	10 (1)	4	10 (2)	8	3
40	6	20	15 (3)	160	13 (1)	8	0	16	17 (1)
80	23 (3)	40	4 (2)	320	7 (1)	16	7 (3)	32	13 (1)
160	1 (1)	80	4 (1)	640	2 (2)	32	2	64	4 (2)
320	7 (1)	> 80	2 (1)	1280	2 (2)	> 32	2 (2)	> 64	5 (3)
640	1 (1)	–	–	> 1280	1	–	–	–	–
1280	1 (1)	–	–	–	–	–	–	–	–
Mean titre	80		26		–		7·15		34

Numbers in parentheses denote number of animals known to have excreted *S. dublin*.

Table 3*a*. *Serum titres in adult cattle, infected farm June* 1970 (6 *months after infection*)

'O'		'H'		CFT (P)		CFT (A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
10	0	< 10	9 (1)	< 4	29 (5)	< 8	3
20	3	10	12 (3)	4	9 (1)	8	4 (1)
40	9 (1)	20	12 (1)	8	4	16	22 (3)
80	24 (4)	40	6 (1)	16	1 (1)	32	11 (2)
160	4 (1)	80	3 (1)	32	0	64	3 (1)
320	2 (1)	> 80	1	> 32	0	> 64	0
640	1	—	—	—	—	—	—
Mean titre	80		23		1·95		21·6

Table 3*b*. *Serum titres in adult cattle, infected farm March* 1971 (15 *months after infection*)

'O'		'H'		CFT (P)*		CFT (A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
10	0	< 10	1	< 4	4	< 8	7
20	0	10	7 (1)	4	0	8	12 (2)
40	3 (1)	20	12 (2)	8	1	16	8 (2)
80	10 (1)	40	12 (3)	16	0	32	5 (1)
> 80	23 (5)	80	2 (1)	32	0	64	4 (1)
		> 80	2	—	—	> 64	0
Mean titre	128		35		—		18

* CFT (P) only carried out on those animals in which CFT (A) inexplicably raised.

Table 4. *Serum titres of cows at slaughter present in herd as adults during infection*

'O'		'H'		CFT(P)		CFT(A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
20	1	10	8	< 4	13	< 4	12
40	2	20	10	4	1	4	4
80	11	80	0	8	0	8	5
160	9	160	2	16	1	16	0
—	—	320	1	—	—	32	1
—	—	—	—	—	—	64	1
Titres of cow G17, tonsillar infection present at slaughter							
160		80		< 4		16	

Young cattle (*court H*)

In this group of 13 fattening cattle, faecal excretion was not detected on the six occasions that the animals were sampled, though infection was present in the animals on either side of this court. The divisions between the courts were of simple tubular construction. Serological testing during March 1970 yielded the results in Table 5.

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Table 5. *Young cattle court H* (*March* 1970)

'O'		'H'		CFT(P)		CFT(A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
< 10	0	< 10	1	< 4	11	4	0
10	0	10	4	4	0	8	1
20	0	20	6	8	1	16	2
40	0	40	1	16	1	32	5
80	9	80	1	32	0	64	5
> 80	4	> 80	0	—	—	—	—
Mean	105		21·5		1·9		40

Table 6. *Serum titres young cattle court F* (*March* 1970)

'O'		'H'		CFT(P)		CFT(A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
< 10	0	< 10	1	< 4	6	8	0
10	0	10	8	4	0	16	1
20	0	20	0	8	3	32	8
40	4	40	1 (1)	16	1	64	0
80	7 (1)	80	3 (1)	32	0	> 64	4 (2)
> 80	2 (1)	> 80	0	64	3 (2)	—	—

Figures in parentheses refer to known infected animals.

All these animals were slaughtered between 5 and 10 months after infection was first identified in the herd, and in no case was the organism recovered from the tissues. At the time of slaughter the mean reciprocal titres for these animals were:

'O', 80; 'H', 23; CFT(P), 2·5; CFT(A), 8.

Young cattle (*courts F and G*)

Infection was present in December 1969 and in March 1970 the serum titres of the animals in court *F* were as shown in Table 6.

The table shows the correlation between the presence of known infection and raised CFT titres, this correlation being less confused by inconclusive reactions than in the case of 'O' titres. Among known infected young cattle the 'H' titres were lower than those encountered among infected adults. Nine months after infection the 'O' titres of the two previously known infected animals were still raised above the group level, whilst the CFT results had fallen within the range encountered in non-infected animals.

The animals from courts *F* and *G* were all examined at slaughter or sampled at calving 10–17 months or 19–26 months, respectively, after active infection. The serum titres are shown in Table 7.

Calves infected as neonates (calf-houses *E* and *C*)

The titres recorded in Table 8 were obtained when calves were sampled 5 months after infection. By this time none had raised 'O' agglutination titres and they could not be differentiated on this basis from a normal non-infected group of calves at

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Table 7. *Serum titres of occupants of courts F and G 10–26 months post-infection period*

'O'		'H'		*CFT(P)		CFT(A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
10	0	< 10	6 (1)	< 4	10 (2)	< 8	12 (1)
20	1	10	5	4	0	8	7 (1)
40	7 (2)	20	5	8	1	16	3
80	12	40	6 (1)	16	1	32	1
> 80	3	80	1	—	—	—	—

* Some animals not tested.

Table 8. *Serum titres of calves May* 1970

(All animals infected 5 months previously.)

'O'		'H'		CFT(P)		CFT(A)	
Titre	No.	Titre	No.	Titre	No.	Titre	No.
< 10	0	< 10	2	< 4	7	< 4	2
10	0	10	3	4	3	4	1
20	2	20	2	8	0	8	4
40	6	40	1	16	0	16	2
80	3	80	1	32	0	32	0
> 80	0	> 80	2	64	1	64	0
						> 64	1

the farm whose 'O' titres ranged from 1/10 to 1/80. In comparison, the range of 'H' titres was markedly raised above that for the normal calves in which the titres were less than 1/10. One animal (U38) had maintained both a high CFT(P) and CFT(A) titre unlike its cohorts in which these tests were both essentially negative.

Ten months after neonatal infection the serum titres of the group fell within the normal range and when sampled at either slaughter or calving, respectively, the titres shown by all the animals fell within the range encountered in the slightly older animals from courts *F* and *G* (Table 6).

As recorded previously *S. dublin* was isolated from the bile of U38 at slaughter (Lawson *et al.* 1974) but not from other individuals of comparable age and similarly infected as neonates. Whilst this calf showed raised CFT(P) and CFT(A) titres persisting for 5 months it could not be differentiated serologically at the time of slaughter from the other members of the group.

DISCUSSION

Serological tests may be employed in a variety of ways in attempting to evaluate the status of animals thought to have been exposed to infection. In salmonellosis of cattle, these tests are likely to be utilized in two main ways: (i) to give some indication of infection within the herd as a whole and, (ii) to indicate those animals in which infection is persisting in order that the threat posed by these carriers may be eliminated.

A serological test has additional epidemiological value if it indicates infected animals for a restricted period of time following infection so that the serological titres can be related to recent events within the herd. The selected test should also indicate infection in a high proportion of infected animals.

Our experience is that the CFT(P) fulfils these requirements and provides a better basis for indicating herd infection in adult cattle than do the other serological tests that have been used previously. In the circumstances of sub-clinical infection in adult cattle it would appear that the CFT(P) generally becomes negative within 6 months of infection. We encountered fluctuations in the mean herd 'O' titres which could not be related to known infection. This observation is in accord with the known gradual acquisition of 'normal' antibody titres observed in young calves as they reach maturity. The CFT(P) titres do not appear to be influenced by the events which bring about these changes in 'O' titres. CFT(A) titres persist longer than do CFT(P) titres and from our results it appears they may be influenced by non-specific factors which stimulate fluctuations in 'O' antibody titres. 'H' titres proved unreliable in detecting proved infection and when raised persisted for a long time.

At slaughter, none of the serological tests identified the two animals in which infection was demonstrated. The persistence of raised CFT(P) or CFT(A) titres beyond the period of active excretion of the organism by calves may give some indication that the organism has not been eliminated from the tissues. With time, however, these titres regress and no longer give an indication of persistent infection. In both the complement fixation tests the amount of complement utilized was kept as low as possible to ascertain if the tests were capable of detecting carrier animals. At this level of complement some difficulty was experienced with anti-complementary sera, and it may be that, as the tests were not capable of detecting carrier animals, a more useful test might utilize a higher dose of complement.

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