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The serology of experimental *Salmonella dublin* infections of cattle

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SUMMARY

Agglutinating antibodies to somatic 'O' and flagellar 'H' antigens of *S. dublin* were measured in the serum of 43 pregnant heifers before intravenous or oral infection with *S. dublin* and in the serum of 21 uninfected control animals. The data from these animals were analysed statistically and a titre of 1/80, to both antigens, has been interpreted as of doubtful significance and a titre of 1/160 to both antigens, has been interpreted as significantly raised. Animals in which fetal infection occurred after challenge by either the intravenous or oral route developed significant increases in 'H' and 'O' titres indicating the value of measuring 'H' titres in the diagnosis of *S. dublin* abortion. In animals which were infected orally and in which infection appeared to be confined to the alimentary tract the 'H' titre did not become significantly raised. Lack of correlation between antibody titres and faecal excretion of *S. dublin* and persistence of infection in carcasses confirms that the serum agglutination test is of no value in detecting latent carriers.

INTRODUCTION

Agglutinating antibodies to *S. dublin* have been measured in serum from naturally infected cows affected with dysentery (Field, 1948) and abortion (Hinton, 1973), and from 'carrier' animals (Field, 1948; Richardson, 1973; Sojka, Thompson & Hudson, 1974). In the last situation they have been found to be of limited diagnostic value. The serology of experimental infections has not been studied. The advantages of experimental infections are that pre-infection titres may be measured, time and route of infection determined and symptoms of disease and excretion of *S. dublin* monitored continually. This paper records the titres of agglutinating antibodies after experimental infections of cattle. These were measured, and the results analysed, to assist in the interpretation of serological data from field cases of *S. dublin* infection.

MATERIALS AND METHODS

Animals

The levels of agglutinating antibodies were measured in sera from 64 pregnant heifers, which had been born and reared at this Institute and were 22–47 months old at the time of infection.

Serum

Samples of serum were obtained from 43 animals before experimental infection; they were stored at -18 °C. In the majority of cases samples were collected daily for 7 days before infection. Daily sampling continued after infection for 21 days and subsequently samples were collected on one or two occasions each week. Serum samples were collected from 21 other pregnant heifers which were not subsequently infected.

Inocula

Animals were inoculated either intravenously with 10 ml of a suspension of *S. dublin* (3246) in 0·85% saline containing approximately 10^7 bacteria/ml or orally with 100 ml of a similar suspension of *S. dublin* containing approximately 10^8 or 10^9 bacteria/ml. The methods of producing the inocula have been described (Hall & Jones, 1976). Thirty-three heifers were inoculated intravenously with 10^8 bacteria, six received an oral dose of 10^{10} bacteria and four received an oral dose of approximately 10^{11} organisms.

Serum agglutination test

Serum was examined using the tube agglutination test. Doubling dilutions of the serum were made in 0·85% saline and incubated in a water bath at 56 °C with an equal volume of a suspension of *S. dublin* 'O' or *S. dublin* 'H' antigen. The method of antigen preparation was as recommended by the Central Veterinary Laboratory and a strain of *S. dublin* (3246) was used to prepare both antigens. The 'H' titre was read after 4 h incubation and the 'O' titre after overnight incubation. The endpoint was taken as the highest dilution giving 50% agglutination and the titre expressed as a reciprocal of that dilution.

Bacteriology

Isolation of *S. dublin* from faeces by enrichment was made by placing approximately 1 g portions of faeces in Rappaport broth (Rappaport, Konforti & Navon, 1956) and Difco selenite brilliant green broth (SBG). The Rappaport broths were incubated at 37 °C and the SBG at 43 °C. After 24 and 48 h incubation, all broths were inoculated on modified brilliant green agar (Oxoid) containing 170 mg/l sulphadiazine (BDH). Plates were incubated at 37 °C and examined after 24 and 48 h.

At slaughter a range of tissue samples was obtained and examined bacteriologically (Hall & Jones, 1977). Samples of rumen, omasum, abomasum, small intestine, caecum, colon and gall bladder were washed twice to remove gross surface contamination; surface contamination was removed from other tissues by dipping in absolute alcohol and igniting. Isolation of *S. dublin* from tissue samples was carried out by enrichment as described above.

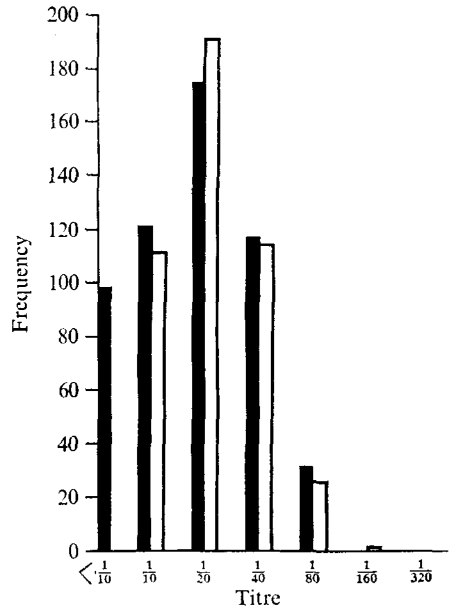

Fig. 1

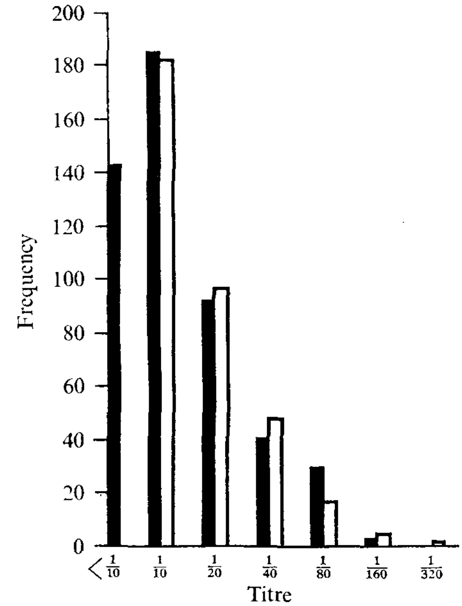

Fig. 2

Fig. 1. Bar chart of observed frequency of distribution of titres of agglutinating antibodies to somatic antigens of *S. dublin* ■ and expected frequency of a negative binomial distribution □.

Fig. 2. Bar chart of observed frequency of distribution of titres of agglutinating antibodies to flagellar antigens of *S. dublin* ■ and expected frequency of a negative binomial distribution □.

Table 1. *The interpretation of the* S. dublin *serum agglutination test in uninfected animals*

	Negative	Test result doubtful	Positive
Somatic O titres	40	80	160
Flagellar H titres	40	80	160

RESULTS

Uninfected animals

The distribution of titres of agglutinating antibodies to *S. dublin* in sera obtained from heifers before infection and from uninfected animals is recorded in Figs 1 and 2. Statistical analysis showed that the data were best fitted by a negative binomial distribution (Figs 1 and 2) and analysis of the expected frequencies showed that 6·0 % of the 'O' titres were ⩾ 1/80 and 2·0 % ⩾ 1/160. Of the 'H' titres 6·4 % were ⩾ 1/80, 2·0 % ⩾ 1/160 and 0·6 % ⩾ 1/320. An interpretation of these results, comparable to that used by Hinton (1973) is given in Table 1.

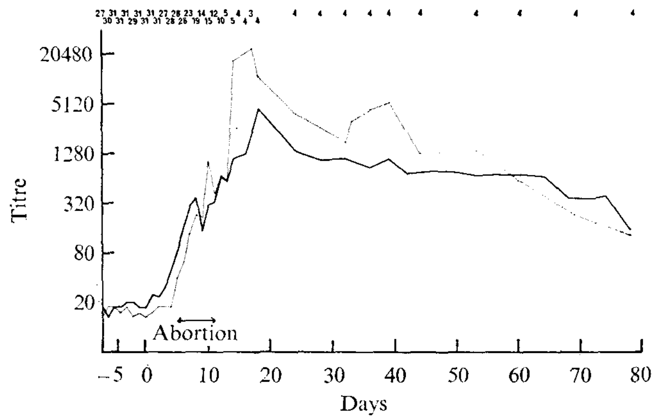

Fig. 3. Mean titres of somatic, ——, and flagellar, ----, agglutinins to *S. dublin* in pregnant heifers intravenously inoculated with 10^8 *S. dublin* at the 180th day of gestation.

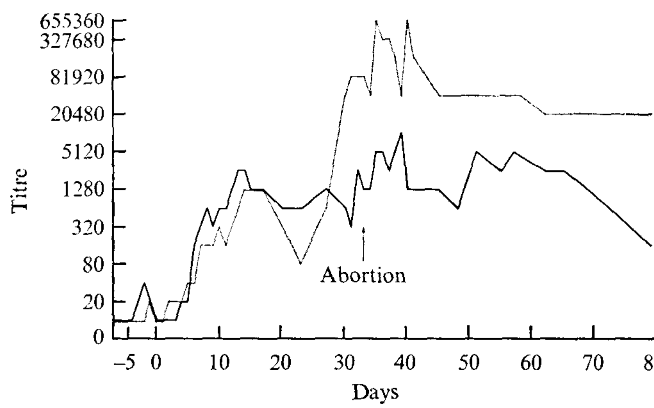

Fig. 4. Titres of somatic and flagellar agglutinins to *S. dublin* in a pregnant heifer intravenously inoculated with 10^8 *S. dublin* at the 180th day of gestation.

Intravenously infected animals

A graph of antibody titre to both antigens, against time, was drawn for each animal and because the curves were similar it was possible to derive mean curves, which are shown in Fig. 3, together with the number of samples from which each mean was derived. The number of samples falls because animals were slaughtered at various intervals after infection. The effect of inoculation of pregnant heifers was abortion (Hall & Jones, 1976) and the occurrence of abortion in relation to the serological changes is also shown. Titres to both 'O' and 'H' antigens became significantly raised and reached their maximum after abortion had occurred. The 'H' titre was much greater than the 'O' titre. The antibody titres in one animal, in which abortion did not occur until 33 days after inoculation, were dissimilar from the majority, and these are shown in Fig. 4.

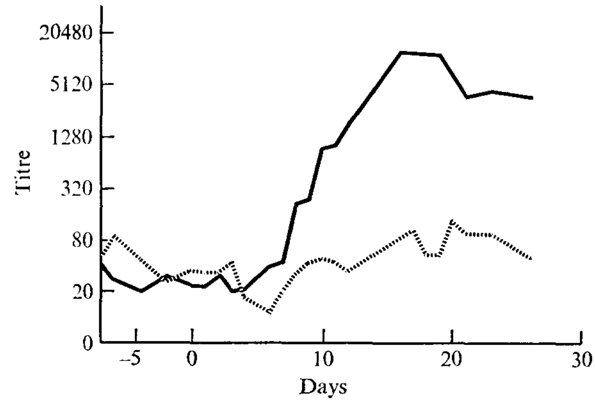

Fig. 5. Mean titres of somatic, ——, and flagellar, ----, agglutinins to *S. dublin* in five pregnant heifers orally inoculated with 10^{10} *S. dublin* at the 180th day of gestation.

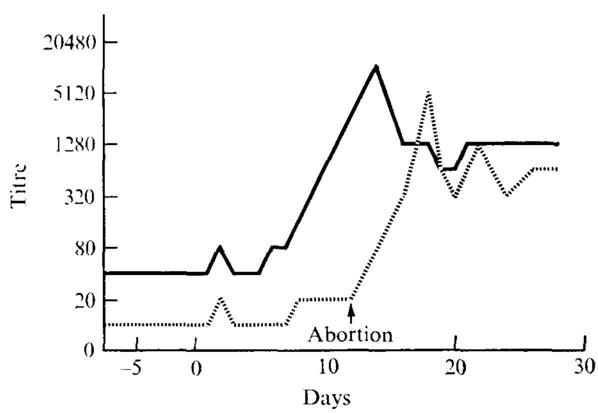

Fig. 6. Titres of somatic, ——, and flagellar, ----, agglutinins to *S. dublin* in a pregnant heifer which aborted following oral inoculations with 10^{10} *S. dublin* at the 180th day of gestation.

Orally infected animals

Graphs were drawn for each animal as above. Similar curves were obtained from five out of six animals infected with 10^{10} *S. dublin* which did not abort, and mean curves were derived (Fig. 5). In these animals the 'O' titre was much greater than the 'H' titre which never became significantly raised. A different antibody response occurred in the remaining animal which aborted (Fig. 6). In this animal the 'O' titre changed in a similar manner to the other five animals, but the 'H' titre rose to a significant level equal to the 'O' titre.

The curves obtained from three out of four animals inoculated orally with 10^{11} *S. dublin*, and which did not abort, were similar and a mean curve was derived (Fig. 7). In this group the 'O' titre was much greater than the 'H' titre which just became significantly raised. A different antibody response occurred in the remaining animal which was slaughtered on the eleventh day after infection, on humane grounds, and was found to contain a dead fetus which was heavily

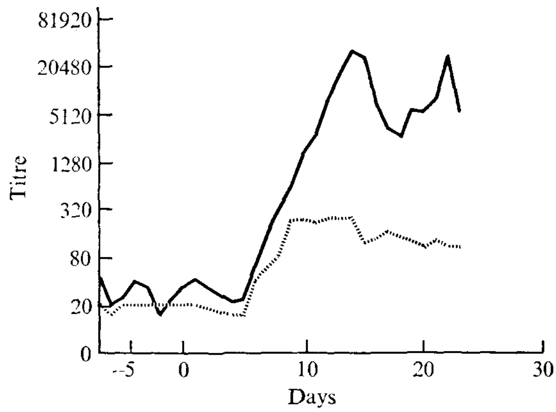

Fig. 7. Mean titres of somatic, ——, and flagellar, ----, agglutinins in three pregnant heifers orally inoculated with 10^{11} *S. dublin* at the 180th day of gestation.

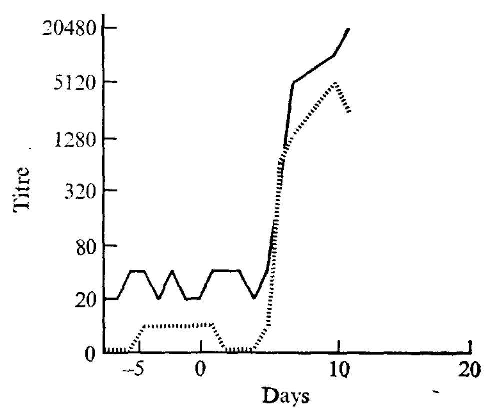

Fig. 8. Titres of somatic, ——, and flagellar, ----, agglutinins to *S. dublin* in a pregnant heifer orally inoculated with 10^{11} *S. dublin* at the 180th day of gestation.

infected with *S. dublin*. The antibody response in this animal (Fig. 8) showed large and significant increases in 'O' and 'H' titres.

Antibody titres in relation to isolation of S. dublin

Isolations of *S. dublin* from faeces from five of the 43 infected heifers are shown, together with mean antibody titres, in Table 2. These five heifers were studied for 500 days after intravenous inoculation. Isolations of *S. dublin* from faeces from a further five heifers, which were studied for 110 days after oral inoculation with 10^{10} or 10^{11} *S. dublin*, are shown together with mean antibody titres in Table 3. These tables contain examples of animals which had not excreted *S. dublin* for long periods of time but still retained significantly raised antibody titres. There are also instances where *S. dublin* was isolated from animals which did not have significantly raised antibody titres at the time of isolation of *S. dublin*.

Isolations of *S. dublin* from carcasses are shown in relation to antibody titres for intravenously infected animals in Table 2 and orally infected animals in Table 3.

Table 2. *Mean 'O' and 'H' titres, and isolations of* S. dublin *from faeces from five heifers studied for 500 days after intravenous inoculation with* 10^8 S. dublin *at the 180th day of gestation*

Animal	Days after inoculation 1–10	11–20	21–40	41–100	101–200	201–300	301–400	401–500	Isolations of *S. dublin* at necropsy
Animal 1									
'O' titre	1/90	1/6, 336	1/1, 344	1/136	1/70	1/196	1/146	1/66	
'H' titre	1/14	1/42, 500	1/5, 120	1/248	1/72	1/84	1/60	1/58	
Isolations of *S. dublin* from faeces	0	9	3	2	0	0	3	0	None
Animal 2									
'O' titre	1/138	1/8, 924	1/4, 142	1/250	1/102	1/48	1/42	1/40	
'H' titre	1/42	1/183, 342	1/40, 734	1/1, 120	1/330	1/160	1/118	1/66	
Isolations of *S. dublin* from faeces	9	9	10	2	0	0	1	0	None
Animal 3									
'O' titre	1/72	1/1, 300	1/5, 120	1/2, 674	1/52	1/38	1/66	1/34	
'H' titre	1/176	1/69, 680	1/59, 734	1/4, 862	1/2, 720	1/338	1/186	1/138	
Isolations of *S. dublin* from faeces	6	10	3	1	3	0	1	1	None
Animal 4									
'O' titre	1/430	1/2, 880	1/5, 760	1/3, 014	1/362	1/140	1/138	1/64	
'H' titre	1/190	1/1, 640	1/384, 504	1/68, 266	1/187, 734	1/27, 648	1/3, 474	1/704	
Isolations of *S. dublin* from faeces	6	10	19	7	1	1	12	1	None
Animal 5									
'O' titre	1/182	1/5, 120	1/1, 528	1/240	1/270	1/414	1/266		Following enrichment from ruminal wall and small intestinal contents
'H' titre	1/118	1/4, 224	1/2, 304	1/1, 041	1/46	1/76	1/76		
Isolations of *S. dublin* from faeces	9	9	4	0	0	9	3		

Table 3. *Mean 'O' and 'H' titres, and isolation of* S. dublin *from faeces from five heifers studied for* 110 *days after oral inoculation with* 10^{10} *or* 10^{11} S. dublin *at the* 180*th day of gestation*

Animal	Inoculum		Days after inoculation 1–10	11–20	21–40	41–60	61–80	81–110	Isolation of *S. dublin* at necropsy
1	10^{10}*	'O' titre	1/304	1/5, 120	1/2, 304	1/320	1/320	1/80	None
		'H' titre	1/26	1/2, 566	1/1, 600	1/640	1/320	1/320	
		Faeces	10	8	2	0	0	0	
2	10^{10}	'O' titre	1/86	1/25, 814	1/12, 288	1/1, 280	1/1, 280	1/1, 280	None
		'H' titre	1/4	1/20	1/4	0	0	0	
		Faeces	9	3	0	0	0	0	
3	10^{10}	'O' titre	1/216	1/2, 880	1/1, 152	1/160	1/120	1/60	None
		'H' titre	1/84	1/56	1/20	1/20	1/10	0	
		Faeces	10	2	2	0	0	0	
4	10^{11}	'O' titre	1/74	1/2, 112	1/826	1/320	1/80	1/40	Following enrichment from seven alimentary tract tissues
		'H' titre	1/30	1/88	1/130	1/20	1/20	1/20	
		Faeces	10	3	0	0	0	0	
5	10^{11}	'O' titre	1/1, 000	1/22, 528	1/40, 960	1/11, 946	1/5, 120	1/2, 560	None
		'H' titre	1/452	1/864	1/374	1/134	1/66	1/80	
		Faeces	10	4	7	2	0	3	

* This animal aborted.

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Isolations were made from one intravenously infected animal which did not have significantly raised antibody titres. Two orally infected animals had significantly raised 'O' titres at slaughter although *S. dublin* was not isolated from their carcasses. One animal, from which isolations of *S. dublin* were made at slaughter, did not have significantly raised antibody titres.

DISCUSSION

Following analysis of titres of agglutinating antibodies to somatic and flagellar antigens in uninfected heifers a titre of 1/80 has been interpreted as of doubtful significance and a titre of 1/160 as a positive result. This interpretation, for titres to somatic antigens, is similar to that of Hinton (1973), who suggested that 1/40 should be considered doubtful and 1/80 positive, and Field (1948) and Clarenburg & Vink (1949) who suggested that in normal cattle the 'O' titre is seldom greater than 1/40. In the present study 96 % of results were less than 1/80, therefore this titre could be regarded as doubtful or positive according to the degree of confidence required. This interpretation, for titres to flagellar antigens is close to those of Field (1948), Gibson (1965) and Hinton (1973) who have interpreted results of 1/80 and 1/160 as doubtful and 1/320 as positive. In the present study a titre of 1/160 has been designated as positive with a high degree of confidence since 98 % of uninfected animals had a titre of less than 1/160.

The slight disagreement as to what constitutes a positive result between the present study and previous studies may be due either to differences in results or to the interpretation of these results. Results may be affected by differences in production of antigen used in the test and determination of endpoint and interpretation of results may depend on their analysis. The results of previous studies (Field, 1948; Clarenburg & Vink, 1949) do not appear to have been analysed statistically.

Le Guilloux (1969) studied the serology of *S. dublin* abortion and concluded that 'O' agglutinins were of greater diagnostic value than 'H' agglutinins and Hinton (1973), whilst finding 'H' titres to be marginally superior for the diagnosis of *S. dublin* abortion, recommended that both should be determined. Field (1948) recommended that 'H' agglutinins were more useful for detecting carrier animals. The results of the present investigation indicate how titres of 'O' and 'H' agglutinins may be affected by the disease process. In intravenously infected animals where bacteraemia was artificially induced the 'H' titre was always greater than the 'O' titre. In two orally infected animals, where fetuses were infected in utero, indicating that bacteraemia had occurred, a significant 'H' titre also developed. These observations suggest that in cases of abortion the 'H' titre is likely to be of greater diagnostic value and confirms the view of Hinton (1971) that examination of paired serum samples after abortion is a useful adjunct to diagnosis. In orally infected animals, where fetal infection did not occur and low 'H' titres developed, it appears that the infection was confined to the alimentary tract where it stimulated a much larger antibody response to somatic than flagellar antigens. These observations may explain why some animals, having recovered from *S. dublin* enteritis, do not have a raised 'H' titre. In cases of enteritis, where spread of infection to the blood has occurred, it must be expected that both titres would rise.

The present study of intravenous inoculations suggests that the 'H' and 'O' titres decline at the same rate since both fell below the significance level at 74 days after inoculation. The present observations of the decline in agglutinin titre in relation to excretion of *S. dublin* and its detection in carcasses show that an infection may persist in an animal whilst agglutinin titres fall to insignificant levels and also that agglutinin titres may persist at significant levels when infection is apparently absent. These observations support the views of Field (1959) and Richardson (1973) that the serum agglutination test is of no value in detecting latent carrier animals.

In all the animals which aborted, significantly raised agglutinin titres were detected. In the intravenously infected animals 'O' and 'H' titres rose almost simultaneously and abortion usually occurred as the agglutinin titres were rising. In the orally infected animal which aborted the 'O' titre started to rise about 6 days before the 'H' titre and when abortion occurred the 'O' titre was significantly raised and the 'H' titre was not increased at all. In an intravenously infected animal with delayed abortion (Fig. 4) there were significantly raised 'O' and 'H' titres before abortion which became much higher after abortion. These variations in serological response may explain the variable titres reported in paired serum samples from naturally occurring cases of *S. dublin* abortion (Hinton, 1973).

The present results and previous results (Hall & Jones, 1977) suggest that abortion results from a bacteraemia which is followed by a prolific growth of *S. dublin* in the placenta and that the processes will initiate a significant rise in antibody titres. Activation of the latent carrier state at abortion, with faecal contamination of the sample or extraneous contamination of the sample, both suggested by Hinton (1973), seem more probable explanations of cases of abortion from which *S. dublin* was isolated but in which significantly raised antibody titres were not detected.

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