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Salmonella dublin abortion in cattle

I. Observations on the serum agglutination test

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SUMMARY

The somatic and flagellar serum agglutinin titre were determined in paired samples obtained from seventy-seven cases of bovine abortion associated with *Salmonella dublin* infection. The cases could be divided into four serological groups with an active infection being demonstrated in most cases. The serum agglutination test was shown to be a relatively specific diagnostic test but was of more limited value in the retrospective identification of convalescent cases.

INTRODUCTION

S. dublin is now a well known cause of bovine abortion. The abortion may be associated with symptoms of dysentery or it may be the only clinical sign. The literature on the subject has been reviewed (Hinton, 1971*b*). During the last decade the incidence of these abortions has increased. Salmonellas, mainly *S. dublin*, were isolated from 9 (0·19 %) of 4785 bovine abortions investigated by the Veterinary Investigation Service in England and Wales between November 1959 and October 1961 (Hughes, 1964) and from 502 (2·7 %) of the 18,382 abortions examined during 1970 (Report, 1971).

Diagnosis is usually based on the isolation of the salmonella from foetal material while a preliminary report has indicated that the serum agglutination test is a useful adjunct to diagnosis especially when both the somatic (O) and the flagellar (H) antibody titres are determined (Hinton, 1971*a*).

This paper records detailed results obtained when using the serum agglutination test.

MATERIAL AND METHODS

Clinical cases

These were encountered during the course of routine investigations of bovine abortion material. The main results are drawn from a total of 111 abortions associated with *S. dublin* infection. In addition, convalescent samples only were collected from a further 10 cases. None of the cows had been inoculated with the live rough *S. dublin* vaccine (Mellavax, Burroughs Wellcome & Co.). Serum O and H agglutination titres were determined in paired samples from seventy-seven

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cases of abortion. Abortion was the only clinical sign in 64 of the cases. Seven were associated with dysentery and six cows were off-colour or had diarrhoea at the time of abortion. The first of the pair of serum samples was obtained within five days of the abortion while at least 7 days elapsed between the samplings though all the second samples were collected within 26 days of abortion.

Convalescent serum samples were obtained, 30–330 days after abortion, from 40 cases. These included 25 out of the 77 cases from which paired samples were examined.

Serum agglutination test

Serum was examined by the tube agglutination test. Doubling dilutions of the serum were made in 0·5 % phenol-saline and these were incubated in a water bath at 48° C with a standard suspension of *S. pullorum* O (1, 9, 12) and *S. dublin* H (gp) antigen obtained from the Central Veterinary Laboratory, Weybridge. The H titre was read after four hours 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. A significant change in titre has been taken to be at least a four-fold change, either up or down.

In order to test the specificity of the agglutination test paired serum samples from 690 abortions from which *S. dublin* was not isolated were screened for *S. dublin* O and H agglutinins. For convenience only the H titres are recorded in the results. The abortions occurred in either Breconshire, Carmarthenshire, S. Cardiganshire or Pembrokeshire between July 1971 and February 1972. *Brucella abortus* was not isolated from any of the cases; the series included 62 cases of mycotic infection and 15 cases associated with *Corynebacterium pyogenes*.

INTERPRETATION OF THE SERUM AGGLUTINATION TEST

In salmonella serology there is no standardization of either techniques or antigen production. This fact makes it difficult, not only to compare the results of other workers, but also to define acceptable diagnostic criteria for the serological diagnosis of infection.

One of the problems of using a salmonella agglutination test in adult cattle is the presence of agglutinating antibody in the sera of apparently normal cattle. The reason for this has not been fully explained though recently Sharpe & Reiter (1971) demonstrated common antigens between the salmonellas and the butyrio-vibrios which are found in the normal ruminal micro-flora. Similarly Protell *et al.* (1971) recorded that, owing to a dysglobinaemia, 11 of 40 human patients with chronic active liver diseases had raised serum salmonella agglutinins. This interesting finding has yet to be evaluated in the context of bovine liver disease (e.g. fascioliasis) but it may prove to be of some significance.

In his review, Gibson (1965) drew on the results of a number of workers and suggested that the sera of normal cattle seldom show an O titre above 40 or an H titre above 160. A suggested guide for interpretation of the serum agglutination test, based on Gibson (1965) and observations in this study is given in Table 1 and this is used throughout this paper.

Table 1. *The interpretation of the* Salmonella dublin *serum agglutination test*

	Test result		
	Negative	Doubtful	Positive
Somatic (O) titres	⩽ 20	40	⩾ 80
Flagellar (H) titres	⩽ 40	80–160	⩾ 320

RESULTS

The examination of paired samples from seventy-seven cases

The full results are listed in Table 2 and summarized in Table 3. These show that the cases can be divided into four groups. In Group I there is a rise in either the O and/or H titre while in Group II a fall in H titre was recorded. In the third group there are diagnostic titres at both samplings but these show no significant change. In group IV diagnostic agglutinin titres do not develop.

About 80 % of cases showed significant changes in O or H titre and were classed in Groups I and II. In the 50 cases in Group I the O and H titre rose significantly in 33, the O only in seven and the H only in 10. The distribution of the O and H titres in both the first and second samples are listed in Table 4 and the changes in O and H titre are summarized in Table 5.

The median change in H and O titre was 16- and 8-fold and was four-fold or more in 43 (86 %) and 40 (80 %) of cases respectively. In the seven cows (nos. 6, 33, 79, 82, 102, 106, 107) in which the H titres did not change significantly the O titres rose between four and 32-fold.

In 10 Group I cases (nos. 19, 21, 31, 37, 51, 56, 65, 69, 97, 100) the changes in O titre were between a two-fold rise and a four-fold fall. The final O titres ranged between 20 and 640. In eight of the 10 it was 80 or more in one or both samples. In the other two both titres were ⩽ 20. The final H titre in no. 37 was 1280 while in no. 100 it was only 160 some 26 days after abortion.

Eleven cases (nos. 18, 23, 24, 30, 36, 40, 47, 60, 63, 71, 76) were classified in Group II. The H titres fell by between four and 64-fold, the median change being eight-fold. The behaviour of the O titres was variable and showed between a four-fold and an eight fold rise in titre. The initial H titres, which exceeded 5120 in nine cases, ranged between 640 and 20,480, while the H titres of the second samples were between 160 and 2560 with a median of 640. In two cases (nos. 36, 47) the final H titres were 160. The corresponding O titres were 80, indicating that if this was the only sample examined, the cases would have been classified as doubtful positives (see Table 1) even though there had been an active infection.

In seven cases in Group III (nos. 2, 39, 42, 44, 46, 61, 92) the H titres were all between 320 and 5120 and the O titres were all 160 or more except in case no. 39 when they were 40 and 80 respectively. In the eighth case (no. 109) the H titres were 160 and 320 and the O titres only 20. These titres indicate that this case falls into an intermediate position between Groups III and IV.

Group IV comprised eight cases (nos. 8, 48, 49, 64, 80, 84, 93, 104) which did not

Table 2. *The serological findings in seventy-seven cases of abortion associated with* Salmonella dublin *infection*

Case no.	Clinical findings*	Day of sampling†		Serum O titre		Serum H titre		Change in titre‡		Sero-logical Group
		1st	2nd	1st	2nd	1st	2nd	O	H	
2	A	1	17	320	320	320	320	0	0	III
3	A	1	16	40	160	80	2,560	+4	+32	I
6	A	4	23	80	320	2,560	2,560	+4	0	I
7	A	4	23	40	320	640	10,240	+8	+16	I
8	A	3	21	20	< 40	20	< 40	0	0	IV
11	A	2	9	< 10	160	20	2,560	+16	+128	I
14	A	1	18	< 20	320	< 20	5,120	+32	+512	I
15	A	1	30	40	160	80	1,280	+4	+16	I
18	D+A	4	24	320	160	10,240	1,280	−2	−8	II
19	A+M	1	20	80	160	40	1,280	+2	+32	I
21	A+S	3	19	80	80	160	640	0	+4	I
22	A	−2	11	80	320	160	5,120	+4	+32	I
23	A	5	19	160	40	20,480	320	−4	−64	II
24	A+M	1	17	320	320	10,240	640	0	−16	II
29	A	1	15	40	160	320	10,240	+4	+32	I
30	A	2	26	80	640	10,240	2,560	+8	−4	II
31	A	1	10	80	160	< 20	640	+2	+64	I
33	A	1	11	80	640	1,280	2,560	+8	+2	I
34	A	1	8	40	160	20	5,120	+4	+256	I
36	D + PLC	1	8	160	80	640	160	−2	−4	II
37	A	1	14	< 20	20	< 20	1,280	+2	+128	I
39	A	4	21	40	80	320	640	+2	+2	III
40	A	5	19	320	80	5,120	1,280	−4	−4	II
41	A	1	19	20	80	160	1,280	+4	+8	I
42	A	1	18	160	320	320	320	+2	0	III
44	A	1	15	320	640	5,120	5,120	+2	0	III
45	A	1	20	80	640	20	2,560	+8	+256	I
46	A	1	24	160	160	640	1,280	0	+2	III
47	A	2	25	40	80	1,280	160	+2	−8	II
48	A	1	13	< 20	< 20	< 20	< 20	0	0	IV
49	A	1	22	< 20	< 20	< 20	< 20	0	0	IV
50	A+S	3	13	40	640	160	1,280	+16	+8	I

Table 2 (*cont.*)

Case no.	Clinical findings*	Day of sampling†		Serum O titre		Serum H titre		Change in titre‡		Sero-logical Group
		1st	2nd	1st	2nd	1st	2nd	O	H	
51	A	1	14	80	160	20	1,280	+2	+64	I
53	A	1	12	40	160	< 20	640	+4	+64	I
54	A	1	20	40	160	80	640	+4	+8	I
55	D+A	1	14	< 20	640	320	1,280	+64	+4	I
56	D+A	1	18	40	80	160	1,280	+2	+8	I
57	A+D	1	11	40	640	20	1,280	+16	+64	I
58	A	1	12	80	640	40	10,240	+8	+256	I
60	A	3	16	320	320	5,120	640	0	−8	II
61	A	5	14	320	320	1,280	1,280	0	0	III
63	A	2	17	640	320	5,120	1,280	−2	−4	II
64	A	1	14	< 20	< 20	< 20	< 20	0	0	IV
65	A	1	25	640	640	640	2,560	0	+4	I
66	A	1	16	40	640	40	1,280	+16	+32	I
69	A	1	14	320	640	80	1,280	+2	+16	I
70	A	3	17	10	80	20	640	+8	+32	I
71	A	1	14	80	40	10,240	2,560	−2	−4	II
73	A	1	15	< 20	160	< 20	2,560	+16	+256	I
75	A	1	15	20	80	80	640	+4	+8	I
76	A	1	14	80	160	5,120	640	+2	−8	II
79	A	3	14	40	320	320	320	+8	0	I
80	A	1	11	< 20	< 20	< 20	< 20	0	0	IV
81	A	1	20	< 20	160	40	640	+16	+16	I
82	A	1	19	40	160	2,560	1,280	+4	−2	I
83	A	1	15	40	320	160	2,560	+8	+16	I
84	A	1	17	< 20	< 20	< 20	< 20	0	0	IV
85	A	1	17	< 20	160	80	640	+16	+8	I
86	A+D	1	15	< 20	80	20	320	+8	+16	I
87	A	1	26	< 20	160	80	2,560	+16	+32	I
88	A	1	18	160	640	320	5,120	+4	+16	I
90	A	1	18	< 20	160	80	640	+16	+8	I
91	A	1	18	80	640	80	1,280	+8	+16	I
92	A	1	17	320	640	640	1,280	+2	+2	III

Table 2 (*cont.*)

Case no.	Clinical finding*	Day of sampling†		Serum O titre		Serum H titre		Change in titre‡		Serological Group
		1st	2nd	1st	2nd	1st	2nd	O	H	
93	A	1	17	< 20	< 20	< 20	< 20	0	0	IV
94	D+A	2	9	< 20	160	< 20	1,280	+16	+64	I
95	A	2	15	< 20	80	80	320	+8	+4	I
96	A+M	1	13	< 20	80	160	2,560	+8	+16	I
97	A	2	17	160	40	640	5,120	−4	+8	I
98	A+M	1	23	40	320	640	2,560	+8	+4	I
99	A	2	11	< 20	640	< 20	5,120	+64	+512	I
100	A	2	26	< 20	20	40	160	+2	+4	I
102	A	1	16	20	80	2,560	1,280	+4	−2	I
104	A	1	10	20	20	40	40	0	0	IV
106	A	3	15	20	320	320	320	+16	0	I
107	A	1	16	20	640	640	1,280	+32	+2	I
109	A	1	15	20	20	160	320	0	+2	III

* A = abortion, D = dysentery, S = scour (diarrhoea), M = malaise, PLC = premature live calf.

† Day 1 is the first 24 hr. after abortion.

‡ Degree of change 2-fold, 4-fold, etc., 0 = no significant change.

Table 3. *A summary of the serological response in seventy-seven cases of abortion associated with* Salmonella dublin *infection*

	Serological response	No. cases	Proportion (%)
Group I	Cases with a significant rise in O and/or H titre	50	65
Group II	Cases with a significant fall in H titre	11	14·2
Group III	Cases with diagnostic titres but which showed no significant change	8	10·4
Group IV	Cases with titres of ⩽ 80 at both samplings	8	10·4

Table 4. *A summary of the O and H titres in the first and second samples from fifty cases of* Salmonella dublin *abortion classified as Group I*

	No. of cases with titres					Proportion (%) of samples*			Titres	
	⩽ 20	40	80	160	⩾320	—	d	+	Range	Median
Initial H titre	14	5	10	7	14	38	34	28	⩽ 20–2,560	80
Final H titre	—	—	—	1	49	—	2	98	160–10,240	1,280
Initial O titre	21	15	10	2	2	42	30	28	⩽ 20–640	40
Final titre	2	1	9	17	21	4	2	94	⩽ 20–640	160

* See Table 1 for interpretation.

Table 5. *A summary of the changes in O and H titres in fifty cases of* Salmonella dublin *abortion classified as Group I*

	Changes in titre between samples											
	−4	−2	0	+2	+4	+8	+16	+32	+64	+128	+256	+512
H titres		2	3	2	6	8	9*	7	5	2	4	2
O titres	1	0	2	7	13	12*	11	2	2			

* = Indicates the median change in titre.

show a serological response as judged by the S.A.T. The placenta was positive on direct culture in two and selenite enrichment was necessary for the isolation of *S. dublin* in the remainder.

Serological findings in convalescent cases

Forty cases were examined between 30 and 330 days after an abortion associated with *S. dublin* infection. The findings are summarized in Table 6.

These results show that as time passes the serum titres generally fall so that by 150 days or more after abortion only 30% have an O titre of ⩾ 80 and 40% an H titre of ⩾ 320. Faeces samples were cultured from 30 of the cases examined 45 days or more after abortion. Seven yielded *S. dublin* and in five of these the H titre was 320 or more.

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Table 6. *The serum titres in convalescent abortion cases from which* Salmonella dublin *was isolated*

Days after abortion	No. of cases	Including cases listed in Table 2	Proportion (%) with titres in the ranges: Somatic O < 20	Somatic O 20–40	Somatic O ⩾ 80	Flagellar H ⩽ 40	Flagellar H 80–160	Flagellar H ⩾ 320
30–40	7	15, 45, 46, 54, 70, 107	—	—	100	—	—	100
45–120	19	2, 18, 24, 36, 47, 53, 73, 81, 88, 90, 91, 95, 102, 106	10·5	31·5	58	10·5	47·5	42
145–330	14	3, 11, 71	21·5	50	28·5	28·5	28·5	43

Table 7. *The screening of paired serum samples from* 690 *cases of abortion which did not yield* Salmonella dublin *on culture*

Material cultured at the first sampling	H titre recorded in the paired sera ⩽ 40*	80–160	160–320	⩾ 320	Total
Foetus and/or placenta	489	10	2(*a*)	1(*a*)	502
Vaginal mucus	122	—	—	2(*b*)	124
No cultures	62	2	—	—	64
Total	673	12	2	3	690

(*a*) and (*b*) see explanation in text, p. 467.

* These include 29 cases in which a titre of 80 was recorded in one sample only.

Screening of paired sera from 690 *cases for* Salmonella dublin *agglutinins*

Paired serum samples from 690 cases of abortion which did not yield *S. dublin* or *Br. abortus* on culture were screened for *S. dublin* agglutinins. The results are listed in Table 7 and these indicate that 97·5% of cases were negative while 2% had doubtful and 0·5% positive titres. In the three cases marked under (*a*) in the Table the titres did not change so that it is possible that these are residual titres. In two of the three cases selenite enrichment was employed. In the two cases marked under (*b*) one showed a Group I and the other a Group II response. In both cases a vaginal swab only was cultured on solid medium and no selenite enrichment was used. In all probability, therefore, there was a failure in the bacteriological technique rather than a lack of specificity of the agglutination test.

DISCUSSION

Loizelier (1945), Sellers & Sinclair (1953), Coutard & De Saint-Aubert (1969) and Harbourne, Randall, Luery & Wallace (1972) all recorded that significant agglutinin titres may be found in the serum of cows after a salmonella abortion. Le Guilloux (1969) and Cottereau, Rancien & Sendral (1970) examined paired serum samples and noted rising titres in four of five and six of nine cases respectively while a preliminary report by the author (Hinton, 1971*a*) proposed that *S. dublin* abortions could be divided into four groups on the basis of the serological findings.

The cases in Groups I and II are relatively easily explained as they obviously confirm an active infection. In Group I the change in H titre usually exceeded that of the O. A significant change in O and H titre was noted in 40 and 43 of the 50 cases respectively. The H titre of the second sample was $\geqslant$ 320 in 49 of 50 cases. The O titre was $\geqslant$ 80 in 47 cases though in three it was $\leqslant$ 40 indicating that on occasions it was of no diagnostic value. In Group II the H titres fell and this is due to the fact that in salmonella infections the flagellar titres often reach high levels fairly soon after infection and then fall again relatively rapidly. This has also been seen in experimental *Br. abortus* abortion (Thomsen 1950). In some Group II cases (e.g. nos. 30, 47, 76) the O titres continued to rise indicating that the O and H agglutinins develop at different rates.

The findings in Groups I and II suggest that the peak in the agglutinin titres develops towards the end of the first week. On the other hand there is evidence to suggest that in cases of dysentery associated with *S. dublin* infection the peak titres are not reached until 10–14 days after the onset of clinical signs (Field, 1948; Gibson, 1958).

In the cases in Group III there are diagnostic titres at both samplings but these do not show any apparent change. In these cases the abortion may have occurred in a carrier cow which had high residual titres and in which *S. dublin* was isolated from the products of conception. On the other hand there may have been an active infection with diagnostic titres developing before abortion, but unlike Groups I and II, they did not change during the sampling period. Alternatively

the titres may have continued to rise for a short while after the abortion but fell again so that by the second sampling they were at the same level as they were originally.

In Group IV the explanations are more speculative. The first is that there may have been an active infection which was not demonstrated by the S.A.T., while secondly the abortion may have occurred in a carrier cow which had low residual titres but in which there was congenital infection of the placenta or foetus. The third explanation is that the abortion may have precipitated a transient activation of the latent carrier state and lastly, extraneous contamination of the sample may have occurred and the isolation has no clinical significance whatsoever.

The explanations propounded for the cases in Groups III and IV indicate that, as with many infectious diseases, diagnosis is not always straightforward and that several factors have to be considered before an opinion can be given.

The screening of paired serum samples from nearly 700 abortions from which *S. dublin* was not isolated indicated that the agglutination test was unlikely to give many false positive results. The interpretation of the titres was discussed in an earlier section though no mention was made of the relative merits of either the O or the H titres for diagnosis. In his study of *S. dublin* abortion Le Guilloux (1969), who used different serological techniques, concluded that the O agglutinins were of more diagnostic value than the H and this view is in agreement with that of Huckstep (1962) in his consideration of the serology of typhoid fever in man. On the other hand the results presented in this paper suggest that though the H titres were marginally superior to the O titre for diagnostic purposes it is important that both O and H titres are always determined.

REFERENCES

COTTEREAU, P., RANCIEN, P. & SENDRAL, R. (1970). Enzooties d'avortements à *Salmonella dublin* sur des vaches du départment de Saône et Loire. *Revue médicale et vétérinaire* **121**, 447.

COUTARD, J. C. & DE SAINT-AUBERT, G. (1969). Avortements salmonelliques à *S. dublin* chez les bovins en Moselle. *Bulletin mensuel de la Société vétéreinaire pratique de France* **53**, 19.

FIELD, H. I. (1948). A survey of bovine salmonellosis in mid and west Wales. *Veterinary Journal* **104**, 251, 294 and 325.

GIBSON, E. A. (1958). *Studies on the Epidemiology of Salmonella Infection in Cattle*. Ph.D. Thesis. University of London.

GIBSON, E. A. (1965). Reviews in the progress of dairy science. Section E. Diseases of dairy cattle. Salmonella infection in cattle. *Journal of Dairy Research* **32**, 97.

HARBOURNE, J. F., RANDALL, R. J., LUERY, K. W. & WALLACE, J. G. (1972). *Salmonella paratyphi B* infection in dairy cows: Part I. *Veterinary Record* **91**, 112.

HINTON, M. (1971*a*). *Salmonella dublin* abortion in cattle: Preliminary observations on the serological response. *Veterinary Record* **88**, 481.

HINTON, M. (1971*b*). Salmonella abortion in cattle. *Veterinary Bulletin* **41**, 973.

HUCKSTEP, R. L. (1962). *Typhoid Fever and other Salmonella Infections*. Edinburgh: E. & S. Livingstone.

HUGHES, L. E. (1964). Infectious diseases of calves in Great Britain. *Bulletin Office International des Épizooties* **62**, 525.

LE GUILLOUX, M. (1969). Avotements des bovins à *Salmonella dublin* serologie – Note conjointe sur un cas d'entérite bovine. *Bulletin mensuel de la Société vétérinaire pratique de France* **53**, 211.

Loizelier, A. B. (1945). Contribución al estudio de las enfermedades paratificas de los animales domésticos en España. *Trabajos del Instituto de biologia animal, Madrid* **8**, 177.

Protell, R. L., Soloway, R. D., Martin, W. J., Schoenfield, L. J. & Summerskill, W. H. J. (1971). Anti-salmonella agglutinins in chronic active liver disease. *Lancet* ii, 330.

Report (1971). *Report on the Animal Health Services in Great Britain 1970*, p. 101. London: H.M.S.O.

Sellers, K. C. & Sinclair, W. B. V. (1953). A case of *Salmonella typhimurium* infection in cattle and its isolation from other sources. *Veterinary Record* **65**, 233.

Sharpe, M. E. & Reiter, B. (1971). In: *Report of the National Institute for Research in Dairying, Shinfield*. 1969–1970, p. 105.

Thomsen, A. (1950). Experimental studies on the incubation period of infectious abortion in cattle. *British Veterinary Journal* **106**, 41.