

***Salmonella dublin* abortion in cattle**

II. Observations on the whey agglutination test and the milk ring test

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SUMMARY

The serum and whey agglutination test were compared on paired samples from thirty-five cases of bovine abortion associated with *Salmonella dublin* infection. The whey test proved nearly as useful as the serum test for confirming an active infection though it was only practicable to examine the whey for flagellar antibodies. *S. dublin* was isolated from nearly half of the milk samples obtained within the first week of abortion but none of those collected after the fourth week. The whey test proved of no value in retrospective identification of abortion cases. The trial using the milk ring test was disappointing.

INTRODUCTION

During the course of clinical investigations of *S. dublin* abortion cases it was noted that high agglutinin titres to the flagellar antigens could often be detected in the whey. The effect of the method of preparation of whey on the *S. dublin* agglutinin titre has been reported elsewhere (Hinton, 1972). This paper records the changes that occur in the whey titres following a salmonella abortion together with details of a milk ring test (M.R.T.) using a stained *S. dublin* antigen.

MATERIALS AND METHODS

Clinical cases

These were encountered during the course of routine investigations of bovine abortion material, as described in the previous paper (Hinton, 1973). Abortion was the only clinical sign in 31 of the cows. In cases 18, 21 and 56 (see Table 1) there were also symptoms of dysentery while case no. 24 was ill after abortion though she did not develop diarrhoea.

When milk was cultured 5 ml. was incubated with 5 ml. double strength selenite F (Oxoid Ltd., London, S.E. 1) at 37° C. One subculture was made after 18–24 hr. onto either deoxycholate citrate agar (Hynes modification) (Oxoid) or MacConkey medium (Oxoid) containing 1/25,000 brilliant green.

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Table 1. *Changes in the serum and whey flagellar agglutination titres in 35 cases of Salmonella dublin abortion*

Case no.	Day collected*		Serum H titre		Whey H titre		Change in titre†	
	1st	2nd	1st	2nd	1st	2nd	Serum	Whey
18	4	24	10,240	1,280	640	160	-8	-4
19	1	20	40	1,280	< 20	160	+32	+16
21	3	19	160	640	20	80	+4	+4
22	-2	11	160	5,120	20	640	+32	+32
23	5	19	20,480	320	1,280	160	-64	-8
24	1	17	10,240	640	1,280	160	-16	-8
31	1	10	< 20	640	< 20	160	+64	+16
34	1	8	20	5,120	< 20	640	+256	+64
36	1	8	640	160	640	20	-4	-32
39	4	21	320	640	80	80	+2	0
40	5	19	5,120	1,280	640	80	-4	-8
41	5	19	5,120	1,280	640	1,280	-4	+2
42	3	18	1,280	320	320	40	-4	-8
44	3	15	10,240	5,120	5,120	160	-2	-32
47	4	25	1,280	160	2,560	40	-8	-64
54	3	20	1,280	640	320	< 20	-2	-32
56	1	18	160	1,280	20	160	+8	+8
60	3	16	5,120	640	320	80	+8	-4
63	4	10	5,120	640	160	< 20	-8	-16
71	1	14	10,240	2,560	320	160	-4	-2
73	3	15	< 20	2,560	< 20	640	+256	+64
79	3	14	320	320	80	160	0	+2
80	5	11	< 20	< 20	< 20	< 20	0	0
81	1	20	40	640	< 20	80	+16	+8
82‡	1	19	2,560	1,280	160	1,280	-2	+8
88	1	18	320	5,120	20	5,120	+16	+256
90	1	18	80	640	< 20	80	+8	+8
91	1	18	80	1,280	80	640	+16	+8
92	1	17	640	1,280	1,280	1,280	+2	0
98	1	23	640	2,560	40	160	+4	+4
100	2	26	40	160	< 20	< 20	+4	0
102	1	16	2,560	1,280	160	80	-2	-2
106	3	15	320	320	160	160	0	0
107	3	16	640	1,280	80	80	+2	0
109	1	15	160	320	< 20	< 20	+2	0

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

† Degree of change two-fold, four-fold, etc. 0 = No significant change.

‡ In the same period the serum O titre rose from 40 to 160.

Whey preparation

The milk was drawn, usually without aseptic precautions, from one or two quarters of the infected cow's udder. The whey was prepared by adding two drops of cheese-making rennet to 2 ml. of milk. This was incubated in a water bath at 52° C for 20-30 min. and then centrifuged at 2000 rev./min. for 10 min. to separate the curd from the whey.

Table 2. Summary of changes in the serum and whey flagellar titres in thirty-five cases of Salmonella dublin abortion

Group	Changes in H titre*		Case no.	Total
	Serum	Whey		
A	Rise	Rise	19, 21, 22, 31, 34, 56, 73, 81, 88, 90, 91, 98	12
B	Fall	Fall	18, 23, 24, 36, 40, 42, 47, 60, 63	9
C	Rise	NSC	100	1
D	NSC	Rise	82	1
E	Fall	NSC	41, 71	2
F	NSC	Fall	44, 54	2
G	NSC	NSC	39, 79, 80, 92, 102, 106, 107, 109	8

NSC = No significant change in titre, i.e. twofold or less.

* Significant rise or fall in titre is equivalent to fourfold or more.

Serum and whey agglutination test

Serum was examined by the tube agglutination test, by the technique described in the previous paper (Hinton, 1973). The whey was examined in a similar manner, but for the presence of flagellar antibodies only.

Milk ring test

A smooth, motile strain of *S. dublin*, isolated from a case of abortion, was seeded into nutrient broth, already at 37° C, and incubated at that temperature for 36 hr. The culture was killed by the addition of 0.5% formalin. The bacterial cells were harvested by centrifugation, stained with a 1 in 5 solution of Harris' haematoxylin and then resuspended in 0.5% phenol-saline to give a solution with a 4% packed cell volume. The test was carried out as recommended by Alton & Jones (1967) on 59 samples in which the whey titre was determined concurrently. About half the samples were from apparently normal cows while the rest were from abortion cases.

RESULTS

It was frequently found that the somatic titres in the whey were very low and that it was difficult to assess the degree of agglutination, consequently no whey O titres are given in these results. However, when they were carried out the titres were usually less than 80 though titres up to 320 were recorded.

A comparison of serum and whey agglutination titres in abortion cases

The full results obtained are given in Table 1 and summarized in Table 2. The cases can be divided into seven groups depending on the changes of the serum and whey titres. In Groups A to F (Table 2) the fact that either a rise or a fall in titre was recorded was related to the time of collection (Table 1) of the first sample relative to the abortion. In 10 of the 14 cases showing a significant rise in serum or whey titre, the first sample was collected within 24 hr. of the abortion while in 10 of the 13 cases, in which there was a significant fall in titre, the first samples were collected within three to five days of the abortion.

Table 3. *Relation between the changes in the serum and whey Salmonella dublin whey flagellar titres in four cases of abortion in which serial samples were examined*

Case no.	Day sample collected*	Serum titre		Whey titre
		O	H	H
47	2	40	1,280	—
	4	40	1,280	2,560
	9	80	320	320
	19	80	320	20
	25	80	160	40
	45	40	160	20
73	1	< 20	< 20	—
	3	< 20	< 20	< 20
	8	20	5,120	1,280
	15	160	2,560	640
	45	160	320	20
	81	1	< 20	40
4		80	320	320
6		160	1,280	320
8		—	—	640
20		160	640	80
90		20	80	< 20
98	1	40	640	40
	3	40	5,120	1,280
	7	160	10,240	320
	23	320	2,560	160
	60	160	640	< 20

* Day 1 is the 24 hr. after abortion.

In the 12 cases in Group A the median rise in whey and serum titre was 16-fold with the changes varying between four and 256-fold. The rise in serum and whey titre was of the same order in five of the cases while in only one case (no. 88) did the degree of change in the whey titre exceed that of the serum, and this was because the whey titre was very low (20) at the time of abortion. In this group the final serum titres ranged between 640 and 5120 with the median at 1280, while in the whey, the range was between 80 and 5120 with the median at 160. In no case did the final whey titre exceed that of the serum though in case no. 88 the titres were the same at 5120.

In Group B both the serum and whey titre showed a significant fall. This ranged between four and 64-fold. The change in titre was greater in the whey in all except four cases (nos. 18, 23, 24, 60). In these the initial serum titres were high, being in the range 5120 to 20,480.

In Groups C, D, E and F only one or other of the serum or whey titres changed significantly. The titres not showing a significant change were in the range 160 to 10,240, excepting in case no. 100. In this no whey agglutinins were detected. The value of examining serum samples for O antibodies was confirmed by case no. 82; the serum H titre showed no significant change while the O titre rose from 40 to 160 during the same period.

Table 4. *The serum and whey flagellar agglutination titres in convalescent Salmonella dublin abortion cases*

Serum flagellar titre	Whey flagellar titre			Total
	≤ 20	40	80	
≤ 40	3			3
80-160	8			8
≥ 320	3	2	1	6
Total	14	2	1	17

Table 5. *A comparison between the Salmonella dublin whey flagellar titre and the milk ring test score using a stained Salmonella dublin antigen*

MRT score	Whey H agglutination titre					Total
	< 20	20-40	80-160	320-640	≥ 1280	
-	8	3		1	2	14
+	9		1			10
++	6	3	3	2	4	18
+++	10	3	3	1		17
Total	33	9	7	4	6	59

In Group G the examination of paired serum and whey titres did not actually confirm an active salmonella infection though in six of the eight cases the maximum serum and whey titres ranged between 320 and 2560, and 80 and 1280 respectively. No serum and whey agglutinins were recorded in case No. 80 despite the fact that *S. dublin* was recovered from the foetus. A number of cases similar to this were identified in the full series of 111 cases and they probably represent transient excretion in the latent carrier animal, possibly activated by the stress of abortion. This feature of *S. dublin* infections will be discussed elsewhere.

Four or more samples of serum and whey were compared in four cases. The results are given in Table 3 and show that the changes in the whey titres follow those of the serum fairly closely though at a lower level.

The whey titre in convalescent cases of Salmonella dublin abortion

Seventeen cows were examined between 40 and 180 days after the abortion. The H titres of the serum and whey are given in Table 4. In 14 of the 17 cases the whey titres were 20 or less while in 11 the serum titre was 160 or less. *S. dublin* was cultured from faeces of four of the cows and in each case the whey titre was 20 or less.

The milk ring test

The results are summarized in Table 5 and show that the correlation between the M.R.T. score and the whey titre was not very good since 25 of the 45 samples with a positive M.R.T. had whey titres of less than 20 while three whey samples, which had titres between 640 and 2560, were negative to the M.R.T.

Table 6. *The isolation of Salmonella dublin from milk, vaginal mucus and faeces from 49 cases of abortion*

<i>S. dublin</i> isolations			Days after abortion				Total
Milk	VMS	Faeces	0-5	6-10	1-25	26†	
+	+	+	11	1	0	0	12
+	+	-	0	1	3	0	4
+	-	+	0	0	0	0	0
+	-	-	0	0	1	0	1
-	+	+	5	4	4	0	13
-	-	+	3	1	4	2	10
-	+	-	1	4	4	0	9
-	-	-	0	3	13	13	29
Total examined			20	14	29	15	78
Proportion of samples positive (%)							
Milk				55	14.3	13.7	—
VMS				85	71.5	38	—
Faeces				95	43	27.3	13.3

Table 7. *The relationship between the presence of Salmonella dublin in milk and the whey agglutination titre*

<i>S. dublin</i> in milk	No. examined	Whey agglutination titres			
		≤ 20	40	80-160	≥ 320
+	14	0	1	4	9
-	58	25*	2	16	15

* Includes 10 samples obtained 40 or more days after abortion and six samples from cows which did not develop serum or whey agglutination titres.

The isolation of Salmonella dublin from milk

Samples of milk, vaginal mucus and faeces were collected on 78 occasions from 49 cases. The results of culture are listed in Table 6. Over half the milk samples yielded *S. dublin* during the first 5 days after abortion, but the proportion of positive samples fell to nil by the end of the fourth week. A similar trend was noted in the case of vaginal mucus and faeces except that in 13.3% of the cases faecal excretion persisted beyond 4 weeks.

The whey agglutination titre was determined in 72 of the 78 milk samples. The distribution of the titres in both culturally positive and negative milks is listed in Table 7. The findings suggest that when *S. dublin* is isolated a whey titre of 80 or more is to be expected. A titre of 80 or more was also found in about half of the samples which were negative for *S. dublin* and this indicates that the presence of the organism is not essential for the appearance of the agglutinins in the milk.

DISCUSSION

The whey agglutination test

The results given in this paper indicate that when paired samples are examined the whey test is often as useful as the serum agglutination test for confirming that an abortion has been associated with an active *S. dublin* infection.

An analysis of the changes in the titres indicate that, when both are increasing, the rate of change of the serum titre often exceeds that of the whey, so that the serum to whey ratio either increases or at least remains the same. Conversely when the titres are falling, the rate of change in the whey was usually greater than that of the serum, except in cases in which the initial serum titre was very high.

A suggested interpretation for the serum agglutination test was given in the previous paper (Hinton, 1973). As far as the agglutinins in whey are concerned there appears to be no information about diagnostic titres. In brucella infections in non-vaccinated cattle, a positive result in the whey is taken at a titre of + +/10 while the corresponding titre in serum is + + + +/40 (Morgan, 1967). This represents an approximately four-fold difference between titres, and if a difference of this order is applied to the salmonella agglutination titres, then a whey titre of 80 or more will be diagnostic, with a doubtful range between 20 and 40.

Apart from the three cases (nos. 80, 100 and 109) in which there was no evidence of agglutinins in either sample, a whey titre of 80 or more was recorded in at least one of each pair of whey samples. This finding coupled with the fact that whey titres of less than 80 were found in only nine (20%) of the 43 samples collected after the third day indicates that the choice of 80 as a diagnostic titre is generally satisfactory from both a clinical and theoretical point of view.

The examination of serial samples of both serum and whey during the first three weeks after abortion show that the whey titres closely follow those of the serum though at a lower level. It is therefore probable that the major source of whey agglutinins is the blood and this suggestion is supported by the observation that titres of 320 or more were frequently recorded in samples which were apparently free of the organism. However, as the immunoglobulins in the serum and whey were not characterized it is not possible to provide a definite opinion on this point.

Sutherland & Berger (1944) suggested that the whey titre may be of value in the detection of faecal *Salmonella* carriers. They examined one cow twice and this had whey titres of 32 and 64. The animal was subsequently observed for a period of months by Rankin & Slavin (1947). By this time the whey titre had fallen to 10, despite the fact that the serum titres remained positive, and that faecal excretion was constant. A possible explanation for the findings in this case is that the cow had recently suffered an active, though possibly subclinical attack of salmonellosis, and that this was reflected in a transient rise in the whey agglutinin level.

Continued excretion of *S. heidelberg*, *S. dublin* and *S. paratyphi B* in milk has been recorded by Davies & Venn (1962), Weigt, Möller & Bleckmann (1972) and Thomas & Harbourne (1972) respectively. Thomas & Harbourne (1972) noted whey titres of O 80 and H 80-160 over a period of several weeks. Weigt *et al.* (1972) recorded a titre with an imprecise end-point of + + + /25, + + /50 and

+ /100 in the milk of the affected quarter. They used the closely related organism *S. enteritidis* as the antigen, and as they gave no details of antigen production it is not possible to assess if this represents the true *S. dublin* titre or not. Nevertheless, these findings indicate that the whey test may be of some help in assisting the identification of cattle, which though thought to be very rare (Davies & Venn, 1962) do continually excrete *Salmonella* in milk. On the other hand the whey test is unlikely to be of much value in the retrospective identification of salmonella abortion cases.

The milk ring test

There have been a number of reports from Russia on the M.R.T. The most encouraging was that of Bodyagin & Leont'eva (1968) who found that the milk of cows immunized with an alum-adjutant salmonella vaccine gave positive M.R.T. reactions even when the sample was diluted 1/124 in normal milk. Similarly the M.R.T. was still positive when bulk milk contained at least 8% of milk from immunized cows. On the other hand Arkhangel'skii & Kartashova (1962) and Kurakina (1967) both record that there were often false positive reactions though Kartashova *et al.* (1969) found that the proportion of false positive reactions could be reduced, if the antigen was suspended in phenol-saline, instead of glycerin.

The small trial using the M.R.T. was disappointing. There were many false positives, even though the antigen was suspended in phenol-saline and, possibly even more important, there were some false negatives in wheys with high agglutinin titres.

Salmonella dublin in the milk

S. dublin was frequently cultured from the milk collected within a week of abortion though excretion had ceased by the end of the fourth week. This finding indicates that under no circumstances should unpasteurized milk be used for either calf feeding or human consumption for at least a month after the abortion.

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REFERENCES

- ALTON, G. G. & JONES, L. M. (1967). *Laboratory Techniques in Brucellosis*. Geneva: World Health Organization.
- ARKHANGEL'SKII, I. I. & KARTASHOVA, V. M. (1962). Rapid methods for demonstrating salmonella in milk. *Veterinariya, Moscow*, no. 9, pp. 74-8 (*Veterinary Bulletin* (1963). **33**, 291).
- BODYAGIN, E. V. & LEONT'eva, N. D. (1968). Technique of examining milk from salmonella carriers (ring test). *Veterinariya, Moscow*, no. 9, pp. 93-4.
- DAVIES, E. T. & VENN, J. A. J. (1962). The detection of a bovine carrier of *Salmonella heidelberg*. *Journal of Hygiene* **60**, 495.

- HINTON, M. (1972). The effect of the method of whey preparation on the clarity of whey and the salmonella whey agglutination titre. *Research in Veterinary Science* **13**, 397.
- HINTON, M. (1973). *Salmonella dublin* abortion in cattle: I. Observations on the serum agglutination test. *Journal of Hygiene* **71**, 459.
- KURAKINA, T. A. (1967). Ring test on milk for bovine paratyphoid. *Veterinariya, Moscow*, No. 12, pp. 105–106.
- KARTASHOVA, V. M., ARKHANGEL'SKII, I. I., MALYAVIN, A. G., ROMIN, A. V. & AGANINA, L. A. (1969). Monovalent antigens for the salmonellosis ring test on cow's milk. *Trudy Vsesoyuznyic Nauchno-issledovatel'skii Institut Veterinaroi Sanitarii* **32**, 181–7. (*Veterinary Bulletin* (1969), **39**, 820.)
- MORGAN, W. J. B. (1967). The serological diagnosis of bovine brucellosis. *Veterinary Record* **80**, 612.
- RANKIN, J. D. & SLAVIN, G. (1947). *Salmonella enteritidis* var. *dublin* infection in a cow resulting in an outbreak of human gastro-enteritis. *Veterinary Record* **59**, 122.
- SUTHERLAND, P. L. & BERGER, F. M. (1944). A milk-borne outbreak of gastro-enteritis due to *Salmonella dublin*. *British Medical Journal* **i**, 488.
- THOMAS, G. W. & HARBOURNE, J. F. (1972). *Salmonella paratyphi* B infection in dairy cows Part II. Investigations of an active carrier. *Veterinary Record* **91**, 148.
- WEIGHT, U., MÖLLER, M. & BLECKMANN, E. (1972). Casuistic contribution of the salmonella infection of the mammary gland. *Deutsche tierärztliche Wochenschrift* **79**, 324.