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The effect of chloroform on *Brucella abortus* agglutinins in whey

By M. HINTON*
Veterinary Investigation Centre, Jobs Well Lane, Carmarthen, S. Wales

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SUMMARY

Defatting milk for the *Brucella abortus* whey agglutination test with chloroform caused some reduction in the titre in i.u./ml. in 42 of 57 samples and this reduction exceeded 50% in seven. Though this procedure will probably rarely affect the final interpretation of the test defatting the milk by centrifugation is preferred.

INTRODUCTION

The Public Health Laboratory Service recommended the use of chloroform to defat milk in the preparation of whey for the brucella whey agglutination test (Report, 1956). Chloroform frequently caused a significant reduction in the titres to *Salmonella dublin* flagellar agglutinins in whey (Hinton, 1972). This communication records the effect of chloroform on the agglutinins to *Br. abortus* in whey.

MATERIALS AND METHODS

The investigation was performed in two parts. In the first milk samples from 50 individual cows in herds in which there was either active or suspected *Br. abortus* infection and which were positive to the Milk Ring Test were examined. In the second, milk samples from 50 bovine abortions, or investigations of full-term calvings carried out in connexion with either the Ministry of Agriculture's Brucella Incentive Scheme or the Brucella Eradication Scheme, in which the serum was positive to the Rose Bengal Plate Test, were tested. It was not the purpose of the investigation to assess the value of the whey agglutination test so no reference was made to the final diagnosis in these cases.

The preparation of the whey has been described (Hinton, 1972). The milk was defatted either by centrifugation or with chloroform. The brucella agglutination test and the other serological tests referred to were performed using standard techniques (Morgan *et al.* 1971). The titres were expressed in international units of antibody and also as the reciprocal of the highest dilution showing 50% agglutination.

* Present address: University of Bristol, Department of Veterinary Medicine, Langford House, Langford, Bristol BS18 7DU.

Table 1. *The effect of chloroform on* Brucella abortus *agglutinins in whey samples from cows in infected herds*

Sample no.	Agglutination titre (i.u./ml)			Reciprocal dilution with 50 % agglutination		
	Untreated whey	Chloroform treated	Reduction (%)	Untreated whey	Chloroform treated	Degree of change
1	20	0	100	<10	<10	0
2	268	134	50	80	40	−2
3	67	34	50	20	10	−2
4	67	34	50	20	10	−2
5	80	47	41	20	20	0
6	53	34	36	20	10	−2
7	2144	1488	30	640	640	0
8	186	134	28	80	40	−2
9	186	134	28	80	40	−2
10	212	186	23	80	80	0
11	536	424	21	160	160	0
12	20	17	15	<10	<10	0
13	1488	1280	14	640	320	−2
14	93	80	14	40	20	−2
15	93	80	14	40	20	−2
16	47	40	14	20	10	−2
17	268	268	—	80	80	0
18	186	186	—	80	80	0
19	106	106	—	40	40	0
20	53	53	—	20	20	0
21	27	27	—	10	10	0
22	20	20	—	<10	<10	0
23	20	20	—	<10	<10	0

RESULTS

The full results are listed in Tables 1 and 2 and are summarized in Table 3. There was a reduction in the titre in i.u./ml. in 42 of the 57 samples in which there were measurable agglutinins. In seven samples the reduction exceeded 50 % and in three the titres were reduced to zero.

When the titres are expressed as the reciprocal of the dilution showing 50 % agglutination there were five fewer samples with recordable titres as those with titres of 17 and 20 i.u. are considered as negative. In the 52 remaining samples there was some reduction in titre in 28. It was twofold in 25 while in three instances it was 4-, 8- and 16-fold respectively.

DISCUSSION

These results show that chloroform may have a detrimental effect on the *Br. abortus* agglutinins in whey though they appear more stable to its action than the *S. dublin* flagellar agglutinins (Hinton, 1972). As the recommended level for a diagnostic titre in whey is 20 i.u./ml. the solvent will have relatively little effect on the final interpretation of the test, especially as it is suggested that this should only be made in the context of the other test results (Morgan *et al.* 1971). However,

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Table 2. *The effect of chloroform on* Brucella abortus *agglutinins in whey. Samples from abortion or full-term calving investigations*

Case no.	Serum* titre (i.u./ml)	Agglutination titre (i.u./ml) Untreated whey	Chloroform treated	Reduction in titre (%)	Reciprocal dilution with 50 % agglutination Untreated whey	Chloroform treated	Degree of change†
1	27*	40	0	−100	10	<10	−2
2	212	27	0	−100	10	<10	−2
3	186	1,696	106	−94	640	40	−16
4	2,976	20,480	4,288	−79	5,120	1,280	−4
5	53*	186	40	−79	80	10	−8
6	212	106	47	−56	40	20	−2
7	1,488	5,952	2,976	−50	2,560	1,280	−2
8	1,696	11,904	6,784	−43	5,120	2,560	−2
9	640	5,952	3,392	−43	2,560	1,280	−2
10	424	2,560	1,488	−42	640	640	0
11	67*	34	20	−41	10	<10	−2
12	67*	34	20	−41	10	<10	−2
13	13,568	13,568	6,784	−37	5,120	2,560	−2
14	320	212	134	−37	80	40	−2
15	134	34	23	−32	10	10	0
16	212	268	186	−31	80	80	0
17	1,280	134	93	−31	40	40	0
18	212	160	106	−30	40	40	0
19	2,560	5,952	4,288	−28	2,560	1,280	−2
20	134	372	268	−28	160	80	−2
21	134	186	134	−28	80	40	−2
22	47	106	80	−26	40	20	−2
23	104	1,072	848	−21	320	320	0
24	40	93	80	−14	40	20	−2
25	168	424	372	−12	160	160	0
26	186	53	47	−11	20	20	0
27	1,488	848	848	—	320	320	0
28	424	848	848	—	320	320	0
29	212	134	134	—	40	40	0
30	93	93	93	—	40	40	0
31	424	47	47	—	20	20	0
32	67*	47	47	—	20	20	0
33	40	20	20	—	<10	<10	0
34	93	17	17	—	<10	<10	0

* In cases marked thus the serum CFT was negative. In the remainder it was ⩾1/10.
† Reduction in titre 2-fold, 4-fold, etc. 0 No significant change.

any procedure which reduces the titre in serological tests is best avoided. Morgan *et al.* (1971) recommended that milk should be defatted by centrifugation and the fact that this regularly produces a clear whey (Hinton, 1972) indicates that this technique is preferable for the production of whey for agglutination tests.

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Table 3. *Summary of the effect of chloroform on* Brucella abortus *agglutinins in whey*

	Milk samples (Table 1)	Abortion/ calvings (Table 2)	Total
Samples tested	50	50	100
No. with agglutinins in untreated whey	23	34	57
Reduction (%) in titres in i.u./ml.			
10–25%	7	4	11
26–50%	8	16	24
51–75%	0	1	1
76–100%	1	5	6
Reduction in titres as reciprocal dilution			
2-fold	10	15	25
⩾4-fold	0	3	3

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