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Comparison of serological methods for the detection of *B. abortus* antibodies in sera from vaccinated and non-vaccinated cattle*

By F. C. HECK

Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Texas A & M University, College Station, Texas 77843

J. D. WILLIAMS, R. P. CRAWFORD and A. I. FLOWERS

Department of Veterinary Public Health, College of Veterinary Medicine, Texas A & M University, College Station, Texas 77843

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SUMMARY

A total of 4551 sera from 863 Strain 19 vaccinated and non-vaccinated adult cattle, independent of disease status, were tested by five serological methods to detect the presence of antibodies to *B. abortus*. Results from Standard Agglutination Tube (SAT), Buffered Brucella Antigen or card (CT), Complement Fixation (CF), Enzyme Linked Immunosorbent Assay (ELISA) and Rivanol (Riv) methods were compared.

There was a 95% probability for agreement among CT negative sera, between serological methods, for all groups of vaccinated and non-vaccinated cattle. The agreement between tests with Riv Positive sera, excluding the calfhood and adult vaccinated group tested by the CF method, was 91–100%. The probability of a serum which was serologically negative by other methods being Riv negative was 98%. The usefulness of serological results from Riv ($\geqslant 1/50$) tests for classifying the reactor status of cattle are of doubtful supplemental value to confirm card test positive results.

Vaccination history is an important consideration when evaluating serological data on cattle sera particularly from SAT and CF methods.

INTRODUCTION

Evaluation of results from serological methods plays an integral role in diagnosis of disease and management of herds and individual cattle exposed to or infected with *Brucella abortus* (*B. abortus*). Data from Standard Agglutination Tube (SAT) Buffered Brucella Antigen or card (CT) and Complement Fixation (CF) tests have been compared to determine agreement among tests, and suitability of serological

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methods, to evaluate the health status of the animal or herd exposed to *B. abortus* (Alton *et al.* 1975; Chappel *et al.* 1978; Morgan, MacKinnon & Cullen, 1969; Morgan & Richards, 1974; Nicoletti, 1967; Rose & Roepke, 1957). Most cattle infected with *B. abortus*, except those which are recently infected, can be identified by the SAT test (Davies, 1971). However, it has been reported that other supplemental serological methods were superior to SAT for detecting *Brucella* antibodies in sera from culture positive cows (Nicoletti, 1969). Recent evidence, suggesting that negative SAT results on specific sera cannot always be confirmed by plate agglutination or CF tests, was interpreted (Morgan & Richards, 1974) as being indicative of the insensitivity of the SAT test.

The immunologic response to antigenic stimulus by *B. abortus* is characterized by the synthesis of different classes and subclasses of immunoglobulins (Allan *et al.* 1976; Patterson, Deyoe & Stone, 1976; Timbs, Digby & Doe, 1978; O'Reilly & Cunningham, 1971). The immune response of individual cattle varies with regard to natural infection and vaccination, and it has been shown that certain immunoglobulins may not be present in serum at specific times, or in proper concentration, to allow for simultaneous positive reactions in different tests (Allan *et al.* 1976).

The purpose of this study was to determine the agreement between serological methods in detecting antibodies to *B. abortus* by comparing results from five different methods, Rivanol (Riv), Enzyme Linked Immunosorbent Assay (ELISA), CF, SAT and CT, on sera from cows under varying vaccination regimens and non-vaccinated cattle. Data were analysed independent of disease status and length of time between vaccination and serum collection.

MATERIALS AND METHODS

Sera

Blood samples were collected at 30- or 60-day intervals from the coccygeal vessels. A total of 8564 sera were obtained from 910 adult dairy cows beginning October 1976 and ending in February 1978. Only individual serum samples from which five serological test results were available were used. There were 4551 sera from 863 cows which satisfied this requirement. Although several serum samples were collected from each cow within 17 months, sera were tested and analysed as independent samples. Some sera obtained from AV cattle were collected 30 days after vaccination.

Experimental groups

The 863 adult cows were divided into four experimental groups: 139 cows were calfhood and adult vaccinated (CVAV), 272 cows were only adult vaccinated (AV), 178 cows were only calfhood vaccinated (CV) and 274 cows were not vaccinated (NV).

Vaccination was accomplished with 1 ml of strain 19 vaccine diluted with sterile peptone solution to contain 3×10^9 viable cells/ml (Crawford, Heck & Williams, 1978).

Serological methods

Card tests were accomplished according to the standard procedure described in the Brucellosis Card Test Kit with accompanying reagents prepared for Veterinary Services, APHIS, U.S.D.A.

Procedures for SAT and Riv were done according to the standard U.S.D.A. procedure. Complement fixation tests were done according to a method previously described (Jones, Hendricks & Berman, 1963), and Enzyme Linked Immunosorbent Assay (ELISA) was done according to the method of Saunders (1977).

CARD, SAT and RIV tests were completed at the State-Federal Brucellosis Laboratory, Austin, Texas. Complement fixation and ELISA tests were completed in the Department of Microbiology and Parasitology, College of Veterinary Medicine, Texas A & M University, College Station, Texas.

Test results from five serological methods on all sera were compared with each other in all possible combinations to determine their relative agreement (%) within and between experimental groups.

Positive reactions were determined by a Riv titre $\geqslant 50$, CF titre $\geqslant 40$, SAT titre $\geqslant 100$. A positive ELISA was determined by an extinction value (EV) $\geqslant 4$.

$$EV = [(100) - (\%T \text{ unknown})] - [(100) - (\%T \text{ of serum control})].$$

RESULTS

Comparison of results between serologic tests and experimental groups

Card test positive

Agreement between 1401 card test positive sera was between 43 and 93% when compared with results by other serological methods (Table 1). The agreement was 43–79% for vaccinated and 82–93% for non-vaccinated cattle.

Card test negative

Agreement between 3150 card test negative sera ranged between 90 and 100% (Table 2). The agreement between sera from non-adult vaccinated cattle was 98–100%.

Rivanol positive

Agreement between 754 Rivanol positive sera was between 84 and 100% when compared with results by other serological methods (Table 3). Except for the 84% agreement between Riv and CF in the CVAV group, the agreement between all tests for all experimental groups was 91–100%.

Rivanol negative

Agreement between 3797 Rivanol negative sera between all tests was from 64 to 99% (Table 4). Agreement in the CV and NV groups ranged from 96 to 99% while the agreement in CVAV and AV groups, that is any cow receiving adult vaccination, ranged between 64 and 83%.

Table 1. *Serological distribution of card test positive sera*

Experimental group	Distribution of test results as a percentage of total samples: ELISA Pos.	ELISA Neg.	CF Pos.	CF Neg.	Riv Pos.	Riv Neg.	SAT Pos.	SAT Neg.	Total card test positive samples
Calfhood and adult vaccinated	70	30	63	37	43	57	71	29	377
Calfhood vaccinated	70	30	74	26	58	42	72	28	43
Adult vaccinated	73	27	65	35	52	48	79	21	796
No vaccination	93	7	88	12	82	18	85	15	185
All groups	75	25	68	32	54	46	77	23	1401

Table 2. *Serological distribution of card test negative samples*

Experimental group	Distribution of test results as a percentage of total samples: ELISA Pos.	ELISA Neg.	CF Pos.	CF Neg.	Riv Pos.	Riv Neg.	SAT Pos.	SAT Neg.	Total card test negative samples
Calfhood and adult vaccinated	10	90	8	92	< 1	> 99	3	97	420
Calfhood vaccinated	2	98	2	98	< 1	> 99	< 1	> 99	831
Adult vaccinated	9	91	6	94	< 1	> 99	6	94	697
No vaccination	2	98	< 1	> 99	0	100	< 1	> 99	1202
All groups	5	95	3	97	< 1	> 99	2	98	3150

Table 3. *Serological distribution of rivanol positive samples*

Experimental group	Distribution of test results as a percentage of total samples: CARD Pos.	CARD Neg.	CF Pos.	CF Neg.	ELISA Pos.	ELISA Neg.	SAT Pos.	SAT Neg.	Total Rivanol positive samples
Calfhood and adult vaccinated	> 99	< 1	84	16	91	9	95	5	162
Calfhood vaccinated	96	4	92	8	92	8	96	4	26
Adult vaccinated	> 99	< 1	91	9	95	5	97	3	415
No vaccination	100	0	95	5	> 99	< 1	97	3	151
All groups	> 99	< 1	90	10	95	5	97	3	754

SAT positive

Agreement between 1141 SAT positive sera ranged between 55 and 98% (Table 5). However, agreement of the card test positive results with SAT positive sera among all experimental groups was 94–98%, while the agreement between CF, Riv and ELISA on sera from vaccinated cattle ranged between 55 and 88%. Agreement with results from CF, Riv and ELISA for sera from non-vaccinated cattle was 91–98%.

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Table 4. *Serological distribution of rivanol negative samples*

Experimental group	Distribution of test results as a percentage of total samples: CARD Pos.	CARD Neg.	CF Pos.	CF Neg.	ELISA Pos.	ELISA Neg.	SAT Pos.	SAT Neg.	Total Rivanol negative samples
Calfhood and adult vaccinated	34	66	21	79	25	75	20	80	635
Calfhood vaccinated	2	98	2	98	3	97	< 1	> 99	848
Adult vaccinated	36	64	17	83	23	77	24	76	1078
No vaccination	3	97	2	98	4	96	1	99	1236
All groups	17	83	10	90	13	87	11	89	3797

Table 5. *Serological distribution of SAT positive samples*

Experimental group	Distribution of test results as a percentage of total samples: CARD Pos.	CARD Neg.	CF Pos.	CF Neg.	Riv Pos.	Riv Neg.	ELISA Pos.	ELISA Neg.	Total SAT positive samples
Calfhood and adult vaccinated	95	5	68	32	55	45	76	24	282
Calfhood vaccinated	97	3	88	12	78	22	84	16	32
Adult vaccinated	94	6	69	31	61	39	77	23	666
No vaccination	98	2	93	7	91	9	98	2	161
All groups	95	5	73	27	64	36	80	20	1141

Table 6. *Serological distribution of SAT negative samples*

Experimental group	Distribution of test results as a percentage of total samples: CARD Pos.	CARD Neg.	CF Pos.	CF Neg.	Riv Pos.	Riv Neg.	ELISA Pos.	ELISA Neg.	Total SAT negative samples
Calfhood and adult vaccinated	21	79	15	85	2	98	17	83	515
Calfhood vaccinated	1	99	2	98	< 1	> 99	3	97	842
Adult vaccinated	20	80	13	87	1	99	16	84	827
No vaccination	2	98	2	98	< 1	> 99	4	96	1226
All groups	9	91	7	93	< 1	> 99	9	91	3410

SAT negative

Agreement between 3410 SAT negative sera ranged between 79 and 99% (Table 6). The agreement within CV and NV groups ranged from 96 to 99%. Agreement within AV and CVAV groups ranged from 79 to 87% for ELISA, CF and CT while the agreement for Riv negative results was 98–99% among all groups.

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Table 7. *Serological distribution of ELISA positive samples*

	Distribution of test results as a percentage of total samples								
	CARD		CF		Riv		SAT		Total ELISA positive
Experimental group	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	samples
Calfhood and adult vaccinated	87	13	69	31	49	51	71	29	303
Calfhood vaccinated	60	40	68	32	48	52	54	46	50
Adult vaccinated	90	10	73	27	61	39	79	21	647
No vaccination	86	14	81	19	74	26	78	22	202
All groups	87	13	73	27	60	40	75	25	1202

Table 8. *Serological distribution of ELISA negative samples*

	Distribution of test results as a percentage of total samples								
	CARD		CF		Riv		SAT		Total ELISA negative
Experimental group	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	samples
Calfhood and adult vaccinated	23	77	12	88	3	97	14	86	494
Calfhood vaccinated	2	98	1	99	< 1	> 99	< 1	> 99	824
Adult vaccinated	25	75	11	89	2	98	18	82	846
No vaccination	1	99	< 1	> 99	< 1	> 99	< 1	> 99	1185
All groups	11	89	5	95	1	99	7	93	3349

Table 9. *Serological distribution of CF positive samples*

	Distribution of test results as a percentage of total samples								
	CARD		ELISA		Riv		SAT		Total CF positive
Experimental group	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	samples
Calfhood and adult vaccinated	88	12	78	22	51	49	71	29	269
Calfhood vaccinated	71	29	76	24	53	47	62	38	45
Adult vaccinated	92	8	83	17	67	33	82	18	564
No vaccination	94	6	95	5	83	17	87	13	173
All groups	90	10	83	17	65	35	79	21	1051

ELISA positive

Agreement between 1202 ELISA positive sera was between 48 and 90% (Table 7). These data vary within groups such that no pattern can be established.

ELISA negative

Agreement between 3349 ELISA negative sera ranged between 75 and 99% (Table 8). There was 98–99% agreement between tests on sera from NV or CV

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Table 10. *Serological distribution of CF negative samples*

Experimental group	Distribution of test results as a percentage of total samples								Total CF negative samples
	CARD		ELISA		Riv		SAT		
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	
Calfhood and adult vaccinated	27	73	18	82	5	95	17	83	528
Calfhood vaccinated	1	99	2	98	< 1	> 99	< 1	> 99	829
Adult vaccinated	30	70	19	81	4	96	22	78	929
No vaccination	2	98	3	97	< 1	> 99	< 1	> 99	1214
All groups	13	87	9	91	2	98	9	91	3500

animals and 97–99 % agreement with Riv negative results between experimental groups.

CF positive

Agreement between 1051 CF positive sera was between 51 and 95 % (Table 9). Agreement among tests in the NV group ranged from 83 to 95 % while agreement between tests on sera from vaccinated cattle ranged from 51 to 92 %.

CF negative

Agreement between 3500 negative sera ranged from 70 to 99 % (Table 10). Agreement among tests within the NV group and CV group ranged from 97 to 99 % while agreement among sera from the AV and CVAV groups ranged from 70 to 96 %. The agreement between groups with Riv negative results ranged from 95 to 99 %.

DISCUSSION

The disparity of results on card test positive sera (Table 1) when tested by other methods was greater between groups of vaccinated cattle than was apparent on sera from non-vaccinated cattle.

Data in Table 2 indicate that if the serum was CT negative, there was at least a 95 % chance, for all groups, that the serum would be negative by other methods. If the animal was not adult vaccinated there was 98 to 100 % probability that the serum was serologically negative by other methods. If the cows were adult vaccinated, there was a 90–99 % probability that the sera were negative by other methods. This agreement between tests for negative animal sera suggests that if the CT is used as the initial herd screening method, those sera which are serologically positive should be tested by supplemental methods to clarify the reactor status of the cattle.

The agreement with rivanol positive sera between methods for all groups is 84–100 %. Excluding the CVAV group sera tested by the CF method, the agreement between methods is 91–100 %. If a serum was positive by any serological

method, a Riv positive result did not increase the basic knowledge relative to reactor status. If a serum was serologically negative by any other method (Tables 2, 6, 8, and 10) the probability, on the average, of it being Riv negative was 98%. Therefore, the value of the Riv procedure as a supplemental method to the card test for the detection of *Brucella* antibodies is of doubtful value when results from CF or SAT tests are known.

Of the 1401 sera that were CT positive, 68% were also CF positive, and of the 1051 sera that were CF positive, 90% were card test positive; therefore, the 32% which were CT positive and CF negative may represent sera which contained immunoglobulins more capable of agglutination (Morgan & Richards, 1974; Allan *et al.* 1976).

Sera from non-adult vaccinated cattle which are negative by the SAT test have a 96–99% probability of being negative by the other serological methods. Twenty per cent of the sera from AV cattle which were SAT negative were positive by the CT, 16% were positive by ELISA and 13% were positive by CF (Table 6). Vaccination history was critical when SAT was considered as the initial screening test for AV cattle, because the variation in results between methods suggest that the SAT did not detect immunoglobulins detectable by the CT and CF methods. However, sera which were positive by SAT have a 94–98% probability of being positive by the CT regardless of vaccination history.

There is a 74% probability that CT positive sera will be positive by CF if the sera were collected from CV cattle (Table 1). These data were in agreement with the conclusions of others (Timbs *et al.* 1978), who indicated that 78·8% of card test positive sera from CV cattle were also CF positive. The agreement of CF negative results on sera from CV and NV cattle was between 97 and 99%, compared with 70 and 96% agreement if the sera originated from AV cows when Riv was included, and 70–83% agreement when Riv test results were excluded (Table 10). These data show the importance of vaccination history when evaluating CF test results on sera from vaccinated cattle.

There was a disagreement between serological test results for sera which were ELISA positive. The variance among all serological test results was so great as to indicate that ELISA reflects specific and non-specific reactions of immunoglobulins not measured by the other methods or, as indicated by others, ELISA results may be affected by affinity characteristics of these immunoglobulins (Butler *et al.* 1978).

The effect of adult vaccination upon the serologic results was apparent when sera which were negative by Riv, SAT, ELISA and CF were compared between methods. Agreement among negative sera from non-adult vaccinated cattle for all methods was between 96 and 100%. Disparity between tests on sera from vaccinated cattle which were positive by any method may reflect serological differences in immunoglobulin classes. Conceivably, soluble immune complexes could influence in vitro reactions between standardized reagents and contribute to apparent variances between serologic methods. The best correlation between methods in sera positive by any test was achieved with sera from non-vaccinated cattle. If sera are card test positive they should be tested by supplemental methods and the

results should be evaluated with consideration being given to vaccination history of the herd.

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