Use of the Brucellosis Card Test for Screening Cattle in Saskatchewan

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ABSTRACT

One group of 28,714 bovine sera were tested by both the brucellosis tube serum agglutination test and the brucellosis card test. The tube serum agglutination test confirmed 99.8% of the negative brucellosis card test results. The brucellosis card test identified 63% of the tube serum agglutination test reactors. In a second group of 496 sera reacting to either the tube serum agglutination test, complement fixation test, plate serum agglutination test or acid antigen serum agglutination test the brucellosis card test identified 99.1% of the complement fixation test positive sera and 91.3% of the sera reacting to any of the other serological tests. The brucellosis card test showed satisfactory agreement with both the complement fixation test and tube serum agglutination test. It appears to be a useful screening test in operations involving large numbers of animals since under these conditions the reactors can be quickly identified and isolated.

RÉSUMÉ

On a procédé à la recherche d'anticorps contre la brucellose sur un lot de 28,714 échantillons sérologiques de bovins. On utilisa à cette fin l'épreuve de l'agglutination lente et celle de la carte; la première permit de confirmer 99.8% des résultats négatifs obtenus par la seconde. Celle-ci permit également d'identifier 63% des réacteurs décelés par l'épreuve de l'agglutination lente. Dans un autre lot de 496 échantillons de sérum réagissant à l'une ou l'autre des épreuves suivantes: agglutination lente, déviation du complément, agglutination rapide et agglutination à l'antigène acide, l'épreuve de la carte permit de déceler 99.1% des échantillons ayant réagi de façon positive à l'épreuve de la déviation du complément et 91.3% de ceux qui avaient réagi à n'importe laquelle des autres épreuves sérologiques. L'épreuve de la carte manifesta une concordance satisfaisante avec celles de la déviation du complément et de l'agglutination lente. Elle semble donc constituer une épreuve utile de dépistage dans les entreprises comptant un grand nombre d'animaux, parce qu'elle permet de déceler et d'isoler rapidement les réacteurs.

INTRODUCTION

The brucellosis card test (BCT) was developed in the United States to meet the need for a rapid field test for the serological diagnosis of brucellosis which would keep the handling of cattle to a minimum under ranch or stockyard conditions. The test is interpreted as either negative or positive without a "suspicious" category. In 1966 the BCT was adopted for use in the United States Brucellosis Eradication Program (5). The BCT antigen is a stained, buffered, whole cell suspension of *Brucella abortus* strain 1119-3 produced by the Animal Health Division, United States Department of Agriculture. The other components are prepared commercially[1].

Trials using the BCT were carried out in Saskatchewan. In that province the problem of holding cattle until laboratory tests have been completed is encountered both on the range and at the entrance to community pastures where herds are mixed during the summer months.

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Submitted August 13, 1974.

[1]Brewer's Brucellosis Card Test, Kit No. 302. Hynson, Westcott & Dunning, Inc., Baltimore, Maryland.

MATERIALS AND METHODS

A total of 28,714 bovine sera were collected by Health of Animals Branch veterinarians, tested by the BCT under field conditions and then shipped to the Saskatchewan Area Laboratory for the tube serum agglutination test (SAT). A second group of 496 bovine sera which reacted to the SAT or other laboratory brucellosis tests were retested in parallel by the SAT, the plate serum agglutination test (PAT), the acid antigen serum agglutination test (AAT), the complement fixation test (CFT) and the BCT.

The sera were processed by the official Canadian standard three tube SAT in 1:50, 1:100 and 1:200 dilutions. The PAT was conducted with its regular antigen in dilutions equivalent to the SAT and the results read after 15 minutes incubation. The AAT was performed in the same manner as the PAT but using antigen buffered at pH 4.0 instead of 6.7. The CFT was performed as a standard six tube test in 1:5, 1:10, 1:25 and 1:50 dilutions and serum controls in 1:5 and 1:10 dilutions. SAT was standardized to give 50% fixation for this test. Hemolysis of 50% was the decisive point for a reaction. The BCT was conducted on serum according to the recommendations of the manufacturer using commercially available kits[1].

The interpretation of tests is shown in Table I. Because of the low incidence of brucellosis vaccinated cattle in the province all sera were interpreted for nonvaccinate status.

RESULTS

The results from 28,714 bovine sera tested by the BCT under field conditions and by the SAT in the laboratory are summarized in Table II. The SAT confirmed 99.8% of the negative BCT and 48% of the positive BCT results.

The results of the comparative testing of the second group of 496 sera is summarized in Table III.

DISCUSSION

Application of the BCT as a screening procedure has proven useful in low incidence brucellosis areas of the United States. Infected cattle were often identified earlier by the BCT than by the SAT. The test appeared to be too sensitive in certain populations (5). In an evaluation of the BCT in Ireland the results of 2,539 bovine sera were found to be in close agreement with the SAT except in the Irish Suspect range (30 to <60 I.U.) where the BCT detected approximately half of the SAT suspect, CFT positive sera. A low correlation was found between the BCT and CFT positive sera with low levels of agglutinins. From this the authors concluded the BCT did not detect complement fixing antibody (6).

In Great Britain, Morgan *et al* investigated the Rose Bengal plate agglutination (RBPT), a modification of the BCT, and found a close agreement between the results of that test and the CFT on 6,424 bovine sera (3). They concluded that the BCT was a useful screening test and recommended that the reactors be subjected to the CFT. In further studies under field conditions, reactions to the RBPT appeared at about the same time as reactions to the SAT (2,4).

The official Canadian brucellosis test is the SAT. In our studies 56 SAT reactors were missed by the BCT (Table II). Twice as many reactors were indicated by BCT as were found by the SAT. Comparison of the results of detailed testing (Table III)

TABLE I. Interpretation of Tests

Agglutination Tests[a]		Complement Fixation Test	
Titre[b]	Interpretation	Titre	Interpretation
< 60 IU	Negative (−)	> 50% hem. at 1/5	Negative (−)
> 60 — < 120 IU	Questionable (±)	⩽ 50% hem. at 1/5, > 50% hem. at 1/25	Suspicious (±)
⩾ 120 IU	Positive (+)	⩽ 50% hem. at 1/25	Positive (+)

[a]Tube serum agglutination, plate serum agglutination and acid antigen serum agglutination tests
[b]Complete agglutination 1:50 — approximately 60 IU
Complete agglutination 1:100 — approximately 120 IU

TABLE II. Comparison of Brucellosis Card Test Field Results with Tube Serum Agglutination Test Laboratory Results

Number Tested	Brucellosis Card Test		Tube Serum Agglutination Test		
	Number Positive	Number Negative	Number Positive	Number Questionable	Number Negative
28,714.....	200	—	46	50	104
	—	28,514	17	39	28,458

TABLE III. Comparison of 496 Reactions of Agglutination Tests or Complement Fixation Test with Brucellosis Card Test Conducted in Laboratory

Reaction		Number of Samples	Brucellosis Card Test Reaction			
Agglutination[a] Tests	Complement Fixation Test		Number Postiive	%	Number Negative	%
+[b]	+	338	336	99.4	2	0.6
+	±[b]	15	5	33.3	10	66.7
+	−[b]	6	1	16.7	5	83.3
±	+	75	74	98.7	1	1.3
±	±	14	7	50.0	7	50.0
±	−	8	2	25.0	6	75.0
−	+	27	26	96.3	1	3.7
−	±	13	2	15.4	11	84.6
Total		496	453	91.3	43	8.7

[a]Reacting to all or any one of tube serum agglutination, acid antigen serum agglutination, plate serum agglutination tests
[b]+ = positive
± = questionable
− = negative

shows a correlation between BCT and CFT. For example, 27 SAT negative sera were positive to CFT, 26 of which also reacted to the BCT. The questionable CFT reactions were either split evenly among the BCT categories or tended to be negative. The 14 sera which were negative for CFT with either positive or questionable agglutination reactions tended to give a negative BCT result. These may reflect vaccinated animals as no detailed records were available to trace them although the general incidence of vaccinated cattle in Saskatchewan was considered small. The 17 positive and 39 questionable SAT results which constitute 0.2% failure of the BCT to detect them (Table II) may also represent those negative to the CFT and thus represent residual vaccination titres. The CFT was not done on these samples.

Corbel showed by fractionation and inhibition test that RBPT activity was associated with immunoglobulins of the IgG_1 class which also contains the major portion of the serum complement fixing activity (1).

Our BCT results, conducted under field conditions on Saskatchewan cattle, show satisfactory agreement with both SAT and with CFT. It appears to be a useful screening test in operations involving large numbers of cattle with limited holding facilities such as herding to community pastures, round-up for sales yards and livestock auction sales. Under these conditions the reactors can be identified immediately and isolated from the rest of the herd. The high sensitivity of the BCT should not be a serious problem. Those animals so identified can be isolated until the laboratory tests become available and then the negative animals released, the number of which should not be excessive.

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