A Comparison of Standard Serological Tests for the Diagnosis of Bovine Brucellosis in Canada

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ABSTRACT

Six agglutination and two complement fixation tests were compared with respect to specificity, sensitivity and relative sensitivity for the serodiagnosis of bovine brucellosis.

Based on 1051 sera from brucellosis free herds, the specificity of the tests was 98.9% for the buffered plate antigen test (BPAT), 99.2% and 99.3% for the standard tube and plate agglutination tests (STAT and SPAT), respectively, and 99.8% for the 2-mercaptoethanol test (2MET). On this small sample, the rose bengal plate test (RBPT), card test (CARD) and the complement fixation test (CFT) correctly classed all sera as negative.

On a sample of 167 culture positive cattle, the sensitivities of the tests were CFT: 79.0%, BPAT: 75.4, RBPT: 74.9%, CARD: 74.3%, SPAT: 73.1%, STAT: 68.9%, and 2MET: 59.9%. All tests combined detected only 82% of these infected cattle.

Analysis of the relative sensitivity of the six agglutination tests gave the following ranking: BPAT > RBPT > CARD > SPAT > STAT. The 2MET ranked between the BPAT and RBPT or between the RBPT and CARD depending on the analysis used.

The use of the BPAT as a screening test is recommended provided that a test of high specificity and sensitivity such as the CFT is used to confirm screening test reactions.

Key words: brucellosis, bovine, diagnosis, *Brucella abortus*, serodiagnosis, agglutination tests, complement fixation test.

RÉSUMÉ

Cette expérience visait à comparer la spécificité, ainsi que la sensibilité individuelle et relative de six épreuves d'agglutination et de deux épreuves de la déviation du complément, pour le diagnostic sérologique de la brucellose bovine.

L'épreuve de 1051 échantillons de sérum, prélevés dans des troupeaux exempts de brucellose, révéla que la spécificité de quatre des six épreuves d'agglutination atteignait les valeurs suivantes: 98,9% pour l'épreuve de l'agglutination rapide avec un antigène-tampon; 99,2% pour celle de l'agglutination lente standard; 99,3% pour celle de l'agglutibnation rapide standard; 99,8% pour celle de l'agglutination lente avec le 2-mercaptoéthanol. Quant à l'épreuve de l'agglutination rapide au rose Bengale, à celle de la carte et aux deux épreuves de la déviation du complément, elles permirent de détecter correctement tous les échantillons négatifs.

L'épreuve du sérum de 167 bovins desquels on avait isolé *Brucella abortus* révéla que la sensibilité des deux épreuves de la déviation du complément atteignait 79%; celle de l'épreuve de l'agglutination rapide avec un antigène-tampon, 75,4%; celle de l'épreuve de l'agglutination rapide au rose Bengale, 74,9%; celle de l'épreuve de la carte, 74,3%; celle de l'épreuve de l'agglutination rapide standard, 73,1%; celle de l'épreuve de l'agglutination lente standard, 68,9%; celle de l'agglutination lente avec le 2-mercaptoéthanol, 59,9%. Dans l'ensemble, ces épreuves ne détectèrent que 82% des bovins atteints de brucellose.

L'analyse de la sensibilité relative des six épreuves d'agglutination donna l'ordre décroissant suivant: l'épreuve de l'agglutination rapide avec un antigène-tampon; l'épreuve de l'agglutination rapide au rose Bengale; l'épreuve de la carte, l'épreuve de l'agglutination rapide standard; l'épreuve de l'agglutination lente standard. L'épreuve de l'agglutination lente avec le 2-mercaptoéthanol se situa entre l'épreuve de l'agglutination rapide avec un antigène-tampon et celle de l'agglutination rapide au rose Bengale, ou entre celle-ci et l'épreuve de la carte, selon l'analyse utilisée.

L'emploi de l'épreuve de l'agglutination rapide avec un antigène-tampon, comme épreuve de tamisage, semble par conséquent recommandable, pourvu qu'on en confirme les résultats avec une épreuve particulièrement spécifique et sensible, telle que l'épreuve de la déviation du complément.

Mots clés: brucellose, bovins, diagnostic, *Brucella abortus*, diagnostic sérologique, épreuves d'agglutination, épreuve de la déviation du complément.

INTRODUCTION

Specificity and sensitivity, respectively, describe the ability of diagnostic tests to correctly identify non-infected cattle as "negative" and infected cattle as "positive" (1).

Complement fixation (CFT) and acidified antigen agglutination tests have been repeatedly shown to offer improved specificity and sensitivity over the standard tube agglutination test (STAT) for the serological diagnosis of bovine brucellosis (2-10). Because of its ability to detect infected cattle that give no agglutination test reactions, the CFT has been used for many years in this country along with standard agglutination methods to test cattle from brucellosis infected herds (2). The importance of using the CFT and other supplemental tests in dealing with herds which posed diagnostic

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TABLE I. Serological Tests Evaluated

Abbreviation	Test	Reference
STAT	Standard Tube Agglutination Test	13,14
SPAT	Standard Plate Agglutination Test	13,14
2MET	2-Mercaptoethanol Test	12
CFT	Complement Fixation Test	15
BPAT	Buffered Plate Antigen Test	16
CARD	Card Test (Hyson, Wescott & Dunning Ltd.)	—
RBPT	Rose Bengal Plate Agglutination Test	17

problems was reemphasized in the 1960s (3-5).

As eradication progressed it became desirable to increase the specificity and sensitivity of the tests used for testing the cattle population. This has been done by the use of an automated CFT (11) or by using an acidified antigen agglutination test for screening sera with the CFT as a confirmatory test (9). Ideally, a screening test should be economical, rapid and highly sensitive, but it need not be highly specific. A confirmatory test, on the other hand, must be sensitive and specific. This paper describes an evaluation of agglutination and complement fixation tests that was conducted from 1975 to 1980 to provide a basis for the selection of screening and confirmatory tests for use under Canadian conditions.

MATERIALS AND METHODS

SEROLOGICAL TESTS

A summary of the tests used is given in Table I. The standard tube (STAT) and plate (SPAT) agglutination tests were performed by procedures essentially similar to the USDA methods described by Alton, Jones and Pietz (12) as detailed previously (13,14) using *Brucella abortus* strain 413 antigens produced by the Animal Diseases Research Institute, Nepean, Ontario. In this STAT, 3+ reactions at dilutions of 1/25, 1/50 and 1/100 represent agglutination activity of approximately 30, 60 and 125 IU/mL, respectively (14). No allowance was made for vaccination status of cattle in interpreting the results of these or other tests, all reactions being interpreted as for nonvaccinates.

The mercaptoethanol agglutination test (2MET) was performed following the USDA method (12) by adding 80, 40, 20 and 10 μL of serum to four tubes. One mL of 0.1 M mercaptoethanol in 0.85 g% NaCl and 1 mL of double strength (1:100) STAT antigen in 0.85 g% NaCl were added. The tubes were shaken, incubated and reactions were read as for the STAT.

A microtiter cold complement fixation test (CFT) and an automated complement fixation test were performed as described previously (15).

The buffered plate antigen test (BPAT) was performed with antigen provided by the National Veterinary Services Laboratory, Ames, Iowa. The preparation and evaluation of this antigen were recently described (16). It is an 11% suspension of *B. abortus* strain 1119-3 stained with crystal violet and brilliant green and buffered to pH 3.63. The test was performed as the SPAT, mixing 80 μL of serum and 30 μL of antigen. The incubation time was eight minutes, with the plate being rotated four times after four minutes of incubation. Reactions were read as ++ for complete agglutination and + for partial agglutination. A negative reaction was a homogenous serum-antigen mixture with no evidence of agglutination.

The card test (CARD) was performed according to instructions of the manufacturer (Hyson, Wescott and Dunning, Inc., Baltimore, Maryland).

The rose bengal plate test (RBPT) was performed manually with antigen obtained from the Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England according to standard procedures (17).

The criteria used in this study for classifying reactors to each test as positive are described in Table II.

TABLE II. Specificity of Seven Serological Tests for Bovine Brucellosis Based on Cattle from Brucellosis-free Herds

Tests	Seropositive Criterion	Specificity (%) Overall[a]	Nonvaccinates[b]	Vaccinates[c]
BPAT	+	98.9	98.9	98.8
STAT	⩾ 3+ at 1/50	99.2	99.5	98.8
SPAT	⩾ 3+ at 1/50	99.3	99.9	98.1
2MET	⩾ 3+ at 1/25	99.9	99.7	100
RBPT	+	100	100	100
CARD	+	100	100	100
CFT	⩾ 1/5	100	100	100

[a]Combined group of 1051 cattle from 24 herds
[b]730 cattle from 24 herds
[c]321 cattle from 15 heres

BACTERIOLOGY

Cattle slaughtered under the national brucellosis eradication program were selected for culture. Tissue collection and bacteriological procedures were described previously (14,18). In several cases, particularly when samples were collected from large abattoirs, the full set of tissues was not submitted.

BRUCELLA INFECTED HERDS

Sixteen herds in Ontario and Quebec were considered infected either after *B. abortus* was isolated in the cases of twelve herds, or because several cattle gave strong reactions to all of the standard serological tests and had serum precipitins to *Brucella* antigen A2 in the cases of four herds (19). Sera from all adult cattle in these herds were tested. Tissues from selected reactor and negative cattle were collected at slaughter.

Twenty herds in Saskatchewan were selected following the isolation of *B. abortus*. Cattle from these herds were selected for study on the basis of reac-

tions to standard agglutination tests. Reactor and negative cattle were slaughtered and a complete set of tissues was collected (18, Forbes, L.B., 1983, "Bacteriological, serological and epidemiological studies of *Brucella abortus* in cattle", M.Sc. thesis, University of Saskatchewan).

BRUCELLA-FREE HERDS

Twenty-four herds from Ontario, Quebec and the Atlantic Provinces were selected for this study in consultation with the regional epidemiologist of the Veterinary Inspection Directorate of Agriculture Canada's Food Production and Inspection Branch. This selection was based upon consideration of the herd's brucellosis test history and management practices to assess the adequacy of evidence for absence of brucellosis.

Fifteen of these herds included no vaccinates, 8 herds had fewer than 10% vaccinates and in one herd about 50% of the animals had been vaccinated as calves. Of a total of 1051 cattle, 730 were nonvaccinates. Calfhood vaccination with the standard dose of strain 19 is the only brucellosis vaccination permitted in Canada. Since 1970 the annual use of vaccine nationally has been less than 100,000 doses in a cattle population of about 15 million.

RESULTS

SPECIFICITY

Table II summarizes the results obtained with tests of sera from 1051 cattle in the 24 brucellosis-free herds. The specificity of the STAT and SPAT was higher for nonvaccinated than for vaccinated cattle (Table II). The mean age of cattle giving reactions to any of these tests was four years (range 3-5 years) for vaccinates (N = 4) and five years (range 1-10 years) for nonvaccinates (N = 16).

SENSITIVITY

The seven tests were ranked (Table III) on the basis of ability to detect 167 cattle from which *Brucella abortus* was isolated. All tests were interpreted in parallel to obtain the results for "All Tests" (Table III). These data were for nonvaccinated cattle from 20 different herds in Saskatchewan. Some may have been very recently infected.

TABLE III. Sensitivity of Seven Serological Tests for Bovine Brucellosis Based on 167 Culture Positive Cattle

Test	Seropositive Criterion	Sensitivity (%)
CFT	⩾ 1/5	79.0
BPAT	+	75.4
RBPT	+	74.9
CARD	+	74.3
SPAT	⩾ 3+ at 1/50	73.1
STAT	⩾ 3+ at 1/50	68.9
2MET	⩾ 3+ at 1/25	59.9
All Tests[a]	—	82.0

[a]Positive on any one or combination of tests

Agglutination tests being considered for use as a screening method were ranked by relative sensitivity (1) because of the risk of biasing estimates of sensitivity by considering only cattle that could be proven infected by culture techniques. Table IV describes the total number of reactions detected by each test with the 2209 sera from 16 infected herds and the estimated number of reactors detected after correction for false positive reactions expected on the basis of the specificity data presented in Table II. Another assessment of relative sensitivity (Table IV) was based on the ability of the tests to detect reactions with 259 and 227 sera from cattle in infected herds which reacted to the CFT and to the CFT + 2MET, respectively. These were selected because of the high specificity of the CFT and of the CFT + 2MET combination and because the CFT + 2MET reactor sera contained both complement fixing and agglutinating anti-*Brucella* activity.

AUTOMATED COMPLEMENT FIXATION TEST

Like the cold fixation microtiter CFT, the automated warm complement fixation test did not give any false reactions with the sera from brucellosis-free herds. The automated test was not performed on the sera from culture positive cattle. Its sensitivity relative to that of the microtiter CFT was determined by comparison of results obtained with sera of cattle from infected herds. Eleven automated test reactor sera were classed negative by the microtiter CFT, while 34 microtiter CFT reactors were negative by the automated test. The discrepancies between the automated and microtiter tests involved only sera with titers of 1/5 or 1/10 with the exception of six sera which were negative by the automated test but had initial microtiter CFT titers ⩾= 1/10. On retest these gave automated test titers of 1/5 and microtiter CFT titers of 1/5 or 1/10.

DISCUSSION

Our sample of 1051 cattle revealed no false reactions to the CFT, CARD or RBPT, but allowed us to detect false reactions to the BPAT, STAT, SPAT and 2MET. With the STAT and SPAT, these reactions were all in the "suspicious" category (reactions

TABLE IV. Relative Sensitivity of Agglutination Tests for the Diagnosis of Bovine Brucellosis Based on Tests of 2209 Sera from 16 Infected Herds

Test	Total Positive (%)	Adjusted Positive (%)[a]	Percent Positive of 259 CFT Reactors	Percent Positive of 227 CFT + 2MET Reactors
BPAT	13.6	12.5	96.9	98.2
2MET	10.9	10.7	95.4	N/A[b]
RBPT	11.2	11.2	93.8	96.0
CARD	9.8	9.8	91.5	94.7
SPAT	10.1	9.5	91.5	93.8
STAT	9.5	8.7	88.8	91.6

[a]Expected false positives (EFP) were calculated on the basis of the specificity estimated in Table II for the sample including vaccinated herds

$$\text{Adjusted Positive (\%)} = \frac{\text{(Total Positive - EFP)}}{\text{Total tested}} \times 100$$

[b]N/A = not applicable

less than 3+ at 1/100). Interestingly, we observed no improvement of BPAT specificity when only nonvaccinated herds were considered, in contrast to the case with the SPAT and STAT (Table II). A larger sample would be required to estimate the specificity of the CFT, CARD and RBPT with more precision.

Only 137 (82%) of the 167 culture positive cattle from Saskatchewan were detected by any of the serological methods. Rapid spread of infection in the herds studied may have contributed to some of these failures, the cattle not having had time to develop serological responses. Even in this context, where many animals might be expected to have IgM antibody in their sera, the STAT performed poorly, detecting only 69% of the culture positive cattle (Table III). With this sample, the CFT detected more infected cattle than any other test, followed by the BPAT and RBPT (Table III).

The six agglutination tests were assessed in terms of their ability to detect reactors with sera from the infected herds in Eastern Canada. An effective screening test would be required to have a good ability to detect such serological indications of infection. Failure to detect CFT or CFT + 2MET reactor sera from cattle in infected herds would be a serious failure to find highly specific indications of the presence of infection. This analysis (Table IV) revealed differences in relative sensitivity between the agglutination tests, with the BPAT clearly being the most effective of the acidified antigen tests in detecting such sera.

The high sensitivity of the BPAT relative to the other plate agglutination tests employed recommends it for use as a screening test. Supplemental tests such as the CFT need to be conducted on all BPAT reactor samples to maintain diagnostic specificity.

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