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Comparison of serological tests for the detection of ovine and caprine antibody to *Brucella melitensis*

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Summary

The indirect enzyme-linked immunosorbent assay (IELISA), the competitive enzyme-linked immunosorbent assay (CELISA) and the fluorescence polarisation assay (FPA) were evaluated with sera from sheep experimentally infected with *Brucella melitensis* and negative Canadian sheep. The sensitivity and specificity of the assays were as follows: IELISA: 91.7% and 97.6%, CELISA: 75.0% and 99.8% and FPA: 91.7% and 89.5%. Sera from the same experimental population were divided according to serological reaction in the rose bengal agglutination test (RBT) and the complement fixation test (CFT). Reactivity relative to the RBT positive and CFT positive sera were as follows: IELISA: 99.7%, CELISA: 93.2% and FPA: 99.1%.

Since sera from goats with proven *B. melitensis* infection were not available, 699 sera from goats judged positive in the buffered antigen plate agglutination test (BPAT) and CFT and 982 BPAT/CFT negative Canadian goats were used. The sensitivity and specificity of the assays relative to the BPAT and CFT positive sera were: IELISA: 99.4% and 98.0%, CELISA: 95.4% and 97.1% and FPA: 92.7% and 99.8%.

Keywords

Brucellosis – Conventional test – Diagnosis – Goat – Primary binding assay – Sheep.

Introduction

Control programmes for brucellosis depend heavily on presumptive diagnosis of infection by serological tests. The accuracy of the serological tests used has considerable impact on the success of a programme. Thus, if the tests are prone to give false positive results, animals may be needlessly condemned, while tests that give false negative results will prolong any control campaign. Other factors are also important, including the test cost, ease of performance, test precision, interference by antibody to vaccine or cross-reacting antigens, and turn around time for results.

A number of serological tests have been developed to detect brucellosis in cattle since the original agglutination test was described (14). These include modifications to the agglutination test to increase specificity and decrease the time required to obtain results (5). The complement fixation test (CFT) was developed for cattle (4) to supplement the agglutination tests but, being complex and expensive, was used mostly as a confirmatory test. The agglutination tests and the CFT have not been validated using statistical analysis for sheep and goats compared with similar studies for cattle.

Indirect enzyme-linked immunosorbent assays (IELISA) were developed to replace the CFT. However, although the IELISA showed better sensitivity, the test lacked specificity due to interference by vaccinal antibody and antibody induced by cross-reacting microorganisms. These problems were largely overcome by the introduction of the competitive enzyme-linked immunosorbent assay (CELISA) (6, 7, 8). The CELISA excluded interfering antibody in most cases and was rapid compared to the CFT. Performing a small number of tests with this method, however, was not cost-effective. This led to the development of the fluorescence polarisation assay (FPA). Use of the FPA for detection of antibody to *Brucella abortus* and *B. suis* has been well documented previously (8, 10).

The premise for the FPA is that a small molecule in solution randomly rotates at a rate inversely proportional to the molecule size. Plane polarised light allows measurement, using an attached fluorochrome, of the rate of rotation through a given angle. Thus, a small molecule rotating at a high rate will revolve through the angle rapidly, resulting in a low polarisation value. If an antibody is attached to the small molecule, the increased size will cause a decrease in the rate of rotation, resulting in a higher polarisation value. Based on this, *B. abortus* O-polysaccharide (OPS), labelled with fluorescein isothiocyanate, was used as an antigen to estimate antibody to *B. abortus* in serum or milk. The FPA is a homogeneous assay which does not require any steps to remove un-reacted reagents and can therefore be performed rapidly in or outside the laboratory and is very cost-effective.

This study compared the results of a number of tests for *B. melitensis* in order to ascertain whether the FPA would be a useful diagnostic tool for the presumptive diagnosis of *B. melitensis* infection in sheep and goats.

Material and methods

Experimental population groups

Sheep were divided into four experimental groups (a total of thirty animals) as follows:

– a first group infected with *B. melitensis* strain H38 (5×10^7 colony forming units [CFU]) was conjunctivally vaccinated 2.5 years before infection (conjuctivally vaccinated [CJ] group = 13) as adults at two to three years old with *B. melitensis* Rev. 1 (4.5×10^8 CFU)

– a second group infected with *B. melitensis* strain H38 (5×10^7 CFU) was subcutaneously vaccinated (SC group = 4) when young (three to six months old) 2.5 years before infection with *B. melitensis* Rev. 1 (1.2×10^9 CFU)

– a third group infected with *B. melitensis* strain H38 (5×10^7 CFU) under the same conditions as the CJ and SC groups was not vaccinated (non-vaccinated [NV] group = 9)

– a non-vaccinated sentinel group (S group = 4) was kept in close contact with the CJ and SC groups after infection.

Positive sheep sera

Seventy-two concurrent serum samples of different days post inoculation (DPI) were collected from the experimental groups NV (n = 46 samples), CJ (n = 7 samples), SC (n = 18 samples) and S (n = 1 sample) from animals in which *B. melitensis* had been isolated in one or more of the following: conjunctival exudate, blood, vaginal exudate, milk or foetus. All the sheep except those from the S group had been experimentally infected with 5×10^7 CFU *B. melitensis* strain H38 via the conjunctival route. The sera were tested with the rose bengal agglutination test (RBT), CFT, IELISA, CELISA and FPA.

Negative sheep sera

Sera from 994 Canadian sheep were randomly selected prior to testing (*B. melitensis* has not been isolated in Canada). These sera were tested with the buffered antigen plate agglutination test (BPAT), CFT, IELISA, CELISA and FPA.

Sera divided according to the rose bengal agglutination test and complement fixation test reactions

One hundred and forty-three samples from the CJ (n = 122), NV (n = 8), SC (n = 7) and S (n = 6) experimental groups tested negative in the RBT, but positive in the CFT. Fourteen sera from the various experimental groups (CJ = 2, NV = 2, S = 9 and SC = 1) tested positive in the RBT but negative in the CFT. A total of 168 sera from each of the experimental groups (CJ = 51, NV = 31, S = 74 and SC = 12) tested negative in both the RBT and CFT.

Goat sera were segregated into two groups: 699 sera from flocks in which *B. melitensis* was isolated from at least one animal and that gave positive reactions in the BPAT and CFT and 982 sera from Canadian goats randomly selected prior to testing and which did not react in the BPAT/CFT.

Serological tests

The BPAT and RBT were performed as prescribed in the OIE (World Organisation for Animal Health) *Manual of Standards for Diagnostic Tests and Vacccines* (11).

The CFT used serum diluted in 96-well plates, incubated at 5°C as described by Samagh and Boulanger (12). A reaction of 50% haemolysis at a dilution of 1:5 (15.6 international CFT units) (1) or higher was considered positive. A number of sera were anti-complementary.

The IELISA and CELISA were described by Nielsen *et al.* (9). For the IELISA, smooth lipopolysaccharide (SLPS) from *B. abortus* strain 1119.3 was used as the antigen, immobilised on polystyrene plates. Sera diluted 1:50 were tested. Reactivity was determined using a mouse monoclonal antibody specific for bovine immunoglobulin G_1 (IgG_1), conjugated with horseradish peroxidase. This monoclonal antibody has been shown to cross react extensively with IgG of sheep and goats (3). For the CELISA, immobilised lipopolysaccharide was used as the antigen. Serum diluted 1:10 was added to each well at the same time as an equal volume of pre-diluted mouse monoclonal antibody specific for a common epitope of the OPS of the SLPS molecule. Reactivity of the mouse antibody was detected using a commercially available goat antibody to mouse IgG, conjugated with horseradish peroxidase. Hydrogen peroxide substrate (H_2O_2) and 2, 2'–azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) chromogen were developed for 10 min. in both enzyme-linked immunosorbent assays (ELISAs) and optical density measurements were obtained without using a stopping reagent. For the IELISA, data were calculated as percent positivity (%P) based on a strongly positive serum from each species included as a control in each plate. For the CELISA, data were calculated based on a buffer control (uninhibited) included in each plate and used to calculate the percent inhibition (%I). All ELISA plates contained the following controls: a strongly positive, a weakly positive and a negative serum, and a buffer control (no serum).

The FPA was detailed by Nielsen *et al.* (8). Briefly, OPS hydrolysed to an average molecular weight of 22 kD was conjugated with fluorescein isothiocyanate and used as an antigen. Sheep sera were diluted 1:40 and those of goats, 1:25. Background activity was measured in each serum or blood sample using a fluorescence polarisation analyser and after incubation for a minimum of 2 min. with antigen, a second reading was obtained. The results were recorded in millipolarisation (mP) units.

Data analysis

For sheep, receiver operator characteristic (ROC) analysis (13) was used to determine the combined sensitivity and specificity of the RBT/BPAT agglutination tests, as well as the sensitivities and specificities of the CFT, IELISA, CELISA and FPA using the 72 sera from which *B. melitensis* had been isolated (from one or more samples) and the 994 Canadian sera. Likewise, ROC analysis was also used to determine the sensitivity (n = 699) and specificity (n = 982) for goats relative to the BPAT/CFT.

Percent reactivity values using the cut-off values determined by ROC analysis were calculated for the IELISA, CELISA and FPA relative to sheep sera classified by the RBT/CFT.

Results

Using ROC analysis of the 72 positive sera and the 994 negative sera, the optimum cut-off values determined for sheep for the IELISA, CELISA and FPA were 9%P, 26%I and 77.8 mP, respectively. The sensitivities and specificities for the combined RBT/BPAT agglutination tests, CFT, IELISA, CELISA and FPA were 76.4% and 99.7%, 81.9% and 99.4%, 91.7% and 97.6%, 75.0% and 99.8%, and

Table I
The sensitivity and specificity values of the combined rose bengal agglutination test (RBT)/buffered antigen plate agglutination test (BPAT), the complement fixation test (CFT), the indirect enzyme-linked immunosorbent assay (IELISA), the competitive enzyme-linked immunosorbent assay (CELISA) and the fluorescence polarisation assay (FPA), based on 72 positive sera of sheep from which *Brucella melitensis* had been isolated from one or more samples and 994 Canadian sheep sera (*B. melitensis* has never been isolated in Canada).
The area under the curve (AUC) represents the area under the receiver operator characteristic (ROC) curve and is an indication of the accuracy of the test

Test	Sensitivity (%)	Specificity (%)	AUC
RBT/BPAT	76.4	99.7	0.880
CFT	81.9	99.4	0.904
IELISA	91.7	97.6	0.990
CELISA	75.0	99.8	0.841
FPA	91.7	89.5	0.973

AUC: area under the curve. An AUC of 0.90 indicates that a randomly selected animal from the positive group has a test value greater than a randomly selected animal from the negative group 90% of the time

Table II

Sera from sheep experimentally exposed to *Brucella melitensis* were divided according to the serological reaction in the rose bengal agglutination test (RBT) and the complement fixation test (CFT). The number of positive sera for the indirect enzyme-linked immunosorbent assay (IELISA), the competitive enzyme-linked immunosorbent assay (CELISA) and the fluorescence polarisation assay (FPA) for each RBT/CFT combination are compared

Group	RBT(+)/CFT (+)			RBT(+)/CFT(–)			RBT(–)/CFT(+)			RBT(–)/CFT(-)		
Exposed groups (CJ/NV/SC)[a] *n* = 559)	*n* = 323			*n* = 5			*n* = 137			*n* = 94		
Tests	IELISA	CELISA	FPA	IELISA	CELISA	FPA	IELISA	CELISA	FPA	IELISA	CELISA	FPA
Number of reactors (percent positive)	322 (99.7)	301 (93.2)	320 (99.1)	5 (100)	0 (0)	3 (60.0)	136 (99.3)	86 (62.8)	136 (99.3)	67 (71.3)	7 (7.4)	64 (68.1)
Non-vaccinated group (sentinel)[b] (*n* = 97)	*n* = 8			*n* = 9			*n* = 6			*n* = 74		
Tests	IELISA	CELISA	FPA	IELISA	CELISA	FPA	IELISA	CELISA	FPA	IELISA	CELISA	FPA
Number of reactors (percent positive)	8 (100)	5 (62.5)	4 (50)	6 (66.7)	0 (0)	3 (33.3)	0 (0)	0 (0)	1 (16.7)	5 (6.7)	3 (4.0)	14 (18.9)
Total (*n* = 656)	*n* = 331 (53 C+)			*n* =14 (2 C+)			*n* = 143 (6 C+)			*n* = 168 (11 C+)		
Tests	IELISA	CELISA	FPA	IELISA	CELISA	FPA	IELISA	CELISA	FPA	IELISA	CELISA	FPA
Number of reactors (percent positive)	330 (99.7)	306 (92.4)	324 (97.9)	11 (78.6)	0 (0)	6 (42.6)	136 (95.1)	86 (60.1)	137 (95.8)	72 (42.8)	10 5.9	78 (46.4)

a) CJ group infected with *B. melitensis* strain H38 (5×10^7 colony-forming units [CFU]) and vaccinated conjunctivally as adults at two to three years old 2.5 years before infection with *B. melitensis* Rev. 1 (4.5×10^8 CFU)
NV group infected with *B. melitensis* strain H38 (5×10^7 CFU) and not vaccinated
SC group infected with *B. melitensis* strain H38 (5×10^7 CFU) and vaccinated subcutaneously at 3 to 6 months of age 2.5 years before infection with *B. melitensis* Rev. 1 (1.2×10^9 CFU)
b) Non-vaccinated sentinel group in close contact with the CJ and SC groups
C+: samples from animals from which *B. melitensis* was isolated

Table III

Comparison of fourteen rose bengal agglutination test (RBT) positive (P) and complement fixation test (CFT) negative (N) sera

Group	ID [a]	DPI [b]	RBT	CFT	IELISA (cut-off = 9%)	CELISA (cut-off = 26%)	FPA (cut-off = 77.8 mP)
CJ [c]	30	364	P	N	28	8	101
CJ	51	455	P	N	40	20	93.7
S [d]	161	203	P	N	3	1	76.1
S	208	266	P	N	17	- 11	75.6
S	208	301	P	N	16	7	78.4
S	161	329	P	N	3	16	75.8
S	161	357	P	N	7	- 18	75.4
S	208	364	P	N	13	3	85.3
S	161	399	P	N	10	- 3	81.9
S	208	406	P	N	16	- 2	62.9
S	208	455	P	N	15	10	75.4
NV [e]	2,422	14 [g]	P	N	21	1	76.7
NV	3,031	14 [g]	P	N	14	5	76.7
SC [f]	2,106	357	P	N	60	16	114.9

a) animal identification
b) days post inoculation
c) group infected with *Brucella melitensis* strain H38 (5×10^7 colony-forming units [CFU]) and vaccinated conjunctivally as adults at two to three years old 2.5 years before infection with *B. melitensis* Rev. 1 (4.5×10^8 CFU)
d) non-vaccinated sentinel group in close contact with the CJ and SC groups
e) non-vaccinated group infected with *B. melitensis* strain H38 (5×10^7 CFU)
f) group infected with *B. melitensis* strain H38 (5×10^7 CFU) and vaccinated subcutaneously at three to six months of age 2.5 years before infection with *B. melitensis* Rev. 1 (1.2×10^9 CFU)
g) Brucella melitensis isolated after fourteen DPI
mP: millipolorisation units

91.7% and 89.5%, respectively, as presented in Table I. The area under the curve (AUC) as determined using ROC analysis for the RBT/BPAT agglutination tests, CFT, IELISA, CELISA and FPA was 0.880, 0.904, 0.990, 0.841 and 0.973, respectively. An AUC of 0.973 as determined for the FPA indicates that a randomly selected animal from the positive group had a test value greater than a randomly selected animal from the negative group 97.3% of the time.

In Table II, the percent reactivity values relative to 331 RBT/CFT positive sera from the combined experimental groups (of which 53 were culture positive) for the IELISA, CELISA and FPA were 99.7%, 92.4% and 97.9%, respectively. Of the 53 sera from which *B. melitensis* was isolated from samples, 39 were from the NV group and fourteen from the SC group. One animal (animal identification [ID] 97 from the NV group) at 14 days post inoculation (DPI) was missed by the IELISA and the CELISA. In addition, animal ID 122 from the NV group (14 DPI) was not detected by the CELISA and the FPA, while animal ID 3,010 from the NV group (14 DPI) was missed by only the CELISA. Similarly, the percent reactivity values relative to 168 RBT/CFT negative sera (of which eleven were culture positive) for the IELISA, CELISA and FPA were 42.8% (seven of the sera were from culture positive samples), 5.9% and 46.4% (nine of the sera were from culture positive samples), respectively, as presented in Table II. Of the eleven sera from which *B. melitensis* was isolated, four (7 DPI) were from the CJ group, four (7 DPI) from the NV group, two (7 DPI) from the SC group and one from the S group (foetus, 399 DPI), respectively.

Of the 143 CFT positive, RBT negative serum samples, six sera were derived from samples from which *B. melitensis* had been isolated (Table II), 136 sera (95.1%) were positive in the IELISA, 86 sera (60.1%) were positive in the CELISA, 137 sera (95.8%) were positive in the FPA and 136 sera (95.1%) were positive in both the IELISA and FPA. Four sera were from culture positive samples which were serologically positive in the IELISA, CELISA and FPA. One serum sample (ID 2548) was negative in all three tests even though *B. melitensis* had been isolated from the animal belonging to the NV group infected with *B. melitensis* strain H38 fourteen days previously (Table II). Of 143 sera, 122 were from the CJ group, eight were from the NV group, six were from the sentinel group and seven from the SC group. The 122 sera of the CJ group were derived from eleven animals that were positive in both the IELISA and the FPA while 86 of the sera were positive in the CELISA.

The eight sera from the NV group were from three animals, four of which were positive in the IELISA, CELISA and FPA, four of which were positive in the IELISA and CELISA and one of which was negative in all the tests (animal ID 2,548, 14 DPI). Similarly, seven sera derived from three animals in the SC group were positive in all three tests.

Of fourteen sera examined from exposed sheep that gave RBT positive and CFT negative reactions, two samples were derived from sheep from which *B. melitensis* was isolated as indicated in Table II. These sheep were infected with *B. melitensis* strain H38 (5×10^7 CFU). Both serum samples were taken fourteen DPI and were positive in the IELISA. Of the total fourteen sera, eleven were positive in the IELISA, six were positive in the FPA and none reacted in the CELISA (Table III). Six samples were positive in both the IELISA and the FPA. Of the fourteen sera tested, nine were from two sentinel sheep that were in close contact with two groups (CJ and SC groups) of sheep infected with *B. melitensis* strain H38 and vaccinated 2.5 years before infection with *B. melitensis* strain Rev. 1. The CFT failed to detect antibodies in all sera (from the CJ, NV, SC and S groups) between 14 DPI and 455 DPI.

Table IV compares the sera that were negative in the RBT, CFT, IELISA, CELISA and FPA relative to each other of the 72 positive sera of sheep from which *B. melitensis* had been isolated from one or more samples. In terms of the total number of sera misdiagnosed, the IELISA and FPA performed consistently better than the RBT, CFT or the CELISA, as shown in Table IV.

As a definitive diagnosis by culturing of the causative organism was not available for the goat sera used in this study, the BPAT/CFT was selected as the assay criterion and the sensitivity and specificity values presented in Table V for the tests are relative to this combination. Using ROC analysis of the 699 positive sera and the 982 negative sera, the optimum cut-off values determined for goats in the

Table IV
Comparison of sera negative in the rose bengal agglutination test (RBT), complement fixation test (CFT), indirect enzyme-linked immunosorbent assay (IELISA), competitive enzyme-linked immunosorbent assay (CELISA) and fluorescence polarization assay (FPA) of 72 defined positive sera of sheep from which *Brucella melitensis* had been isolated from one or more samples

Test negative sera	RBT negative	CFT negative	IELISA negative	CELISA negative	FPA negative
RBT negative ($n = 17$)	–	11	5	13	3
CFT negative ($n = 13$)	11	–	4	13	4
IELISA negative ($n = 6$)	5	4	–	6	2
CELISA negative ($n = 18$)	13	13	6	–	6
FPA ($n = 6$)	3	4	2	6	–
Total *	32	32	17	38	15

* in terms of misdiagnosed sera, both the FPA and IELISA, perform better than the other tests

IELISA, CELISA and FPA were 12%P, 26%I and 88 mP, respectively. The IELISA performed slightly better than the CELISA and the FPA with relative sensitivity and specificity values of 99.4% and 98.0%, 95.4% and 97.1%, and 92.7% and 99.8%, respectively. The AUCs of the IELISA, CELISA and FPA were 0.996, 0.987 and 0.986, respectively.

Table V
Sensitivity (%) and specificity (%) values for the indirect enzyme-linked immunosorbent assay (IELISA), competitive enzyme-linked immunosorbent assay (CELISA) and fluorescence polarisation assay (FPA) for the serological diagnosis of caprine brucellosis relative to the buffered antigen plate agglutination test (BPAT) and the complement fixation test (CFT)

Test	Sensitivity percentage	Specificity percentage	AUC
IELISA	99.4	98.0	0.996
CELISA	95.4	97.1	0.987
FPA	92.7	99.8	0.986

AUC: area under the curve. An AUC of 0.90 indicates that a randomly selected animal from the positive group has a test value greater than a randomly selected animal from the negative group 90% of the time

Discussion

Some of the results presented are based on data obtained using the two serological tests prescribed by the OIE (the CFT and the buffered *Brucella* antigen test (11).

The first step in data analysis when comparing serological tests is to establish the most suitable cut-off value for each test. This can be performed in various ways (2), but ROC analysis presents the advantage of allowing sensitivity and specificity to be calculated for a number of different cut-off values. The cut-off value that yields the greatest sum of the percent sensitivity and percent specificity is also calculated and is generally considered the optimum value for that assay. In addition, ROC analysis enables the AUC to be calculated, which is an indication of the accuracy of a test. For example, an AUC of 0.9 indicates that a randomly selected animal from the positive group has a test value greater than a randomly selected animal from the negative group 90% of the time. For each assay, the optimum cut-offs for sheep and goats were determined to be 9% and 12%P for the IELISA, 26%I for the CELISA and 77.8 and 88 mP for the FPA, respectively.

To be accepted for the presumptive diagnosis of brucellosis, a new test would be required to perform as well as, or better than, the current tests, or possess a major advantage. The data reported for the diagnosis of *B. melitensis* in sheep indicates that the IELISA, outperforms the FPA, CFT, RBT/BPAT and CELISA, the AUCs being 0.990, 0.973, 0.904, 0.880 and 0.841, respectively (Table I). However, the differences in performance are due mostly to lower sensitivity values for the other tests compared to the IELISA, except for the FPA. The sensitivity and specificity values of the IELISA and FPA were 91.7% and 97.6% and 91.7% and 89.5%, respectively. Of the 168 RBT/CFT negative sera, eleven were from animals from which *B. melitensis* had been isolated. The IELISA detected seven of those sera as positive, the FPA detected nine of the eleven sera as positive and six sera were detected in both tests. A total of 72 sera were obtained from animals from which *B. melitensis* had been isolated. The RBT did not detect antibody to *B. melitensis* in 17 out those 72 sera (23.6%), while 13 of the 72 sera (18.1%) were CFT negative. The IELISA, CELISA and FPA did not detect antibody to *B. melitensis* in 6 out of 72 (8.3%), 18 out of 72 (25.0%) and 6 out of 72 (8.3%) sera, respectively. Both the IELISA and FPA performed better than the RBT, CFT and CELISA.

Of the seventeen RBT negative sera from culture positive animals, the IELISA missed five, the CELISA missed thirteen and the FPA missed three. Similarly, for the thirteen CFT negative sera from infected sheep, the IELISA, CELISA and FPA missed four, thirteen and four, respectively. The RBT, CFT, CELISA and FPA missed five, four, six and two of the six IELISA negative sera. Similar data is presented for the CELISA and FPA in Table IV, showing that the IELISA and FPA perform consistently better than the other tests. Based on the data presented, the two currently used tests, namely, the RBT and the CFT, are inadequate for the diagnosis of brucellosis in sheep compared with the IELISA and the FPA since the former tests in combination missed more sera from sheep infected with *B. melitensis*, most of which were obtained 7 DPI, i.e. at an early stage of infection. At 4 to 7 DPI, the isotype IgG_1 antibody is produced, demonstrating that both the RBT and CFT which were developed to detect IgG_1 are incapable of early detection compared to the IELISA and FPA. Based on the data, the performance of both the IELISA and FPA proved to be as good as, or better than, both the RBT and CFT. The true usefulness of the CELISA and FPA is difficult to assess since the majority of the infected animals were also vaccinated (i.e. fourteen RBT positive/CFT negative in close contact with the CJ and SC groups, the 143 CFT positive, RBT negative sera were derived mostly from the CJ group [n = 122] and the SC group [n = 7]), thereby complicating the serological picture. The CELISA and FPA were able to differentiate between animals that were infected and animals that were vaccinated in most cases (8). The lack of sensitivity of the CELISA compared with the other tests could be partly due to the capacity of the test to differentiate these animals. Both ELISA techniques are relatively complicated and expensive laboratory assays that cannot be used cost-effectively in the field. The FPA presents the advantage of being very simple and inexpensive compared to the CFT and ELISAs and can be adapted to performance outside the laboratory setting.

The data for goat sera also indicate that both the FPA and CELISA perform marginally less well than the IELISA (Table V). This is probably due to some sera selected as positive, based on the BPAT, being derived from animals with vaccinal antibody titres or non-specific antibody. Using this selection criterion has a negative impact on tests that perform better. However, sera from goats with confirmed *B. melitensis* infection were not available. This would also explain the lower sensitivity of the CELISA and FPA.

It is possible that the reported specificity values of the IELISA, CELISA and FPA, obtained using sera from Canadian goats, may be inflated. In most areas where *B. melitensis* infection occurs, vaccination with *B. melitensis* Rev1 is used as a means of controlling the disease. Residual vaccinal antibody would not cause reactivity in most cases in the CELISA and FPA. Assuming lower specificity values for these tests, the respective AUC values would undoubtedly be lower.

Based on the data presented, the FPA is as efficient as ELISAs for serological diagnosis of brucellosis in small ruminants. All the primary binding assays diagnosed brucellosis in sheep more efficiently than did the combined RBT/BPAT and to a certain extent, the CFT. This and the other attributes of the FPA make the test a useful tool for the presumptive diagnosis of *B. melitensis* infection in small ruminants.

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Comparaison d'épreuves sérologiques pour le dépistage d'anticorps dirigés contre *Brucella melitensis* chez les ovins et les caprins

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Résumé

La méthode immuno-enzymatique indirecte (IELISA), la méthode immuno-enzymatique de compétition (CELISA) et le test de polarisation en fluorescence (FPA) ont été évalués sur des sérums appartenant à des ovins infectés expérimentalement par *Brucella melitensis* et à des ovins canadiens négatifs. Les taux de sensibilité et de spécificité des différentes épreuves étaient respectivement de 91,7 % et 97,6 % (IELISA), de 75,0 % et 99,8 % (CELISA) et de 91,7 % et 89,5 % (FPA). Les sérums appartenant aux ovins infectés expérimentalement ont été répartis selon la réaction sérologique à l'épreuve rose Bengale (RBT) et à l'épreuve de fixation du complément (CFT). La réactivité par rapport aux sérums ayant présenté une réaction positive aux épreuves RBT et CFT était de 99,7 % (IELISA), 93,2 % (CELISA) et 99,1 % (FPA).

Faute de sérums de chèvres présentant une infection confirmée à *B. melitensis,* les auteurs ont utilisé 699 sérums de chèvres jugées positives par l'épreuve d'agglutination sur lame à l'antigène tamponné (BPAT) et par l'épreuve de fixation du complément, ainsi que 982 sérums de chèvres canadiennes sans anticorps détectables par ces mêmes épreuves. La sensibilité et la spécificité des épreuves réalisées sur des sérums possédant des anticorps détectés par les épreuves BPAT et CFT étaient respectivement de 99,4 % et 98,0 % (IELISA), de 95,4 % et 97,1 % (CELISA) et de 92,7 % et 99,8 % (FPA).

Mots-clés
Brucellose – Caprin – Diagnostic – Épreuve conventionnelle – Immunodétection – Ovin.

Comparación de pruebas serológicas para detectar anticuerpos contra *Brucella melitensis* en ovejas y cabras

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Resumen
Los autores describen la evaluación de tres ensayos, el inmunoenzimático indirecto (I-ELISA), el inmunoenzimático de competición (C-ELISA) y la prueba de polarización de fluorescencia (FPA) con sueros de ovejas infectadas experimentalmente con *Brucella melitensis* y de ovejas canadienses negativas. Se obtuvieron los siguientes valores de sensibilidad y especificidad: I-ELISA: 91,7% y 97,6%; C-ELISA: 75,0% y 99,8%; FPA: 91,7% y 89,5%. Se dividieron los sueros de la misma población experimental según su reacción serológica a las pruebas de aglutinación en rosa de Bengala (RBT) y de fijación del complemento (CFT). Con los sueros positivos a ambas pruebas, los ensayos evaluados ofrecieron los siguientes porcentajes de reactividad: I-ELISA: 99,7%; C-ELISA: 93,2% y FPA: 99,1%.
Ante la falta de sueros caprinos con infección comprobada por *B. melitensis,* se utilizó el suero de 699 cabras cuya reacción a las pruebas de aglutinación en placa del antígeno tamponado (BPAT) y de CFT se consideró equivalente a un resultado positivo. Por otra parte, se utilizó el suero de 982 cabras canadienses que dieron negativo a estas dos pruebas. Tras aplicarlos a sueros positivos para la BPAT y la CFT, los tres ensayos evaluados arrojaron los siguientes valores de sensibilidad y especificidad: I-ELISA: 99,4% y 98,0%; C-ELISA: 95,4% y 97,1%; FPA: 92,7% y 99,8%.

Palabras clave
Brucelosis – Caprino – Diagnóstico – Ovino – Prueba convencional – Prueba de unión de anticuerpos.

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