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Evaluation of North American Antibody Detection Tests for Diagnosis of Brucellosis in Goats

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The sensitivities and specificities of 17 antibody detection tests for brucellosis in goats were estimated. Tests evaluated included the U.S. Department of Agriculture (USDA) card test with 8% cell concentration (8%Card), USDA rapid automated presumptive test (RAP), Mexican rose bengal plate tests with 8 and 3% cell concentrations (8%RB and 3%RB), French rose bengal plate test with 4.5% cell concentration (4.5%RB), USDA standard plate test (SPT), USDA buffered acidified plate agglutination test (BAPA), USDA and Mexican rivanol tests (URIV and MRIV), USDA standard tube tests with *Brucella abortus* and *Brucella melitensis* antigens (SATA and SATM), serum enzyme-linked immunosorbent assay (ELISA), USDA cold-fixation complement fixation tests with *B. abortus* and *B. melitensis* antigens (CFA and CFM), USDA and Mexican milk ring tests (UBRT and MBRT), and a milk ELISA. Test sensitivity was evaluated by using two groups of 10 goats experimentally infected with *B. melitensis* or *B. abortus* and monitored for 24 weeks. Specificity was evaluated by using 200 brucellosis-free nonvaccinated goats from 10 California herds. The 3%RB was considered a good screening test because of high sensitivity at week 24 postinfection (90%), ease of performance, and low cost. The cold-fixation CFA and CFM had 100% specificity in the field study and were considered appropriate confirmatory tests. The milk ELISA was significantly more sensitive ($P < 0.05$) than the UBRT and significantly more specific ($P < 0.05$) than the MBRT. The milk ELISA also had the advantage of objectivity and ease of interpretation.

Brucellosis is a frequent public health and food safety problem in Latin America, with the highest numbers of cases occurring in Mexico, Argentina, and Peru (1, 3). Serologic tests for brucellosis have been used widely for cattle; however, there is less experience with the same tests for small ruminants (11, 12, 14). Accurate diagnostic tests for brucellosis of small ruminants are necessary for control of *Brucella melitensis*, the most frequent cause of human brucellosis in Mexico (9). The majority of brucellosis test reagents are made with *Brucella abortus* antigen, and although there is serologic cross-reactivity between *B. abortus* and *B. melitensis*, the validity of assays that use North American *B. abortus* test antigens in goats is unknown. Also, optimal cutoff titers for interpretation may differ among the ruminant species.

Research and application of brucellosis testing of small ruminants have primarily been done in Mediterranean countries, especially in France and Spain (5, 6, 10, 12). Published European data cannot be directly applied to similar serologic tests in the Americas because of differences in reagent pH, *Brucella* cell concentrations, strains of *Brucella* used, composition, production methods for test antigens, and testing protocols. Examples of differences in test protocols which can alter sensitivity and specificity include serum-to-reagent ratios, test incubation times, testing surfaces, and origins of species-specific reagents, such as complement. A study of 15 different rose bengal (U.S. Department of Agriculture [USDA] card test with 8% cell concentration [8%Card]) test antigens for diagnosis of sheep and goats showed wide variation in composition and differences in sensitivity, with cell concentrations ranging from 3 to 20%, pH ranging from 3.63 to 3.95, and sensitivities for sheep from 44 to 93% (5). Based on work in Europe, the complement fixation (CF) test was the most accurate serologic test for small ruminants, having high sensitivity and specificity (14, 16). However, among 25 laboratories surveyed, no two locations used identical methods (14).

In North America, the performance of brucellosis tests in goats has not been critically evaluated, to our knowledge. In the United States, there are no federally approved official tests or guidelines for the diagnosis of brucellosis in goats; thus, the tests and diagnostic protocols for cattle are usually applied to goats unless a particular state has its own guidelines. In Mexico, the federally approved official screening test for brucellosis in goats is the 3% rose bengal plate test (3%RB), and the CF test is the official confirmatory test (7). This poses a problem because most regional laboratories in Mexico are not equipped to perform the CF test. For Mexican cattle, which are screened only with the 8% rose bengal test (8%RB), the Mexican rivanol test (MRIV) is also an approved confirmatory test (7). Increased knowledge of the sensitivities and specificities of diagnostic tests for brucellosis in goats is needed in order to develop appropriate guidelines for the United States and to evaluate the validity of the guidelines in effect in Mexico. Appropriate testing guidelines are vital for the success of *B. melitensis* control in Mexico and for minimizing the risk of

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TABLE 1. Cell concentration, pH, and dye composition of 15 *Brucella* whole-cell test antigens

Test	Manufacturer[a]	% Cells	pH	Dye
CFA	USDA	0.009	7	
CFM	USDA	0.009	7	
SATA	USDA	0.045	7	
SATM	USDA	0.045	7	
SPT	USDA	11	6.4–7.0	Brilliant green, crystal violet
BAPA	USDA	11	3.65	Brilliant green, crystal violet
8%Card	USDA	8	3.65	RB
RAP	USDA	8	3.65	RB
8%RB	SAGAR	8	3.65	RB
3%RB	SAGAR	3	3.65	RB
4.5%RB	Rhône-Merieux	4.5	3.65	RB
URIV	USDA	4	5.8–6.2	Brilliant green, crystal violet
MRIV	SAGAR	4	5.8–6.2	Brilliant green, crystal violet
UBRT	USDA	4	4.0–4.3	Hematoxylin
MBRT	SAGAR	4	4.0–4.3	Hematoxylin

[a] USDA, Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, Ames, Iowa; SAGAR, PRONABIVE, Secretaría de Agricultura, Ganadería, y Desarollo Rural, Colonia Lomas Altas, México, Distrito Federal.

its introduction into the United States through the movement of breeding animals.

The objective of this study was to evaluate the sensitivities and specificities of 17 antibody detection tests for diagnosis of brucellosis in goats and to identify appropriate cutoff titers for their interpretation. In addition, this study compared common diagnostic tests for brucellosis (the rose bengal [RB] test, the rivanol test, and the brucella ring test [BRT]) using reagents made in the United States and Mexico.

MATERIALS AND METHODS

Experimental study. The experimental study consisted of an experimental infection of goats with *B. melitensis* and *B. abortus* for evaluation of the sensitivities of selected diagnostic tests.

(i) Study animals. Forty yearling Nubian goats, 8 males and 32 females, were obtained in Imperial, California, in order to ensure no prior exposure to *B. melitensis*. California is considered free of caprine brucellosis. After the goats tested negative by the USDA standard plate test (SPT) for brucellosis, they were transported to the Universidad Autonoma de Baja California, Mexicali, Mexico. Goats were assigned randomly by sex to one of three groups, each consisting of two males and eight females. Goats were housed in separate, goatproof pens (5 m by 5 m), and sanitary precautions were taken to prevent cross-transmission of infection between groups. Goats were acclimated for 4 months before the start of the study.

(ii) Experimental infection. Ten goats were inoculated with approximately 10^8 CFU of field strain *B. melitensis* biotype 1 by placing 50 μl of a physiologic saline suspension containing the bacteria into the left conjunctival sac (2, 4). The isolate was obtained from a goat milk sample from an infected herd associated with an outbreak of human brucellosis in Mexicali in 1994. The second group was inoculated conjunctivally with 10^8 CFU of field strain *B. abortus* biotype 1 isolated from a cow. The conjunctival route was used to mimic natural infection through a mucosal surface. The third group was kept as noninfected controls.

(iii) Blood samples. Blood samples were collected for serological testing 1 day before inoculation and at weeks 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 postinoculation. Blood samples were also collected from *B. melitensis*-infected goats before euthanasia at 32 to 33 weeks postinoculation. Fourteen serologic tests for brucellosis were performed on 370 samples collected over 12 sampling periods. Eleven serologic agglutination tests and the enzyme-linked immunosorbent assay (ELISA) were also performed on the samples collected before euthanasia. Samples were numerically coded and analyzed in a blinded manner.

(iv) Milk samples. Milk samples were collected every 2 weeks for 12 weeks after parturition from the 23 lactating does. Milk was stored frozen at −20°C for culture and testing for antibody.

(v) Bacteriologic culture. Culture for isolation of *Brucella* spp. was performed on milk, postpartum vaginal swabs, aborted fetuses, and tissues collected at necropsy to verify the infection status of the goats. Aborted fetuses were necropsied, and samples of the liver, the lung, and abomasal fluid were taken for culture. At approximately 8 months postinoculation, the 20 experimentally infected goats were euthanatized. At necropsy, tissues taken and frozen for culture included lymph nodes (supramammary or superficial inguinal, submandibular, medial and lateral retropharyngeal, parotid, and iliac), spleen, thymus, liver, mammary gland, uterus, ovaries, testicles, and seminal vesicular glands.

Culture for isolation and identification of *Brucella* spp. was carried out by standard techniques, including use of selective media (tryptose with serum and antibiotics, tryptose with serum and antibiotics and ethyl violet, Farrell's medium) (17). Presumptive identification of *Brucella* was based on colony morphology, Gram stain, and biochemical tests (CO_2 requirement, catalase, oxidase, urease, dye inhibition tests with 1:50,000 basic fuchsin and thionin, and H_2S) and was confirmed by agglutination with monospecific antisera (17).

Field specificity study. A convenience sample of 10 herds was selected from moderate-to-large-sized dairy goat herds in the central valley and coast of California. Twenty adult female goats were selected from each of the 10 herds: randomly from 6 herds, randomly from two milking groups in another 3 herds, and from a single milking group in the 10th herd. Blood samples were collected from the 200 does for serologic testing, and milk was collected for immunodiagnostic testing from all selected lactating does ($n = 182$).

Serologic tests performed. Agglutination tests performed on goat sera included the 8%Card, 8%RB, 3%RB, French rose bengal test (4.5%RB) (Rhône-Merieux, Lyon, France), SPT, USDA buffered acidified plate agglutination test (BAPA), USDA rivanol test (URIV), MRIV, and USDA standard tube tests with *B. abortus* antigen (SATA) and with *B. melitensis* antigen (SATM). Other serologic tests performed included an experimental ELISA (IDEXX Laboratories, Westbrook, Maine), the *B. abortus* cold-fixation CF test (CFA), and the *B. melitensis* cold-fixation CF test (CFM). Additionally, the USDA rapid automated presumptive (RAP) test was performed on all California samples, baseline experimental samples, *B. melitensis* experimental samples, and *B. abortus* experimental samples from weeks 2 through 4 and at weeks 12 and 24 in order to compare it with the 8%Card test. The RAP test, designed as an automated alternative to the 8%Card test, uses the same antigen and was expected to produce similar results. USDA and Mexican *B. abortus* antigens were prepared with *B. abortus* 1119-3, while the 4.5%RB antigen was prepared with *B. abortus* 99. USDA *B. melitensis* antigens were prepared with *B. melitensis* 16M. Test antigens are described in Table 1 (7, 22). The immunologic principles behind serologic tests have been reviewed elsewhere (21). Positive- and negative-control sera were used with each test run.

Serologic test protocols. (i) Agglutination tests. Protocols for agglutination tests are given in Table 2. For tests performed on glass plates, transmitted light was used to evaluate the agglutination reaction after incubation. SATA and SATM results were recorded as negative or positive with complete or incomplete

TABLE 2. Protocols for brucellosis agglutination tests

Test	Serum (μl)	Antigen (μl)	Test surface	Incubation	Agitation
SATA	80, 40, 20, 10, 5	2,000	Glass tube	48 h @ 37°C	Mix once at beginning
SATM	80, 40, 20, 10, 5	2,000	Glass tube	48 h @ 37°C	Mix once at beginning
SPT	80, 40, 20, 10	30	Glass plate	8 min in box	Swirl 4× pre, mid, post
BAPA	80	30	Glass plate	8 min in box	Swirl 4× pre, mid, post
8%Card	30	30	Opaque paper card	4 min	Rock 16×/min[a]
RAP	30	20	Microtiter plate	30 min	Rotate first 10 min[a]
8%RB	30	30	Glass plate	4 min	Rock 16×/min
3%RB	30	30	Glass plate	4 min	Rock 16×/min
4.5%RB	30	30	Opaque plastic plate	4 min	Rotate[a]
URIV	80, 40, 20, 10[b]	30	Glass plate	12 min in box	Swirl 4× pre, mid, post
MRIV	80, 40, 20, 10[b]	30	Glass plate	12 min in box	Swirl 4× pre, mid, post

[a] Mechanical agitation.
[b] Serum was pretreated by dilution and a standard plate test was performed with equal amounts of 1% rivanol solution (supernatant used).

agglutination at a 1:25, 1:50, 1:100, 1:200, or 1:400 dilution; however, only the results of complete agglutination are reported here. SPT, URIV, and MRIV results were similarly reported as negative or positive at a 1:25, 1:50, 1:100, or 1:200 dilution, although these titers do not represent the actual dilutions of the sera (they are named for the standard tube test dilution which uses the same volume of serum). The RAP test was scanned with an automated plate reader pre- and postincubation, and samples with agglutination of >5% were reported as positive. For the other RB tests and the BAPA, any agglutination observed was considered a positive result.

(ii) CF tests. The CFA and CFM tests were performed by the cold-fixation method used at the National Veterinary Services Laboratories. Sera were heat treated at 56°C for 30 min before testing, and guinea pigs were the source of complement. Evidence of CF at a sample dilution of ≥1:10 was considered positive.

(iii) ELISA. An ELISA was performed as directed by the manufacturer (IDEXX Laboratories), except that an anti-small ruminant immunoglobulin G (IgG) conjugate was used. Lipopolysaccharide-coated wells were used. Results were reported as negative, suspect, or positive when the ratio of the sample optical density to the positive-control optical density (S/P ratio) was <0.04, 0.4 to <0.75, or ≥0.75, respectively.

Milk tests performed. Three immunodiagnostic tests were performed on milk from goats in the specificity study (n = 182) and milk collected every 2nd week from experimental goats (n = 107). The tests were the USDA BRT (UBRT), the Mexican BRT (MBRT), and an experimental modification of the HerdChek milk ELISA (IDEXX Laboratories). Milk test antigens were prepared with *B. abortus* 1119-3. For each test, equal quantities of milk from the right and left teats were pooled. Positive and negative controls were used with each test run.

Milk test protocols. (i) BRTs. The UBRT and MBRT were performed on individual 1-ml milk samples. Three hundred microliters of brucella-negative cow cream (Crystal heavy whipping cream) and 30 μl of test antigen were added to each milk sample in a test tube, mixed, and incubated at 37°C. Tests were read after 1, 3, 4, and 8 h of incubation. A positive sample was defined as one in which precipitation of the dyed antigen complex allowed clearing or partial clearing of color from the milk or in which any clumping of dyed antigen occurred in the milk column. Weak positive reactions on the UBRT and MBRT were considered positive.

(ii) Milk ELISA. The milk ELISA was performed on 100 μl of milk as recommended by the manufacturer for the HerdChek ELISA but was modified by using an anti-small ruminant IgG conjugate. Milk ELISA results were recorded as negative, suspect, or positive when the S/P ratio was ≤0.24, 0.25 to 0.74, or ≥0.75, respectively. Suspect results of the milk ELISA were considered positive.

Analysis. All experimentally inoculated goats were considered infected in the analyses. For goats testing negative at the baseline time period, time to seroconversion was the number of weeks elapsed postinoculation at the first positive test result. For individual goats with positive baseline titers, time to seroconversion was the number of weeks elapsed when a fourfold increase in titer from the baseline value was reached. Median time to seroconversion (MTC) was determined by using the Kaplan-Meier product limit estimator (BMDP 1L, BMDP DYNAMIC Release 7; BMDP Statistical Software, Inc., Los Angeles, Calif.). Diagnostic sensitivity with exact 95% confidence intervals (CI) (Epi Info 6; Centers for Disease Control and Prevention, Atlanta, Ga.) was determined for each test at different time periods for the experimental goats. Diagnostic specificity with Fleiss quadratic 95% CI (Epi Info 6) was determined for each test for the California goats. Specificity was also determined for baseline samples (n = 40) from the experimental goats. McNemar's chi-square test was used to test for significant differences ($P < 0.05$) in sensitivity and specificity between tests. Receiver-operator characteristic curves (ROC curves) were determined for the titered tests SPT, SATA, and SATM by plotting each test's true-positive rate versus its false-positive rate, using sensitivities obtained from the experimental study and specificities obtained from the field study (8) (Corroc2 Program, IBM-PC version 1.2.1; C. E. Metz, Department of Radiology and Franklin McLean Memorial Research Institute, University of Chicago, Chicago, Ill.). Areas under the curves were compared to check for significant differences in accuracy between tests, with the more accurate test having the larger area (Corroc2).

RESULTS

Experimental study. (i) Reproductive outcomes. Breeding began 6 weeks before inoculation. *B. abortus* was isolated from twins aborted 8 weeks postinoculation by a doe in the *B. abortus* group, and *B. melitensis* was isolated from a fetus aborted 10 weeks postinoculation by a doe in the *B. melitensis* group. These fetuses were of approximately 12 weeks gestational age, and no other fetuses were aborted. Thirty-three kids were born live in the three groups; 1 died within the 1st week of life, and the remaining 32 survived to the end of the study.

A probable embryonic death occurred 4 weeks postinoculation, evidenced by a bloody vaginal discharge from a doe of the *B. abortus* group. An assay for pregnancy-specific protein B (Bio Tracking, Moscow, Idaho) confirmed the loss of this pregnancy, and a *Brucella* sp. was isolated from a vaginal specimen of this doe, the only one which did not give birth during the study.

Birth records indicated that seven of eight does in the *B. melitensis* group and four of eight does from the *B. abortus* group were pregnant when inoculated; they were between 2 and 5 weeks of gestation. Only two of the eight control does were pregnant at the start of the study.

TABLE 3. Sensitivities of 14 serological tests determined with 10 goats experimentally infected with approximately 10^8 CFU of *B. melitensis* biotype 1

Test	Sensitivity (%) (95% CI) at:		
	Wk 3	Wk 12	Wk 24
8%Card	60 (26, 88)	90 (55, 100)	50 (19, 81)
RAP	60 (26, 88)	90 (55, 100)	50 (19, 81)
8%RB	90 (55, 100)	90 (55, 100)	70 (35, 93)
3%RB	100 (69, 100)	90 (55, 100)	90 (55, 100)
4.5%RB	60 (26, 88)	90 (55, 100)	70 (35, 93)
BAPA	90 (55, 100)	90 (55, 100)	80 (44, 97)
CFA	50 (19, 81)	90 (55, 100)	80 (44, 97)
CFM	50 (19, 81)	90 (55, 100)	80 (44, 97)
ELISA	60 (26, 88)	90 (55, 100)	90 (55, 100)
SATA 1:25	80 (44, 97)	90 (55, 100)	70 (35, 93)
SATA 1:50	60 (26, 88)	90 (55, 100)	50 (19, 81)
SATA 1:100	40 (12, 74)	60 (26, 88)	10 (.2, 45)
SATM 1:25	90 (55, 100)	90 (55, 100)	100 (69, 100)
SATM 1:50	90 (55, 100)	90 (55, 100)	80 (44, 97)
SATM 1:100	60 (26, 88)	90 (55, 100)	60 (26, 88)
SPT 1:25	90 (55, 100)	90 (55, 100)	70 (35, 93)
SPT 1:50	70 (35, 93)	50 (19, 81)	30 (7, 65)
SPT 1:100	50 (19, 81)	40 (12, 74)	0 (0, 31)
URIV 1:25	60 (26, 88)	70 (35, 93)	40 (12, 74)
URIV 1:50	50 (19, 81)	60 (26, 88)	30 (7, 65)
MRIV 1:25	60 (26, 88)	70 (35, 93)	40 (12, 74)
MRIV 1:50	50 (19, 81)	60 (26, 88)	40 (12, 74)

(ii) Serologic test results. For *B. melitensis*-infected goats, the MTC for 13 of 14 tests was 3 weeks; the SPT had an MTC of 2 weeks. Similarly, for *B. abortus*-infected goats, most tests had MTCs of 3 weeks. Exceptions were SPT, SATM, and 3%RB (2 weeks) and CFA and CFM (4 weeks).

Tables 3 and 4 list the sensitivities for the *B. melitensis*-infected and *B. abortus*-infected goats, respectively, for weeks 3, 12, and 24 postinoculation. Initial test specificities determined for baseline sera from 40 goats were similar to results from the California field study and are not reported here.

(iii) Milk test results. For *B. melitensis*-infected does, sensitivity ranged from 86 to 100% for the milk ELISA, 14 to 50% for the UBRT read at 4 h, and 14 to 88% for the MBRT read at 4 h (Table 5). The milk ELISA was significantly more sensitive ($P < 0.05$) than the UBRT read at 1 h for all six samplings of *B. melitensis*-infected does. The milk ELISA was significantly more sensitive than the UBRT read at 3, 4, and 8 h at the second milk sampling of *B. melitensis*-infected does. The milk ELISA was significantly ($P < 0.05$) more sensitive than the MBRT read at 1 h at the first three samplings. The mean time to parturition and commencement of lactation in *B. meli-*

TABLE 4. Sensitivities of 14 serological tests determined with 10 goats experimentally infected with approximately 10^8 CFU *B. abortus* biotype 1

Test	Sensitivity (%) (95% CI) at:		
	Wk 3	Wk 12	Wk 24
8%Card	90 (55, 100)	60 (26, 88)	40 (12, 74)
RAP	90 (55, 100)	60 (26, 88)	40 (12, 74)
8%RB	90 (55, 100)	80 (44, 97)	40 (12, 74)
3%RB	90 (55, 100)	90 (55, 100)	60 (26, 88)
4.5%RB	90 (55, 100)	80 (44, 97)	40 (12, 74)
BAPA	90 (55, 100)	90 (55, 100)	50 (19, 81)
CFA	40 (12, 74)	70 (35, 93)	30 (7, 65)
CFM	40 (12, 74)	70 (35, 93)	30 (7, 65)
ELISA	60 (26, 88)	80 (44, 97)	30 (7, 65)
SATA 1:25	90 (55, 100)	80 (44, 97)	50 (19, 81)
SATA 1:50	90 (55, 100)	50 (19, 81)	30 (7, 65)
SATA 1:100	40 (12, 74)	30 (7, 65)	20 (2, 56)
SATM 1:25	90 (55, 100)	90 (55, 100)	80 (44, 97)
SATM 1:50	90 (55, 100)	60 (26, 88)	50 (19, 81)
SATM 1:100	40 (12, 74)	40 (12, 74)	30 (7, 65)
SPT 1:25	90 (55, 100)	90 (55, 100)	50 (19, 81)
SPT 1:50	90 (55, 100)	40 (12, 74)	40 (12, 74)
SPT 1:100	80 (44, 97)	20 (2, 56)	20 (2, 56)
URIV 1:25	70 (35, 93)	50 (19, 81)	30 (7, 65)
URIV 1:50	60 (26, 88)	30 (7, 65)	20 (2, 56)
MRIV 1:25	90 (55, 100)	50 (19, 81)	30 (7, 65)
MRIV 1:50	70 (35, 93)	30 (7, 65)	20 (2, 56)

TABLE 6. Specificities of 14 brucellosis serologic tests in California field study goats

Test	Specificity (%) (95% CI)	*n*
8%Card	100 (97.7, 100)	200
RAP	100 (97.6, 100)	199
8%RB	100 (97.7, 100)	200
3%RB	99.5 (96.8, 100)	200
4.5%RB	100 (97.6, 100)	197
BAPA	99.5 (96.8, 100)	200
CFA	100 (97.5, 100)	191
CFM	100 (97.5, 100)	191
ELISA	100 (97.6, 100)	197
SATA 1:25	92 (83.8, 97.4)	200
SATA 1:50	99.5 (96.8, 100)	200
SATA 1:100	100 (97.7, 100)	200
SATM 1:25	73 (61.8, 82.0)	200
SATM 1:50	96 (91.7, 98.2)	200
SATM 1:100	98.5 (94.0, 99.8)	200
SATM 1:200	100 (97.7, 100)	200
SPT 1:25	92.5 (85.5, 96.4)	200
SPT 1:50	99 (96.1, 99.8)	200
SPT 1:100	100 (97.7, 100)	200
URIV 1:25	100 (97.6, 100)	198
MRIV 1:25	99.0 (96.6, 100)	193
MRIV 1:50	100 (97.6, 100)	193

tensis-infected does was 19.4 weeks after inoculation (range, 10.3 to 28.6 weeks). Six of eight does gave birth at approximately 19 to 20 weeks after inoculation. The milk ELISA was not significantly more sensitive than the tests performed on serum samples collected at the corresponding time period. It did, however, identify some infected goats as positive that other serologic tests incorrectly identified as negative at weeks 20 and 24 postinoculation.

(iv) Bacteriologic examination. *B. melitensis* biotype 1 was isolated from 4 of 10 goats experimentally infected with the organism. Isolates were from lymph nodes of one male and two females and from an aborted fetus and milk of a third female. *B. abortus* biotype 1 was isolated from aborted fetuses and milk of a goat from the group infected with the organism. An isolate from a vaginal swab of a second female with embryonic death was presumptively identified as *B. abortus.*

(v) Pathologic examinations. Nonspecific histopathologic changes consistent with brucellosis were observed in some placentas and selected tissues collected at necropsy from experimentally infected goats and aborted fetuses (data not shown).

Field specificity study. (i) Serologic test results. Specificities and 95% CI for serologic tests are reported in Table 6. The 8%Card, RAP, 8%RB, 3%RB, 4.5%RB, BAPA, URIV at a 1:25 dilution (URIV 1:25), MRIV 1:50, ELISA, CFA, and CFM tests had significantly ($P < 0.05$) greater specificities than the SPT 1:25, SATA 1:25, and SATM 1:25 to 1:50. The SPT and SATA specificities were not statistically different from one another when compared at the same titer values; however, at titers of ≥1:25, SPT and SATA had significantly ($P < 0.05$) higher specificities than SATM at 1:25. At titers of ≥1:50, SPT and SATA had significantly ($P < 0.05$) higher specificities than SATM at 1:50. MRIV 1:25 had significantly ($P < 0.05$) higher specificity than SPT 1:25, SATA 1:25, and SATM 1:25 to 1:50.

(ii) Milk test results. Specificities and 95% CI for each milk test are reported in Table 5. The milk ELISA had significantly ($P < 0.05$) greater specificity than the MBRT read at 3, 4, and 8 h and the UBRT read at 8 h.

Test accuracy assessed by ROC curves. The SATM test was significantly ($P < 0.05$) more accurate than the SATA for discriminating noninfected from infected goats at weeks 3 and 32 after inoculation with *B. melitensis*. The SATM was significantly ($P < 0.05$) more accurate than the SPT at weeks 20, 24, and 32 after inoculation with *B. melitensis*. The SPT was significantly ($P < 0.05$) more accurate than the SATM for discriminating noninfected from infected goats at weeks 4, 6, and 8 after inoculation with *B. abortus*. The SATA was significantly

TABLE 5. Specificities and sensitivities of three brucellosis milk tests determined with 182 California field study goats and 8 goats experimentally infected with *B. melitensis* biotype 1

Test (time of reading [h])	Field study, specificity (%) (95% CI)	Exptl study, sensitivity (%) at the following wk postparturition (*n*)					
		1 (8)	3 (8)	5 (8)	7 (7)	9 (7)	11 (7)
Milk ELISA	99.5 (95.3, 100)	100	100	88	86	100	86
UBRT (1)	99.5 (96.5, 100)	13	0	0	0	0	0
UBRT (3)	97.3 (91.4, 99.3)	50	25	25	14	29	29
UBRT (4)	96.2 (89.2, 98.9)	50	25	38	14	29	43
UBRT (8)	91.8 (83.2, 96.3)	63	38	50	29	43	43
MBRT (1)	99.1 (95.0, 99.9)	0	0	13	14	21	14
MBRT (3)	94.5 (88.7, 98.1)	63	38	38	14	43	43
MBRT (4)	92.8 (85.7, 97.3)	88	38	38	14	43	43
MBRT (8)	83 (65.1, 93.7)	88	50	63	43	57	43

($P < 0.05$) more accurate than the SATM at weeks 6 and 8 after inoculation with *B. abortus.*

DISCUSSION

Seroconversion. The median time from inoculation to seroconversion ranged from 2 to 3 weeks in *B. melitensis*-infected goats, ranged from 2 to 4 weeks in *B. abortus*-infected goats, and was 3 weeks for the majority of tests evaluated with goats infected with either species of *Brucella*. These estimates correlate well with knowledge of natural infection, where a serological response is usually detected within 2 to 4 weeks of infection with *B. melitensis* (3). The earliest seroconversions were evident with the SPT and SATM, both of which are agglutination tests that detect IgM, the first antibody isotype produced after infection or vaccination (14). Later seroconversion occurred with the CF tests in the *B. abortus*-infected goats. The USDA CF tests detected principally IgG1, produced subsequent to IgM (14).

Sensitivity evaluations. In this study, we used experimental infections to estimate sensitivity because of lack of access to field samples from goats known to be infected with *B. melitensis*. Even if samples had been available from naturally infected goats identified as culture positive for *B. melitensis*, evaluation using this population would likely overestimate sensitivity. Low diagnostic sensitivity of *Brucella* sp. culture would result in negative cultures for some infected goats. We used a dose of 10^8 CFU of field strain *B. melitensis* biotype 1 to infect goats experimentally. At inoculation, it was not known how many goats were pregnant, and pregnant goats would have been in early gestation. A high dose was used to ensure infection of all the goats, since we believed they would be more resistant to infection if they were not pregnant or in early gestation. In retrospect, we now know that seven of eight does were in early gestation at the time of inoculation with *B. melitensis*. Because of the high infective dose used, this study may overestimate the sensitivity of these tests for naturally infected goats. Nevertheless, the sensitivity estimates determined in this study remain useful as relative comparisons. Because only one doe aborted, we believe that the selected dose was appropriate. Goats also were infected with *B. abortus* because the agent is endemic in cattle in Baja California (18–20), where goats sometimes share pastures with cattle and are exposed to *B. abortus.*

The MRIV and URIV tests performed similarly in the experimental study, as did the CFA and CFM tests. We did not find substantial differences to justify use of the CFM test over the CFA. Also, the RAP and 8%Card tests performed similarly, showing that the RAP test is a reasonable automated substitute for the 8%Card test. The Mexican 8%RB test, however, identified 24 sera as positive that the USDA 8%Card test identified as negative, probably due to an increased ability to detect small amounts of agglutination by using light transmitted through a plate. These sera were from goats in the *B. melitensis* and *B. abortus* groups. Because of small sample sizes, we found few statistically significant differences in sensitivity. Accordingly, we emphasize that these estimates should be considered preliminary until they can be validated with large numbers of representative naturally infected goats.

Tests that were more sensitive at both early and late times (3 and 24 weeks) in the experimental study included SATM 1:25 to 1:50, SATA 1:25, SPT 1:25, and the buffered plate tests (3%RB, 8%RB, 4.5%RB, and BAPA). These tests could be considered for use as screening tests for *B. melitensis*. The CFA, CFM, and ELISA were more sensitive only later in the study (24 weeks). Less-sensitive tests, including SPT 1:50, SATA 1:50, SATM 1:100, URIV 1:25, and MRIV 1:25, would not be good candidates for screening tests.

The milk ELISA was more sensitive ($P < 0.05$) than the UBRT read at 3 and 4 h at one sampling and more specific ($P < 0.05$) than the MBRT read at 3 and 4 h, the best times to read these tests. A study with larger sample sizes would likely find the ELISA more sensitive than either ring test. A major advantage of the milk ELISA over the BRTs is the objectivity of the former test. Since the BRTs do not usually result in ring formation in goat milk, many results may subjectively be considered positive. These include the formation of small dyed clumps under the cream layer, clumps in the cream layer, and occasional true rings, but most commonly, different degrees of clearing of the milk as the dye precipitates. Some antibody-positive milk samples will not have complete clearing but a gradient of light to dark purple from top to bottom. The problem is that all milk samples have some precipitate, so a subjective decision is made of how much whitening constitutes clearing. Also, milk samples, including antibody-negative samples, will accumulate more precipitate of the dyed antigen with increasing incubation times. The BRTs, therefore, pose substantial problems in standardization and have inadequate sensitivity and specificity. These tests were evaluated only in individual milk samples. Low concentrations of antibodies in goat milk have been cited as a reason for the lack of sensitivity of the BRT in pooled herd samples (13). This could be a problem for the milk ELISA as well, and evaluation of this test for pooled herd samples is warranted.

Specificity evaluations. We evaluated test specificity only for goats not vaccinated for brucellosis. The most specific tests in the field study included the buffered agglutination tests (BAPA, 8%Card, RAP, 8%RB, 4.5%RB, and 3%RB), URIV, MRIV 1:50, both CF tests, and the serum ELISA. Buffering agglutination tests at an acid pH facilitates agglutination of IgG1 and reduces agglutination of IgM. In cattle, the presence of IgG1 antibodies to *Brucella* spp. is considered more indicative of infection than other antibody isotypes (14). The rivanol test detects principally IgG1, and to a lesser extent IgG2, because initial treatment of sera with rivanol removes IgM by precipitation, reduces the reactivity of IgG2, and promotes the reactivity of IgG1. This gives the rivanol test low sensitivity but high specificity. The USDA CF tests detect principally IgG1, since heat treatment of the sera inactivates most of the IgM, and IgG2 cannot fix the guinea pig complement as it would bovine complement (14, 15). Antibody isotypes detected by various ELISAs depend on the isotype specificity of the conjugate used. Both ELISAs used in this study detected IgG antibody isotypes.

The least specific tests, at least at lower titers, were the SPT, SATA, and SATM. These tests detect principally IgM and IgG2, which are both agglutinating at the neutral pH of the tests. IgM is associated with nonspecific cross-reactions with other gram-negative bacteria (14). Both IgM and IgG2 can be associated with persistent vaccinal antibody titers in cattle (14), although vaccinated goats were not evaluated in this study. For these reasons, the SPT, SATA, and SATM are considered to have the highest sensitivities but lowest specificities with cattle. With goats infected with *B. melitensis*, the SATM was more accurate than the SATA and SPT at particular times, while the opposite was true for goats infected with *B. abortus*. This is probably attributable to the increased abilities of these tests to detect antibodies to the homologous antigen and suggests that there may be some advantage in preparing homologous test antigens for detecting infection with *B. melitensis* biotype 1.

Agreement with other studies. The sensitivity estimates of this study are similar to those reported by Diaz-Aparicio et al.

(10). Using naturally infected goats culture positive for *B. melitensis*, they found sensitivities of 100% for the CF, 90% for an RB plate test performed by standard procedures with antigen produced by Rhône-Merieux, and 100% for a modified procedure using the same RB antigen. They also reported sensitivities of 80, 100, and 100% for three indirect ELISAs prepared differently. Relative sensitivities were 103.6% for the modified RB test and 93, 72, and 59% for the ELISAs, compared to the CF test.

Our results differ from those of another study (5), which found total agreement in sensitivity among four different RB tests with cell concentrations ranging from 3 to 9%. Our study found total agreement in test sensitivity for the RB tests only from weeks 6 through 12 of infection, with discrepancies between tests at times earlier and later in infection. Since the stage of infection of the goats in the second study (5) was not known, our results are not necessarily conflicting. The second study reported sensitivities of 100% and 67 to 72% in two different cold-fixation CF tests relative to the total number of sera with any positive CF test result. Antigens in the second study were prepared with *B. abortus* 99. The specificity estimates of our study are consistent with those of both studies (5, 10), which found 100% specificity for all CF tests, RB tests, and ELISAs evaluated.

Selection of tests for use in surveillance. For *B. melitensis* surveillance, it is important to have a screening test of high sensitivity to detect chronically infected goats with waning antibody responses in order to minimize the risk of disease transmission. Good test specificity is also necessary to avoid the high costs of confirmatory testing on false-positive samples. Tests shown to have a desirable combination of good sensitivity (>79%) at 24 or 32 weeks after infection and high specificity (>95%) included the 3%RB, CFA, CFM, and ELISA. The CFA and CFM, however, are not practical screening tests because they are complicated, costly, and time-consuming. The 3%RB seems to be an appropriate screening test, based on simplicity, time requirement, and high sensitivity and specificity. The ELISA was comparable to the 3%RB for goats infected for 24 weeks, although it failed to detect 10 sera identified as positive by the 3%RB during the first 8 weeks of infection.

A confirmatory test should be chosen not only on the basis of high specificity, but on the basis of adequate sensitivity as well. This is particularly important if the test is to be used as a safeguard against the introduction of infection into a nonvaccinated, brucellosis-free region. If a test of lower sensitivity is used, infected goats could be misclassified and allowed to remain as a source of infection for an immunologically naive population. The CFA or CFM tests are well suited as confirmatory tests, with the possible exception of use for goats infected less than 4 weeks. Anticomplementary reactions were observed in only 3 of 194 California samples tested by the cold-fixation CFA and CFM. It appears that this problem is rare in goats and would not impede the usefulness of the CF as a confirmatory test.

The MRIV test, often used as an official confirmatory test for brucellosis in Mexican cattle, is not an official test for goats (7). Our data indicate that this test should not be used as a confirmatory test for goats. For the nonvaccinated goats of the field study, MRIV 1:25 had no advantage in specificity over 3%RB, the official screening test, and it had poor sensitivity with goats infected longer than 12 weeks. At a cutoff of 1:50, where the MRIV had better, but not significantly ($P > 0.05$) better, specificity than 3%RB, it had only 10% sensitivity with goats infected for 32 weeks.

Based on the results of this study, an appropriate serologic testing protocol for surveillance of *B. melitensis* in nonvaccinated goats would involve screening with 3%RB, followed by confirmation of positive samples with a cold-fixation CF test. This protocol would result in a high rate of detection of infected goats and low numbers of confirmation tests required for noninfected, nonvaccinated goats. This agrees with previously published recommendations of screening protocols for caprine brucellosis (5, 13, 14) but additionally specifies the cell concentration of the RB test and the method of fixation. For lactating goats, individual milk samples could be screened with the milk ELISA and positive results could be confirmed serologically by the CF test or by bacterial culture. It would be advisable to repeat negative confirmatory tests on samples taken at a later date from goats testing positive on the screening test. Since serologic tests for brucellosis are more effective as herd tests than as individual tests for goats (3, 13), any goat sample that tests positive to a screening test should be considered a positive reactor if it comes from a nonvaccinated herd in which other goats have given positive confirmatory tests.

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