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Validation of the Abbreviated *Brucella* AMOS PCR as a Rapid Screening Method for Differentiation of *Brucella abortus* Field Strain Isolates and the Vaccine Strains, 19 and RB51

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The *Brucella* AMOS PCR assay was previously developed to identify and differentiate specific *Brucella* species. In this study, an abbreviated *Brucella* AMOS PCR test was evaluated to determine its accuracy in differentiating *Brucella abortus* into three categories: field strains, vaccine strain 19 (S19), and vaccine strain RB51/parent strain 2308 (S2308). Two hundred thirty-one isolates were identified and tested by the conventional biochemical tests and *Brucella* AMOS PCR. This included 120 isolates identified as *B. abortus* S19, 9 identified as *B. abortus* strain RB51, 57 identified as *B. abortus* biovar 1, 15 identified as *B. abortus* bv. 2, 1 identified as *B. abortus* bv. 2 (M antigen dominant), 7 identified as *B. abortus* bv. 4, and 22 identified as *B. abortus* S2308 and isolated from experimentally infected cattle. The *Brucella* AMOS PCR correctly identified each isolate as RB51/S2308, S19, or a field strain of *Brucella*.

The *Brucella* AMOS PCR assay (2, 3) was developed at the National Animal Disease Center to identify and differentiate *Brucella abortus*, *B. melitensis*, *B. ovis*, and *B. suis* bacteria (AMOS is an acronym for the *Brucella* species identified). An abbreviated multiplex AMOS PCR assay was developed to differentiate *B. abortus* into three categories: field strains, vaccine strain 19 (S19), and vaccine strain RB51 and the RB51 parental strain, U.S. Department of Agriculture challenge strain 2308 (S2308) (3).

The abbreviated AMOS assay is based on the insertion of the genetic element IS*711* at a unique chromosomal locus in *B. abortus* bv. 1, 2, and 4 and the double insertion of IS*711* at a specific locus in *B. abortus* RB51 (2, 3). One PCR primer is anchored within the IS*711* sequence, while the differentiating primers are localized in the unique chromosomal DNAs adjacent to the insertion. The primers were selected to amplify up to three products of different sizes. The primers amplify a 498-bp product present in *B. abortus* bv. 1, 2, and 4 plus two vaccine strains, and they also amplify a 364-bp product from *B. abortus* RB51. Identification of S19 is based on a PCR primer pair which amplifies a short sequence (178 bp) (3) of the *eri* gene (essential for erythritol catabolism), present in all *Brucella* strains except *B. abortus* S19 (4). Thus, the identification of S19 is based on the absence of amplification of this target.

The classical method of identifying the species and biovars of *Brucella* strains requires a minimum of 5 days. A PCR procedure can differentiate the vaccine strains from the field strains in 24 h and will provide useful, early information to regulatory officials. The purpose of this paper is to report the results of using the abbreviated *Brucella* AMOS PCR as a rapid screening test for *B. abortus* field strains and vaccine strains.

Isolates from tissue and milk samples submitted to the National Veterinary Services Laboratories and cultures submitted from state, federal, and university laboratories were identified to *Brucella* species and biovar level by the conventional methods (1). The following tests were performed on the isolates: growth in the presence of basic fuchsin (1:25,000 and 1:100,000), thionin (1:25,000 and 1:100,000), thionin blue (1:500,000), penicillin (5 U/ml), and erythritol (1 mg/ml and 2 mg/ml plus 5% bovine serum); urease and catalase activity; lysis by the Tbilisi phage; H_2S production; and CO_2 dependence. The dominant antigen was determined by the microagglutination test. Rough isolates were tested for susceptibility to rifampin in order to identify RB51 isolates (5).

The isolates were also tested by the abbreviated *Brucella* AMOS PCR. With a sterile inoculating loop, a small quantity of inoculum was suspended in 0.5 ml of 0.85% sterile saline. The cell suspension (2.5 μl) was added to 22.5 μl of the master mix consisting of 60 mM Tris-HCl (pH 9.0), 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 250 μM concentrations of each of the four deoxynucleoside triphosphates, 1 U of *Taq* polymerase, and five-primer cocktail (0.2 μM each), as previously described (3). The mixture was cycled 35 times through a regimen of 1.2 min at 95°C, 2.0 min at 55.5°C, and 2.0 min at 72°C under the conditions previously described (2). The amplified products were separated by electrophoresis in a 2.5% Metaphor agarose gel (FMC) in the presence of 0.5× Tris-borate-EDTA and were visualized by staining with ethidium bromide under UV light. The results were recorded by photographic methods.

Figure 1 shows that the results for the *B. abortus* field strain, *B. abortus* vaccine strains, and representatives of the classical *Brucella* species were as expected. As designed, the typical *B. abortus* PCR amplifies two fragments, 498 and 178 bp. PCR using vaccine strain RB51 amplifies these two fragments plus the 364-bp fragment, while PCR with vaccine strain S19 produces only the *B. abortus*-specific 498-bp fragment. PCR with all other *Brucella* species except *B. ovis* amplifies only the 178-bp fragment from the *eri* gene. PCR with *B. ovis*, which has not been reported to infect cattle, also amplifies the 364-bp fragment associated with tandem copies of IS*711*, but this species is readily distinguished by the absence of the *B. abortus*-specific 498-bp fragment.

Two hundred thirty-one isolates from cattle and bison located in various states including Alabama, California, Florida, Kansas, Massachusetts, Texas, Vermont, Wisconsin, and Wyoming were identified and tested by the conventional biochem-

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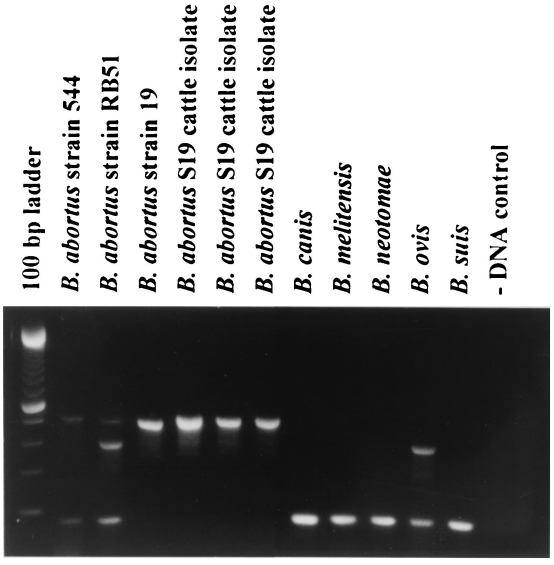

FIG. 1. Amplification of DNA fragments from different *Brucella* strains. DNA was amplified by the abbreviated AMOS PCR assay described in the text. Eight microliters of amplicons was separated by electrophoresis, treated with ethidium bromide, and visualized under UV light. Strain 544 is biovar 1; *B. abortus* S19 in lane 4 is from a lab passage; *B. abortus* S19 in lanes 5 to 7 was reisolated from vaccinated cattle. For other species, the strains used were as follows: *B. canis* strain RM 6/66, *B. melitensis* bv. 1 strain 16M, *B. neotomae* strain 5K33, *B. ovis* strain 63/290, and *B. suis* bv. 1 strain 1330.

ical tests and abbreviated *Brucella* AMOS PCR. This included 120 isolates identified as *B. abortus* S19, 9 identified as *B. abortus* strain RB51, 57 identified as *B. abortus* bv. 1, 15 identified as *B. abortus* bv. 2, 1 identified as *B. abortus* bv. 2 (M antigen dominant), 7 identified as *B. abortus* bv. 4, and 22 identified as *B. abortus* S2308. The 22 *B. abortus* S2308 samples had all been reisolated from experimentally challenged cattle. The abbreviated *Brucella* AMOS PCR was in 100% agreement with the conventional biochemical identification procedures in identifying the *Brucella* isolates tested (Table 1).

The abbreviated *Brucella* AMOS PCR correctly identified each isolate as either RB51 or S2308, S19, or the field strain of *Brucella*. This PCR procedure has high potential as a rapid screening test for differentiating the two *Brucella* vaccines from the virulent field strains of *Brucella*. *B. abortus* strains RB51 and S2308 are not differentiated from each other by the abbreviated *Brucella* AMOS PCR. *B. abortus* strain S2308 is the U.S. Department of Agriculture challenge strain and is distinct from the virulent field strains in both its PCR pattern and biochemical characteristics. It also differs from RB51 because it forms smooth, colonies, while RB51 forms rough ones. *B. abortus* strain RB51 is resistant to rifampin while growth of the rest of the *B. abortus* strains is inhibited by rifampin.

TABLE 1. Results of the conventional identification method and the enhanced abbreviated *Brucella* AMOS PCR

B. abortus biovar or strain	No. identified (classification) by[a]:		Lower 95% CI limit (%)[b]
	Conventional tests	PCR results	
Biovar1	57	57 (Field strain)	94.9
Biovar2	16	16 (Field strain)	82.9
Biovar4	7	7 (Field strain)	65.2
S19	120	120 (S19)	97.5
RB51	9	9 (RB51/S2308)	71.7
S2308	22	22 (RB51/S2308)	87.3
Total	231	231	98.7

[a] All results showed 100% agreement between the two methods.
[b] CI, confidence interval.

In this study, PCR was shown to be a valuable tool for differentiating the vaccine strains from the field strains of *Brucella*. The conventional methods of identification require a minimum of 5 days to identify an isolate to *Brucella* species and biovar level. This can delay the movement of cattle between different owners and have a negative impact on the owners' financial planning. This study indicates that Brucellosis eradication program personnel could reliably use the abbreviated *Brucella* AMOS PCR to supplement other diagnostic and epidemiological data (such as herd history and serological test results) to release sale animals from quarantine before the conventional identification methods are completed.

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