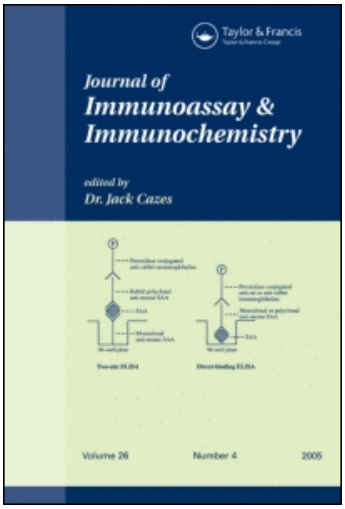

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Detection of Anti-*Brucella* Antibodies in Llama (*Lama glama*)

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Abstract: Seven llamas were immunized with killed *Brucella abortus* S1119.3 cells and bled sequentially, resulting in 64 samples. An eighth llama was kept as a negative control. In addition, 299 llama and 2075 alpaca sera, submitted for diagnostic testing, were included. Sera from all llamas were tested by the buffered antigen plate agglutination test, the complement fixation test, and the indirect enzyme immunoassays using smooth and rough lipopolysaccharides. A competitive enzyme immunoassay and fluorescence polarization assays were also performed. The sensitivity values for llama sera ranged from 92.2% to 100% and the specificity values from 89.6% to 100%. No alpacas were immunized. The specificity values for alpaca sera ranged from 94.8% to 100% specific although some sera gave an 'agglutination like' reaction after about 10 minutes of incubation. The complement fixation test could not be used, as 31% of the sera were anticomplementary and 4% were false positive.

Keywords: *Brucella abortus*, Llama, Alpaca, Serological diagnosis, Primary binding assays

INTRODUCTION

Canada, like many other countries, imports camelids as pets, for wool production, and as breeding stock. Because of the zoonotic nature of brucellosis and Canada's freedom from the disease in domestic animals, testing of imported animals for anti-*Brucella* antibody is done as a routine. However, no serological tests have been validated for testing camelids for brucellosis.

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Hence, sera are tested in traditional tests which may or may not provide the correct result.

Very little information is available on the serological diagnosis of brucellosis in llamas and alpacas. Experimental infection has been shown to cause abortion in at least one llama.[1] Widespread distribution of the organism was observed in the dam and the fetus, as well as in the fetal membranes, indicating typical dissemination of the bacteria would be a reasonable assumption. Experimental infection using high and low doses of *B. abortus* S2308 resulted in measurable serum antibody responses, approximately two weeks post exposure using agglutination, CFT, IELISA, and particle counting fluorescence immunoassay.[2] A similar observation was made with llamas vaccinated with *B. abortus* S19. In addition, Hilbink et al.[3] noted that *Yersinia enterocolitica* serotype O:9 was isolated from an alpaca in New Zealand, although no cross reaction in serological tests for brucellosis were observed.

This study was designed to determine which serological tests could be reliably used to detect antibody to *B. abortus* in llamas.

EXPERIMENTAL

Animals and Serum Samples

Eight mature llamas were purchased from a local establishment. No evidence of brucellosis had ever been observed in the establishment. The llamas were prebled once and seven were then injected intramuscularly with 10^9 heat-killed *B. abortus* S1119.3 incorporated into Freund's incomplete adjuvant. The eighth llama received saline in adjuvant. Blood samples were collected at intervals, starting day 7, up to 113 days post exposure ($n = 64$).

Llama sera ($n = 299$) and alpaca sera ($n = 2075$) submitted for testing for brucellosis were included as a presumed negative population.

Serological Tests

The BPAT, CFT, IELISA (SLPS), CELISA, and FPA (OPS) serological tests were performed as described for cattle in the OIE Manual.[4,5] *B. abortus* S1119.3 or RB51 cells or cell extracts were used as antigen for all tests. The protocol of Galanos et al.[6] for extraction of RLPS was used for preparing antigen for the IELISAs (RLPS and S/RLPS). A preparation of the RLPS was hydrolysed with 2% phosphoric acid (100°C for 60 minutes) and labelled with fluorescein isothiocyanate[5] for use as the antigen in the FPA (core) and FPA (OPS/core). Sera were tested using a Sentry 1000 (single tube) analyzer (Diachemix LLC, Wisconsin, US) and a Polarion 96-well analyzer (Tecan Polarion, Austria). A serum dilution of 1:100 was used in the FPA single tube analyzer, except for the core antigen which was

tested with a 1:25 serum dilution while the dilution was 1:10 in the 96-well FPA format.

Data

Cutoff values were determined using ROC analysis.[7] The percent sensitivity and specificity were determined for each assay. For ease of comparison, a performance index (% sensitivity plus % specificity) was determined for each test for the llama sera. As no alpacas were immunized, only specificity data are presented.

RESULTS AND DISCUSSION

The various serological tests with llama and alpaca sera gave the results depicted in Table 1. The prebled samples from the immunized animals reacted below the cutoff in all tests. The BPAT provided sensitivity and specificity values of 100 and 99.3%, indicating a very accurate diagnostic test; however, it was noted that, if samples were observed a few minutes after the normal 8 minute incubation period, many appeared to produce a fine agglutinate which could be mistaken for a positive reaction (false). In the CFT, the serum reacted non-specifically with the antigen in over 30% of the samples tested and, in addition, 4% of the sera gave false positive reactions. Thus, the CFT is not useful for brucellosis serology with sera from llamas and alpacas.

The IELISA, using the SLPS antigen, resulted in 100% sensitivity and 99.7 and 99.0% specificity values with llama and alpaca sera, respectively, making it a very useful screening test. The performance using RLPS antigen was less, 92.2% sensitivity and 89.6% specificity, while the combined antigens gave results virtually identical to those obtained with SLPS antigen only.

The CELISA was slightly less sensitive than the SLPS-IELISA, but slightly more specific (100%) with sera from both species.

The two different FPAs gave almost perfect specificity values for both llamas and alpacas, but some variation was observed in the sensitivity values, ranging from 92.2% using OPS antigen with the field test analyzer to 100% using the 96-well format. Overall, the performance of the 96-well format was better than using the field analyzer.

Because *Brucella sp.* are classified as biosecurity level 3 organisms, it was not possible to secure facilities to infect the llamas with viable bacteria. Immunization with killed *Brucella* bacteria does not mimic actual infection; however, incorporation of the bacteria into a depot adjuvant results in the slow release of antigen over an extended period, similar to infection. The antibody response of the immunized llamas may or may not resemble that induced by infection, but all the immunized animals seroconverted,

Table 1. Reactivity of llama (64 positive samples collected sequentially from 7 animals and 299 negative sera from different animals) and alpaca sera (2075 negative sera) in BPAT, IELISA, CELISA, FPA (portable format) and FPA (96 well format). The CFT data was not included due to a large number of AC reactions. The PI value (the sum of % sensitivity and % specificity) gives an indication of the overall performace of each test. Because no positive alpaca were available, the PI values could not be calculated

		LLAMA				ALPACA	
Test	Antigen	% Sens	% Spec	PI	Cut-off	% Spec	Cut-off
BPAT	Whole cell	100	99.3	199.3	+/−	100	+/−
CFT	Whole cell	NI	NI			NI	
IELISA	SLPS	100	99.7	199.7	10%P	99.0	>10%P
	RLPS	92.2	89.6	181.8	12%P	94.8	>10%P
	S/RLPS	100	100	200	10%P	98.7	>10%P
CELISA	SLPS	96.9	100	196.9	30%I	100	>30%I
FPA-1	OPS	92.2	100	192.2	>90 mP	100	>90 mP
	Core	100	100	200	>80 mP	98.9	>97 mP
	OPS/Core	95.3	100	195.3	>98 mP	100	>98 mP
FPA-2	OPS	100	100	200	>77 mP	99.1	>77 mP
	Core	100	99.0	199.0	>73 mP	98.3	>86 mP
	OPS/Core	98.4	100	198.4	>100 mP	98.8	>95 mP

% Sens = % sensitivity; % Spec = % specificity; PI = performance index; Cut-off = value below which the sample is considered negative; BPAT = buffered antigen plate agglutination test; CFT = complement fixation test; IELISA = indirect enzyme immunoassay; SLPS = smooth lipopolysaccharide; RLPS = rough lipopolysaccharide; S/RLPS = mix of smooth and rough lipopolysaccharide; CELISA = competitive enzyme immunoassay; FPA-1 = fluorescence polarization assay with mobile analyzer; OPS = O-polysaccharide from SLPS; Core = core region of RLPS; FPA-2 = fluorescence polarization assay in 96 well format; NI = not included; +/− positive or negative; % P = percent positivity = optical density test sample/optical density strong positive control × 100; % I = percent inhibition = 100 − optical density test sample/optical density uninhibited control (buffer control) × 100 mP = millipolarization units.

providing a series of samples that were useful for assessing the performance of the various serological tests commonly used for presumptive diagnosis.

Because an immunization schedule was used, the onset of the antibody response was rapid, within 7 days of injecting the antigen. Sera collected at intervals up to 113 days after immunization were tested and any sensitivity discrepancies were due to tests not detecting sufficient antibody levels in the first bleeding. All tests were positive with subsequently collected samples. The saline injected llama did not produce antibody to *B. abortus.* Based on the results, the BPAT, the SLPS and S/RLPS IELISAs, the field FPA with core antigen, and the 96-well FPA with OPS or core antigens all

detected 100% of the serum samples from immunized llamas. Of the 299 llama diagnostic samples tested, the S/RLPS IELISA, the CELISA, and all the FPAs except the 96-well core antigen test did not detect any false positive reactions. The other tests detected 4 or less false positive reactions, except for the core IELISA which detected 31 false positive reactions. Of some 2075 diagnostic alpaca samples, the PBAT, the CELISA, and the field FPA with OPS or OPS/core antigen did not detect any false positive reactions, while the other tests detected between 19 and 108 false positive reactions. The IELISA using RLPS antigen detected 108 reactions above the cut-off, giving the lowest specificity as it did with llama sera. The RLPS and core serological tests are not intended to be used as screening tests for brucellosis, but rather for eliminating reactions due to cross-reacting antibody resulting from exposure to, for example, *Y. enterocolitica* serotype O:9.[5]

CONCLUSIONS

Based on the data presented, all the tests used, except for the CFT and the RLPS IELISA detect antibody to *B. abortus* efficiently in llama sera, with excellent specificity values, with both llama and alpaca sera. The CFT appeared not to be useful as a diagnostic test because of the large number of sera capable of activating complement in the absence of antigen. These results are in agreement with the findings of Gilsdorf et al.[2]

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