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EVALUATION OF THE FLUORESCENCE POLARIZATION ASSAY AND COMPARISON TO OTHER SEROLOGICAL ASSAYS FOR DETECTION OF BRUCELLOSIS IN CERVIDS

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ABSTRACT: The complement fixation test (CFT), competitive enzyme immunoassay (CELISA), indirect enzyme immunoassay (IELISA) and fluorescence polarization assay (FPA) were evaluated for the detection of antibodies to *Brucella abortus* and *Brucella suis* biotype 4 in caribou (*Rangifer tarandus caribou*), elk (*Cervus elapus*), red deer (*Cervus elapus*), and reindeer (*Rangifer tarandus tarandus*). When combining the data the FPA and the CELISA were determined to be the most suitable tests for serodiagnosis of Cervidae. The overall actual sensitivity of the CFT and the IELISA was 100%. The overall actual sensitivity for the CELISA and FPA was 99%. The overall relative specificity of the CFT (including treatment of anti-complementary data as positive or negative for analysis), the CELISA, the IELISA and the FPA were 65%, 93%, 99%, 99%, and 99%, respectively. The specificities of the buffered plate agglutination test (BPAT), the CFT, the CELISA, the FPA and the IELISA for 55 elk vaccinated with *B. abortus* strain 19 and tested 4 mo post vaccination were 14%, 31%, 51%, 84%, and 2%, respectively. The FPA is the diagnostic test of choice because it has sensitivity and specificity values comparable to the CELISA; it has the capability to distinguish vaccinal antibody and antibody resulting from exposure to cross-reacting organisms such as *Yersinia enterocolitica* 0:9 from antibody to *Brucella* spp. in most cases; it is technically simple to do; it is adaptable to field use and it is relatively inexpensive.

Key words: *Brucella* spp., buffered antigen plate agglutination test, Cervidae, comparative serologic diagnostic tests, competitive ELISA, complement fixation test, fluorescence polarization assay, serologic evaluation.

INTRODUCTION

Serological tests developed for the detection of antibody to *Brucella abortus* in cattle have been used for the diagnosis of brucellosis in non traditional species such as caribou (*Rangifer tarandus caribou*), elk (*Cervus elapus*), red deer (*Cervus elapus*), fallow deer (*Dama dama*), and reindeer (*Rangifer tarandus tarandus*) without validation. The existence of game ranches next to zones of traditional agriculture and the proximity of cattle ranches to national parks have raised concerns about transmission of brucellosis from Cervidae to cattle and pigs and vice versa (Cohn, 1991). Survey results of captive ungulates in Canada in 1998 were 125,000 animals of which approximately 75,000 were Cervidae (Inch, 1998). Having accurate methods for the serological detection of antibodies to *B. abortus* and *B. suis* biotype 4 in Cervidae was therefore important.

Primary binding assays for the detection of antibodies to *Brucella* spp. in cattle were developed to improve the test sensitivity and specificity over those of the traditional tests such as the Buffered Antigen Plate Agglutination test (BPAT), the tube agglutination test (TAT) and the Complement Fixation Test (CFT). The indirect enzyme immunoassay (IELISA) is highly sensitive and has been modified for use with sera from Cervidae using a monoclonal antibody produced against bovine immunoglobulin light chain which cross-reacts with immunoglobulins of other species (Henning and Nielsen, 1992). How-

ever, the IELISA cannot differentiate vaccinal antibody or antibody elicited by cross-reacting organisms from antibody resulting from field infection with *Brucella* spp. The competitive enzyme immunoassay (CELISA) and the fluorescence polarization assay (FPA) can often distinguish antibodies due to vaccination with *B. abortus* strain 19 from antibodies elicited by exposure to the pathogenic strains (Nielsen et al., 1996a) and can detect antibodies to *Brucella* spp. in Cervidae. This is important in areas, such as Wyoming (USA), where vaccination of elk was conducted (Cohn, 1991). The FPA, unlike the CELISA, could be adapted for serological testing in the field with a subsequent reduction in submission costs, turnaround time and animal handling.

In this study, the sensitivity and specificity values of the CELISA and the FPA were compared with other tests for use in the presumptive diagnosis of brucellosis in Cervidae. These data support the use of the CELISA and FPA for diagnosis of brucellosis in Cervidae. Present conventional tests (BPAT, CFT, TAT) have not been validated for domestic livestock or for wildlife. The assumption that conventional serological tests would work identically in wildlife and domestic livestock were incorrect (Gardner et al., 1996). The primary binding assays have been validated for domestic livestock, standardized and have extensive built-in quality control. This validation, standardization and quality control has been adapted and extended to wildlife. In addition, the CELISA and the FPA could differentiate antibodies caused by exposure to cross-reacting organisms and *B. abortus* strain 19 in domestic livestock and the data presented in this study demonstrates a similar capability in Cervidae.

MATERIALS AND METHODS

Serological tests

The BPAT was performed as described in the Office International Des Epizooties Manual of Standards for Diagnostic Tests and Vaccines (1996).

The CFT was done as described by Samagh and Boulanger (1978).

The IELISA was done as described by Nielsen et al. (1994). The IELISA uses smooth lipopolysaccharide (sLPS) from *B. abortus* strain 1119.3 as the antigen, adsorbed onto a polystyrene microplate (NUNC 2-69620 from Gibco-BRL, Burlington, Ontario, Canada) and followed stepwise by the application of the diluted serum samples, murine monoclonal antibody to anti-bovine light chain (M4-1) conjugated with horseradish peroxidase and substrate/chromogen. Divalent cation chelating agents (EDTA/EGTA) were added to the serum diluent to reduce nonspecific interactions (Nielsen et al., 1994) and the microplate was washed between each step with 0.01 M, pH 7.2 phosphate buffer saline containing 0.15 M NaCl and 0.05% Tween-20 (PBS/T). Optical density readings at 414 nm were obtained after 10 min and the data presented as a percentage of a positive control serum included in each plate.

The CELISA was performed as described by Nielsen et al. (1996a). As in the IELISA, the CELISA uses sLPS antigen adsorbed onto a polystyrene microplate. After incubation and washing of the adsorbed microplate, serum samples were added immediately followed by the addition of a murine monoclonal antibody specifically for a *B. abortus* O-polysaccharide epitope (M84). Before addition, serum samples were diluted in PBS/T containing EDTA/EGTA (Nielsen et al., 1994). The serum samples and the monoclonal antibody were mixed for 3 min in the microplate and incubated for 30 min. After incubation and washing, commercially available goat anti-mouse IgG horseradish peroxidase-conjugated antibody (heavy and light chain specific, Jackson Immuno-Research Labs. Inc., West Grove, Pennsylvania, USA) was added, followed by the addition of substrate chromogen after incubation and washing. Optical density readings at 414 nm were obtained after 10 min and the data presented as percent inhibition relative to the buffer (uninhibited control) included in each plate.

The FPA was performed as described by Nielsen et al. (1996b). The assay used *B. abortus* O-polysaccharide conjugated with fluorescein isothiocyanate (FITC). The assay involved the addition of serum at 1:100 dilution in 1 ml of 0.01 M sodium phosphate, pH 7.4, containing 0.15 M NaCl, 0.1% sodium azide and 0.05% lithium dodecyl sulfate (PBSAL). The sample was measured in a fluorescence polarization analyzer (FPM-1, Jolley Consulting and Research Inc., Grayslake, Illinois, USA) to obtain a baseline measurement. A predetermined amount of conjugated-antigen in 0.01 M sodi-

um phosphate, pH 7.4 containing 0.15 M NaCl and 0.1% sodium azide was added, mixed and incubated for approximately 2 min to allow for the interaction between the antigen and any antibody present. After incubation, the sample was again measured in a fluorescence polarization analyzer. In the presence of antibody, a high millipolarization (mP) result was achieved, while without an anti-brucella antibody, a low mP value was obtained.

Serum samples

Serum samples were collected and assays evaluated in three non blind studies conducted in 1991, 1994 and 1998. Normally, a test should be compared with a "gold standard" which should be a biologically independent method showing absence of disease in one set of animals and presence of disease in another set of animals. This method could be another test, procedure or set of tests or procedures and must not include the test under evaluation or the results would be biased (Martin, 1988). The first study determined the sensitivity and specificity compared with another test; the second study determined the specificity to a procedure (no clinical or epidemiological evidence) and the sensitivity to actual culture results. The third study combined the use of a test to select data for specificity, sensitivity and actual culture results for the sensitivity.

In the first study (1991), samples from Tuktoyaktuk North West Territories (Canada) were selected from two herds of reindeer submitted for ancillary testing. Brucellosis had been documented in these herds. Consequently, the samples used for specificity determination were selected based on BPAT negative results (n = 1,000). The samples used for sensitivity determination were selected based on BPAT positive results (n = 160). This classification method does not need to be perfect for the principles of sensitivity and specificity to be valid (Martin, 1988). The BPAT was considered the best available test at the time for comparison (Martin, 1977, 1988).

In the second study (1994), 372 negative samples from the same region were tested in the BPAT, CELISA and the IELISA. The reindeer herds in this area were declared free of brucellosis in 1992 and have been extensively monitored since then. The 58 reindeer positive sera in this comparison were collected from reindeer from which *B. suis* biotype 4 had been isolated from various tissues in 1991.

Negative samples for the third study (1998) were obtained from areas where no clinical or epidemiological evidence of brucellosis was apparent and tested negative in the BPAT. These samples were collected from 1990 to 1997 and stored at −20 C. Immunoglobulins have been successfully stored long term in a frozen state with little apparent loss of activity (Montoya et al., 1987). Reindeer samples that tested positive in the BPAT were obtained from the same areas. Sera from the following species were tested in the CELISA, FPA and the IELISA: 308 woodland caribous from Quebec (Canada), 351 elk from Ontario and Quebec (Canada), 658 reindeer from the same region as indicated above and 1,314 red deer from New Brunswick (Canada), Quebec and Ontario that included some fallow deer from Ontario and Quebec. The fallow deer were included with the red deer because not enough samples were available to analyze them as a separate group. Sera from the following species were tested using the CFT: 154 caribou from the same regions as indicated above, 306 elk from the same regions as indicated above, 243 reindeer from the same regions as indicated above and 1,314 red deer from the same regions as indicated above including fallow deer.

Positive samples (n = 102) for the third study (1998) were obtained from 54 positive sera selected from the original 58 collected in 1991 from reindeer from which *B. suis* biotype 4 had been isolated from various tissues and 48 sera collected 4 to 12 wk post challenge from elk that had been experimentally infected with *B. abortus* strain 2308 (1×10^7 CFU). *Brucella abortus* was isolated from various tissues in 44 of the 48 elk at necropsy. The phylogenetic difference between various North American deer species, based on DNA analysis, was 5 to 10% (Polziehn and Strobeck, 1998). It was therefore reasonable to assume that their immune responses would be similar as well.

Fifty-five elk were experimentally inoculated with *B. abortus* strain 19 low dose (9×10^8 CFU) or a high dose (6×10^9 CFU) and serum collected approximately 4 mo post vaccination.

Data handling and analysis

Using receiver operating characteristics (ROC), the data was analyzed (Schoojans et al., 1995). Receiver operating characteristic curves were used to compare the diagnostic performance of two or more diagnostic tests. This analysis determined the cutoff value between positive and negative results to achieve the optimum sensitivity and specificity estimates for each species and for the data combined for all species (combined data). In addition, a range of cutoffs with associated sensitivities and specificities were calculated.

The results of each sample tested by the IELISA were expressed as a percentage (%

positivity) of the test sample optical density (OD) reading of the mean of the positive control included on each microplate at a specific development time. Percent positivity (%P) was calculated as follows: %P = (OD of the test sample/mean OD of a positive control) × 100.

The results of each sample tested by the CELISA were expressed as a percent inhibition (%I) of the test sample optical density (OD) reading of the mean of the buffer control included on each microplate at a specific development time. Percent inhibition was calculated as follows: %I = 100 − ((OD of the test sample/mean OD of the buffer control) × 100).

The ELISA software used for the calculation of %P and %I was developed at the Animal Diseases Research Institute (ADRI) and is available upon request from W. Kelly (Canadian Food Inspection Agency, Animal Diseases Research Institute, Nepean, Ontario, Canada).

Each sample tested by the FPA was expressed as a millipolarization unit (mP) of serum activity against the antigen. The mP value for each sample was calculated as follows: mP = (Iv − (Ih × G)/Iv + (Ih × G)) × 1,000; where Iv = vertical intensity of light, Ih = horizontal intensity of light, and G = FP factor.

RESULTS

The data presented in Figure 1 compare the optimal cutoffs of the CELISA, FPA and the IELISA as determined by ROC analysis of the different species with the optimal cutoff of the combined data of all the species. The phylogenetic difference between various North American deer species, based on DNA analysis, was 5 to 10% (Polziehn and Strobeck, 1998). Therefore it was reasonable to assume that their immune responses would be similar as well. From Figure 1, the cutoff of 11% positivity for the IELISA was consistent regardless of species and for the combined data. The FPA was almost as consistent as the IELISA ranging from 83 to 87 mP but the cutoff was not as variable as for the CELISA, which ranged from 16% inhibition for caribou to 34% inhibition for reindeer. The data suggests that both the IELISA and the FPA would be better for testing Cervidae. However, the IELISA was incapable of distinguishing antibody elicited by vaccination or cross-reacting organisms such as *Y. enterocolitica* 0:9 (Nielsen, 1990) leaving the FPA as the most suitable assay. The sensitivity and specificity values at other cutoffs were also determined from the data. For example, the relative specificity of the IELISA varied considerably at 3% P. The CELISA cutoff at 10% I resulted in less variation for relative specificity while the FPA cutoff of 80 mP was the most consistent regardless of species.

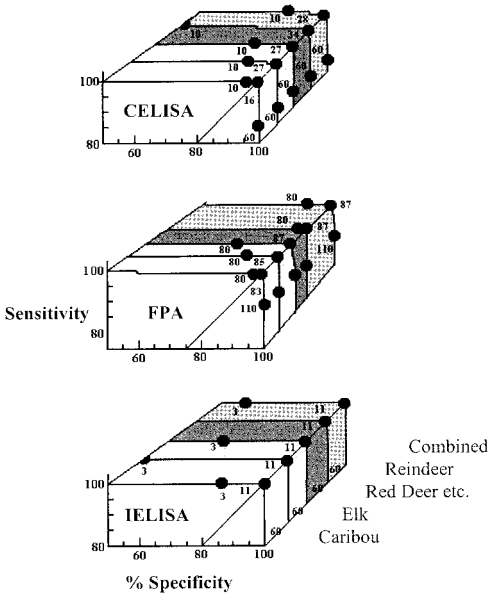

FIGURE 1. The Receiver Operating Characteristic curves represent each species and the combined data for all the species for the competitive ELISA, the fluorescence polarization assay, and the indirect ELISA. The optimal cutoff for each species and the combined data is in the upper right-hand corner of each curve. The CELISA was 16% (caribou) to 28% (combined data). The IELISA was 11% positivity no matter the species and the FPA was 83 (caribou) to 87 mP (combined data).

The specificity estimates of the data collected in 1998 for the detection of antibody to *Brucella* spp. is compared with previous unpublished data collected in 1991 and 1994 (Table 1). In 1991, the only assay evaluated for detection of antibody to *Brucella* spp. in reindeer sera was the IELISA. The relative specificity of the IELISA was assessed using sera from herds in which brucellosis was documented. The relative specificity was determined from BPAT negative results resulting in a

TABLE 1. Comparison of relative specificity for the detection of antibody to *Brucella* spp. for various Cervidae sera collected in 1991, 1994, and 1998.

Year	Species	Number of sera tested	BPAT[a] (%)	CFT(ACs+)[b] (%)	CFT(ACs−)[c] (%)	CELISA (%)	FPA (%)	IELISA (%)
1991	Reindeer	1,000	NA[d]	ND[e]	ND	ND	ND	96[f]
1994	Reindeer[g]	372	78	ND	ND	99	ND	98
1998	Caribou[h]	308	NA	86	100	99	99	99
	Elk[i]	351	NA	72	93	99	99	99
	Red deer[j]	1,314	NA	54	91	100	99	99
	Reindeer[k]	658	NA	97	100	99	100	99
	Combined[l]	2,613	NA	65	93	99	99	99

[a] Definitions were as follows: buffered antigen plate agglutination test (BPAT), complement fixation test (CFT), anti-complementary (AC), competitive enzyme immunoassay (CELISA), fluorescence polarization assay (FPA) and indirect enzyme immunoassay (IELISA).

[b] The anti-complementary results were treated as positive.

[c] The anti-complementary results were treated as negative.

[d] Not applicable.

[e] Not done.

[f] Relative specificity determined on the basis of 1000 BPAT negative results. Cutoff for the IELISA was 25% positivity.

[g] Relative specificity estimates of the sera submitted for diagnostic testing. Cutoff for CELISA was 26% inhibition and 10% positivity for the IELISA.

[h] Relative specificity estimates for caribou determined on the basis of BPAT negative results. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 16% inhibition; for the FPA 83 mP and for the IELISA 11% positivity.

[i] Relative specificity estimates for elk determined on the basis of BPAT negative results. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 27% inhibition; for the FPA 85 mP and for the IELISA 11% positivity.

[j] Relative specificity estimates for red deer determined on the basis of BPAT negative results. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 27% inhibition; for the FPA 87 mP and for the IELISA 11% positivity. The red deer category included some fallow deer samples. There was an insufficient number for a separate analysis.

[k] Relative specificity estimates for reindeer determined based on BPAT negative results. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 34% inhibition; for the FPA 87 mP and for the IELISA 11% positivity.

[l] Relative specificity estimates for the combined data. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 28% inhibition; for the FPA 87 mP and for the IELISA 11% positivity.

relative specificity of 96% for the IELISA. Because the samples were selected based on the BPAT, it was possible that the relative specificity was higher than estimated (Martin, 1977). The relative specificity estimate of the IELISA (98%) determined in 1994 on sera submitted for routine diagnostic testing after the reindeer herds in the same area were declared free of brucellosis in 1992 confirms this point. However, the relative specificity of the CELISA was 99%, 1% higher than the IELISA. As an example, this would result in 750 fewer false positives out of 75,000 animals tested. As the CELISA was capable in most cases of distinguishing antibody from cross-reacting organisms such as *Yersinia enterocolitica* 0:9 from antibody to *Brucella* spp., this may explain its higher specificity. This also explains the low specificity estimate of the BPAT (78%) which was unable to distinguish antibody from cross-reacting organisms to antibody elicited by infection to *Brucella* spp. (Samartino et al., 1999). The relative specificity estimate of the IELISA for reindeer in 1998 was 99%. The improvement in relative specificity was at least partly due to the addition of EDTA/EGTA in the dilution of sera reducing nonspecific protein interaction (Nielsen et al., 1994) resulting in a relative specificity comparable to the CELISA (99%) and the FPA (100%). If the anti-complementary data of the CFT were treated as negative data then the relative specificity of the CFT was 100%. If the anti-complementary data of the CFT were treated as positive, the relative specificity was 97%. The relative specificity estimates of the data for the other species and combined data were

TABLE 2. Comparison of relative sensitivity determined from positive BPAT sera collected in 1991 with sensitivity based on the isolation of *Brucella* spp. for Cervidae sera collected in 1994 and 1998.

Year	Species	Number of sera tested	BPAT[a] (%)	CFT[b] (%)	CELISA (%)	FPA (%)	IELISA (%)
1991	Reindeer	160	NA[c]	ND[d]	ND	ND	99[e]
1994	Reindeer[f]	58	98	ND	98	ND	100
1998	Caribou[g]	102[h]	NA	100	100	99	100
	Elk[i]	102	NA	100	99	99	100
	Red deer[j]	102	NA	100	99	99	100
	Reindeer[k]	102	NA	100	98	99	100
	Combined[l]	102	NA	100	99	99	100

[a] Definitions were as follows: buffered antigen plate agglutination test (BPAT), complement fixation test (CFT), anti-complementary (AC), competitive enzyme immunoassay (CELISA), fluorescence polarization assay (FPA) and indirect enzyme immunoassay (IELISA).

[b] No sera were anti-complementary.

[c] Not applicable.

[d] Not done.

[e] Relative sensitivity determined from 160 BPAT positive results. Cutoff for the IELISA was 25% positivity.

[f] Sera collected from reindeer in which *B. suis* had been isolated from various tissues. Cutoff for CELISA was 26% inhibition and 10% positivity for the IELISA.

[g] Sensitivity estimate for caribou. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 16% inhibition; for the FPA 83 mP and for the IELISA 11% positivity.

[h] Sera collected from reindeer in which *B. suis* had been isolated from various tissues and sera collected from elk inoculated with *B. abortus* strain 2308 and reisolated in all but four. The same data ($n = 102$) set was used for elk, red deer, reindeer and combined data.

[i] Sensitivity estimate for elk. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 27% inhibition; for the FPA 85 mP and for the IELISA 11% positivity.

[j] Sensitivity estimate for red deer. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 27% inhibition; for the FPA 87 mP and for the IELISA 11% positivity. The red deer category included some fallow deer samples. There was an insufficient number for a separate analysis.

[k] Sensitivity estimate for reindeer. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 34% inhibition; for the FPA 87 mP and for the IELISA 11% positivity.

[l] Sensitivity estimate for the combined data. The cutoff for the CFT was greater than or equal to a 1:5 dilution; for the CELISA 28% inhibition; for the FPA 87 mP and for the IELISA 11% positivity.

comparable for the CELISA, FPA and the IELISA. There was increased variability in the CFT relative specificity estimates depending on the treatment of anti-complementary data.

The sensitivity estimates of the data collected in 1998 for the detection of antibody to *Brucella* spp. is compared with previous unpublished data collected in 1991 and 1994 (Table 2). The relative sensitivity of the IELISA in 1991 was 99% based on BPAT positive results. This compares with the sensitivity of the IELISA in 1994 determined with 58 sera collected in 1991 from reindeer in which brucellosis had been documented. The sensitivity of the IELISA in the 1998 was 100% based on the previous serum collection augmented with 48 sera from elk inoculated with *B. abortus* strain 2308 and in which *B. abortus* was reisolated from various tissues in all but four. The sensitivity estimates of the assays in 1998 regardless of species were determined using ROC analysis, which explains the consistency of the sensitivity results.

A comparison of the specificity estimates of the BPAT, CFT, CELISA, FPA and IELISA for 55 elk vaccinated with *B. abortus* strain 19 low dose (9×10^8 CFU) and high dose (6×10^9 CFU) was done with sera collected 4 mo post vaccination. The highest specificity was 84% for the FPA followed by the CELISA at 51%. The lowest specificity estimate was the IELISA at 2%. Both the BPAT and the CFT had low specificities of 14% and 31%, respectively. The data suggests that both the FPA

and the CELISA could distinguish some elk 4 mo after vaccination with *B. abortus* strain 19. Since the number of elk tested was small ($n = 55$), the specificity estimate could be higher than presented in this study. The number of false positives was almost evenly divided between low dose and high dose vaccination.

Relative percent agreement of BPAT positive reindeer sera ($n = 55$) with results obtained with the other assays was done. The BPAT and the CFT results were the original data collected in 1991. Both the CFT (with the anti-complementary results included) and the IELISA agree with the BPAT (100%). However, if the anti-complementary results were treated as negative, the agreement for the CFT was 45%. The number of anti-complementary results was 54%. The relative percent agreement between the BPAT and both the CELISA and FPA was 94%.

DISCUSSION

The CFT was developed for the detection of an antibody to *B. abortus* in cattle and has been evaluated for the detection of antibody to *Brucella suis* in pigs. The sensitivity in culture positive and the specificity in culture negative pigs was 49% and 91%, respectively (Rogers, 1989). However, it had not been previously evaluated for detection of antibody to *Brucella suis* in Cervidae. The relative specificity of the CFT varied from 54% to 100%, depending on the treatment of anti-complementary results. If the anti-complementary results were treated as negative, then the relative specificity range of the CFT was 91% to 100%. However, if the anti-complementary results were treated as positive, then the relative specificity range changed from 54% to 97% depending on the species tested. The inability of diagnostic interpretation of anti-complementary results and the labor intensity of the CFT test, hinders the adoption of the CFT for the diagnosis of *Brucella* spp. in Cervidae. The majority (90%) of the caribou samples tested, was plasma, which also reduces the usefulness of the CFT but not the IELISA, CELISA or the FPA. Heparin from the collection tubes for plasma interferes with the CFT by reducing the binding of the C1q to immune complexes (Allan et al., 1979) which was essential for the stability of the C1 complex, preventing the activation of the complement cascade.

The BPAT was developed for the detection of serum antibody to *Brucella* spp. in bovine and porcine sera (Angus and Barton, 1984) and not plasma. The test uses an acidified antigen with a pH of 3.65 to 3.66. This reduces the final pH of the plasma and antigen mixture to approximately pH4 that causes the fibrinogen in the plasma to form fibrin. The fibrin may interfere with the agglutination sometimes or could be falsely interpreted as agglutination by inexperienced personnel.

The CFT identified most BPAT positive sera providing the sera were not anti-complementary. The anti-complementary samples were from reindeer and were haemolysed, further reducing the usefulness of the CFT. Hemolysis does not interfere with the IELISA, CELISA or the FPA for the detection of serum antibody. There was 100% agreement between the IELISA and the BPAT positive samples. There was less agreement between the BPAT positive samples and the CELISA or the FPA data because of the higher specificity of the latter and their capability of distinguishing animals infected with cross-reacting organisms from animals infected with *B. abortus* or *B. suis*. This probably resulted in fewer false positive reactions.

The sensitivity and relative specificity of the IELISA (1998) was 100% and 99%, respectively regardless of the species. However, unlike the CELISA or the FPA, the IELISA detects vaccinal and cross-reacting antibodies. The specificity estimate of the IELISA for elk vaccinated with *B. abortus* strain 19 was 2%. Consideration of nonspecific reactions is important as spread of cross-reacting microorganisms may occur from Cervidae to traditional livestock or vice versa and confound dis-

ease surveillance programs. For instance, Cervidae were susceptible to yersiniosis. Both the CELISA and the FPA can distinguish cross-reacting antibodies to organisms such as *Yersinia enterocolitica* 0:9 (Nielsen, 1990) from antibody to *Brucella* spp. reducing the number of false positive reactors in brucellosis tests in cattle. The CFT does not distinguish between antibodies produced by *Y. enterocolitica* 0:9 and antibodies produced by *B. suis* or *B. abortus* in 70% of sera tested while the CELISA was positive in only 14% of the cases (J. Hansen and P. Heegarrd, unpubl. data). The FPA is also capable of differentiating antibodies caused by exposure to cross-reacting organisms such as *Y. enterocolitica* 0:9 (K. Nielsen, unpubl. data).

The sensitivity (1998) of the CELISA and the FPA ranged from 98% to 100% and 99%, respectively and the relative specificity (1998) ranged from 99% to 100% for both the CELISA and FPA (Table 1). The specificity estimates of the CELISA and the FPA for elk vaccinated with *B. abortus* strain 19 was 51% and 84% respectively, in comparison to the BPAT at 14% and the CFT at 31%.

Since 1992 the number of Cervidae ranched in Canada has increased from 69,805 to 75,000, an increase of 6.9%. This makes the possibility of the transmission of brucellosis between species (Nicoletti, 1980) more likely as ranching of wildlife continues to grow in both Canada and the USA.

From the results the best overall test for the diagnosis of brucellosis in Cervidae was the FPA because of its accuracy and because it could be adopted for field use to improve the speed of diagnosis and decrease animal handling and cost.

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