Evaluation of the fluorescence polarization assay for the detection of Brucella abortus antibodies in bison in a natural setting

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\textbf{A B S T R A C T}

Bison and elk in the greater Yellowstone area are the last-known reservoir of \textit{Brucella abortus} in the United States. Diagnosis of brucellosis is challenging as there is no perfect reference test. The objectives of this study were to estimate the accuracy of the fluorescence polarization assay (FPA) for the screening of \textit{B. abortus} antibodies in bison in a natural setting. Serum and tissue samples were collected and analyzed from the known brucellosis-infected bison herd in Yellowstone National Park (YNP). Additionally, serum samples from privately owned bison were serologically tested for brucellosis. While the FPA and five other tests had perfect sensitivity, all tests had substantially lower specificity in the YNP herd. However, a Bayesian analysis showed that as many as 59–74% of the culture-negative animals were most-likely truly infected. A decision-tree analysis showed that the expected cost of FPA testing was comparable to the cost of other serologic tests. The FPA was shown to be highly sensitive but may not be able to differentiate culture-positive and culture-negative animals. There is a need for long-term longitudinal studies to estimate diagnostic accuracy of tests for \textit{B. abortus} in bison.

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\section{1. Introduction}

\textit{Brucella abortus} causes disease in several domestic and wildlife species, including cattle, bison (\textit{Bison bison}), and elk (\textit{Cervus elaphus}) \cite{1,2}. Infection with \textit{B. abortus} causes variable clinical signs in ungulates, including late-gestation abortions, stillbirths, infertility, decreased milk production, loss of condition, and lameness \cite{3}. Such losses in livestock production led the federal and state governments to start a Cooperative State-Federal Brucellosis Eradication Program in 1934. During its 75-year history, the program has limited the impact of brucellosis in cattle throughout the United States \cite{4}. However, recent outbreaks of cattle brucellosis in the three states surrounding Yellowstone National Park – Idaho, Montana, and Wyoming – have highlighted the importance of wildlife brucellosis. Currently, wild, free-ranging bison and elk in the Greater Yellowstone Area (GYA) are the last-known reservoir of \textit{B. abortus} in the United States and wild elk have been implicated in the GYA cattle outbreaks.

Several federal and state agencies are involved in research initiatives designed to identify appropriate strategies to eradicate \textit{B. abortus} from the GYA while maintaining healthy and genetically diverse populations of bison and
elk. The YNP bison are desirable for the conservation of the species because the population is derived from the original wild herd and an introduced herd containing widely diverse genetics [5]. In addition, the GYA bison have had no evidence of cattle-hybridization [6]. Therefore, disease management activities, including the future potential for movement of individual bison into other herds, are of special interest in this population.

Management alternatives for brucellosis – animal translocations, test and cull operations, immuncontra-
ception, vaccination, etc. – are predicated on correct classification of infection status. This is difficult in the case of brucellosis, where no perfect reference test exists. Although culture of tissues or fluids such as milk is frequently used as a standard for *B. abortus* diagnosis, culture is also imperfectly sensitive [7]. There are often few detectable bacteria and no obvious signs of infection (i.e., subclinical or latent infection). If an individual clears the infection, it is likely to test-positive on serologic tests yet not shed bacteria. Also, collection and handling of samples and culture techniques can affect the success of culture [8–10]. Because of the difficulties with culture, serologic testing is frequently used to determine infection status. The ideal serologic test should correctly classify an animal's infection status, be performed animal-side and yield rapid results. However, under field conditions, where an individual has the opportunity for exposure to *Brucella* organisms, it is impossible to determine whether serologic test-positive but culture-negative individuals are either exposed but non-infected, or infected with undetectable bacteria due to the lack of sensitivity of culture. Alternatively, those same animals could have antibodies to other bacterial species that cross-react on standard brucellosis serologic tests [11].

In 2000, Gall et al. validated a fluorescence polarization assay (FPA) for use in detecting serum antibodies for *B. abortus* in bison [12]. The authors estimated the specificity of FPA and other serologic tests in a population with no epidemiologic evidence of the presence of brucellosis. Few studies, however, are performed in field conditions where the disease is endemic and animals have an opportunity for exposure to *B. abortus*. The objective of this study was to determine the diagnostic accuracy (sensitivity and specificity) of the FPA in the detection of *B. abortus* antibodies in unvaccinated bison in a natural setting and to compare the FPA to other tests used to screen for or confirm *B. abortus* serologic responses.

2. Materials and methods

2.1. Subject selection

Bison samples were acquired from two different sources: the brucellosis-infected bison herd in Yellowstone National Park (YNP) and a collection of privately owned bison from the Western USA states. Bison are prohibited from having contact with cattle grazing in the GYA by the Interagency Bison Management Plan [13]. In order to prevent this contact, bison that exit park boundaries are hazed back into the park or captured and slaughtered based on their numbers and brucellosis serostatus. For this study, YNP animals were taken from a convenience sample of 190 bison that exited the confines of YNP between February 1996 and March 2001 and were subsequently tested and slaughtered according to state bison management plans. State bison management plans differed during the study, but tended to target animals that tested positive on brucellosis culture. Evidence of seropositivity was also noted in a number of YNP bison, and these animals were used to calculate the specificity of the FPA.

The privately owned bison were part of a collection of animals that existed as a large meta-population. The bison were from locations in 4 states, but belonged to a single owner. There had been periodic mixing of animals from the different locations so the bison were considered, epidemiologically, to be from a single herd. Animals were handled periodically for disease testing and other studies. For this study, privately owned bison were enrolled when they were sampled for various reasons unrelated to brucellosis. Serum samples were collected and tested from December 2002 through October 2003. The 189 animals sampled were not selected for any reason that would affect their brucellosis status. Periodic testing for brucellosis has been performed over several years since the establishment of the herd had not revealed sero-reactor animals, nor was clinical evidence of brucellosis ever noted. In the absence of any indication of infection in the herd, culture of animals for *B. abortus* had not been attempted. Although some of these bison had been vaccinated against brucellosis, all tests were evaluated using standards for non-vaccinated animals [18]. These standards are more conservative than those for vaccinated animals.

2.2. Sample processing and testing

For the YNP herd, serum samples were taken from all animals ante-mortem or immediately post-mortem for serologic testing. Animals were humanely euthanized and organ samples were taken on necropsy for bacteriologic culture. Forty-two of the 151 bison were killed by gunshot, an additional 18 animals were euthanized using xylazine (0.5 mg/kg by IM injection) and captive bolt, and the remaining 91 animals were processed through corrals and a chute. Blood samples were collected from the jugular or caudal vein of these latter animals, and they were transported for slaughter at a local processing plant. A comprehensive culture technique was used on 23 animals (14 of 18 euthanized; nine of 42 killed by gunshot) [15]. Tissue specimens collected included vaginal, rectal, and uterine swabs, mammary gland (5 cm × 5 cm × 5 cm), uterus (7.6 cm length), spleen (10 cm × 2.5 cm × 2.5 cm), blood (30 ml, synovial fluid (1–3 ml from the stifle), liver and kidney (50–100 g), bone marrow (5 ml), ileum (7.6 cm length), and the following whole lymph nodes: medial
and lateral retropharyngeal, tracheobronchial, mediastinal, hepatic, mesenteric, lumbar, medial and lateral iliac, superficial inguinal, superficial cervical, prefemoral, popliteal, mandibular, and parotid. Specimens were homogenized individually in sterile distilled water and the whole volume of the homogenates was then plated onto large Brucella-selective culture media. Standard bacteriologic culture was performed on all slaughtered animals as well as the remaining euthanized and gunshot-killed animals \((n = 128)\). The standard culture tissues and fluids included blood (10 ml), whole retropharyngeal, iliac, and superficial inguinal lymph nodes, and male genitalia (swabbed seminal vesicles, 25–30% of a testicle, and whole epididymis). Blood samples for serology were immediately chilled and frozen at \(-70^\circ\) C. Tissue specimens, fluids and swabs for culture were immediately chilled and frozen at \(-70^\circ\) C within 8 h of collection. Some samples may have been stored for as long as 5 years under these conditions. After freezing, tissue and serum specimens were shipped on dry ice to the National Veterinary Services Laboratories (NVSL) for bacteriologic and serologic examination. At NVSL, tissues were homogenized in sterile distilled water and the homogenates were swabbed and then plated on standard Brucella-selective culture media.

FPA was performed on most samples at the Montana Veterinary Diagnostic Laboratory (MVDL), although for 5 samples the FPA was done at NVSL. Ten serologic tests were performed on all samples, including fluorescence polarization assay (FPA), standard plate (SPT), standard tube agglutination (STT), card, rivanol, complement fixation (CF), competitive enzyme-linked immunosorbent assay (D-TEC), particle concentration fluorescence immunoassay (PCFIA), rapid automated presumptive (RAP), and buffered acidified plate antigen (BAPA) tests using methods previously described \([19,20]\). Serum and tissue samples were handled separately from each other and laboratory personnel were unaware of the results of other tests when performing the analysis.

Serological results were interpreted according to standards specified in the United States Department of Agriculture, Brucellosis Uniform Methods and Rules \([18]\) or standards suggested by the manufacturers (FPA). A spreadsheet was constructed with results from the ten serologic tests and bacteriologic culture \([9]\). Some animals were missing results from one or more tests, but all had results from at least nine tests, in addition to the FPA. Serum samples from each of the 189 privately owned bison were evaluated for brucellosis at the MVDL, using a battery of serologic tests including SPT, STT, card, rivanol, CF, FPA, and BAPA. No samples were taken for culture from any of these animals.

2.3. Statistical analysis

Test performance characteristics (sensitivity (Se) and specificity (Sp)) and 95% confidence limits were calculated using culture as the criterion for infection \([21]\). Suspect test results were included in the calculations but were classified as neither test-positive nor test-negative, thus penalizing both sensitivity and specificity values. Median ages for the culture-positive and -negative animals were compared for statistical significance using the Mann–Whitney test \([22]\). Test results for all animals were evaluated to determine whether the sampling method (target tissues or full set) had a significant effect on the culture results and test performance using Pearson’s chi-square test. Comprehensive and standard sampling of tissues for bacteriologic culture were compared for differences in performance of all 10 serologic tests. Pairwise covariances between FPA and comparison tests were calculated for both sensitivity and specificity as a measure of correlation in results.

A Bayesian analysis of the sensitivity and specificity of the FPA was then performed using a 2-test in two-population model allowing for conditional correlations as described in Section 3.3 of Branscum et al. \([23]\). The private bison herd was not used for this analysis because the serologic tests would presumably have had different sensitivities (due to differing vaccination histories) and different specificities (due to potential differences in exposure to Yersinia and other cross-reacting environmental pathogens). Also, because the private herd had a presumed zero prevalence it could not be used to evaluate the prevalence of the Yellowstone herd. Instead, the 2 groups for the Bayesian analysis were both derived from the Yellowstone herd and were divided based on culture results into culture-positive and culture-negative populations. Beta distributions for the informative priors of the 2 population prevalences were created from the test results of the culture-negative Yellowstone population with a non-informative prior for the correlation coefficients for positive and negative tests. This analysis was performed four times, comparing FPA with rivanol, STT, card, and PCFIA tests. Posterior inferences were based on 50,000 iterations of each model. A copy of the Winbugs code used for the Bayesian analysis is available from the first author on request.

2.4. Decision analysis

Decision-tree analysis was used to evaluate the costs of various testing scenarios, accounting for the effects of prevalence and the values or costs of test outcomes (true-positive, false-positive, true-negative and false-negative) \([24]\). The tree structure used the sensitivity and specificity results from the Bayesian analysis for the FPA, rivanol, card, STT, and PCFIA tests, which ranked highly on the initial analysis of test accuracy. Also, the PCFIA is considered a highly accurate laboratory test while the card test is the only currently available animal-side test aside from the FPA (Steve Henninger, personal communication). The predictive values of diagnostic test results are related to prevalence since, when prevalence is very high, test-negative animals are more likely to be false-negatives and when prevalence is very low, test-positive animals are more likely to be false-positives. Because of this relationship, the test accuracies were evaluated for hypothetical prevalences of 2%, 50%, and 70%. The 70% prevalence was used to represent the highest reported seroprevalence of brucellosis in the GYA \([25]\). The 50% prevalence represents the mid-point seroprevalence typically found in the Yellowstone bison population and the 2% prevalence represents a scenario where brucel-
losis is close to eradication. The costs of the various tests were based on values on the USDA website [26]. The decision tree was linked to an intervention targeted to remove bison either reproductively or physically from a population based on their serologic status.

Costs of false-positive and false-negative test results were calculated for three different assumptions of the cost for inaccurate results. For the baseline, the cost of a true-positive or true-negative test would be the personnel and drug/equipment costs for the capture ($600/capture; Rick Wallen, personal communication). The cost of a false-negative result was the unnecessary need to capture another animal to achieve the quota for the intervention ($1200). The cost of a false-positive result was the removal an animal from the population with an unnecessary intervention. This was taken from the approximate yearly budget for bison-related activities in the GYA ($3,000,000) divided by the approximate herd size (3500) to yield a total cost for each bison ($857). For other assumptions, we doubled the cost of a false-negative or false-positive test result, since some individuals might place a much higher economic value on an individual bison and some might place a much higher economic cost on delaying decreases in the seroprevalence of brucellosis in the herd. Examples of calculations used in the decision analysis are in Fig. 1.

3. Results

The bison sampled from the YNP herd included 58 male and 93 female adult bison ranging from 1 to 15 years in age (median = 4.0). The privately owned bison consisted of 151 adult female bison, one 9-month-old female, and thirty-seven 1.5-year-old males. None of the 189 privately owned bison tested positive for brucellosis on any of the tests performed, based on guidelines defined for animals not vaccinated against brucellosis [18]. However, 3 bison were classified as suspect on FPA and a different individual tested suspect on STT. Because there was no history of disease in this closed population and no abortions had been were noted, we were confident in classifying all bison from among the privately owned animals as *B. abortus* negative. However, suspect test results were penalized because they would be non-definitive and would require further testing. Therefore, the specificity of the FPA in the non-infected herd was 98.4% (95% CI = 95.4–99.7) compared to 99.5% for STT (95% CI = 96.2–99.9) and 100% for the other tests performed (95% CI = 98.1–100).

*B. abortus* was isolated from tissues from 31 adult animals from the YNP herd. Culture-positive animals were statistically significantly younger (median age = 2) than culture-negative animals (median age = 5; p < 0.0005). Six tests – FPA, Rivanol, BAPA, RAP, STT, and PCFIA – were 100% sensitive, correctly identifying all the culture-positive animals as infected (Table 1). Six of the 10 tests had one or more suspect results. The tests had substantially lower specificity when using *Brucella* culture results as the criterion for infection. Sixty-three culture-negative animals were positive to at least 6 serological tests. Of all the culture-negative animals, eight of the 15 comprehensively cultured animals were positive on at least 6 serological tests as were 55 of the 105 animals with a reduced set of tissues cultured. A higher proportion of animals with a full set of tissue samples collected were culture-positive (8 of 23; 34.8%) than with target tissues only (23 of 128; 18.0%). The difference was substantial but not statistically significant (p = 0.154). There were no statistically significant differences between sensitivities and specificities among tests run on samples that had a full set of tissues cultured versus those that were run on samples with only target tissues cultured.

The pairwise specificity covariances between the FPA and other tests ranged from 0.073 to 0.144 (median 0.106). Estimates of pairwise covariance for sensitivity were zero or negligible. On the basis of the Bayesian analysis (Table 2),
Table 1
Sensitivity and specificity estimates (with 95% exact binomial confidence intervals; CI) and number of suspect test results (Sus.) for 10 tests used to detect *Brucella abortus* infection in serum samples from 31 culture-positive and 120 culture-negative bison (*Bison bison*) from Yellowstone National Park.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity % (CI); Sus.</th>
<th>Specificity % (CI); Sus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence polarization assay (FPA)</td>
<td>100 (88.8–100) 19.2 (12.6–27.4); 3</td>
<td>98.8 (95.5, 99.8) 64.7 (60.0–69.4)</td>
</tr>
<tr>
<td>Rivanol</td>
<td>100 (88.8–100) 61.7 (52.4–70.4)</td>
<td>97.8 (93.4, 99.7) 50.5 (46.0–55.0)</td>
</tr>
<tr>
<td>Buffered acidified plate antigen (BAPA)</td>
<td>100 (88.8–100) 36.7 (28.1–46.0)</td>
<td>36.9 (31.0–43.2) 10.0 (6.0–15.0)</td>
</tr>
<tr>
<td>Rapid automated presumptive (RAP)</td>
<td>100 (88.8–100) 36.5 (27.7–46.0)</td>
<td>97.6 (93.3, 99.7) 24.2 (18.0–31.0)</td>
</tr>
<tr>
<td>Standard tube agglutination (STT)</td>
<td>100 (88.8–100) 33.3 (25.0–42.5); 15</td>
<td>98.8 (95.5, 100.0) 61.4 (56.0–66.6)</td>
</tr>
<tr>
<td>Particle concentration fluorescence immunoassay (PCFIA)</td>
<td>100 (88.8–100) 32.5 (24.2–41.7); 16</td>
<td>97.6 (93.3, 99.7) 13.2 (9.0–19.0)</td>
</tr>
<tr>
<td>Card</td>
<td>96.8 (83.3–99.9) 44.2 (35.1–53.5)</td>
<td>45.0 (39.9–54.4); 32</td>
</tr>
<tr>
<td>Standard plate (SPT)</td>
<td>83.9 (66.3–94.6); 5</td>
<td>34.2 (25.8–43.4); 7</td>
</tr>
<tr>
<td>Complement fixation (CF)</td>
<td>83.9 (66.3–94.6); 2</td>
<td>34.2 (25.8–43.4); 7</td>
</tr>
<tr>
<td>Competitive enzyme-linked immunosorbent assay (D-TEC)</td>
<td>60.2 (48.2–85.7); 4</td>
<td>55.1 (45.2–64.8); 15</td>
</tr>
</tbody>
</table>

Table 2
Summary of a series of Bayesian analyses assuming a two-population model comparing FPA test results for detecting *Brucella abortus* infection in serum samples from culture-negative bison with the corresponding results obtained by four other tests.

<table>
<thead>
<tr>
<th>Tests compared with FPA</th>
<th>% Iterations FPA higher specificity</th>
<th>% Iterations FPA higher sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rivanol</td>
<td>21.5</td>
<td>100</td>
</tr>
<tr>
<td>STT</td>
<td>27.4</td>
<td>51.6</td>
</tr>
<tr>
<td>Card</td>
<td>21.4</td>
<td>100</td>
</tr>
<tr>
<td>PCFIA</td>
<td>100</td>
<td>83.9</td>
</tr>
</tbody>
</table>

Table 3
Costs of five different testing schemes used under three prevalences and three different assumptions of the cost of imperfect test results for *Brucella abortus* exposure testing in bison (*Bison bison*) from Yellowstone National Park.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Cost of testing scheme ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FPA</td>
</tr>
<tr>
<td>Prevalence = 70%</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>636</td>
</tr>
<tr>
<td>Assumed increased false-positive costs</td>
<td>645</td>
</tr>
<tr>
<td>Assumed increased false-negative costs</td>
<td>646</td>
</tr>
<tr>
<td>Prevalence = 50%</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>638</td>
</tr>
<tr>
<td>Assumed increased false-positive costs</td>
<td>652</td>
</tr>
<tr>
<td>Assumed increased false-negative costs</td>
<td>645</td>
</tr>
<tr>
<td>Prevalence = 2%</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>643</td>
</tr>
<tr>
<td>Assumed increased false-positive costs</td>
<td>671</td>
</tr>
<tr>
<td>Assumed increased false-negative costs</td>
<td>643</td>
</tr>
<tr>
<td>Mean cost</td>
<td>647</td>
</tr>
</tbody>
</table>

The sensitivity and specificity of the FPA ranged from 97.7 to 98.8% and from 97.5 to 98.1%, respectively. The FPA had greater sensitivity than all other tests and also had a higher specificity than the PCFIA. The potential prevalence of *B. abortus* in the culture-negative animals from the brucellosis-endemic YNP herd was also high, ranging between 59.4 and 74.1%.

3.1. Decision-tree analysis

The STT had the lowest expected value of test cost for prevalences of 50% and 70% regardless of the assumption relating to false-positive and false-negative test results (Table 3). At 2% prevalence, the STT still had the lowest expected test cost from the regulator perspective (higher false-negative cost), while the card test had the lowest cost for conservationist (higher false-positive cost) and baseline perspectives. Mean cost showed the STT as the lowest ($620) with the FPA ranked second ($647). Most of this difference was attributable to the higher test cost for the FPA ($26/test) compared to the STT ($7/test). Expected mean costs for the other tests were $703 for card, $770 for rivanol, and $943 for PCFIA.

4. Discussion

The sensitivity of the FPA in this field evaluation of GYA bison was excellent. Only one FPA-negative animal had serologic evidence of brucellosis infection on any other test (a suspect on the CF test), and all culture-positive
animals tested positive on the FPA. The Bayesian analysis agreed with this finding (sensitivity 97.7–98.8%) and was only different from the 100% values for the traditional calculation because it was a weighted average of the data and a non-informative prior. However, this study highlighted the difficulties of field diagnostic test evaluations. Similar to traditional specificity studies, the serologic tests showed very high specificity when the privately owned animals from a population without brucellosis were evaluated. Where B. abortus exposure was common, however, the FPA and other serologic tests were not completely accurate in differentiating culture-positive from culture-negative animals.

There are several explanations why bison that tested positive on the FPA or other tests were culture-negative. Their test results could have been false-positives, indicating a problem with the test either through test failure, or cross-reaction with antibodies to a structurally similar bacterium. The animals could have been exposed but no longer harboring any viable bacteria in their tissues. Finally, sampling and bacterial culture might have missed the infected tissue or section of tissue, or failed to detect B. abortus because of contaminant overgrowth. The Bayesian analysis provided evidence in support of this latter explanation by estimating a potential prevalence of B. abortus in the culture-negative animals of 59–74%.

Specificity is not usually evaluated in infected herds because of the difficulties in determining whether culture-negative animals are truly infection-free. B. abortus culture, although considered the reference test, has a sensitivity of less than 100%, which affects the apparent specificity of any test to which it is compared. It is possible that the FPA detected animals previously exposed to B. abortus, incubating infection, or latently infected with the bacterium, but with low antibody concentrations undetectable by less sensitive tests.

In this study, 63 culture-negative animals were positive on at least 6 of the serological tests. This finding is consistent with prior studies in cattle [27] and elk [28] and is likely attributable to the inherent difficulties of culturing brucella from chronically infected animals [17]. More animals with a full complement of tissues taken on necropsy had positive cultures for B. abortus and hence, it is likely that more of the animals with only target tissues cultured would have been culture-positive if a complete set of tissues had been collected. The difference between the culture-positive percentages for both groups, however, was not statistically significant, nor were there differences in sensitivity and specificity of any of the tests between the two groups. Younger animals sampled from the YNP bison herd were more likely to be culture-positive than older animals. Although the bison sampled were not a representative sample from the whole population, this is consistent with the finding that transmission rates are higher among juveniles than older animals [16].

In the decision-tree analysis, the FPA was comparable with other tests but had a higher expected cost than the STT to which it was compared. This was mainly accounted for in the higher relative testing costs of the FPA. Field-side testing and future technological development may allow the FPA to better compete with the STT and other tests due to reduced costs and improvements in test performance. The additional advantage of the FPA that was not factored into the decision-tree was its ability to be performed animalside. Other tests, such as the rivanal and STT are cost- and time-efficient; however, they must be performed at room temperature, which is often not possible in field settings. The card test was also comparable in certain scenarios although it failed to detect one culture-positive animal and has been problematic anecdotally with particulate matter causing false-positive reactions during windy conditions. All tests were run under laboratory conditions which may have improved the calculated performance characteristics of the card test over its field performance.

Findings from the present study indicate the need for further evaluation to assure that current tests are well-suited for their intended purpose. The development of other assays or culture techniques that resulted in a more accurate reference standard would improve diagnostic test evaluations in YNP. Brucellosis testing in the Yellowstone herd requires a test with high sensitivity and reasonable specificity. The emphasis on sensitivity is necessary because the consequences of releasing infected animals are serious, potentially exposing uninfected herd mates as well as domestic animals to B. abortus. Animals cannot be held for additional testing, so there is a need to decide immediately, based on an accurate and reliable test. The specificity must be reasonable to prevent identifying uninfected animals as positive, because there is no opportunity to hold those animals and retest later. The FPA is perhaps the most sensitive test for detecting serum antibodies to B. abortus in bison but may be unable to differentiate exposed individuals from those with active infections or sequestered bacteria. Further work needs to be performed evaluating the repeatability of the FPA with different operators and under different environmental conditions. A comparison between animal-side testing and bench-top machines is also needed. For evaluation of tests under field conditions, however, longitudinal studies should be performed, testing animals throughout the study period and harvesting a subset of subjects at various points to determine their culture status.

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References

Kittelberger R, Hilbank F, Hansen MF, Penrose M, de Lisle GW, Letes-

T, et al. Pathogenesis and epidemiology of brucellosis in Yel-
lowstone bison: serologic and culture results from adult females
729–39.

Brucellosis in the greater Yellowstone area. Washington, DC:

[18] USDA Animal and Plant Health Inspection Service. Brucellosis erad-
cation: uniform methods and rules, effective October 1, 2003. Ames,
IA: USDA National Veterinary Services Laboratories; 2003.

al. A homogeneous fluorescence polarization assay for detection
of antibody to Brucella abortus. Journal of Immunological Methods

Brucellosis Laboratory. Paris: Institute National de la Recherche
Agronomique; 1988.


[22] Mann HB, Whitney DR. On a test of whether one of two random vari-
ables is stochastically larger than the other. Annals of Mathematical

sensitivity and specificity through Bayesian modeling. Preventive

[24] Collins MT, Morgan IR. Economic decision analysis model of a para-
tuberculosis test and cull program. Journal of the American Veterinary


[26] USDA Animal and Plant Health Inspection Service. Diagnostic
Testing, National Veterinary Services Laboratories. 2009 [cited

[27] McCullough NB, Eisele CW, Byrne AF. Incidence and distribution of
Brucella abortus in slaughtered Bang’s reactor cattle. Public Health

and bacteriologic survey in Wyoming. Journal of Wildlife Diseases