EVALUATION OF THE SENSITIVITY AND SPECIFICITY OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DIAGNOSING BRUCELLOSIS IN AFRICAN BUFFALO (SYNCERUS CAFFER)

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ABSTRACT: Brucellosis is a disease of veterinary and public health importance worldwide. In sub-Saharan Africa, where the bacterium Brucella abortus has been identified in several free-ranging wildlife species, successful disease control may be dependent on accurate detection in wildlife reservoirs, including African buffalo (Syncerus caffer). We estimated the sensitivity and specificity of a commercial enzyme-linked immunosorbent assay (ELISA) (IDEXX Brucellosis Serum Ab test, IDEXX Laboratories, Westbrook, Maine, USA) for B. abortus based on a data set of 571 serum samples from 258 buffalo in the Kruger National Park, South Africa. We defined a pseudogold standard test result as those buffalo that were consistently positive or negative on two additional serologic tests, namely, the rose bengal test (RBT) and the complement fixation test (CFT). The ELISA’s cutoff value was selected using receiver operating characteristics analysis, the pseudogold standard, and a threshold criterion that maximizes the total sensitivity and specificity. Then, we estimated the sensitivity and specificity of all three tests using Bayesian inference and latent class analysis. The ELISA had an estimated sensitivity of 0.928 (95% Bayesian posterior credibility interval [95% BCI] = 0.869–0.974) and specificity of 0.870 (95% BCI = 0.836–0.900). Compared with the ELISA, the RBT had a higher estimated sensitivity of 0.986 (95% BCI = 0.928–0.999), and both the RBT and CFT had higher specificities, estimated to be 0.992 (95% BCI = 0.971–0.996) and 0.998 (95% BCI = 0.992–0.999), respectively. Therefore, no single serologic test perfectly detected the antibody. However, after adjustment of cutoff values for South African conditions, the IDEXX Brucellosis Serum Ab Test may be a valuable additional screening test for brucellosis in Kruger National Park’s African buffalo.

Key words: African buffalo, Bayesian, brucellosis, enzyme linked immunosorbent assay, latent data, sensitivity, specificity.

INTRODUCTION

Brucellosis is an important veterinary public health issue and one of the most common zoonotic diseases worldwide (McDermott and Arimi 2002). Brucella abortus, the pathogenic bacteria responsible for bovine brucellosis, has been isolated from many ungulate species, including African buffalo (Syncerus caffer), elk (Cervus elaphus), American bison (Bison bison), eland (Taurotragus oryx), waterbuck (Kobus ellipsiprymnus), impala (Aepyceros melampus), and cattle (Bos taurus; Godfroid 2002). Transmission of B. abortus occurs primarily when bacteria are shed from infected animals around birthing periods. Bacteria are shed in birth products, aborted fetuses, and intermittently through unpasteurized milk (Rhyan et al. 2009). Infection is characterized by abortions, high morbidity rates, and context-dependent reductions in survival (Joly and Messier 2005) and, as a leading cause of cattle morbidity worldwide, accurate identification of infection is essential for public health (Godfroid et al. 2011). These concerns have motivated successful ‘test-and-slaughter’ programs in industrialized countries that have virtually eliminated the disease except in areas adjacent to wildlife reservoirs. Research efforts aimed at understanding infection in wildlife and minimizing transmission between wildlife...
and livestock are essential for disease management (Kilpatrick et al. 2009; Gomo et al. 2012) and the development and evaluation of reliable diagnostic tests for brucellosis in wildlife is a priority.

Brucellosis has been maintained enodemically in African buffalo in Kruger National Park (KNP), South Africa (Chapparo et al. 1990), since its speculated introduction from European cattle (Gradwell et al. 1977). In African buffalo, diagnosis is based on three indirect diagnostic tests that measure the host’s antibody response rather than the presence of *B. abortus* organisms: the rose bengal test (RBT), the complement fixation test (CFT), and the serum agglutination test (SAT; Herr and Marshall 1981; Chapparo et al. 1990). We restrict our analysis to those tests routinely used in African buffalo (Chapparo et al. 1990), although additional diagnostic tests have been used for brucellosis testing in cattle and American bison (Gall et al. 2000). Experimental infections in cattle have provided information on the host antibody response to *B. abortus* infection (Nielsen et al. 1984). The SAT was one of the first serologic tests for *Brucella* antibody and is based primarily on immunoglobulin M antibodies because they are the most active agglutinins (Nielsen 2002). This test produces many false-positives and has been discontinued by the World Organisation for Animal Health (OIE 2008). The RBT and CFT are often used in combination for accurate diagnosis, with the RBT used as a screening test and the CFT used as a confirmatory test. However, application of the CFT requires precise measurements and specialized reagents, making it difficult to implement under field conditions. As a result, it is being replaced by enzyme-linked immunosorbertent assays (ELISAs) (Godfroid et al. 2010). All three tests (RBT, CFT, ELISA) are recommended by the OIE as valuable livestock diagnostic tests (2008), but the direct application of these tests from cattle populations to African buffalo populations is problematic. This is because test sensitivity (Se) and specificity (Sp) will vary among species, and none of these tests has been validated in African buffalo.

Traditional estimates of diagnostic test Se and Sp are based on direct comparisons against an established gold standard test (detection of *Brucella* organisms by culture methods). Because true gold standard test results are often costly or impractical to obtain, especially in wildlife systems, a new test’s accuracy is commonly estimated by comparing it to a reference test with a known error rate (Buck and Gart 1966) or by comparison to multiple imperfect diagnostic tests (Enoe et al. 2000). Techniques that estimate test accuracy or the test’s ability to detect antibody to the pathogen when there is uncertainty in the test’s Se or Sp are called latent class analyses because they use the observed frequency of diagnostic test results to estimate a latent variable, the true infection status, from which the new diagnostic test can be evaluated (Branscum et al. 2005). Accurate estimates of a test’s Se and Sp with latent class analysis requires correctly representing whether the outcomes of two tests for a given animal are independent or correlated (conditional upon the true state of the animal; Georgiadis et al. 2003). Therefore, we consider potential correlations among tests in this analysis. This analysis also follows an increasing trend in the use of a Bayesian inference with latent class analysis; examples include the estimation of test accuracy for exposure to the agents of foot and mouth disease (Engel et al. 2008), tuberculosis (Alvarez et al. 2012), and brucellosis in cattle (Matope et al. 2011). Bayesian inference could also be useful for diagnostic test evaluation in wildlife because it incorporates uncertainty about model parameters based on independently collected, or prior information. These techniques are recommended by the OIE to estimate Se and Sp but represent only one step in the validation process.
The assumptions and modifications used in latent class analyses have been reviewed in general for latent class techniques (Enoe et al. 2000) and, more specifically, for latent class techniques with Bayesian inference (Branscum et al. 2005).

We evaluate the utility of an ELISA (IDEXX Brucellosis Serum Ab Test, IDEXX Laboratories, Westbrook, Maine, USA) for diagnosis of brucellosis in an important wildlife host, African buffalo. First, we selected an ELISA cutoff value, based on a pseudogold standard created from a subset of sampled buffalo consistently found to be antibody-positive or antibody-negative on both the RBT and CFT. Next, we used latent class modeling to estimate the Se and Sp of the ELISA, RBT, and CFT based on the entire data set of diagnostic test results.

**MATERIALS AND METHODS**

**Animal captures and test methods**

Serum samples were collected from a cohort of 202 female African buffalo from herds in two areas of southern Kruger National Park, South Africa: the Lower Sabie and the Crocodile Bridge area. Buffalo were captured approximately every 6 mo between 2008 and 2010 as part of an ongoing disease study. Fifty-two animals died throughout the study period and were replaced with additional buffalo, resulting in 571 samples collected from 254 buffalo. No buffalo were sampled <6 mo apart. We collected samples for diagnostic test evaluation between June 2008 and August 2009 and again between March 2010 and October 2010. All buffalo captured in those periods were tested with each diagnostic test. Animals were chemically immobilized by research veterinarians and South African National Parks (SANParks) staff with M99 (etorphine hydrochloride) and ketamine (Pharm4Game, Pretoria, South Africa). Jugular blood was collected from each animal into blood tubes and immediately stored on ice for transportation back to the laboratory. The blood was centrifuged at 6,000 × G for 10 min, and serum samples were separated and stored at −20 C for subsequent antibody testing. Animal capture and data collection protocols were approved by Oregon State University, the University of Georgia, and SANParks' Institutional Animal Care and Use committees.

We used three serologic measures of *Brucella* antibody. The RBT and CFT were conducted by the diagnostic laboratories of Onderstepoort Veterinary Institute (Pretoria, South Africa) according to OIE specifications (OIE 2008). Briefly, the RBT is conducted by monitoring the agglutination response after mixing serum with rose bengal–stained *B. abortus* cells. The CFT is conducted by monitoring the degree of hemolysis after incubating inactivated test serum, antigen, and exogenous complement with sensitized sheep red blood cells (OIE 2008). The Brucellosis Serum Ab ELISA tests (IDEXX P04130) were conducted in the field laboratory at KNP according to kit instructions. This assay detects antibodies to the lipopolysaccharide (LPS) antigen of smooth *Brucella* strains. Test results are determined by a sample’s optical density (OD) read at 450 nm and compared with the positive and negative controls according to the equation:

\[
\text{Cut-off} = 100 \times \left( \frac{\mu(\text{OD}_{450} \text{ of the paired sample wells})}{\mu(\text{OD}_{450} \text{ of negative control wells})} \right) - 100 \times \left( \frac{\mu(\text{OD}_{450} \text{ of positive control wells})}{\mu(\text{OD}_{450} \text{ of negative control wells})} \right)
\]

The cutoff value for determination of antibody-positive status in cattle recommended by IDEXX is 120%. However, we explored test Se and Sp at additional cutoff values because we were testing sera from a different species.

**Selection of ELISA cutoff values with receiver operating characteristic curve analysis**

To select ELISA cutoff values, we defined a pseudogold standard that estimated true antibody prevalence. We combined the results from the CFT and RBT into a composite reference standard (Alonzo and Pepe 1999). Buffalo were identified as antibody-positive only if they remained positive by both RBT and CFT during a 6-mo period and antibody negative only if they remained negative on both tests during a 6-mo period. Of the 254 individuals tested with all three diagnostic tests, 153 buffalo were sampled twice during a consecutive 6-mo period and returned concordant test results using the RBT and CFT tests. The ELISA results at the end of the period were compared with this pseudogold standard.

We used receiver operating characteristic (ROC) curves to select the ELISA cutoff value and two-graph ROC (TG-ROC) curves to display the relationship between Se and Sp for
various cutoff values (Gardner and Greiner 2006). Selection of test cutoff values remains dependent on the intended use of the test, which may vary for different decision-making situations (e.g., test-and-cull programs vs. surveillance). For example, lower cutoff values may be advisable when there are consequences for false-negative test results, whereas higher cutoff values may be preferred when there are high costs for false-positive test results (Greiner et al. 2000). We report the cutoff value that maximizes the total Se and Sp. The ROC analysis and the TG-ROC plot were conducted with the package DiagnosisMed (Brasil 2010) for R statistical software (R Core Team 2012). Clopper-Pearson binomial confidence intervals were drawn for test accuracy in the ROC curve analysis (Brasil 2010). Because estimates from the pseudogold standard analysis include only a subset of the animals with concordant test results on the RBT and CFT tests, the analysis may overestimate ELISA accuracy. This could occur if the reduced data set excluded animals with lower antibody responses or animals that became infected during the study. Thus, we used latent class models to estimate ELISA Se and Sp from the test results of all collected samples.

**Latent class analysis and prior estimation**

Latent class analysis allows evaluation of diagnostic tests in the absence of a gold standard. The simplest model presented here assumed that the outcomes of the tests for a given animal were independent, conditional upon the true state of the animal. This model is referred to as the conditional independence model and is described in detail in Supplementary Appendix 1. Complete model specifications and a review of Bayesian approaches to estimation is provided by Branscum et al. (2005); the model’s initial descriptions in two and three populations are provided by Hui and Walter (1980) and Walter and Irwig (1988), respectively. The validity of assuming two tests are conditionally independent requires further justification (Vacek 1985). The results of diagnostic tests that measure similar biological processes are likely to be correlated (conditional on the animals’ true infection status; Gardner et al. 2000) and assuming independence may result in incorrect estimates of test accuracy (Georgiadis et al. 2003). The RBT, CFT, and ELISA all measured the hosts’ antibody response to *Brucella* smooth LPS, but they used different methods of antibody detection (Nielsen 2002; Godfroid et al. 2010). Therefore, because we had little prior knowledge about the potential correlation between test outcomes, we considered models by assuming both conditional independence and conditional dependence.

We used model selection based on deviance information criteria (DIC) to compare the fit of models assuming conditional independence and conditional dependence (e.g., Rahman et al. 2013). Deviance information criteria are model assessment tools based on model fit and the effective number of parameters (Link and Barker 2010). Models with lower DIC values provide a better fit to the data, and we chose the model with the lowest DIC value (Spiegelhalter et al. 2002). Prior distributions for diagnostic test Se and Sp were represented as beta distributions and were defined using published results from test validations in cattle (Greiner et al. 2009; Table 1). The prior distributions for each parameter are displayed in Table 1, and details of their specification are provided in Supplementary Appendix 1. This prior information was combined with the full data set of 571 samples (Supplement 2). Median and 95% Bayesian credible intervals are presented for all parameters in the best-fitting model. We conducted sensitivity analy-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mode</th>
<th>Lower limit</th>
<th>Beta distribution (a, b)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA&gt;159</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>0.976</td>
<td>&gt;0.60</td>
<td>6.29, 1.13</td>
<td>a</td>
</tr>
<tr>
<td>Sp</td>
<td>0.975</td>
<td>&gt;0.60</td>
<td>6.31, 1.14</td>
<td>a</td>
</tr>
<tr>
<td>RBT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>0.981</td>
<td>&gt;0.21</td>
<td>1.94, 1.02</td>
<td>a, b</td>
</tr>
<tr>
<td>Sp</td>
<td>0.998</td>
<td>&gt;0.688</td>
<td>8.08, 1.01</td>
<td>a, b</td>
</tr>
<tr>
<td>CFT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>0.960</td>
<td>&gt;0.23</td>
<td>2.08, 1.05</td>
<td>a, b</td>
</tr>
<tr>
<td>Sp</td>
<td>0.998</td>
<td>&gt;0.306</td>
<td>2.56, 1.00</td>
<td>a, b</td>
</tr>
<tr>
<td>Prevalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>0.30</td>
<td>&gt;0.10</td>
<td>2.35, 4.14</td>
<td>c</td>
</tr>
<tr>
<td>CB</td>
<td>0.35</td>
<td>&gt;0.10</td>
<td>1.96, 2.78</td>
<td>c</td>
</tr>
</tbody>
</table>

a-Grenier et al. 2009.
b-Nielsen 2002.
c-Chapparo et al. 1990.
ses on this model by 1) increasing the mode and lower bounds of prior distributions of each tests’ Se and Sp by 5 percentage points, 2) decreasing the mode and lower bounds of prior distributions of each tests’ Se and Sp by 5 percentage points, and 3) specifying uninformative priors between the interval of zero to one, modeled as beta(1,1), for each tests’ Se and Sp parameter. We also compared estimates generated from models fit with only the first sample point for each of the 254 buffalo sampled to explore whether the pseudoreplication in our data set influenced the estimates of test accuracy.

**RESULTS**

**Selection of ELISA cutoff values with ROC curve analysis**

The pseudogold standard defined 28 positive and 123 negative animals (Table 2). Within this subset of buffalo, the Se and Sp estimates when using the kit’s defined cutoff of 120% were 1 (95% confidence interval [CI]=0.82–1.00) and 0.87 (95% CI=0.80–0.92), respectively. The ROC curve analysis shows that ELISA Sp was improved at higher cutoff values with minimal reductions in Se. The cutoff value with the highest Se and Sp was 159% (Fig. 1). That cutoff was associated with a Se of 1 (95% CI=0.82–1) and a Sp of 0.93 (95% CI=0.87–0.97).

**Latent class analysis**

The diagnostic test results used for latent class models were calculated based on the ELISA cutoff value of 159% and all 571 samples (Table 3). The model assuming conditional dependence between the ELISA and CFT had the lowest DIC value (59.24). Neither the model assuming conditional independence (DIC=63.24) nor the models with additional dependence parameters had lower DIC values (Supplementary Table S1). We, therefore, report the results of that model based on parsimony and model fit.

Test accuracy varied among the diagnostic tests (Fig. 2). The ELISA’s Se and Sp were estimated to be Se=0.928 (95% CI=0.80–1.00) and Sp=0.93 (95% CI=0.87–0.97).

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**Table 2.** Pseudogold standard test result frequencies and 95% confidence estimates of test accuracy. Results were calculated with the enzyme-linked immunosorbent assay (ELISA) cutoff value recommended for cattle (cutoff=120) and the cutoff value selected based on receiver operating characteristic analysis (cutoff=159). Test accuracy was improved with a higher cutoff value.

<table>
<thead>
<tr>
<th>Pseudogold standard status</th>
<th>ELISA cutoff&gt;120</th>
<th>ELISA cutoff&gt;159</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>107</td>
</tr>
<tr>
<td>No. misclassified/accuracy (%)</td>
<td>16 (89.4%)</td>
<td>9 (94.0%)</td>
</tr>
<tr>
<td>Sensitivity (range)</td>
<td>1 (0.82–1.00)</td>
<td>1 (0.82–1.00)</td>
</tr>
<tr>
<td>Specificity (range)</td>
<td>0.87 (0.80–0.92)</td>
<td>0.93 (0.87–0.97)</td>
</tr>
</tbody>
</table>

**Figure 1.** Two-graph receiver operating characteristic (ROC) curve that plots sensitivity (Se), specificity (Sp), and their nonparametric confidence bands as a function of test cutoff value. Vertical dashed lines show the cutoff value selected by ROC analysis for further investigation (cutoff=159%).
Bayesian posterior credibility interval [BCI]=0.869–0.974) and Sp=0.870 (95% BCI=0.836–0.900). The RBT had the highest estimated Se (Se=0.986, 95% BCI=0.928–0.999), and both the ELISA and RBT had significantly higher sensitivities than the CFT did (Se=0.374, 95% BCI=0.294–0.460). However, both the RBT and CFT had significantly higher specificities than the ELISAs did, with estimated values of 0.992 (95% BCI=0.971–0.996) and 0.998 (95% BCI=0.992–0.999), respectively. Prevalence in the Lower Sabie region was estimated as 0.235 (95% BCI=0.183–0.292) and in the Crocodile Bridge region as 0.228 (95% BCI=0.183–0.277).

Sensitivity analyses showed that decreasing the mode of the ELISA prior distribution by five percentage points decreased the median of the posterior distributions from Se=0.928 (95% BCI=0.869–0.974) to 0.925 (95% BCI=0.867–0.971) and from Sp=0.870 (95% BCI=0.836–0.900) to 0.869 (95% BCI=0.835–0.900), with similar results when prior information was also relaxed to 70% (Table 4). Increasing the mode of the ELISA prior distributions by 5 percentage points resulted in only a minor increase to Se=0.930 (95% BCI from 0.871–0.976) and Sp=0.870 (95% BCI=0.836–0.901). The estimates of ELISA accuracy also remained similar when the prior values for RBT and CFT accuracy were relaxed (Table 4). When the model was fit to data with one test result per buffalo, test Sp remained similar, but Se increased slightly to 0.960 (0.887–0.993). The 95% credible intervals overlapped despite these perturbations, suggesting that the estimates of ELISA Se and Sp were influenced by the frequency of the test results and, to a lesser extent, the prior information.

<table>
<thead>
<tr>
<th>ELISA/RBT/CFT</th>
<th>+/+/+</th>
<th>+/+/-</th>
<th>+/-/+</th>
<th>+/-/+</th>
<th>-/+/-</th>
<th>-/+/+</th>
<th>-/+/-</th>
<th>-/+/-</th>
<th>-/+/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Sabie</td>
<td>24</td>
<td>28</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>161</td>
<td></td>
</tr>
<tr>
<td>Crocodile Bridge</td>
<td>21</td>
<td>47</td>
<td>0</td>
<td>39</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>75</td>
<td>0</td>
<td>58</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>381</td>
<td></td>
</tr>
</tbody>
</table>

* + = positive; − = negative.

Table 3. Enzyme-linked immunosorbent assay (ELISA), rose bengal test (RBT), and complement fixation test (CFT) results classified for the Lower Sabie and Crocodile Bridge regions, Kruger National Park, South Africa.

Figure 2. Summary of prior and posterior distributions for latent class analysis of enzyme-linked immunosorbent assay (ELISA), rose bengal test (RBT), and complement fixation test (CFT) accuracy. Prior information for the sensitivity and specificity of each test is summarized by the median and 95th percentile of their distribution. Median parameter estimates and 95% Bayesian credible intervals for (a) sensitivity and (b) specificity are displayed for the model assuming conditional dependence between the ELISA and CFT.
DISCUSSION

The IDEXX ELISA was estimated to have a Se of 0.928 (95% BCI from 0.869–0.974) and Sp of 0.870 (95% BCI from 0.836–0.900) when using the cutoff value of 159%. At that cutoff value, the ELISA had a higher median Se than the CFT did, similar but lower Se to the RBT, but a lower Sp than both the RBT and the CFT had. The estimates of test accuracy in this study are based on the selected ELISA cutoff value. The cutoff value that maximized the total Se and Sp was 159%. Because test Se and Sp are inversely related at a given cutoff value, a different cutoff would result in altered estimates of test accuracy. The selected cutoff value should be taken into account when comparing diagnostic tests (Greiner et al. 2000). For example, at the suggested cutoff value for cattle, 120%, the ELISA had a lower Sp and a higher Se. This result emphasizes the importance of test optimization for each population and species to which it is applied.

In addition to species-specific differences, three nonexclusive factors explain why the cutoff value for cattle resulted in a higher number of misclassified results. First, the test is being applied under field laboratory conditions. Serum samples for these analyses were collected and frozen in the field at $-20^\circ$C for 1–3 yr, with temperature fluctuations possible because of a somewhat variable power supply (although to our knowledge no outright freezer failure occurred during the storage period). Ideally, sample storage would use consistent and colder ($-80^\circ$C) temperatures; as such, suboptimal storage conditions might have degraded the samples to some degree. Second, brucellosis is endemic in this buffalo population, and our sampling may have resulted in animals with a wider range of times since infection than those used for the tests’ validation in cattle. Finally, all diagnostic tests are susceptible to cross-reactive antibodies. *Yersinia enterocolitica* O:9 shares common antigenic epitopes with *B. abortus* and is known to cross-react during diagnosis, but little is known about the presence of *Yersinia* in buffalo populations (Godfroid et al. 2002). The evaluation presented
here allows these sources of variability to be incorporated into the estimates of test accuracy, allowing the estimates to be robust to problems inherent in most field conditions.

Latent class analysis allows the quantification of test variability and accuracy in the absence of a gold standard. Like all model-based analyses, its implementation involves a trade-off between the model complexity (number of parameters) and parsimony. The model selection performed in this study shows that models including covariance between the ELISA and CFT had a better fit to the data compared with the model assuming conditional independence. The lack of support for models representing dependence between the ELISA/RBT and the RBT/CFT, based on DIC values, is supported by the low conditional correlations among those tests. Because the tests measure antibodies through different mechanisms (Nielsen 2002), it is plausible that the tests are conditionally independent of each other, given the true state of the animal. However, those models also may have had higher DIC values because there were minimal data to estimate the conditional dependence terms; there were few samples with ELISA\(^-\), RBT\(^-\), and CFT\(^+\) test results or ELISA\(^+\), RBT\(^+\), CFT\(^+\) test results. Previous work on brucellosis in sheep represented conditional dependence between the RBT and the ELISA and between the RBT and SAT (Rahman et al. 2013). Other systems, however, have found the conditional independence model to be most appropriate (Muma et al. 2007; Rahman et al. 2013). The results of this analysis show similar estimates of Se and Sp in all models regardless of the test correlation assumptions (Supplementary Table S1) and suggest that these estimates were robust to model assumptions.

The uncertainty in how any of the diagnostic tests relate to active infection in wildlife represents a major hurdle to accurate diagnostic methods (Treanor et al. 2011). Because of these limitations, the results of this evaluation serve as a comparison among the serologic tests historically used. Additional assays for brucellosis, including the fluorescence polarization assay (Gall et al. 2000), polymerase chain reaction assays (Bricker 2002), and alternative ELISA techniques (Nielsen 2002) have shown improved accuracy in other systems and should be considered for future testing in African buffalo. Further, the estimates of test Se and Sp presented in this analysis include prior information (Fig. 2). Rather than a limitation, incorporating this information could be a valuable tool for wildlife studies given the sample size requirements and potential identifiability problems with latent class models (Dendukuri et al. 2010). Our analysis also assumes that test Se and Sp are consistent throughout the course of brucellosis infection and among populations. As more information develops about the course of brucellosis in buffalo, future diagnostic tests evaluations should incorporate variation in detection rates between different stages of infection (Engel et al. 2010; Caraguel et al. 2012) or different populations (Munoz et al. 2012).

The benefits of the ELISA are that it is relatively inexpensive, is easy to perform in field conditions, and results in quantitative test results. The choice of an appropriate diagnostic test, however, is dependent on its intended use. For example, with a Sp of 87\%, the ELISA may not present an ideal diagnostic tool for screening of commercial buffalo herds because it would result in many false-positive animals being removed at an undesirably high cost to the farmer. However, its use in combination with the RBT could improve current diagnostic methods by avoiding misclassifications. For large-scale disease surveys, the ELISA’s 93\% Se and ease of use may make it a valuable screening tool for African buffalo. Given the importance of brucellosis for public health in sub-Saharan Africa, further work establishing and validating improved diagnostic meth-
odds is needed for detection of *B. abortus* in one of its wildlife reservoirs, the African buffalo.

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**SUPPLEMENTARY MATERIAL**

Supplementary material for this article is online at http://doi:10.7589/2013-12-334.

**LITERATURE CITED**


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