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Evaluation of the sensitivity and specificity of the lateral flow assay, Rose Bengal test and the complement fixation test for the diagnosis of brucellosis in cattle using Bayesian latent class analysis

Davies M. Pfukenyi[a], Eleftherios Meletis[b], Boitumelo Modise[c], Masimba Ndengu[a], Faith W. Kadzviti[d], Kopano Dipuo[c], Kago Moesi[c], Polychronis Kostoulas[b], Gift Matope[d,*]

[a] Department of Clinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe
[b] Laboratory of Epidemiology, Biostatistics and Animal Health Economics, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, 224 Trikalon St., Greece
[c] Botswana National Veterinary Laboratory, Private Bag 0035, Gaborone, Botswana
[d] Department of Paraclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe

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ABSTRACT

This study was conducted to evaluate the sensitivity (*Se*) and specificity (*Sp*) of the Rose Bengal test (RBT), complement fixation test (CFT), the serum lateral flow assay (LFA_{serum}) and the blood lateral flow assay (LFA_{blood}) for the detection of antibodies to *Brucella* spp. using Bayesian latent class models (BLCMs). Sera and whole blood were collected from naturally infected cattle reared in smallholder, small-scale commercial and large-scale commercial farms in Zimbabwe (n = 1022) and Botswana (n = 770). The BLCMs were fitted under the assumption that conditional dependences existed between the tests. Based on the conditional dependence model, the RBT had the highest *Se* of 0.897 (95 % Probability Intervals: 0.854; 0.932) compared to 0.827 (0.773; 0.872), 0.812 (0.76; 0.858) and 0.809 (0.785; 0.832) for the LFA_{serum}, LFA_{blood} and CFT, respectively. The CFT recorded a higher *Sp* of 0.999 (0.995; 1.000) than the LFA_{serum} 0.996 (0.99; 1.000), the LFA_{blood} 0.984 (0.976; 0.991) and the RBT 0.969 (0.959; 0.978). The data indicated that both the *Se* and *Sp* of RBT and CFT and the *Sp* of LFA_{serum} and LFA_{blood} were conditionally independent, while the *Se* appeared to be conditionally dependent. These results indicated that none of the evaluated tests had perfect *Se* and *Sp* and consequently could not be used alone for the diagnosis of brucellosis in cattle from the studied farming sectors. Thus, based on high *Se* and *Sp*, respectively, a brucellosis testing regimen using the RBT (screening) and the LFA (confirmatory) may be considered.

1. Introduction

Brucellosis is a disease of animals caused by Gram-negative coccobacilli and facultatively intracellular bacteria of the genus *Brucella*. Currently, there are 11 species that have been delineated (Whatmore et al., 2014), recognised mainly due to their pathogenicity and host preference; four of these are classical zoonoses. Bovine brucellosis is primarily caused by biovars of *B. abortus* and occasionally by *B. melitensis* in farms where cattle are co-kept with infected goats and/or sheep (OIE, 2017). It is a disease of both economic and public health importance in many countries world-wide and it is endemic especially in low income countries in Sub-Saharan Africa including Zimbabwe (Matope et al., 2011a). In such low income countries, the majority of the population lives in rural areas where their livelihood is largely dependent on subsistence farming and cattle rearing. Thus, in these rural communities, bovine brucellosis remains a threat to not only losses in cattle production, but also to public health. Further, people in rural communities lack awareness of the disease and often engage in cultural practices that place them at risk of exposure to *Brucella* spp. and other zoonoses (Gadaga et al., 2016; Ndengu et al., 2017). Therefore, it is imperative for State Veterinary Services to place priority towards building capacity for diagnosis, control and surveillance programmes for bovine brucellosis.

The clinical diagnosis of the disease in the field is often unrewarding primarily due to the absence of apparent clinical signs except for abortion in the third trimester. While culture and isolation is long regarded as the reference standard diagnostic test for brucellosis, brucellae are fastidious organisms that are difficult to grow, and once grown they pose an immense risk of infection to the laboratory personnel. This usually requires an upgrade of laboratory facilities to at

* Corresponding author.
E-mail address: giftmatope@gmail.com (G. Matope).

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least biosafety level 3 (BSL-3) which is expensive and mostly not available in many low income countries. Thus, serological tests are often relied upon for the diagnosis of brucellosis as these are relatively safer for laboratory personnel and are more cost-effective and sustainable (Arif et al., 2018).

To date, many serological tests have been used for the diagnosis of bovine brucellosis, but all have shortcomings in different epidemiological settings especially in screening individual animals, due to the variation in the diagnostic test accuracy and predictive values with varying disease prevalence (Godfroid et al., 2002). Given that none of these tests are perfect, use and interpretation of tests in combination is common in order to minimise diagnostic errors. In Zimbabwe, Botswana and other southern African countries, the Rose Bengal test (RBT) is used for screening, while the complement fixation test (CFT) is used as a confirmatory test for the diagnosis of bovine brucellosis (Madsen, 1989). Both tests have been prescribed for the diagnosis of bovine brucellosis by the World Organisation for Animal Health (OIE) (OIE, 2017). Although, a previous study evaluated the sensitivity (*Se*) and specificity (*Sp*) of RBT in conjunction with the c-ELISA and fluorescence polarisation assay (FPA) in cattle in Zimbabwe (Matope et al., 2011b), the evaluation of RBT together with the CFT as the approved confirmatory test, has not been done under our local conditions. Further, following the arguments presented in a previous report (Matope et al., 2011b), it is important to evaluate the performance of a field test kit like the lateral flow assay (LFA) that is cow-side, easy to use and relatively cheap.

The LFA is a cow-side field immunochromatographic test that is designed to detect both IgM and IgG in blood of animals exposed to smooth *Brucella* spp. using specific anti-bovine antibodies labeled to gold nanoparticles (Life Assays). The LFA has been reported to be highly sensitive and specific and offers several advantages over other conventional serological tests, but has not been evaluated for the detection of antibodies to *Brucella* spp. in cattle under field conditions of Botswana and Zimbabwe. Once evaluated, such a test kit will be handy for brucellosis eradication as it does not require automation and is likely to improve turnaround time. Although a number of methods have been used to estimate the *Se* and *Sp* of diagnostic tests (Joseph et al., 1995; Hui and Zhou, 1998), Bayesian latent class models (BLCMs) have been the most frequently used, especially when the true infection status of animals is unknown (Hui and Walter, 1980). Therefore, the objective of this study was to estimate the *Se* and *Sp* of the four tests; RBT, CFT, blood LFA (LFA_{blood}) and serum LFA (LFA_{serum}) for the detection of antibodies *Brucella* spp., and to estimate the true prevalence of brucellosis in cattle from selected farming sectors in Zimbabwe and Botswana.

2. Materials and methods

2.1. Ethics statement

This work was approved by the National Animal Research Committee under the Department of Veterinary Services (Zimbabwe), registration Number NARC/002/19. In Botswana the work was approved by the Department of Veterinary Services under Ministry of Agricultural Development and Food Security. The methods of handling, restraint and after-care treatment of animals were carried out in order to minimise stress, as recommended in the Terrestrial Animal Health Code for use of animals in research and education (OIE, 2011). The purpose of the project was explained well to the cattle owners and they all gave consent to the bleeding of their animals for testing.

2.2. Study areas

The study was conducted in selected communal, small scale commercial and large scale commercial farms in Zimbabwe and Botswana. The selection of the study areas for Zimbabwe was based on brucellosis outbreak data from the respective areas obtained from the Department of Veterinary Services, and the Department of Animal Production and Veterinary Services of Zimbabwe and Botswana, respectively. In Zimbabwe, based on the available brucellosis outbreak data and cattle management practices, study areas were divided as follows; communal farming areas (sub-population1), small scale commercial (sub-population2) and commercial (sub-population 3) farms, where in the latter (sub-population 3), some farms used *B. abortus* S19 vaccine. Previous studies reported that the mean prevalence of brucellosis was 16.7 %, 5.6 % and 15 % for communal, small-scale and commercial (Mashonaland Provinces) farms, respectively (Matope et al., 2011; Ndengu et al., 2017; Vhoko et al., 2018). The study areas in Botswana were selected from mainly smallholder cattle farms where brucellosis status was largely unknown and with no history of *B. abortus* S19 vaccine use.

2.3. Study design

A cross-sectional study was designed targeting cattle populations in the three groups of the study areas. For sampling, the areas were visited once between March and August 2018. In Zimbabwe, animal health regulations compel all cattle owners in communal farms to dip their cattle weekly during the rainy season and fortnightly during the dry season for control of ticks and tick-borne diseases (Chikerema et al., 2013). Therefore, for easy access to cattle, the sampling was made to coincide with the dipping sessions in the respective areas.

In the smallholder farms, four areas, namely Chizvirizvi, Chomupani, Domboshava and Sivezvili were selected for sampling based on brucellosis outbreak data. Eight small-scale commercial farms; Bulawayo, Chikwaka, Chinhoyi, Fern Valley, Hodzi, Marirangwe, Mvuma and Serima, while two commercial farms, one from Mount Hampden and another from Mazowe areas were recruited for the study. The management system and breeds of cattle in the small scale farms were similar. Thus, the farms were assumed as one population. One dip tank serving each of these respective areas was selected as a sampling frame from each of the selected smallholder areas. For sampling, the animals were selected by a systematic random procedure by taking every tenth animal as they passed through the dip tank.

In Botswana, the study was conducted in three out of ten districts being South East, Southern and Chobe districts between April and August 2018. Sampling coincided with the National Surveillance programme for FMD and CBPP in Chobe District, while an active surveillance was initiated for Southern and South East Districts. Systematic random sampling was used to select animals to be sampled, taking every tenth animal as they passed through the crush.

2.4. Blood sampling

The sera (n = 1022) and whole blood (n = 1022) were collected from all cattle from the three sub-populations in Zimbabwe as follows: sub-population 1 (sub1) (n = 487), sub2 (n = 315) and sub3 (n = 220). From Botswana, sera (n = 770) and whole blood (n = 770) were also collected from individual cattle selected for the study. Briefly, individual animals were restrained in the crush and the cow side test administered immediately by pricking the ear vein and collecting a drop of blood to run the LFA. Approximately 10 mL of blood was then collected by jugular venipuncture into EDTA blood sample collection tubes and IMPROVACUTER® evacuated blood collection tubes. The blood was preserved on ice in a cooler box and transported to the laboratory for further analysis. Further analysis was carried out at the University of Zimbabwe Microbiology Laboratory and the Central Veterinary Laboratory (CVL) (Zimbabwe) and the National Veterinary Laboratory in Gaborone (Botswana).

2.5. Laboratory tests

In the laboratory, to collect serum, blood in the IMPROVACUTER® evacuated blood collection tubes was centrifuged at 3000 revolutions per minute for 10 min and approximately 2 mL serum was collected and stored in cryo-tubes at -20 ˚C until laboratory tests were performed. Personnel that carried out each test were blinded to the results of the other tests.

2.5.1. The Rose Bengal test (RBT)

The RBT was performed according to the guidelines provided in the World Organisation for Animal Health (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2017). The buffered *Brucella abortus* (S-99) antigens, positive and negative control sera were procured from IDVet, France. The test was performed on round-bottom-welled plastic plates and white ceramic tile. Briefly, 30 µL of RBT antigen and 30 µL of the test serum were placed alongside on the plate / tile, and then mixed thoroughly. The plate/tile was shaken for 5 min and the degree of agglutination reactions was recorded. The test was read as positive if there were visible agglutinations (agglutinations visible by the unaided eye) and negative if no visible agglutinations appeared within 5 min of running the test (Matope et al., 2010).

2.5.2. Complement fixation test (CFT)

All the serum samples were also subjected to the complement fixation test. The test was performed according to the guidelines provided in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2017). The antigens, positive and negative control sera were procured from IDVet, France. Briefly, all dilutions were made in commercial veronal buffered saline. The indicator system was a 3% suspension of fresh sheep red blood cells (SRBC) (CVL, Zimbabwe) sensitised with an equal volume of rabbit anti-sheep red blood cells (SRBC) serum (haemolysin). This was prepared at a dilution previously determined to contain five times the minimum haemolysin concentration required to produce 100 % lysis of the SRBC in the presence of a 1/10 dilution of fresh guinea-pig complement (CVL, Zimbabwe). The latter was independently titrated to determine the minimum concentration required to produce 100 % lysis of the sensitised SRBC suspension; this was defined as the minimum haemolytic dose (MHD). The complement was used at 1.25 MHD in the test. Test sera and appropriate working standards were diluted with veronal buffered saline in small tubes and incubated at 58 ˚C ± 2 ˚C for 30 − 50 min in order to inactivate the native complement. The test was performed on standard 96-well U-bottom microtitre plates where 25 µL of diluted test serum were placed and double dilutions made with equal volumes of veronal buffered saline followed by adding 25 µL of complement at 1.25 MHD and 25 µL of antigen. The plates were incubated at 4 ˚C for 14–18 h and / or 37 ˚C for 30 min with gentle shaking. To each well, 25 µL of sensitised SRBC suspension were added, and the plates were re-incubated at 37 ˚C for 30 min with agitation at least for the first 10 min. The results were read after the plates had been left to stand at 4 ˚C for up to 1 h and / or after centrifuging plates at 1500 rpm for 4 min to allow unlysed cells to settle. A positive result was read as the formation of a button of red blood cells at the bottom of the well whereas a negative result was a homogenous solution indicating hemolysis.

2.5.3. The lateral flow assay (LFA)

The lateral flow was performed using the Test-it® developed by Life Assay Diagnostics (Pvt) Limited (Cape Town, South Africa) to detect IgM and IgG produced against smooth *Brucella* spp. The kit was used according to the guidelines developed by the manufacturer and essentially as described in other studies elsewhere (Smits et al., 2003; Abdoel et al., 2008). Briefly, 5 µL serum/ or blood was added to the sample pad of the assay kit followed by the addition of 130 µL sterile running fluid consisting of phosphate-buffered saline, pH 7.6, containing 1.67 % bovine serum albumin and 3% Tween 20. Test results were read after 15 min by visual inspection for staining of the antigen and control lines. Tests were scored negative when no staining was observed at the test line and scored positive when the test line was stained visibly and detected with an unaided eye. LFA_{blood} was performed on site in the field and LFA_{serum} at the veterinary laboratories.

2.6. Bayesian latent class analysis

In this analysis, STARD-BLCM reporting guidelines on the design, conduct and results of diagnostic accuracy studies that use BLCMs were followed (Kostoulas et al., 2017).

2.6.1. Definition of infection status

BLCMs create their own probabilistic definition of infection that depends on the test under evaluation. Here, all tests detect humoral immune response, after natural infection, of the host: the LFA and RBT detect both IgM and IgG, while the CFT detect mainly IgG. In this study, “infection” means that the brucellae organism is present intracellularly and persistent within an animal long enough to produce a detectable humoral immune response at any time during their life (Rahman et al., 2019).

2.6.2. Model and assumptions

In the absence of a reference standard, BLCMs may be used to estimate the *Se* and *Sp* of two or more diagnostic tests and the prevalence of a disease in two or more populations (Hui and Walter, 1980). Models can be considered under different assumptions. The main ones are: (i) the prevalence of the true infection/exposure differs between populations; (ii) measures of diagnostic accuracy of each test are constant across the populations; (iii) and the tests are conditionally independent or dependent given the disease/infection status (CID) (Matope et al., 2011b). Two tests are conditionally independent, given the disease/infection status, when the result of the first test does not modify the expectation about the result of the second test. However, conditional dependence cannot be ignored if the tests are based on the same biological principles (Gardner et al., 2000). Thus in this study, BLCMs that adjusted for the potential conditional dependence between tests were used to estimate the *Se* and *Sp* of four tests for bovine brucellosis: LFA_{serum}, LFA_{blood}, RBT and CFT. All four tests were applied in three sub-populations (Zimbabwe) and LFA_{serum} and RBT in one additional sub-population (Botswana). Estimations were based on the cross-classified results of the tests (Table 1).

The BLCMs assumed that (i) and (ii) hold while instead of assumption (iii) they captured the biologically plausible conditional dependence between LFA_{serum} and LFA_{blood} as well as RBT and CFT by introducing relevant covariance terms. Under this setting, the parameters to be estimated were 16 (i.e. four *Se*s, four *Sp*s, four prevalences (*p*) and four covariance terms), while the degrees of freedom offered by the data were 48, thus essential identifiability criteria are met.

2.6.3. Priors

Informative beta distributions *Be(a, b),* were used as priors for the parameters of interest (*Ses, Sps*, prevalences). Prior beliefs for the mode and the 95th percentile and the resulting Beta distributions, *Be* (a, b), as well as the *a priori* prevalence for the three cattle subpopulations in Zimbabwe are shown in Table 2. Scientific literature were used to derive estimates for priors for the test parameters (Bronsvoort et al., 2009; Matope et al., 2011b; Gusi et al., 2019) and prevalence of brucellosis in the three cattle sub-populations in Zimbabwe (Matope et al., 2011a; Ndengu et al., 2017; Vhoko et al., 2018). The Beta distributions were calculated using the PriorGen R Package (Kostoulas, 2018).

2.6.4. MCMC convergence and software

The latent class models were run using the freeware program OpenBUGS (Spiegelhalter et al., 2007). The models use a Markov Chain Monte Carlo (MCMC) sampling to obtain a Monte Carlo sample from

Table 1
Cross-classified results of LFA_{serum}, LFA_{blood}, RBT and CFT in Zimbabwe and Botswana.

Zimbabwe

		RBT				Total
		Positive		Negative		
		CFT		CFT		
		Positive	Negative	Positive	Negative	
LFA_{serum}	Positive	63	3	5	4	75
	Negative	24	56	4	863	947
		87	59	9	867	1022
LFA_{blood}	Positive	61	5	5	7	78
	Negative	26	54	4	860	947
Total		87	59	9	867	1022

Botswana

		RBT		Total
		Positive	Negative	
LFA_{serum}	Positive	6	0	6
	Negative	1	763	764
Total		7	763	770

LFA_{serum}, Lateral Flow Assay in serum; LFA_{blood}, Lateral Flow Assay in blood; RBT, Rose Bengal plate test; CFT, Complement Fixation test.

Table 2
Prior beliefs for the mode and the 95th percentile and the resulting Beta distributions, *Be*(a, b).

Test		Parameter	Mode	95th percentile	*Be*(a, b)
LFA_{serum}		*Se*	0.9	0.93 – 0.94	*Be*(77.22, 9.47)
		Sp	0.98	0.99	*Be*(208.05, 5.23)
LFA_{blood}		*Se*	0.88	0.91 – 0.92	*Be*(102.02, 14.78)
		Sp	0.97	0.98	*Be*(469.17, 15.48)
RBT		*Se*	0.9	0.93	*Be*(165.21, 19.25)
		Sp	0.999	0.999	*Be*(64.87, 1.65)
CFT		*Se*	0.8	0.82	*Be*(788.89, 197.97)
		Sp	0.999	0.999	*Be*(10000, 11.01)
Prevalence					
Zimbabwe	Sub1	P	0.167	0.4	*Be*(2.45, 8.23)
	Sub2	P	0.056	0.4	*Be*(1.25, 5.22)
	Sub3	P	0.15	0.4	*Be*(2.15, 7.52)

Se, sensitivity; *Sp*, Specificity; Sub, subpopulation (Sub1 = smallholder; Sub2 = small scale commercial; Sub3 = Commercial); *p*, prevalence.

the posterior distribution of the parameters. As part of the MCMC method, parameter estimates were based on analytical summaries of 100,000 iterations of one chain, after a burn-in phase of 5000 iterations. Convergence was assessed by visual inspection of Gelman–Rubin diagnostic plots as well as time series plots of selected variables. All checks suggested that convergence occurred and autocorrelations dropped-off fast.

2.6.5. Sensitivity analysis

Models, assuming conditional dependence and independence, based on non-informative prior values (*Be*(1,1)) were also introduced for comparisons. An alternative scenario where conditional dependences were ignored was also considered for comparisons. The DIC Tool dialog box in OpenBUGS environment was used to estimate the deviance information criterion (DIC) for the independence (DIC = 446) and the dependence (DIC = 358) model. The model with the smallest DIC is the model that best fits the data (i.e. the model that would best predict a replicate dataset of the same structure as the currently observed

Table 3
Posterior medians and 95 % probability intervals (PrIs) for the sensitivity (*Se*) and the specificity (*Sp*) of each diagnostic test and the true prevalence of disease in each subpopulation, assuming pairwise conditional dependences between LFA_{serum} and LFA_{blood} as well as RBT and CFT[a], using informative priors.

Test		Parameter	Median (PrIs)
LFA_{serum}		*Se*	0.827 (0.773; 0.872)
		Sp	0.996 (0.99; 1.000)
LFA_{blood}		*Se*	0.812 (0.76; 0.858)
		Sp	0.984 (0.976; 0.991)
RBT		*Se*	0.897 (0.854; 0.932)
		Sp	0.969 (0.959; 0.978)
CFT		*Se*	0.809 (0.785; 0.832)
		Sp	0.999 (0.995; 1.000)
LFA_{serum} & LFA_{blood}		cdp[b]	0.127 (0.096; 0.158)
		cdn[c]	0.003 (-0.002; 0.008)
		covcdp[d]	2.215 (1.83; 2.569)
		covcdn[e]	2.576 (0.25; 5.238)
RBT & CFT		cdp	0.022 (-0.012; 0.065)
		cdn	0 (-0.003; 0.004)
		covcdp	0.187 (-0.108; 0.5)
		covcdn	1.634 (-0.111; 5.303)
Prevalence			
Zimbabwe	Sub1	*p*	0.127 (0.099; 0.159)
	Sub2	*p*	0.08 (0.053; 0.114)
	Sub3	*p*	0.086 (0.049; 0.138)
Botswana	Sub1	*p*	0.009 (0.002; 0.018)

Sub1 = Smallholder (communal) cattle farming areas where brucellosis is endemic.
Sub2 = Small scale commercial farms (low prevalence).
Sub3 = Commercial farms.
[a] The RBT/CFT; $_{LFAserum/blood}$ are based on parallel testing.
[b] cdp refers to conditional dependence between sensitivities.
[c] cdn refers to conditional dependence between specificities.
[d] covcdp refers to covariance between sensitivities.
[e] covcdn refers to covariance between specificities.

(Spiegelhalter et al., 2002). The model suggesting independence between the tests had a greater DIC than the dependence model, therefore, the results were calculated, by adopting the dependence model.

3. Results

The cross-classified positive and negative results of LFA_{serum}, LFA_{blood}, RBT and CFT for the detection of antibodies to *Brucella* spp. in cattle from Zimbabwe and Botswana are shown in Table 1. Approximately 6% (61/1022) of samples from Zimbabwe were positive to all the four tests while 84 % (860/1022) were negative to all the tests. A total of 0.8 % (6/770) and 99.1 % (763/770) of the samples from Botswana were positive and negative to both the LFA_{serum} and RBT, respectively.

The medians of the posterior distributions and the 95 % posterior probability intervals (PrIs) for the sensitivity (*Se*) and the specificity (*Sp*) of each diagnostic test, relevant covariance terms and prevalences, assuming conditional dependence between LFA_{serum} and LFA_{blood} as well as RBT and CFT are shown in Table 3.

The RBT showed the highest mean for *Se* of 0.897 (95 % PIs: 0.854; 0.932), compared to 0.812 (0.76; 0.858) for LFA_{blood}. The *Se* of LFA_{blood}, 0.812 (0.76; 0.858), was marginally lower than that of LFA_{serum} 0.827 (0.773; 0.872). The highest *Sp* was seen for CFT 0.999 (0.995; 1.000) and LFA_{serum} 0.996 (0.99; 1.000). The RBT had the least *Sp* of 0.969 (0.959; 0.978). It is noteworthy that the *Se* and *Sp* of both LFA_{serum} and LFA_{blood} were approximately similar to that of the CFT. The estimated true prevalence of brucellosis for the three cattle subpopulations in Zimbabwe were 0.127 (0.099; 0.159), 0.08 (0.0053; 0.114) and 0.086 (0.049; 0.138) for sub-population 1 (communal), sub-population 2 (small-scale commercial) and sub-population 3 (large-scale commercial) respectively, while that for Botswana was 0.009

Table 4
Posterior medians and 95 % PrIs for *Se* and *Sp* of each diagnostic test and the true prevalence of disease in each subpopulation, assuming pairwise conditional dependences between LFA_{serum} and LFA_{blood} as well as RBT and CFT[a], using non-informative priors.

Test		Parameter	Median (PrIs)
LFA_{serum}		*Se*	0.477 (0.399; 0.557)
		Sp	0.999 (0.994; 1.000)
LFA_{blood}		*Se*	0.474 (0.396; 0.554)
		Sp	0.994 (0.987; 1.000)
RBT		*Se*	0.904 (0.834; 0.955)
		Sp	0.999 (0.993; 1.000)
CFT		*Se*	0.593 (0.512; 0.672)
		Sp	0.998 (0.992; 1.000)
LFA_{serum} & LFA_{blood}		cdp[b]	0.222 (0.2; 0.237)
		cdn[c]	0 (-0.003; 0.005)
		covcdp[d]	1.799 (1.63; 1.914)
		covcdn[e]	2.383 (0.082; 8.933)
RBT & CFT		cdp	0.004 (-0.022; 0.039)
		cdn	0 (-0.003; 0.004)
		covcdp	0.026 (-0.161; 0.229)
		covcdn	6.53(0.226; 31.31)
Prevalence			
Zimbabwe	Sub1	*p*	0.14 (0.111; 0.175)
	Sub2	*p*	0.088 (0.06; 0.124)
	Sub3	*p*	0.296 (0.235; 0.363)
Botswana	Sub1	*p*	0.01 (0.003; 0.02)

Sub1 = Smallholder cattle farming areas where brucellosis is endemic.
Sub2 = Small scale commercial farms (low prevalence).
Sub3 = Commercial farms.
[a] The RBT/CFT; $_{LFAserum/blood}$ are based on simultaneous testing.
[b] cdp refers to conditional dependence between sensitivities.
[c] cdn refers to conditional dependence between specificities.
[d] covcdp refers to covariance between sensitivities.
[e] covcdn refers to covariance between specificities.

(0.002; 0.018).

The data indicated that both the *Se* and *Sp* of RBT and CFT were conditionally independent as shown by the 95 % PrIs for the covariance terms that included zero. The *Sp* of the LFA_{serum} and LFA_{blood} were conditionally independent, while their *Se* were conditionally dependent (Table 3).

Sensitivity analysis showed that the estimates were dependent on the specified priors, but the results were qualitatively similar. Table 4 summarizes the obtained results using non-informative priors.

4. Discussion

This study used BLCMs to estimate the *Se* and *Sp* of RBT, CFT, LFA_{serum} and LFA_{blood} in the absence of a reference standard for the detection of antibodies to *Brucella* spp. in cattle from different managements systems in Botswana and Zimbabwe and estimate the true prevalence of brucellosis in these areas. Bovine brucellosis caused by biovars of *Brucella abortus* is a chronic disease which may undergo periods of quiescence, depending on host factors (Poester et al., 2013). The propensity of the bacteria to colonise the female and male reproductive tracts accounts for the most common clinical signs of abortion in pregnant cows and epididymitis/orchitis in bulls. Infection activates both cell-mediated and humoral immune response, where specific immunoglobulin M (IgM) antibodies start to develop during the acute stages and remain present for several weeks to months (Smit et al., 2003). The immunoglobulin G (IgG) antibodies (both IgG1 and IgG2 subtypes) appear during the chronic phase of infection and remain present for months after the initial infection (Lamb et al., 1979). In recurrent infections, IgG but not IgM antibodies usually are present (Smits et al., 2003). The RBT detects mainly the agglutinating antibodies (mainly IgM), but when used at acidic pH, it may be able to detect both agglutinating and non-agglutinating antibodies (Luwumba, 2019), while the CFT predominantly detects non-agglutinating antibodies (IgG). While the LFA may be designed to detect either the IgM, or the IgG (Smits et al., 2003), the assay used in this study was a composite kit designed to detect both the IgM and IgG antibodies. Therefore, it is likely that the tests used in our study were able to detect antibodies to *Brucella* spp. at all stages of the infection process. However, the failure of these tests to discern the IgM of field *Brucella* spp. from some Gram-negative bacteria like, *Escherichia coli* O:157, *Salmonella* spp., and *Sternotrophomonas maltophilia* (Nielsen, 2002; Nielsen et al., 2004) is of major concern. Similarly, the use of the *B. abortus* S19 vaccination is likely to result in serological cross-reactions due to the residual effect of IgM antibodies (Lamb et al., 1979). It has been argued that these serological cross reactions may be reduced or completely eliminated by testing cows above two years where calfhood *B. abortus* S19 vaccination was practiced (Madsen, 1993), or by using specific tests such as c-ELISA or the CFT detecting IgG antibodies (Nielsen et al., 2004). Further, given that the majority of our positive cattle (87.4 %; 76/87) were sampled from smallholder and small-scale commercial farms where *B. abortus* S19 vaccine was not used, then it is likely that most of the positive reactive tests were due to antibodies produced by field strains of *Brucella* spp.

In our study, the RBT showed the highest point estimate for *Se*, compared to the other tests. Although the observed *Se* of RBT is slightly lower, it is comparable with what has been reported in previous studies for the diagnosis of brucellosis in cattle, namely 0.945 (Matope et al., 2011b), 0.93 (Muma et al., 2007), and 0.96 (Samartino et al., 1999). But, there have been reports documenting low *Se* of RBT, including 0.41 (Arif et al., 2018), 0.57 (Ahasan et al., 2017) and 0.78 (Sutherland, 1984), indicating that this parameter may be influenced by other factors such as animal populations, the status of the disease in the population, and the presence of cross-reacting antibodies from closely related Gram-negative bacteria (Greiner and Gardner, 2000). Although such discrepancies may call for a review as suggested (Arif et al., 2018), studies in Southern Africa (Muma et al., 2007; Matope et al., 2011b; Chisi et al., 2017) have consistently reported high *Se* of RBT, thereby justifying its use as a screening test in brucellosis control programmes. The current sensitivities of the LFA_{serum} and the LFA_{blood} are lower than what was previously documented in Portuguese cattle (0.9) (Abdoel et al., 2008), Egypt (0.944) (Elshemey and Abd-Elrahman, 2014) and Cameroon (0.87) (Bronsvoort et al., 2009). In Portugal/Spain, the *Se* of 0.90/0.966, 1.00/0.957, 0.9/0.94 and 0.73/0.923 were reported in cattle, goats, sheep and pigs, respectively and was found to vary according to animal species (Abdoel et al., 2008; Gusi et al., 2019). Similarly, the *Se* of 0.87 and 0.96 were reported in water buffalo and humans, respectively (Smits et al., 2003; Shome et al., 2015). While these data seem to suggest that animal species is an important determinant of the *Se* of the LFA, the role of other factors such as the epidemiology of the disease and the characteristics of the animals need to be explored further. It has been reported for the RBT (Muñoz et al., 2012) and recently for the LFA (Gusi et al., 2019), that the method of analysis could influence the observed test parameters, especially the sensitivity. When the RBT was assessed with reference sera as the reference standard, both the *Se* and *Sp* of RBT were 1.00 but, the *Se* was significantly lower (0.75) while the *Sp* was almost the same (0.99) when BLCMs were applied on sera from naturally infected cattle in the field (Muñoz et al., 2012). As reiterated elsewhere (Arif et al., 2018), this potential discrepancy in the *Se* suggest that further studies are required to determine the explicit factors that may influence the test parameters before the LFA can be recommended as a screening test for bovine brucellosis.

The CFT showed the highest (though not statistically significant) *Sp* compared to the other tests. The observed high specificity of the CFT agrees well with what has been documented in literature (OIE, 2017; Stemshorn et al., 1985). Similarly, it is noteworthy that the high *Sp* of the LFA observed in this study corroborates other studies in Spain (1.00) (Bronsvoort et al., 2009), Egypt (1.00) (Elshemey and Abd-

Elrahman, 2014) and Cameroon (0.97) (Bronsvoort et al., 2009). The LFA has been reported to have a superior *Sp* in other animals as well as in humans (Smits et al., 2003; Gusi et al., 2019). Based on its high *Sp*, the CFT has been used as a confirmatory test in many brucellosis eradication campaigns, and is one of the prescribed tests for bovine brucellosis for the purpose of international trade of livestock and products (OIE, 2017). However, similar to the observations of Matope and coworker (Matope et al., 2011b), the CFT is cumbersome and requires experienced technicians, which may preclude its wide use in resource-limited developing countries. Many brucellosis test regimens require tests that are simple, cheap, cost effective and technically less demanding. Thus, given, the high *Sp* of the LFA, and coupled with its simplicity and the ability to be adapted for field use as it can be used to test whole blood, then it provides a suitable option for use as a confirmatory test for bovine brucellosis. This is also supported by the fact that the *Sp* of LFA is approximately equal to that of the CFT, which is an OIE approved confirmatory test (OIE, 2017). Thus, a brucellosis testing regimen which uses the RBT and LFA as screening and confirmatory tests, respectively, needs to be carefully considered.

The assumption of constant *Se* and *Sp* implies that the distribution of the various infection stages is similar across the different subpopulations. If this is not true, the assumption of constant *Se* and *Sp* does not hold. However, in our case, the low prevalence estimates across all subpopulations is an indirect indication that this assumption holds (Kostoulas et al., 2017). A major advantage of Bayesian latent class methods is that these models capture all latent stages of infection and thus provide unbiased diagnostic accuracy estimates and true disease prevalence estimates. This was crucial as the cross sectional study design resulted in collection of samples from animals at different levels of the disease. Considering that most of the study animals from Zimbabwe and all from Botswana were drawn from areas where *B. abortus* S19 vaccine was not used, thus, the serological reactions were likely to be due to exposure to field *Brucella* spp. In these herds, antibodies are likely to persist for a long time after seroconversion and can easily be detected by serological tests. Infections where antibody responses persist for a long period are ideal for the application of BLCMs (Branscum et al., 2005).

In this study, we used a model that assumed conditional dependence among the tests because they were based on the same biological principle: detection of antibodies. The data suggested that the RBT and CFT were conditionally independent as the 95 % PIs of the covariance terms for Se and *Sp* included zero. However, the *Se* of the LFA_{serum} and LFA_{blood} were conditionally dependent, while their *Sp* were not. In addition, to check the assumption of constant test accuracy across subpopulations separate analyses on two populations (Zimbabwe and Botswana) were run (Georgiadis et al., 2003). These analyses revealed similar results from those obtained with four subpopulations.

The estimated true prevalence of brucellosis in cattle sub-population 1, sub-population 2 and sub-population 3 in Zimbabwe and in Botswana is generally low. Similarly, the observed brucellosis seroprevalence data in the present study agree with previous reports in Zimbabwe (Matope et al., 2011a; Gomo et al., 2012; Ndengu et al., 2017) and Botswana (Alexander et al., 2012), indicating that the disease definition has neither been altered by the inclusion of the LFAs in the testing regimen, nor the use of BLCMs. Therefore, the BLCMs may be used for estimating true prevalence of disease in addition to an advantage of concurrently evaluating the *Se* and *Sp* parameters of the tests. BLCMs have been used in similar studies for the diagnosis of brucellosis in cattle and other species both in Africa and Europe (Abdoel et al., 2008; Muma et al., 2007; Matope et al., 2011b; Gorsich et al., 2002). To our knowledge this appears to be the first field BLCM evaluation of the *Se* and *Sp* of the LFA for the detection of antibodies to *Brucella* spp., in cattle in southern Africa.

5. Conclusion

In conclusion, the BLCMs revealed that the RBT exhibited the highest *Se* for the detection of antibodies to *Brucella* spp. in cattle from predominantly smallholder farms in Zimbabwe and Botswana, in comparison with the CFT and the two LFAs (LFA_{serum} and LFA_{blood}). The CFT exhibited a higher (but not statistically significant) *Sp* compared to the LFA_{serum} and the LFA_{blood}. None of the tests evaluated had perfect *Se* and *Sp*. Consequently they could not be used alone for the diagnosis of brucellosis in cattle from smallholder areas. Thus, a brucellosis testing regimen using the RBT (screening) and the LFA (confirmatory) is proposed.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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