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REGULAR ARTICLES

Bovine and Caprine Brucellosis in Bangladesh: Bayesian evaluation of four serological tests, true prevalence, and associated risk factors in household animals

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Abstract A cross-sectional study was carried out to estimate the true prevalence of *Brucella* spp. and identify allied risk factors/indicators associated with brucellosis in the Dinajpur and Mymensingh districts of Bangladesh. A total 320 stratified random blood samples were collected and tested in parallel for *Brucella* antibodies using Rose Bengal (RBT), slow agglutination (SAT), and indirect and competitive ELISA. In addition, a structured questionnaire was administered to each household herd owner to gather information regarding potential risk factors. Both univariate and multivariate logistic regression analyses were used to identify potential risk factors or indicators at animal level. A Bayesian approach was used to estimate the true prevalence of brucellosis along with the test performances (Se and Sp). The estimated animal level true prevalence in cattle was 9.70 % (95 % CPI 5.0–16 %) and in goat 6.3 % (95 % CPI 2.8–11.0 %). The highest sensitivity was achieved by SAT ranges from 69.6 to 78.9 %, and iELISA was found to be more specific (97.4 to 98.8 %) in comparison with other tests. On the other hand, a significant level of ($P < 0.05$) *Brucella* seropositivity was found in cattle that breed naturally compared with those that undergo artificial insemination. In goats, exotic breeds were significantly associated ($P < 0.05$) with *Brucella* seroprevalence compared with indigenous breeds. Goats with a previous records of abortion and/or retained placenta were also found to have significant levels ($P < 0.05$). Cows with previous abortion records showed higher odds (18 times) of being seropositive. None of the evaluated tests can be recommended to apply alone for the diagnosis of bovine and caprine brucellosis.

Keywords Brucellosis · Seroprevalence · Bayesian analyses · Test characteristics · Risk factors · Goats · Cattle

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Introduction

Brucellosis is recognized as one of the most important zoonotic infections to have accompanied human civilization for over 2.5 million years (D'Anastasio et al. 2011). The disease is caused by bacteria under genus *Brucella*, comprising at least 10 species, each with a preferred natural host (Olsen and Palmer 2014). The most prevalent species in livestock include *B. abortus* (preferred host cattle), *B. melitensis* (preferred host small ruminants), and *B. suis* (preferred host pigs). The importance of the disease is widely known because of its vast economic impact on the livestock industry and severe human health hazards (Ariza et al. 2007). Brucellosis is ubiquitous in a wide host range except in those countries where bovine brucellosis (*B. abortus*) has been eradicated, with no reported cases for at least 5 years (Seleem et al. 2010).

Isolation and identification of *Brucella* organisms are considered the gold standard for confirmatory and accurate diagnosis of brucellosis (Alton et al. 1988). However, this technique is quite impractical in Bangladesh because of the absence of sophisticated laboratory facilities (BSL 3), adequate

safety for laboratory personnel, and limited resources, as well as the huge costs involved when a large number of samples need to be processed. Therefore, conventional serological tests and primary binding tests have been used worldwide, including in Bangladesh, for screening as well as confirmatory diagnosis. Problems that interfere with the frequent use of these serological tests are their wide range of sensitivity and specificity (Sanogo et al. 2014). To overcome this situation, a combination of tests is usually performed together to improve test performance and outcome of the study. The test performance (Se and Sp) of any diagnostic test can be evaluated using different approaches; Bayesian analysis is one of them (Speybroeck et al. 2013). This approach has gained considerable acceptance in scientific communities as it allows test results to be combined with prior information to draw inferences regarding diagnostic sensitivity (Se) and specificity (Sp) of multiple imperfect tests.

Brucella infection in animals is believed to be influenced by a number of epidemiological factors (Godfroid et al. 2011). These factors have been classified into one of three categories related to the biology of disease, animal population characteristics, or animal management practices. Although the importance of brucellosis is widely known, the benefits of determining, understanding, and interpreting the risk factors associated with *Brucella* seroprevalence in different demographical and environmental situations need to be assessed. Accurate identification and evaluation of the impact of allied intimate risk factors are essential for the development of a cost-effective and efficient brucellosis control program.

Although previous sero-surveys in Bangladesh have indicated variable levels of seropositivity (Islam et al. 2013; Rahman et al. 2009; Rahman et al. 2011; Uddin and Rahman 2007) for brucellosis, no vaccination has been yet considered in any animal species. Normally, seropositivity of a disease often indicates the presence of a larger problem than immediately shown by the absolute number of serologic reactors detected in current conditions. Moreover, risk factors associated with brucellosis seropositivity are not well described and identified. Although a number of risk factors (age, abortion record, retention of placenta) have been identified in certain parts of the country, prevalence of the disease may vary depending on different geographical and environmental conditions (Ahasan et al. 2010; Amin et al. 2005; Islam et al. 2013; Uddin and Rahman 2007). Therefore, the aim of the current study was to estimate the true exposure prevalence of brucellosis in cattle and goats along with diagnostic test characteristics (sensitivity and specificity) of performed serological tests in Bangladesh. Identifying the potential risk factors associated with *Brucella* infection was also a priority. The results of the study may serve to improve the public health and livestock economy of the country through consideration and implementation of effective control strategies for brucellosis. In addition, the study may play an important role in increasing food security and improving the value of trade in livestock and their products.

Materials and methods

Study areas

A cross-sectional study was carried out in two major districts of Bangladesh including Dinajpur (Old Himalayan Piedmont Plain) and Mymensingh (Old Brahmaputra Flood plain) between June 2009 and October 2009.

Dinajpur is situated in the northern part of Bangladesh between 25.38° N, 88.39° E and 25.63° N, 88.65° E and has around 0.99 million cattle and 0.76 million goats. The Mymensingh District accommodates approximately 1 million cattle and 0.72 million goats and is located between 24.6343° N, 90.2677° E and 24.634167° N, 90.2675° E. Both districts are the highest rearing areas of cattle and goats in the Rangpur and Dhaka divisions of Bangladesh. Most people in these two districts live in rural areas and keep a small number of household animals (<15).

Survey design and selection of animals

A stratified random sampling was carried out where each individual district was considered as strata (Dohoo et al. 2003). The formula $n = 1.96^2 p(1-p)/d^2$ was used to calculate the sample size with a confidence level of 95 %, desired absolute precision of 5 %, expected seroprevalence p of 10 % in both cattle and goats based on previous studies (Amin et al. 2005; Uddin et al. 2007). Approximately 138 cattle and 138 goat samples would be needed for the study. But, after the addition of a 10 % sample as a reserve supply, the sample size had a total of approximately 160 for both cattle and goats. An identical number of samples (160 for both cattle and goats) were considered for each stratum in order to minor variation in cattle and goat population in between the strata. At first, lists of household herds (cattle and goats) were obtained from the respective district's veterinary departments. Thereafter, a random selection of 160 animals was sampled for each stratum using Microsoft® Office Excel 2010. One animal was sampled randomly from each household herd through a mechanical procedure. Animals of each herd were assigned with a unique number in a separate strip. The strips were then put in a bowl and mixed thoroughly. Each strip was extracted with closed eyes to select individual animal. Importantly, true random sampling for every animal inside the herds was not achievable for each situation because of the unwillingness of some owners to allow sampling especially from calf and pregnant animals. In that situation, the strip was extracted for the second time.

Questionnaire and created variables

An interview-based, structured questionnaire was administered to each household herd owner by the author to gather information on potential risks during blood collection (Appendix C). The animal level covariates including species, herd size, location, age, sex, breed, abortion records, retention of placenta, and breeding type were recorded by face-to-face interview and visual inspection. The farmers' memories were also used to obtain information about age, retention of placenta, abortion, and breeding type. All household animals in the study areas were not exposed to any vaccination against brucellosis.

Blood collection, processing, and preservation

Blood samples were collected for serological diagnosis of *Brucella* specific antibodies. About 5–7 ml of blood was collected from the jugular vein of each animal with the help of sterile disposable syringes and needles in a properly labeled screw capped test tube without anticoagulant. After collection of blood, the test tube was kept undisturbed on a tray for 1 h at room temperature in a slightly inclined position to facilitate the clotting and separation of serum. The test tube was further refrigerated at 4–8 °C overnight. Later, the sera were separated from blood clots and centrifuged at 2500 rpm for 10 min to obtain clear sera free from blood cells. Finally, sera were transferred into a sterilized Eppendorf tube and stored at −20 °C until used.

Serological tests

Four serological tests were used in parallel for diagnosis of *Brucella*-specific antibodies in cattle and goats, including Rose Bengal Test (RBT), slow agglutination test (SAT), and enzyme-linked immunosorbent assay (both indirect and competitive).

The RBT was performed as described by Alton et al. (1988). All control sera (negative and positive) and the buffered *Brucella abortus* S99 antigens used in this study were obtained from the Veterinary Laboratory Agency (VLA), Weybridge (UK). Any visible agglutination was considered as positive and graded according to the degree of agglutination, namely grade 1(+) as low, grade 2(+) medium, and grade 3(+) high agglutination.

SAT was carried out using ethylene-diamine-tetraacetic acid (EDTA) according to the protocol described by Garin et al. (1985). The SAW (Synbiotics, concentrated suspension of *B. abortus*, Weybridge, strain 99) buffer was prepared by adding 0.93 g EDTA (5 mM, Triplex®) with 500-ml phosphate-buffered solution (PBS), which in turn was prepared by adding five tablets of PBS (DULBECCO-A Oxoid) in 500-ml distilled water (at 1 tablet/100 ml distilled water). The antigen SAW was diluted at 1 ml antigen with 19-ml SAW buffer solution. For every group of samples tested, a positive control serum was included. Reaction was observed using a magnifying mirror against an illumination source. Serum antibody titers ≥25 IU/ml were considered as positive following EU guidelines.

Both competitive ELISA (cELISA) and indirect ELISA (iELISA) were performed and interpreted using commercial kits following the manufacturer's instructions (Svanova Biotech AB, article number 10-2701-02 and 10-2700-10, SE-751 83 Uppsala, Sweden).

Statistical analysis

Statistical data from the questionnaires on animal level factors and laboratory test results were stored in Microsoft Excel 2010 before the data was transferred to STATA/IC 12.0 for Windows (StataCorp. College Station, Texas, USA), WinBUGS (Windows version of Bayesian Analysis Using the Gibbs Sampler) 14 (version 1.4), and R software (version 3.0).

Bayesian analysis

Model building

In the absence of a "gold standard" test, the true prevalence of brucellosis and diagnostic test characteristics (Se and Sp) of performed serological tests were estimated separately for cattle and goats through a Bayesian framework in WinBUGS 1.4 (Spiegelhalter et al. 2003) applying models described by Branscum et al. (2005) and Berkvens et al. (2006). In a four-test scenario, under the assumption of conditional independence, a model based on multinomial distribution, a total of nine parameters need to be estimated, including the prevalence, sensitivities (Se), and specificities (Sp) of the four tests. Alternatively, under the assumption of conditional dependence, a multinomial model including all possible interactions among the four tests required 31 parameters to be estimated. These included the prevalence, sensitivity and specificity of the first test, two conditional sensitivities and two conditional specificities of the second test, four conditional sensitivities and four conditional specificities of the third test, and eight conditional sensitivities and eight conditional specificities of the fourth test. However, this model is in fact inestimable as the data (16 classes of test results) allows only 15 degrees of freedom. Hence, the model building strategy of incorporating prior knowledge was applied to reduce the number of parameters to be estimated and make the model identifiable. In this regard, specificity of a test is equal to 1, meaning that all parameters including conditional term 1 can be dropped. Moreover, constraints can be imposed on a subset of

parameter to reduce the possible ranges of values of the specific parameter. This reduction may furthermore affect the possible range of values of other parameters. Assuming and/or estimating the level of reduction in the numbers of parameters to be estimated due to the complexity of the model is impossible. However, as the four tests are based on a common principle (detection of the antibody against *Brucella* infection), the test characteristics were assessed considering all conditionally independent (Pouillot et al. 2002) as well as conditionally dependent (Berkvens et al. 2006).

To evaluate the influence of entry order of different tests on the posterior estimates of different models (Gyorke et al. 2011), a total of three different permutations were used in the study. The entry order was varied between entering the test with the highest or lowest positive test results at first and other places between the tests randomly with other remaining tests.

Criteria for assessing the best fit between the test results and prior information were based on the deviance information criterion (DIC), the number of parameters effectively estimated by the model (pD), and the Bayesian *P* value (Bayesp) found in WinBUGS 1.4. The DIC and pD values obtained from WinBUGS 1.4 were compared with the DIC and pD values found in R software (version 3.0) for increasing accuracy. Models with a smaller DIC got preference compared to those with a larger DIC (Berkvens et al. 2006). A Bayesian *P* value is the posterior predictive and illustrates lack-of-fit of the model to the data by calculating the difference between the deviance of the observations and deviance of the observations that are randomly generated from fitted model. Hence, the Bayesian *P* value was always expected to be around 0.50, as the posterior probability for the multinomial probabilities would be flat, indicating that the model had been overspecified perfectly (Kelly and Smith 2011). Posterior inference was done by calculating means and 95 % posterior credible intervals (95 % CIs) of the sensitivity and specificity of four tests. The WinBUGS and R codes used are presented in Appendix A and B.

Prior derivation

As the Bayesian approach allows combining prior information on test sensitivity and specificity with the diagnostic test results at hand, both deterministic and probabilistic constraints (prior information) were used during the analysis. Prior information relating to RBT (Se, 0.21- 1; Sp, 0.68- 1), SAT (Se, 0.291- 1; Sp, 0.992- 1), iELISA (Se, 0.925- 1; Sp, 0.905- 1), and cELISA (Se, 0.905-1; Sp, 0.997- 1) was obtained from available literature and kit Manufacturer Companies (VLA, Weybridge, UK; Svanova Biotech AB, Uppsala, Sweden). In addition to these, expert opinion sought from the Department of Biostatistics and Epidemiology, Institute of Tropical Medicine, Belgium, was also a priority. During the analyses, specificity (Sp) of SAT and cELISA was assumed as 100 % (one) in order to reduce the number of estimable parameters and make the model identifiable.

Convergence diagnostics

The models were implemented in WinBUGS, which uses Markov Chain Monte Carlo (MCMC) sampling to obtain the joint posterior distribution of the model. All models were run using three MCMC chains. The first 10,000 samples of MCMC chain as initial burn-in, later the following 10,000 iterations, were used for computing posterior inference. Convergence of the chain after initial burn-in was determined by visual inspection on the trace dynamic simultaneously combined with autocorrelation plots, time series plots, or history of the variables (Ntzoufras 2011). Another formal test, the Brooks–Gelman–Rubin (BGR) statistic, was also applied to explore convergence (Gelman and Rubin 1992).

Descriptive epidemiology For risk factor analyses, an individual animal was considered *Brucella* seropositive if it was tested positive in at least two serological tests. This condition aided to minimize the occurrence of misclassification and enhance the chance to detect *Brucella*-specific antibodies when present in a given serum. The outcome (dependent) variable was animal level seroprevalence status for each test coded as 1 (positive animal) versus 0 (negative animal). All the independent variables used in analyses were categorical: information on locality of origin (Dinajpur, Mymensingh), species, age (≤4 years versus >4 years), sex (male, female), breed (indigenous, exotic), herd size (≤5, 5–10, >10 animals), abortion record (no, yes), retention of placenta (no, yes), and breeding type (for cattle: naturally and AI; for goat: inbreeding and outbreeding). The percentage of *Brucella* seropositives was calculated by taking into account the proportion of positive sera against the total number sampled.

Logistic regression analyses Two logistic regression models were built separately for cattle and goats data to test significant associations with *Brucella* seropositivity. At first, univariable association was analyzed between the binary outcome and all explanatory variables. Only the explanatory variables that were statistically significant at the 10 % level in the univariate analyses were considered for multivariable logistic regression analysis. Significant correlation between the explanatory variables was assessed through cross tabulation using Fisher's exact test. Where two variables were found to be highly correlated, only the preferred one was included in the multivariable logistic regression model, based on the one believed to have more biological relevance of correlation (Degefa et al. 2011).

Multivariable logistic regression analysis was performed using a model building strategy described by Hosmer and Lemeshow (1989). A preliminary reference model was

estimated with all explanatory variables that were selected based on the univariate analysis. Later, a backward elimination procedure was used by applying an iteration maximum-likelihood estimation procedure followed by dropping the least significant explanatory variable until all remaining predictor variables were significant ($P < 0.05$). The explanatory variable was reentered which was not in the model and remained if significant. The statistical significance of explanatory variables was tested using a likelihood-ratio test (Dohoo et al. 2003). Interaction between variables was assessed by constructing two-way interaction product terms for the main significant effects of variables and forcing the variables into the model for examining the changes in the coefficient and *P* values. Odds ratio with 95 % confidence intervals were estimated to interpret the results of all categorical explanatory variables.

Results

Bayesian estimation

A total of 320 (160 cattle and 160 goats) individual sera samples were tested in parallel for *Brucella* spp.-specific antibodies by RBT, SAT, cELISA, and iELISA respectively. Bayesian estimation was conducted using dichotomized tabulated results of four different tests in three different permutations. The results shown in Table 1 reveal that 4.37 % (7/160) of all cattle were positive for all four tests, whereas 89.38 % (143/160) tested negative. Likewise, 3.12 % (5/160) goats were found positive for all four tests and 91.88 % (147/160) tested negative.

DIC, pD, and Bayesian *P* value of well-converged models in both conditions (tests conditionally independent and conditionally dependent) are shown in Table 2. Under the assumption of conditional independence for both species in three different permutations, the Bayesian *P* value of 1 or around 1 suggested a lack-of-fit of the model indicating conditional independence did not exist. However, with regard to conditional dependence, the Bayesian *P* value was found to be around 0.50, suggesting a good model fit. Moreover, the lowest DIC value was obtained in the models with the assumption that the tests were conditionally dependent compared to models with tests that were conditionally independent. Therefore, later, Bayesian estimation was carried out assuming that the tests were conditionally dependent.

True prevalence

The true prevalence of brucellosis was calculated using a Bayesian approach, where cattle showed the highest seroprevalence with a mean 9.70 % (95 % CI 5, 16 %) compared with goats with a mean of 6.30 % (95 % CI 2.8, 11 %) (Table 3).

Table 1 The tabulated dichotomized results of four diagnostic tests in three different permutations

Test status[a]	Permutation 1		Permutation 2		Permutation 3	
	Cattle	Goats	Cattle	Goats	Cattle	Goats
1111	7	5	7	5	7	5
1110	0	0	0	0	0	0
1101	0	0	0	0	0	0
1100	1	1	1	1	1	1
1011	0	0	0	0	0	0
1010	1	0	0	0	0	0
1001	0	0	0	1	1	0
1000	3	1	1	0	3	1
0111	0	0	0	0	0	0
0110	0	0	1	0	0	1
0101	0	1	0	0	0	0
0100	1	0	3	1	1	0
0011	1	0	1	0	1	0
0010	1	4	1	4	2	1
0001	2	1	2	1	1	4
0000	143	147	143	147	143	147

[a] 1: test positive and 0: test negative; permutation 1: SAT, cELISA, RBT, iELISA; permutation 2: cELISA, SAT, RBT, iELISA; and permutation 3: SAT, cELISA, iELISA, RBT

Diagnostic test characteristics (Se and Sp)

Using dichotomized tabulated results of four (4) different tests in three different permutations, the diagnostic test characteristics (Sensitivity and Specificity) were estimated using Bayesian analyses along with prior information. Well-converged models were selected based on the DIC, pD, and Bayesian *P* values of the models (Table 2). The posterior mean with 95 % credibility intervals (CI) of the test sensitivities (Se) and specificities (Sp) is presented in Table 3. Results show that different entry orders of the tests were not able to affect the posterior estimates. iELISA exhibited the highest mean Sp, ranging from 97.4 to 98.8 %, while SAT was the most sensitive (Se 69.6 to 78.9 %) among the four tests. In SAT, no consistent difference was observed in the posterior sensitivity estimates between species. In cattle, the mean estimate ranged from 73.7 to 78.9 % and was slightly lower in goats at 69.6 to 72.8 %. In goats, a consistently higher sensitivity was revealed in cELISA (69.2–72.5 %), iELISA (60.8–61.2 %), and RBT (58.3–58.9 %) compared with cattle (58.8–59.6, 53.8–54.9, 56.7–58.2 %). For RBT, cattle exhibited high posterior estimates where mean Sp ranges from 98.3 to 98.5 % and in goats 97.5 to 97.6 %.

Table 2 Bayesp, pD, and DIC values of well-converged models for three different permutations of four tests assuming conditional independence and conditional dependence

	Permutation	Model	WinBUGS			R software	
			Bayesp	pD	DIC	pD	DIC
Conditional independence	1	Cattle	1.0	4.853	75.854	4.868	75.869
		Goat	0.980	4.691	42.246	4.677	42.232
	2	Cattle	0.999	5.151	61.642	5.154	61.646
		Goat	0.980	4.691	42.246	4.666	42.221
	3	Cattle	1.0	4.863	75.882	4.859	75.878
		Goat	0.980	4.645	42.177	4.625	42.157
Conditional dependence	1	Cattle	0.555	6.355	39.824	6.497	39.966
		Goat	0.556	4.829	31.984	4.901	32.056
	2	Cattle	0.553	6.261	39.918	6.347	40.003
		Goat	0.560	4.808	32.001	4.910	32.104
	3	Cattle	0.510	5.904	38.473	6.027	38.596
		Goat	0.550	4.773	31.816	4.957	32.000

Bayesp Bayesian *P* value, *pD* number of parameters effectively estimated by the model, *DIC* Deviance Information Criterion

Table 3 The posterior mean for prevalence and test characteristics together with 95 % posterior credibility intervals of four (4) tests in three different permutations

Permutation	Test	Parameters	Posterior mean (95 % posterior credible interval)	
			Cattle	Goat
1	SAT	Prevalence	0.097 (0.050–0.16)	0.063 (0.028–0.11)
		Se	0.786 (0.47–0.971)	0.728 (0.37–0.96)
		Sp	1	1
	cELISA	Se	0.596 (0.33–0.82)	0.698 (0.37–0.92)
		Sp	1	1
	RBT	Se	0.578 (0.33–0.79)	0.587 (0.31–0.82)
		Sp	0.985 (0.96–0.998)	0.976 (0.96–0.994)
	iELISA	Se	0.549 (0.33–0.75)	0.608 (0.35–0.82)
		Sp	0.974 (0.94–0.994)	0.987 (0.96–0.998)
2	cELISA	Prevalence	0.102 (0.05–0.18)	0.063 (0.03–0.12)
		Se	0.588 (0.29–0.84)	0.725 (0.37–0.96)
		Sp	1	1
	SAT	Se	0.737 (0.42–0.94)	0.696 (0.37–0.93)
		Sp	1	1
	RBT	Se	0.567 (0.31–0.78)	0.583 (0.31–0.82)
		Sp	0.985 (0.96–0.999)	0.975 (0.96–0.994)
	iELISA	Se	0.545 (0.31–0.75)	0.612 (0.36–0.83)
		Sp	0.975 (0.94–0.995)	0.987 (0.96–0.998)
3	SAT	Prevalence	0.097 (0.05–0.16)	0.064 (0.03–0.12)
		Se	0.789 (0.48–0.97)	0.721 (0.36–0.96)
		Sp	1	1
	cELISA	Se	0.589 (0.34–0.82)	0.692 (0.36–0.93)
		Sp	1	1
	iELISA	Se	0.538 (0.31–0.76)	0.612 (0.32–0.85)
		Sp	0.977 (0.95–0.997)	0.988 (0.96–0.999)
	RBT	Se	0.582 (0.36–0.78)	0.589 (0.35–0.80)
		Sp	0.983 (0.96–0.997)	0.976 (0.96–0.994)

SAT slow agglutination test, *cELISA* competitive ELISA, *iELISA* indirect ELISA, *RBT* Rose Bengal test, *Se* sensitivity, *Sp* specificity

Logistic regression analyses

The differences between *Brucella* seropositivity per each risk factor categories and their associations are summarized in Table 4 and in Table 5. The results for univariate logistic regression analyses (ULRA) in cattle showed that no statistically significant difference was observed between brucellosis seropositivity and gender of cattle ($P = 0.313$), breed ($P = 0.143$), location ($P = 0.516$), herd size ($P = 0.552$, 0.936), or animals with a history of retention of placenta ($P = 0.306$). On the other hand, seroprevalence is significantly higher in cattle aged over 4 years ($P = 0.037$) that breed naturally ($P = 0.007$) and with a previous abortion record ($P = 0.002$).

In multivariable logistic regression analyses (MLRA), breeding type ($P = 0.042$) and previous abortion record ($P = 0.052$) remained significant risk factors/indicators for *Brucella* infection, while age ($P = 0.261$) was no longer significant. The odds for the cattle that breed naturally and with a previous abortion record to be infected with *Brucella* sp. were respectively 6.0 and 7.3 times higher than those bred by artificial insemination and with no previous record of abortion.

In goats, during univariate logistic regression analyses, a highly significant association was revealed between *Brucella* seropositivity and breed ($P = 0.040$) of the animal. Goats with a previous records of abortion ($P = 0.029$) and retention of placenta ($P = 0.011$) also exhibited significant *Brucella* seroprevalence. Moreover, when explanatory variables were accessed for collinearity by cross tabulation using Fisher's exact test, a highly significant correlation ($P = 0.001$) was revealed between goats with a previous record of abortion and retention of placenta. In goats, the variables of age, sex, herd size, and breeding type showed no significant association in univariate regression models.

In multivariable logistic regression analyses, exotic breeds of goat ($P = 0.042$) and goats with a previous abortion record ($P = 0.012$) remained significant risk factors/indicators for *Brucella* infection at the animal level. The possibility of exotic breeds being infected was 11.17 times higher than indigenous goats. Moreover, goats with a previous abortion record showed 26.02 times higher odds of *Brucella* infection compared with goats with no previous abortion record.

Discussion

Establishing a successful disease control or eradication program entails knowing the true status of the disease. Taking this into consideration, the main objective of this study was to estimate the true prevalence of brucellosis in cattle and goats and to determine diagnostic test characteristics (Se and Sp) of performed serological tests in the context of Bangladesh. In this study, the estimated true prevalence of brucellosis was respectively 9.7 % (95 % CI 5, 16 %) in cattle and 6.3 % (95 % CI 3, 12 %) in goats. No record of true prevalence exists for brucellosis in cattle in Bangladesh; however, this present finding is in agreement with Rahman et al. (2006) who reported an individual level apparent prevalence of brucellosis in cattle of up to 8.4 %. A lower level of seroprevalence (7.5 and 5 %) was also recorded by Rahman et al. (2009) and Rahman et al. (2010). In goats, the estimated true prevalence (6.3 %) is considerably higher than the 1 % reported previously by Rahman et al. (2013). This difference might be due to the variation in rates of infection in different parts of the country. This present study tested the caprine population of the Dinajpur and Mymensingh districts of Bangladesh, which vary from the study area of Rahman et al. (2013). Similarly, a higher rate of brucellosis seroprevalence (14.8 %) was recorded by Rahman et al. (1988) in caprine species in different areas of Bangladesh.

The characteristics of the four serological tests applied in the study were estimated using Bayesian analyses. This Bayesian approach is frequently used in human and veterinary medical science and has gained considerable acceptance for evaluating diagnostic tests' characteristics (Meyer et al. 2009). The most desirable advantages of Bayesian analysis are that it allows prior information (knowledge) to be taken into account to estimate posterior parameters (Se and Sp) and also provides a true probability interval. Prior information on the estimable parameters can be fixed in a deterministic way or through probability distribution. The prior distribution that was considered in the analyses might not be ideal, but it reflects the central tendency with variation of the sensitivity (Se) and specificity (Sp) of the respective tests from previous studies.

In the study, the assumption of test conditional independence was not true as clearly shown in Table 2. Model that was built considering the tests conditionally independent with or without prior information showed a consistently higher DIC value in comparison with the model developed with the assumption of tests conditionally dependent. The Bayesian *P* value of the models for tests conditionally independent always stayed at or around 1, indicating a poor model fit. On the contrary, well-converged models with tests conditionally dependent showed constantly a desirable (around 0.50) Bayesian *P* value, agreed Berkvens et al. (2006). Moreover, the antibody response was detected in the study against *Brucella* antigens prepared by smooth lipopolysaccharide (SLPS). Basically, all the tests used in this study are based on same biological basis (Nielsen 2002) in detecting anti-*Brucella* SLPS antibodies and therefore could be expected as tests are conditionally dependent (Gardner et al. 2000). Another important approach was the entry order of the test in models, which was thought to influence the posterior estimates. The results revealed that the entry order of the respective tests was not able to influence the test characteristics because no notifiable differences were identified in the posterior estimates for the three different permutations of four diagnostic tests (Table 3), contrary to Engel et al. (2006).

Table 4 Potential risk factors or indicators of Cattle Brucellosis based on Univariate and Multivariate Logistic Regression Model (ULRM)

Variable	Category	Frequency	Positive no. (%)	Univariate analysis			Multivariate analysis		
				OR	*P* value	95 % CI	OR	*P* value	95 % CI
Age	≤4 years	101	3 (2.97)	1	–	–	1	–	–
	>4 years	59	7 (11.87)	4.38	0.037[a]	1.09–17.7	2.46	0.261	0.51–11.81
Sex	Male	38	1 (2.63)	1	–	–			
	Female	122	9 (7.38)	2.95	0.313	0.36–24.0			
Breed	Exotic	53	1 (1.87)	1	–	–			
	Indigenous	107	9 (8.81)	4.78	0.143	0.59–38.73			
Location	Dinajpur	80	4 (5.00)	1	–	–			
	Mymensingh	80	6 (7.50)	1.54	0.516	0.42–5.68			
Herd size	*A* = ≤5	102	7 (6.86)	1	–	–			
	B = 6–10	27	1 (3.70)	0.52	0.552	0.06–4.44			
	C ≥ 10	31	2 (6.45)	0.94	0.936	0.18–4.76			
Retention of placenta	No	108	7 (6.48)	1	–	–			
	Yes	14	2 (14.29)	2.40	0.306	0.45–12.92			
Breeding type	AI	84	2 (2.38)	1	–	–	1	–	–
	Naturally	38	7 (18.42)	9.26	0.007[a]	1.82–47.01	6.00	0.042[b]	1.07–33.65
Abortion record	No	116	6 (5.17)	1	–	–	1	–	–
	Yes	6	3 (50.0)	18.33	0.002[a]	3.03–110.79	7.30	0.052[a]	0.99–54.34

OR odd ratio, *CI* confidence interval

[a] Variables found significant at 10 % level and included in multiple logistic regression

[b] Variables found significant at 5 % level model

Table 5 Potential risk factors or indicators of Goat Brucellosis based on Univariate and Multivariate Logistic Regression Model (ULRM)

Variable	Category	Frequency	Positive no. (%)	Univariate analysis			Multivariate analysis		
				OR	*P* value	95 % CI	OR	*P* value	95 % CI
Age	≤4 years	114	6 (5.17)	1	–	–			
	>4 years	46	1 (2.17)	0.4	0.403	0.05–3.42			
Sex	Male	64	1 (1.65)	1	–	–			
	Female	96	6 (6.25)	4.2	0.189	0.49–35.75			
Breed	Indigenous	124	3 (2.42)	1	–	–	1	–	–
	Exotic	36	4 (11.11)	5.04	0.040[a]	1.07–23.68	11.17	0.042[b]	1.09–113.88
Location	Dinajpur	80	3 (3.75)	1	–	–			
	Mymensingh	80	4 (5.0)	1.35	0.700	0.29–6.24			
Herd size	*A* = ≤5	44	3 (6.82)	1	–	–			
	B = 6–10	77	2 (2.60)	0.36	0.279	0.06–2.27			
	C ≥ 10	39	2 (5.13)	0.74	0.748	0.12–4.67			
Retention of placenta	No	91	4 (4.40)	1	–	–			
	Yes	5	2 (40.0)	14.5	0.011[a]	1.87–112.7			
Breeding type	Inbreeding	10	1 (10.0)	1	–	–			
	Outbreeding	86	5 (5.81)	0.56	0.609	0.06–5.30			
Abortion record	No	89	4 (4.50)	1	–	–	1	–	–
	Yes	7	2 (28.5)	8.5	0.029[a]	1.24–58.10	26.8	0.012[b]	2.06–349.00

OR odd ratio, *CI* confidence interval

[a] Variables are significant at 10 % level and included in multiple logistic regression model

[b] Variables found significant at 5 % level model

Considering the estimates of test characteristics (Se and Sp) in Bayesian analyses, highest sensitivity (Se) was achieved by SAT in cattle, ranging from 73.7 to 78.9 % compared to RBT (56.7 to 58.2 %), cELISA (58.8 to 59.6 %), and iELISA (53.8 to 54.9 %). The highest Se of SAT could be due to consideration of a cutoff value ≥25 IU agglutination as positive during test performance. Another possible explanation of increased Se of SAT might be the inability to detect IgM efficiently, produce in response to cross-reacting bacteria including *Yersinia enterocolitica* O:9, *Salmonella* spp., *Escherichia coli* O: 157 (Nielsen and Yu 2010). A low Se of RBT was also recorded by Muma et al. (2007b) in comparison with cELISA and the Fluorescent Polarization Assay. On the other hand, RBT as a qualitative test for diagnostic characteristics could be affected by the external environment including temperature, status of disease in a population, and even technician expertise. The Se of RBT can also vary depending on the type of antigen used, and recently, it was approved for use in a trial conducted in Europe for standardizing of brucellosis diagnostic test and reagents (Munoz et al. 2012).

In this study, a remarkably high Sp was achieved in goats by iELISA ranges from 98.7 to 98.8 % although an almost similar Sp (98.3 to 98.5 %) was recorded by RBT in cattle. This finding agrees with the findings of others. Highest Sp by iELISA and concurrently by RBT in comparison with competitive ELISA and blocking ELISA was recorded by Munoz et al. (2012). A high Sp by RBT compared to competitive ELISA and FPA was found by Matope et al. (2011). To minimize the bias due to nonspecific sero-reactors, high specificity of a serological test is very important, especially when working on small household herds areas where individual animal seroprevalence of *Brucella* spp. has been established to be low (Matope et al. 2010). On the other hand, sensitivity to any serological test can be increased by lowering the cutoff value. However, of the four tests used in this study, none was sensitive and specific enough to use alone to determine the true status of bovine or caprine brucellosis in Bangladesh. Due to low specificity, the OIE has recommended the discontinuation of SAT for the diagnosis of bovine brucellosis (OIE 2009). However, it is simple, cheap, and if used simultaneously with IgG, detecting test like iELISA will help to determine the stage of infection (acute infection if simultaneous positive in SAT and iELISA) in animals (Godfroid et al. 2010).

Another objective of the study was to identify the potential risk factors or indicators of *Brucella* infection in small household herds of cattle and goats in Bangladesh. In cattle, risk factors/indicators identified for *Brucella* infection using multivariable logistic regression analyses were the breeding type and previous abortion record, although increasing age (≥4 years) was found to be significant in univariable logistic regression analyses. Cattle bred naturally by bulls showed significant association with *Brucella* seropositiviy. This finding is in line with the results of others Muma et al. (2007a) and Rahman et al. (2009). Because of the unwillingness of the farmers to use AI techniques due to the cost, they are more likely to share a stud bull with neighbors, and that could be a potential source of *Brucella* infection, a most common scenario among small household herds farmers in Bangladesh. In this study, when female animals were considered separately, the higher odds (18.33 times) of test seropositive were found in animals with a history of abortion. This finding is in agreement with the observations of others that brucellosis is one of the most important causal agents of abortion, especially in third trimester, and abortion is the most typical outcome of the disease in female animals (Carvalho Neta et al. 2010; Xavier et al. 2009). Among all the reproductive disorders investigated in this study, the highest prevalence of brucellosis was associated with a history of abortion.

In goats, Brucellosis seropositiviy was found to be higher in exotic/cross breeds ($P < 0.05$) compared to local indigenous breeds, which correlates with the observation of Jergefa et al. (2009). Actually, indigenous Black Bengal goats are a highly proliferative multiparous breed and resistant to extreme environmental conditions. Conversely, exotic breeds like Jamuna Pari or others found difficulties acclimatizing to adverse environmental conditions in the country and this could be the reason for high brucellosis seroprevalence in exotic/crossbreeds. However, Radostits et al. (2007) reported no association between the breed of animals. Another important risk indicator, goats with a history of retained placenta or abortion, showed significant association ($P < 0.05$) with *Brucella* infection. In our analyses, a strong correlation was revealed between the history of retained placenta and previous abortion records ($P < 0.002$). The reason is clear as the two variables are believed to have more biological relevance of correlation. Cotyledons are not capable of maturing due to premature delivery of the fetus leading to failure or incomplete removal of fetal membrane after birth and/or abortion. However, this is consistent with the biology of *Brucella* sp. and supports earlier observations (Degefa et al. 2011; Tesfaye et al. 2011).

For both cattle and goats, no significant association was found in terms of gender of the animals. However, the trends of seroprevalence observed in this study agree with Rahman et al. (2011) where an increased level seroprevalence was reported in females compared to males. Basically, this relationship has been shown to vary with different subpopulations of animals in different geographic areas around the world (Chimana et al. 2010; Muma et al. 2006).

In this study, herd size did not have any statistically significant effect on *Brucella* infection, which contradicted many other findings (Chand and Chhabra 2013; Chiebao et al. 2015). Infection is more likely to persist in larger herds because of frequent contact with infected animals and difficulties in controlling exposure to infectious excretions (Al-Talafhah et al. 2003; Kabagambe et al. 2001). Significant association could be related to the typical herd size of animals. A more extensive

farm has large numbers of animals compared to the small household herds in our study. On the other hand, the lack of significant difference in brucellosis seroprevalence between the districts could be due to similarities in livestock husbandry.

In conclusion, although the true prevalence of bovine and caprine brucellosis at the animal level was not high, it deserves immediate attention because of its zoonotic importance. To estimate the actual burden of the disease, a detailed statistically sound surveillance program is recommended among domestic animals and high-risk group occupations throughout the country. Our study showed that, among the four serological tests, none was sensitive and specific enough to apply alone. Therefore, the suggested test regimen of SAT together with iELISA is more likely to identify acutely infected animals and is thus recommended for diagnosis and control of brucellosis in Bangladesh. Moreover, the study explored distinct risk factors linked to the independent association with *Brucella* seropositivity in cattle and goats that are assumed to contribute to establishing an effective brucellosis control program.

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Compliance with ethical standards

Statement of animal rights All National and institutional animal ethics guidelines were followed. Animals were subjected to minor procedures without anesthesia for blood collection. Minimal distress may occur as a result of animal handling.

Conflict of interest statement None of the authors of this article are involved with any personal and or financial relationship with others or any organizations that could inappropriately influence the contents of the article.

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