J. Vet. Med. OH Res. (2022). 4(2): 115-124 p-2664-2352 : ISSN : e-2664-2360
Website: www.lepvmbj.org DOI: 10.36111/jvmohr.2022.4(2).0034

PREVALENCE AND ASSOCIATED RISK FACTORS OF BOVINE BRUCELLOSIS IN SMALLHOLDER DAIRY COWS OF MYMENSINGH DISTRICT IN BANGLADESH

N. S. Tithy, S. M. Salahuddin Islam,[1] SMAK Hussaini,[1] S. T. Sharmy, A. Maruf, F. Yeasmin, A. C. Das, M. M. Rahman,[1] M. M. Hasan, A. Chakrabartty, A. K.M.A. Rahman, M. I. Mokbul,[2] and M.S. Rahman*

Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202; [1]Bangladesh Army; [2]Dhaka Medical College Hospital, Bangladesh
Corresponding author(s): *siddique.medicine@bau.edu.bd

Academic Editor: M. A. Samad

ABSTRACT

Background: Most of the smallholder animal farmers in rural Bangladesh depend on livestock for their livelihoods but significant percentage of these animals do not achieve their potential mainly due to inadequate nutrition and disease and occasionally transmit zoonotic disease like brucellosis. Brucellosis has been recognized as a neglected zoonotic disease in the low-income countries that produce few or no clinical signs in the affected animals making it more difficult for the dairy farmers to use preventive measures. However, sero-monitoring could help to detect the occurrence of Brucella infection in smallholder dairy farm management system.

Objective: This study aimed to determine the prevalence and associated risk factors for positivity of bovine brucellosis by using sero-screening and milk ring test supported with questionnaire

Materials and Methods: A cross-sectional study on bovine brucellosis was conducted in smallholder dairy cows in the district of Mymensingh during the period from August to December 2019. Serum samples of 460 lactating cows along with their milk samples were collected randomly. Serum samples were screened for brucellosis with Rapid Antigen Kit Test and Rose Bengal Test (RBT), whereas milk samples were tested with Milk Ring Test (MRT). Farm and animal level demographic and risk factor data were collected using a questionnaire and analyzed using univariable and multivariable logistic regression.

Results: The overall sero-prevalence was found to be 3.9% (95% CI 2.4-6.2) using RBT and Rapid Antigen Kit Test and 2.8% (CI 1.5-4.9) using Milk Ring Test, respectively. The odds of brucellosis was 7.4 times (95% CI: 2.5-21.5) higher in cows with repeat breeding that without repeat breeding. Moreover, the sero-prevalence of brucellosis was significantly higher (Odds ratio: 15.7; 95% CI: 5.2-47.4) in cows with retention of fetal membranes than without retention of fetal membranes.

Conclusions: The prevalence of Brucella infection in smallholder dairy farms with no adaptation of any preventive measures against this disease in Bangladesh. The sero-prevalence of brucellosis recorded in this study should be interpreted with caution and confirmatory diagnosis is needed to know the accurate status of brucellosis in smallholder dairy farms. The prevalence of Brucella infection in smallholder farms by using sero-test and milk ring test warrants further molecular test prior to embarking on a control program.

Keywords: Prevalence, Risk factors, Bovine brucellosis, Smallholder dairy farms, Rapid antigen kit, Rose Bengal test, Milk ring test

Article Info: Article Code No. © LEP: JVMOHR/0033/2022
Received: 10 October 2022 Revised: 25 November 2022 Accepted: 25 December 2022 Published: 30 December 2022

Citation: Tithy NS, Islam SMS, Hussaini SMAK, Sharmy ST, Maruf AA, Yeasmin F, Das AC, Rahman MM, Hasan MM, Chakrabartty A, Rahman AKMA, Mokbul MI and Rahman MS (2022). Prevalence and associated risk factors of bovine brucellosis in smallholder dairy cows of Mymensingh district in Bangladesh. J. *Vet. Med. OH Res.* 4 (2): 115-124 [doi: 10.36111/jvmohr.2022.4(2).0033]

INTRODUCTION

Smallholder animal farmers in developing low-income countries like Bangladesh depend on their animals for their livelihoods. Most smallholder dairy farms are critical because they provide food, income, social status and are financial reserve for the family but most of their animals do not achieve their productive potential or even die due to disease including zoonotic pathogens, causing public health problems.[1] Brucellosis is a widespread economically important bacterial zoonotic disease with impact on human and animal health associated with reproductive disorders in both male and female animals in low to medium income countries of the developing world including Bangladesh. Bovine brucellosis is primarily caused by *Brucella abortus* and occasionally by *B. melitensis* where cattle are kept together with infected small ruminants.[2] The disease is usually asymptomatic but it causes abortion at first gestation, retained placenta, metritis and decreased lactation. After the first abortion, subsequently pregnancy are usually normal, however, some cows may shed the organism in milk and uterine discharges. Epididymitis, seminal vesiculitis, orchitis and testicular abscess are sometimes reported in bulls.[3] Brucellosis has been reported as 'multiple burdens' disease with economic impacts attributable to human, livestock and wildlife.[2] Brucellosis has been successfully controlled or eliminated in livestock populations in high income countries, whereas its persistence in wildlife populations have become the main reservoirs. In low-income countries, brucellosis is endemic and neglected disease associated with livelihood burdens in animals and people and almost no effective control measures.[2] The prevalence of *B. abortus* infection in animals and humans in Bangladesh are largely confined to serological surveys in different species of domestic animals. Based on serological surveys performed during the last 50 years, the estimated prevalence of bovine brucellosis has reported from 2.4 to 18.4%[4,5] with an average of 3.7%[4,6] in cattle from Bangladesh. These results provide strong evidence that brucellosis is a problem in low-income country Bangladesh. In addition, assessment on the status of sero-prevalence and associated risk factors of brucellosis in the districts of Dinajpur and Mymensingh mainly based on hospital and village cases[7] and commercial dairy farms of Chittagong Metropolitan area[8] and humans in Sylhet district[9] have also been reported from Bangladesh. Mostly the smallholder dairy farms located in the rural areas which supply raw milk and milk products to the consumers either directly or through milk processors organization in Bangladesh. The demand for consumption of meat and milk has recently been increased due to rapid urbanization that may cause the increase rate of transmission of zoonotic diseases like brucellosis. Therefore, this study aimed to determine the prevalence of bovine brucellosis and its associated risk factors in smallholder dairy lactating cows in Bangladesh.

MATERIALS AND METHODS

About 5 to 10 ml of blood from 460 randomly selected lactating cows were collected by using the jugular vein, of which 290 samples were collected from Mymensingh Sadar and 170 samples from Bhaluka Upazila, Mymensingh. The tubes contained blood were kept vertically at room temperature for one hour and then refrigerated at 4^0C overnight before centrifugation at 3,000 rpm for 10 minutes for separation of serum samples. The separated serum samples were transferred into sterile Eppendrof tubes and kept at -20^0C until tested. Milk samples were also collected from the same cows for performing milk ring test. Palacenta and vaginal swabs or aborted fetus in case

of any abortion case (where available) were collected when the authority informed.

A standard questionnaire was used to collect data mainly on history of lactating cows and their production and breeding history. Questionnaire based data of age, sex, breeds, location, calving status, disease history were also recorded beside sample collection. For each sample, the following data were also collected (a) history of abortion, (b) history of retained placenta [yes, no], (c) History of repeat breeding (d) age group [≤6 years, ≥6 years] and (e) parity.

Laboratory evaluation

Two serological tests (a) Rapid Brucella Antibody Test (RBT) and (b) Rapid Kit Test were performed for antibody screening of the collected sera and milk samples were tested with Milk Ring Test (MRT) as follows:

Rose Bengal Test (RBT)

The test serum samples *Brucella abortus* antigen (William James House, Cowley Rd. Cambridge, CB4 OWX,UK) were kept 1 hour at room temperature before beginning the test. 30 µl of each serum to be tested was placed on a glass plate circled approximately 2 cm in diameter. Then the vial of antigens was shaked gently and 30 µl of antigen was put beside each of the sera and were mixed on the plate with a stirrer. Then the plate was placed on a mechanical rotator at 80 to 100 rpm for 4 minutes and the reading was taken immediately. Any sign of agglutination was considered as positive case of brucellosis in the tested serum and graded to be positive with clear agglutination (Fig 1) and negative if there was no sign of agglutinations.

Rapid Brucella Antibody Test Kit

The serum samples of cattle were subjected to antigen Rapid Brucella Antibody test Kit (Senspert® Brucella Ab Test Kit, Korea) to detect the antibodies of *B. abortus*. The test was performed as recommendation of the manufacturer. One drop (10 µl) was dispensed into the specimen well. When the fluid was completely absorbed into the specimen well 2 drops (80 µl) of buffer was added. Result was read within 5-10 min. The purple band should appear on the control line regardless of the test result. The presence of another band on the test line determines the result.

Milk Ring Test (MRT)

Antigen was kept at room temperature (18 to 23^0C) for 1 hour before starting the test. After proper mixing, 1.0 ml of milk sample and 50 µl of MRT antigen reagent were added in each tube. The milk and MRT reagent was mixed with vortex and incubated for 1 hour at +37 ^{0}C and then between +2 to +8 ^{0}C for 18 to 20 hours. Milk in the middle tube indicates positive result showing ring of cream more colored than underlying milk (Fig. 2). Milk in the two corner tubes indicate negative result shows ring of cream less colored than under lining milk.

Data analysis

The data were entered into a spreadsheet (Microsoft Excel 2010) and transferred to R 4.0.2 for analysis.[10] Age was converted to categorical variable based on median. The frequency and

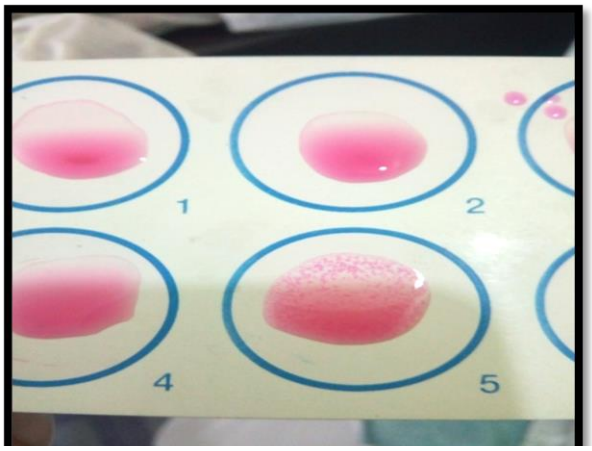

Fig. 1. Rose Bengal Plate Test showing positive for antibodies for Brucella infection in lactating cows.

An + Ab reaction form a ring (middle tube)

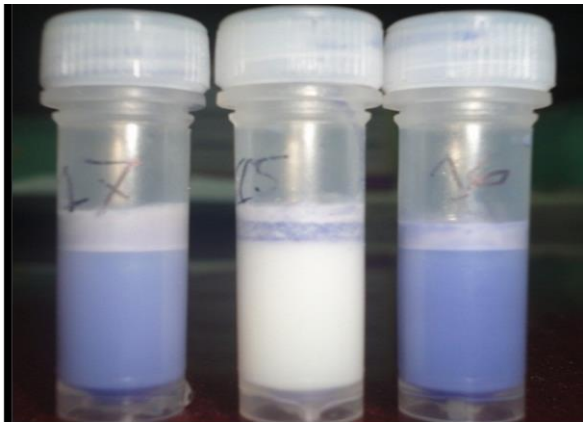

Fig.2. Milk Ring Test showing positive for Brucella antibodies in milk of smallhoder dairy cows.

proportion of bovine brucellosis in each category of independent variable were calculated using "tabpct" function of the R package 'epi Display.'[11]

Pearson Chi-square Test

The brucellosis sero status (Yes/No) was considered as the response and other hypothesized risk factors were used as explanatory variables. Pearson Chi-square test was used to assess the univariable association between dependent and independent variables. The R functions "table" and "Chi-square test" were used to construct contingency tables and to perform Chi-square tests, respectively. Any explanatory variable associated with hTB status with a p-value of (≤ 0.10) was selected for multiple logistic regression analysis. Co-llinearity among explanatory variables was assessed by Cramer's phi-prime statistic (R package "vcd," "assocstats" functions). A pair of variables was considered collinear if Cramer's phi-prime statistic was >0.70.[12]

Multivariable Logistic Regression Analysis

A stepwise (both forward and backward) multiple logistic regression model was used to identify risk factors for bovine brucellosis sero-prevalence. The best multivariable model was automatically selected by the software and it had the lowest Akaike's information criterion (AIC) value. The overall model fit was assessed by Hosmer-Lemeshow goodness-of-fit tests[13] using "hoslem.test" function of the R package 'Resource Selection.'[14] Confounding was checked by observing the change in the estimated coefficients of the variables that remained in the final model by adding a non-selected variable to the model. If the inclusion of this non-significant variable led to a change of more than 25% of any parameter estimate, that variable was considered to be a confounder and retained in the model.[15] The two-way interactions of all variables remaining in the final model were assessed for significance based on AIC values, rather than significance of individual interaction coefficients.[15]

RESULTS

Out of 460 randomly collected serum samples 18 serum samples 3.9% (95% CI 2.4-6.2) were found positive using RBT and Rapid Antigen Kit Test and 13 of the samples 2.8% (CI 1.5-4.9) were positive in Milk Ring Test, respectively. The results of the univariable association between brucellosis sero status (RBT) and explanatory variables are presented in Table 1.

Table 1. Univariable association of Rose Bengal test results with explanatory variables

S/N	Variables	Categories	Results +ve	-ve	Chi-square test p-value
1.	Age (years)	≤ 7	05 (02.0)	244	0.04
		> 7	13 (06.2)	198	
2.	Parity	1-2	11 (04.5)	236	0.68
		3-4	07 (03.3)	206	
3.	Abortion	Yes	11 (09.3)	107	0.001
		No	07 (02.0)	335	
4.	Repeat breeding	Yes	12 (11.4)	093	<0.001
		No	06 (01.7)	349	
5.	Retention of placenta	Yes	13 (17.3)	349	<0.001
		No	05 (01.3)	380	
6.	Area	Mymensingh Sadar	11 (03.8)	279	1
		Bhaluka upazila	07 (04.1)	163	

Table 2 shows the results of the univariable association between brucellosis sero-status (RBT) and explanatory variables. Only age, repeat breeding and retention of fetal membranes were significantly associated with brucellosis.

Table 2. Risk factors retained in the final multivariable logistic regression model

Variable	Categories	Coefficients	SE	Odds ratio (95% Confidence Interval)	p-value
Repeat breeding	Yes	1.99	0.57	7.4 (2.5-21.5)	< 0.001
	No	-	-	Reference	
Retention of placenta	Yes	2.76	0.56	15.7 (5.2-47.4)	< 0.001
	No	-	-	Reference	

Finally repeat breeding and retention of fetal membranes were associated with brucellosis. The odds of brucellosis was 7.4 times (95% CI: 2.5-21.5) higher in cows with repeat breeding that without repeat breeding. Moreover, the sero-prevalence of brucellosis was significantly higher (Odds ratio: 15.7; 95% CI: 5.2-47.4) in cows with retention of fetal membranes than without retention of fetal membranes.

DISCUSSION

Brucella is an intracellular zoonotic bacterium that causes abortion, retained placenta and metritis in female, and orchiepididymitis and infertility in males, resulting in reduced fertility and decrease milk production.[2] The intracellular Brucella has the ability to avoid recognition by the immune system of the host and promote its survival and replication. Brucellae reside mostly within phagocytes and other cells including placental trophoblasts. The 'gold organs' for nesting Brucella, in which Brucella replicates in cells of the reticular endothelial system include the spleen, lymph nodes, liver, bone marrow, epididymis and placenta.[16] Low to medium sero-prevalence rate of Brucella infection have been reported in animals and humans in developing low-income countries in the world including Bangladesh.

Diagnosis of clinical brucellosis in animals is initially made by the use of appropriate serological or other immunological tests, and confirmed by bacteriological isolation and identification of the agent.[17] The sensitivity based on culture positivity accepted as the gold standard ranged from 87 to 100% for Rose Bengal test and still efficient methods for brucellosis serodiagnosis for IgG but ELISA for IgA and IgG antibody has been reported more specific and sensitive.[18,19] Though serological tests for brucellosis have been in use for over 100 years, none has optimal sensitivity or specificity, leading to multiple modifications and development of new tests.[20] In order to be able to screen a large number of animals, the diagnostic tests should be inexpensive, easy to perform, rapid, highly sensitive and fairly specific.[21] Rapid Antigen Test Kit, Milk Ring Test (MRT) and Rose Bengal Test (RBT), all the three tests are easy to be done and do not require specialized training or equipment and the components are stable and rapid in the management of large numbers of blood and serum samples, these factors make the test ideal for developing countries and rural settings especially in smallholder farms. The present study represents identification of various risk factors for brucellosis in cattle in two selected areas in the district of Mymensingh in Bangladesh.

The overall prevalence of brucellosis in lactating cows was estimated to be 3.9% (95% Confidence Interval (CI): 2.4-6.2) and 2.8% (95% CI: 1.5-4.9) based on RBT and Milk Ring Test, respectively. The 3.9% sero-prevalence rate of Brucella infection in smallholder dairy lactating cows recorded in this study found comparatively lower than the earlier reports of 8.9% in Chittagong Metropolitan area,[8] 7.6% Chittagong,[22] 8.5% in commercial dairy cattle in Sirajgonj,[23] 4.20% in selected dairy cattle[24] but it correlates with the 3.7% sero-prevalence reported based on analysis of 50 years reports.[4,6] The difference in sero-prevalence among different reports might be due to differences on the geographical area, husbandry practices, animal species, infection status of the hosts, sample size and type of tests used.

Both Milk ring test (MRT) and Rapid Antibody Kit test are not considered sensitive because it is found that small number of infected animals and low grade infection may be overseen using the tests. However, this lack of sensitivity is compensated by the fact that the test can be repeated, usually monthly, due to its very low cost and gives a good reflection of serum antibody. False positive reactions may also occur due to abnormal milk like mastitic milk, colostrum and late lactation cycle milk. Variations in sensitivity depending on antigens of various sources and the use of good quality antigens made by experienced or reference laboratories, has been occasionally considered as a weakness of RBT. RBT overcomes false negative results because of prozones and

blocking and non-agglutinating antibodies.[19] This is the basic test for twenty-three of the thirty countries where it is used. There is therefore a large measure of agreement on the use of this test, which is justified to the extent that the RBT is economical, simple and rapid, and gives few false negative or false positive results.

Commonly used tests for brucellosis in these studies detect antibodies produced in response to infection. A combination of tests may be used to improve accuracy or ability to detect. Given the endemic nature of brucellosis, positive test results are strongly correlated with infection burden. Depending on the sensitivity and specificity of individual and combined testing regimes and the true prevalence of disease within a country, test results may under or over-estimate the true prevalence but usually provide a rough guide.[2]

Brucellosis associated risk factors

About 20 different risk factors have been reported that contribute / predispose to occurrence of bovine brucellosis and these risk factors have been classified in four groups: (a) host factors, (b) farmer's factors, (c) management factors and (d) agro-ecological factors.[25] The results of this study suggest that the presence of brucellosis related symptoms, abortion, repeat breeding and retained placenta are significantly associated with brucellosis sero-positivity in Mymensingh Sadar and Bhaluka Upazila of Bangladesh. These findings are in conformity with the earlier reports[8,22,26] who reported significant association between reproductive disorders and sero-positivity of Brucella infection from Bangladesh. However, comparatively higher sero-prevalence of brucellosis reported in cows with no history of abortion (38.5%) than with history of abortion (17.0%), whereas higher sero-prevalence reported in cows with retained placenta (36.0%) than without retained placenta (2.0%) elsewhere.[27]

CONCLUSIONS

This study indicates that the brucellosis has an important impact on animal health associated with reproductive disorders in smallholder dairy farms in Bangladesh. Brucellosis is a neglected disease in low-income countries including Bangladesh that can be transmitted to humans through consumption of unpasteurized milk and milk products and direct contact with animal birth materials. However, serological tests could be used monitor natural Brucella infection in smallholder farms to prevent and control brucellosis especially reproductive disorders to minimize the economic losses and human health.

ETHICAL APPROVAL

Collection of blood samples and data were made from all smallholder farm owners by using verbal consent. This study was conducted under the approved research project No. 2021/1019/BAU which is approved by the Animal Ethical Committee of the University.

CONFLICT OF INTEFREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The study was supported by Krishi Gobeshona Foundation (KGF), Bangladesh Agricultural Research Council campus, Farmgate, Dhaka from the research project-"Livestock and Human Brucellosis: Molecular diagnosis, treatment and control (TF44-L/17)" and the research project of Bangladesh Agricultural University Research System- "Development of treatment strategy against brucellosis in dairy cattle" (Project No. 2021/1019/BAU).

REFERENCES

01. Donadeu M, Nwankpa N, Abela-Ridder B and Dungu B (2019). Strategies to increase adaptation of animal vaccines by smallholder farmers with focus on neglected diseases and marginalized populations. *PLoS Neglected Tropical Diseases* 13(3): e0007279 [doi: 10.1371/journal.pntd.0006989]

02. McDermott J, Grace D and Zinsstag J (2013). Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique (International Office Epizootics)* 32: 249-261

03. Lopes LB, Nicolino R and Haddad JPA (2010). Brucellosis- risk factor and prevalence- A review. *Open Veterinary Science Journal* 4: 72-84

04. Rahman MS, Sarker RR, Melzer F, Sprague LD and Neubauer H (2014). Brucellosis in human and domestic animals in Bangladesh: a review. *African Journal of Microbiology Research* 8: 3580-3594 [doi: 10.5897/AJMR2014.7074]

05. Rahman MS, Faruk MO, Her M, Kim JYS, Kang Sung-II and Jung SC (2018). Prevalence of brucellosis in ruminants in Bangladesh. *Veterinarni Medicina* 56: 379-385 [doi: 10.17221/1555-VETMED)

06. Islam MA, Khatun MM, Were SR, Sriranganathan N, and Boyle SM (2013). A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control program. *Veterinary Microbiology* 166: 317-326 [doi: 10.1016/j.vetmic.2023.06.014]

07. Ahasan S, Rahman MS and Song HJ (2010). A sero-surveillance of Brucella spp. antibodies and individual risk factors of infection in cattle in Bangladesh. *Korean Journal of Veterinary Service* 33: 121-128

08. Islam S, Barua SR, Moni SP, Islam A, Rahman AKMA and Chowdhury S (2020). Seroprevalence and risk factors for bovine brucellosis in the Chittagong Metropolitan area of Bangladesh. *Veterinary Medicine and Science* 7: 86-98 [doi: 10.1002/vms3.348]

09. Akhtar J, Chowdhury OA, Das P, Sinha SP (2020). Sero-prevalence of human brucellosis among high risk and normal individuals of Sylhet district in Bangladesh. *Bangladesh Medical Research council Bulletin* 46: [doi: 10.3329/bmrcb.v46i1.47467]

10. Team RC (2013). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria [http:www.R-project.org/

11. Chongsuvivatwong V (2018). Package 'epiDisplay' Epidemiological Data Display Package (R package version 3.5. 0.1)

12. Meyer D, Zeileis A, Hornik K, Gerber F and Friendly M (2021). Package 'vcd.' Visualizing Categorical Data. *R package version***:** 1.4-9.

13. Hosmer DW, Hosmer T, Le Cessie S and Lemeshow S 1998: A comparison of goodness-of-fit tests for the logistic regression model. *Statistics in Medicine* **16:** 965-980 [doi: 10.1002/(SICI)1097-0258(19970515)16:9<965::AID-SIM509>3.0.CO;2-0]

14. Subhash RL, Jonah LK and Solymos P (2019). Package 'ResourceSelection'- Resource Selection (probability) Functions for Use-Availability Data. R package version 0.3-0.

15. Dohoo I, Martin W and Stryhn H (2003). Veterinary Epidemiological Research. AVC Inc., Charlottetown, Prince Edward Island, Canada pp. 706

16. Gonzalez-Espinoza G, Arce-Gorvel V, Memet S and Gorvel JP (2021). Brucella: Reservoirs and niches in animals and humans. *Pathogens* 10 (2), 186 [doi: 10.3390/pathogens10020186]

17. Renukaradhya GJ, Isloor S, Crowther JR, Robinson M and Rajasekhar M (2001). Development and field validation of an avidin-biotin enzyme-linked immunosorbent assay kit for bovine brucellosis. *Revue scientifique et technique (International Office of Epizootics)* 20: 749-456 [doi: 10.20506/rst.20.3.1304]

18. Dias M and Dias E (2015). Comparative evaluation of various serological tests in the laboratory diagnosis of brucellosis. *CHRISMED Journal of Health and Research* 2: 136-139 [doi: 10.4103/2348-3334.153258]

19. Díaz R, Casanova A, Ariza J, Moriyon I (2011). The Rose Bengal Test in human brucellosis: a neglected test for the diagnosis of a neglected disease. *PLoS Neglected Tropical Diseases* 5(4): e950 [doi: 10.1371/journal.pntd.0000950]

20. Nielsen K (2002). Diagnosis of brucellosis by serology. *Veterinary Microbiology* 90: 447-459.

21. Bricker BJ (2002). PCR as a diagnostic tool for brucellosis. *Veterinary Microbiology* 90: 435-446.

22. Sikder S, Rahman AKMA, Faruque MR, Alim MA, Das S, Gupta AD, Das BC, Uddin MI, and Prodhan MAM (2012). Bovine brucellosis: An epidemiological study at Chittagong, Bangladesh. *Pakistan Veterinary Journal* 32: 499-502

23. Belal SMSH and Ansari ARMIH (2013). Seroprevalence of Brucella abortus antibodies in the cattle population in the selected upazilas of Sirajgonj district. *Bangladesh Journal of Veterinary Medicine* 11: 127-130

24. Hassan AA, Uddin MB, Islam MR, Cho HS and Hossain MM (2014). Serological prevalence of brucellosis of cattle in selected dairy farms in Bangladesh. *Korean Journal of Veterinary Research* 54: 239-243 [doi: 10. 14405/kjvr.2014.54.4.239]

25. Deka RP, Magnusson U, Grace D and Lindahl J (2019). Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- a review. *Infection Ecology and Epidemiology* 8: [doi: 10.1080/20008686.2018.1556548]

26. Rahman MS, Han J. Park J, Lee J, Eo S and Chae J (2006). Prevalence of brucellosis and its association with reproductive problems in cows in Bangladesh. *Veterinary Record* 159: 180 [doi: 10.1136/vr.159.6.180]

27. Ndazigaruye G, Mushonga B, Kandiwa E, Samkange A and Segwagwe BE (2018). Prevalence and risk factors for brucellosis seropositivity in cattle in Nyagatare district, Eastern province, Rwanda. *Journal of South African Veterinary Association* 89: 1625 [doi: 10.4102/jsava.v89i0.1625]