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HUMORAL IMMUNE RESPONSE IN CROSS-BRED HEIFERS IMMUNIZED WITH *BRUCELLA ABORTUS* STRAIN RB51 VACCINE IN MILITARY DAIRY FARM OF BANGLADESH

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ABSTRACT

Background: Brucellosis is a chronic zoonotic disease with negligible mortality rate that might be the reason not to attract the concerned authority to prevent and eradicate it in low income endemic countries. Recently, it has been recognized as a re-emerging zoonotic disease not only in low income countries but also its eradicated developed world.

Objective: The main objective was to determine the humoral immune response (HIR) in crossbred dairy heifers immunized with *Brucellaabortus*strain RB51 vaccine by using indirect ELISA

Materials and Methods: Each of the 20 randomly selected *B. abortus*sero-negative crossbred (Holstein-Friesian × Local) dairy heifers aged between 4 to 8 months old at the Military Dairy Farm received 2.0 ml imported commercial *B. abortus* SRB51 strain vaccine subcutaneously in the neck region at day 0 and then booster dose at 60 days after first vaccination with similar dose and route during the period from June to October 2020. Each of the collected serum samples of 20 heifers at day 0, 7, 14, 21, 28, 60, 90, 120 and 150 was tested to detect the antibody status by using commercial indirect ELISA kit.

Results: The humoral immune response (HIR) in terms of antibody levels detected by OD values in the serum of immunized cross-bred dairy heifers by using *B. abortus* strain RB51 commercial vaccine resulted 0.097 ± 0.0032 (mean ± SE) OD value at 0 day (i.e. pre-immunization) and 0.108 ± 0.0032 at 7th day. After that, the OD value started to rise from day 14 (OD value 0.124 ± 0.0032) and reached to a peak level at 60 days (OD value 0.223 ± 0.0032) with the initial vaccination. Booster vaccination inoculated at 60 days resulted peak antibody level in terms of OD value (0.313 ± 0.0032) at the day 90 and then the antibody level started to decline from 120 days (OD value 0.242 ± 0.0032) to 150 days (OD value 0.199± 0.0032) in cross-bred dairy heifers.

Conclusions: This study suggests that the commercial *B. abortus* RB51 strain vaccine has induced satisfactory HIR with initial inoculation and significantly higher HIR produced with a booster dose in crossbred heifers by using commercial I-ELISA. The presence of Brucella antibodies have importance on sero-diagnosis whereas the cell mediated immunity (CMI) plays major role in protection against brucellosis which needs further investigation in cross-bred heifers in Bangladesh.

Keywords: Brucellosis, SRB51 vaccine, Humoral immune response, I-ELISA, Cross-bred heifers, Bangladesh

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INTRODUCTION

Brucellosis is one of the major zoonosis that affects livestock and wildlife animal species as well as humans leading to significant impact on public health and animal industry.[1,2]The Brucellaorganism is responsible for a variety of disease conditions having zoonotic significance and reported worldwide causing abortion, infertility, retained placenta, endometritis in cows and to a smaller extent, orchitis and infection of the accessory sex glands in bulls.[3]Although the death rate associated with brucellosis is negligible both in animals and humans, the prevalence is approximately 10.0% in endemic countries.[4]However, WHO estimates only half a million cases annually being registered as human brucellosis distributed in more than 170 countrieswitha quarter of cases are unreported with non-specified clinical symptoms which is 10 times higher, and accordingly, the true prevalence is estimated at 5.0 to 12.5 million cases annually which indicates brucellosis is one of the most important widespread zoonotic diseases in public health concern.[1,5,6]Although brucellosis is the most widespread zoonosis worldwide, it remains severely neglected as a potential cause for chronic, debilitating maladies, due to non-descript clinical presentation in human populations.[6] In addition, recently it has been recognized as a re-emerging zoonotic disease in both developing and eradicated developed nations.[4,7-10]It appears that the control of animal brucellosis will lead to the control of human brucellosis. This can be achieved in one of the several ways including vaccination, culling of infected animals, surveillance testing or a combination of any of these.[6] There are three main vaccines used for control brucellosis, of which RB51 and S19 are directed at *Brucella abortus* infections in bovid, while Rev 1 is used for *B. melitensis* in small ruminants.[6,11]Currently, S19 and RB51 are the *B. abortus* vaccine strains have been more widely used to prevent brucellosis in cattle.[12] Both vaccines are effective in the prevention of abortion and infection, besides offering long lasting protection.[12-16]The *B. abortus* S19 is a stable smooth attenuated organism with high immunogenicity and antigenicity.[17]The RB51 vaccine is a lipopolysaccharide O-antigen deficient naturally occurring rough mutant derived from the virulent smooth strain, *B. abortus* 2308. The RB51 does not induce antibodies against smooth lipopolysaccharide (LPS) detectable by routine serological tests.[18] This feature allows RB51 vaccination to be performed at any age, while vaccination with S19 is normally restricted to calves between 3 and 8 months of age to avoid interference in the routine serological tests.[2]There are several reports on sero-prevalence, sero-epidemiology, sero-molecular epidemiology, risk factors, zoonotic importance and review of brucellosis in human and animals have been published from Bangladesh.[19-22]In addition, molecular detection of *Brucella* sp. from milk of sero-negative cattle,[23]isolation and genetic characterization of *B. abortu*s biovar 3 from dairy cattle,[24,25]haemato-biochemical and therapeutic responses of chronic brucellosis in crossbred dairy cows,[26]comparison of humoral immune response (HIR) in indigenous cattle immunized with heat inactivated *B. abortus* biovar 3 and RB 51 vaccines[27,28]and HIR between indigenous cattle and indigenous buffaloes immunized with RB 51 vaccine[29] have been reported from Bangladesh.Report on the immunization and immunological response with B. abortus RB51 strain vaccine in cross-bred heifers is lacking in inland literature. Moreover, the complete understanding of the immune response triggered by the worldwide used *B. abortus* vaccines in

cattle is still undefined. This paper describes the HIR in crossbred heifers immunized with *B abortus* strain RB51 vaccine in the Military Dairy Farm, Chattogram,Bangladesh.

MATERIALS AND METHODS

This study was conducted on the Holstein-Frisian × Local cross-bred heifersin the Military Dairy Farm, Chattogram, Bangladesh during the period from June to October 2020.A total of 1050 dairy cattle and 120cross-bred heifers of four to eight months of age were available in the farm, of which brucellosis sero-negative 20 cross-bred heifers were randomly selected for this study (Photo 1). The *B. abortus*strain RB51vaccinewas imported from Spain (CZ Veterinaria SA, Spain) and 2.0 ml of the vaccine contains 10-34 $\times 10^9$ cfu organisms, one vial contains 25 doses in powder forms and suspension is made by mixing with diluent supplied with the vaccine as directed by the manufacture instructions. Each of the experimental heifers was inoculated strain RB51 vaccine @ 2.0 ml subcutaneously in neck region (Photo 2). The immunized animals were observed for six months with especial emphasis to two to three hours post-vaccination for any immediate untoward reactionsand boosting at the days of 60 with same dose and route.Then vaccinated heifercalves were observed for 150 days.

Collection of blood samples

Blood was collected from each of the selected and vaccinated heifers before vaccination (0 day) and on 7, 14, 21, 28, 60, 90, 120 and 150 days at post-vaccination. The calves were restrained properly, the injection site was disinfected with 70% alcohol and 10 ml of blood was collected from each of the calves from jugular veins. The collected blood was kept undisturbed in syringe in a slightly inclined position on a tray for one hour to facilitate clotting and separation of serum. The separated serum was taken in a tube and then centrifuged at 2500 rpm for 10 minutes.The sera were transferred to the sterile and labeled eppendorf tube. The sera samples were stored at -20° C until tested with Indirect ELISA.[22]

Photo 1.A group of crossbred heifers used for the study of HIR immunized with *B. abortus* strain RB51 vaccine

Photo 2. Shows s/c inoculation of RB51 vaccine in a heifer

Enzyme Linked Immunosorbent Assay (ELISA)

Level of antibody was detected by Antibody I-ELISA Test Kit (IDEXX Montpellier SAS, France) according to the protocol of the manufacturer and reading was performed by automated ELISA reader.[22]Briefly, microplates are coated with 50µl *Brucella*lipopolysaccharide (LPS). Coated plate were wrapped in plastic to seal and incubated for 2 hour at 37^0 C. Upon incubation of the test samples in the coated wells, *Brucella* specific antibodies form immune complexes with *Brucella* LPS. Unbound materials were washed away with PBS. The solutions or washes were removed by pipetting. 200µl blocking buffer was added for blocking the remaining protein binding sites in coated wells and incubated 30 minutes at room temperature. The solution was discarded and coated well was washed away. Then 50µl antibody solution is added using micropipette. Plate were wrapped in plastic and incubated for 2 hour at room temperature. The plate was washed away. Blocking and washing steps were repeated. 50µl secondary antibodyreagent was added to wells. After wrapping, it was incubated for 2 hour in room temperature. After washing, 75µl substrate solution was added on micro titer plates. The plate was wrapped with plastic and incubated for 1 hour at room temperature. 25µl of stop solution was added on micro titer plate. The result is obtained by comparing the sample optical density at 450 nm with the positive control mean optical density.

Statistical analysis

The data was entered in Microsoft Excel and transferred to R 4.0.1[30] for statistical analysis. Repeated measure ANOVA model was built using "nlme" package[31] considered animal ID as random variable and date of sample collection as fixed effect variable. The pairwise means of OD values among different dates of sampling were compared in Post-hoc analysis using"lsmeans" function of "lsmeans" package.[32]

RESULTS

The mean values of antibody level in terms of OD values in different age groups of heifers immunized with commercial *B. abortus* strain RB 51 vaccine did not differ significantly (Table 1). Table 1 also shows that the antibody levels in terms of OD values at day 14, 21, 28, 60, 90 and 150 days post-immunization in cross-bred heifers with commercial *B. abortus* strain RB51 vaccine were significantly ($p < 0.05$) different from those of pre-vaccination values at days 0. It also appears from Fig.1 that the OD values in heifers immunized with commercial *B. abortus* strain RB 51 vaccine increased gradually from day 7 up to 60 days and then administration of the booster dose of the vaccine at day 60 resulted a peak OD level at 90^{th} day (0.313 ± 0.0032) and then started to decline the antibody titer gradually with a lowest level at day 150 of post-vaccination.

DISCUSSION

Brucellosis is a disease of socio-economic and public health importance associated with abortions and reduced fertility in ruminant animals in many countries in the world[1,2]but it is not a serious problem either in animals or in humans in Bangladesh.[33] However, it is maintained at a low level in different livestock species and humans in Bangladesh[19-22]and possibility to exist

Table 1. Comparison of antibody OD values at different age and days after vaccination with *Brucellaabortus* strain RB51 vaccine in heifers

S/N	Age (months)	No. of animals	OD values Mean ± SD	S/N	Day PV	No. of animals	OD values Mean ± SE	95% CI
1.	4	1	0.19 ± 0.077	1.	000	20	0.097^{a} ± 0.0032	0.087-0.11
2.	5	7	0.18 ± 0.069	2.	007	20	0.108^{a} ± 0.0032	0.098-0.12
3.	6	7	0.18 ± 0.068	3.	014	20	0.124^{b} ± 0.0032	0.114-0.13
4.	7	2	0.18 ± 0.072	4.	021	20	0.138^{c} ± 0.0032	0.128-0.15
5.	8	3	0.18 ± 0.075	5.	028	20	0.157^{d} ± 0.0032	0.147-0.17
				6.	060	20	0.223^{c} ± 0.0032	0.189-0.21
				7.	090	20	0.313^{f} ± 0.0032	0.213-0.23
				8.	120	20	0.242^{g} ± 0.0032	0.232-0.25
				9.	150	20	0.199^{h} ± 0.0032	0.303-0.32

SD = Standard deviation SE = Standard error PV = Post-vaccination
Different superscript indicates significant difference at ($p < 0.05$)

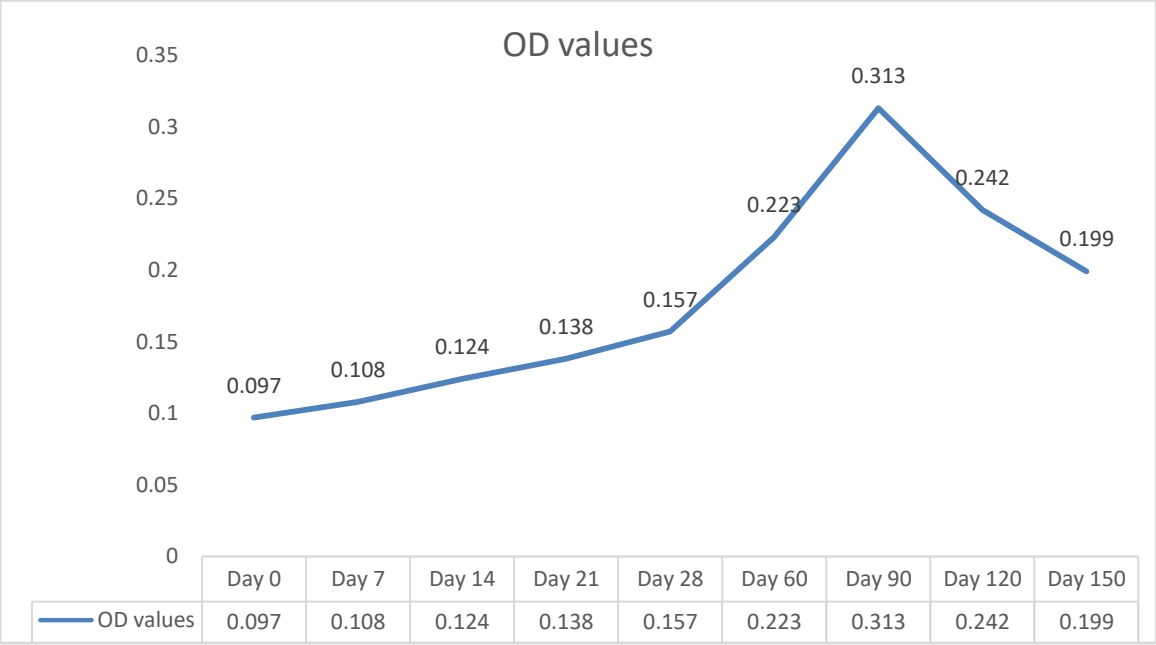

Figure 1. Comparison of mean OD valuesin cross-bred heifers at different days of immunization with *Brucellaabortus*strain RB51 vaccine

chronic zoonotic infection in humans as *Brucella* sp. has been detected*B. abortu*s biovar 3 from dairy cattle.[24,25]The internationally approved methods for eradication of brucellosis include vaccination, culling of infected animals, surveillance testing or a combination of any of these.[6]Only culling method of the brucellosis infected animals from the herd might be possible to eradicate this disease at this very low level of infection in Bangladesh.

Vaccination method is most suitable to control brucellosis in animals but there is currently no licensed vaccine for brucellosis in human and the available animal vaccines may cause disease and considered unsuitable for use in humans. As human brucellosis originates essentially from livestock and livestock products, the human brucellosis would be automatically controlled if brucellosis is controlled in livestock. Mass vaccination of livestock against brucellosis in endemic countries would be cost effective and would result in net economic benefit if interventions cost are shared between the different beneficiaries.[34]

The antibody responses and efficacy of RB51 vaccine have been evaluated in cattle, buffaloes and elk elsewhere[35-37]and in indigenous adult cattle and buffaloes in Bangladesh[28,29]but not in cross-bred heifers. Understanding immune responses of immunized cross-bred heifers with SRB51 may be beneficial for development of an efficacious brucellosis vaccine.

The result of the present study confirms the possibility of using commercial I-ELISA to evaluate RB51 vaccinated of heifers (booster at 60 days) of Bangladesh, monitor antibody responses to RB51 vaccine up to 150 days. These observation supports with the earlier report on RB51 vaccinated adult indigenous cattle and buffaloes in Bangladesh.[28,29]

This study has recorded the rise of antibody level from the second weeks of vaccine administration and highest OD (0.223 ± 0.0032) at 60 days after initial vaccination and the peak ODat 90 days (0.313 ± 0.0032) after booster vaccination and then the antibody level started to decline from 120 to 150 days in heifers. These results are in conformity with earlier reports of immunized indigenous adult cattle and buffaloes.[28,29]

All animals were sero-negative for Brucella antibodies before immunization which indicates that experimental animals were neither infected nor vaccinated, whereas satisfactory immune responses produced after immunization indicates inoculation of vaccine produced satisfactoryHIR in heifers. The antibody titer has been reported to be significantly increased in the vaccinated calves after one month and the titer declined but remained positive up to six months and then negative throughout the 12 months study periods.[38]These observations could not be compared with the present study because this study did not continue after 150 days of vaccination.

It appears from this study that the HIR is produced both in cattle and buffaloes but it is comparatively higher in cattle than buffaloes immunized with *B. abortus* RB 51 vaccine. This suggests that there may be differences between cattle and buffaloes in their immunologic responses to infection with virulent field strains of *B. abortus*. In addition, a major challenge in the development of an ideal vaccine lies in evoking robust CMI in the host. Vaccines that evoke a strong CMI response confer a better level of protection. Therefore, targeting the CMI branch of host immunity via induction of IL-12 and INF-γ should prove to be useful.[39]

CONCLUSIONS

Results of this study showed that the *B. abortus*RB51 strain vaccine has induced satisfactory antibody response with initial doseand significantly higher HIR(OD level) obtained at day 90 with booster dose inoculated at 60 days in crossbred dairy heifers. This indicates that this vaccine is potent enough to induce HIR in inoculated animals. However, the CMI has been reported to be played a major active role in protection against brucellosis and therefore there is a need to investigate CMI response along with HIR in *B. abortus* RB51 vaccinated animals with challenge studies to the detect the typesof immunological responses induced by brucellosis with its protection efficacy in Bangladesh.Currently, very low level of Brucella infection exists in Bangladesh and accordingly surveillance testing and culling of infected animals would be the choice to prevent and eradicate this disease under local conditions. As it is an important zoonotic disease, itrequires an interdisciplinary and collaborative ('One Health') approach that consists of public education and awareness, the development of an infrastructure for disease surveillance and reporting in both medical and veterinary medical, and campaigns for prevention and eradication in livestock and wildlife species.

ETHICAL APPROVAL

The study protocol of Ethical statement was peer reviewed and approved by the Ethical Review Committee of appropriate authority.Animal research was approved by the Faculty of Veterinary Science of Bangladesh Agricultural University and concern Military authority of Bangladesh Army.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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REFERENCES

01. Corbel MJ (2006). Brucellosis in Humans and Animals. Geneva: World Health Organization. who.int/csr/resources/publications/Brucellosis.pdf

02. OIE (2019). Infection with *Brucellaabortus*, *Brucellamelitensis* and *Brucellasuis*. Chapter 8.4.Terrestrial Animal Health Code- 28/06/2019. oie.int/fileadmin/Home/eng/Health_standards/tahc/ current/chapitre_bovine_brucellosis.pdf

03. Olsen S and Palmer M (2014).Advancement of knowledge of *Brucella*over the past 50 years.*Veterinary Pathology* 51: 1076-1089.

04. Ghanbari MK, Gorji HA, Behzadifar M, Sanee N, Mehedi N and Bragazzi L (2020). One health approach to tackle brucellosis: a systematic review. *Tropical Medicine and Health* 48, 86 (2020). [doi: 10.1186/s41182-020-00272-1]

05. Pal M, Gizaw F, Fekadu G, Alemayehu G and Kandi V (2017). Public health and economic importance of bovine brucellosis: an review. *American Journal of Epidemiology* 5: 27-34

06. Hull NC and Schumaker BA (2018). Comparisons of brucellosis between human and veterinary medicine.*Infection Ecology Epidemiology* 8(1): 1500846 [doi: 10.1080/20008686.2018.1500846]

07. Morens DM, Folkers GK and Fauci AS (2004). The challenge of merging and re-emerging infectious diseases.*Nature* 430(6996): 242-249

08. Franc KA, Krecek RC, Hasler BN and Arenas-Gamboa AM (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health* 18 : 25 [doi: 10.1186/s12889-017-5016-y]

09. Pappas G, Papadimitriou P, Akritidis N, Christou L and Tsianos EV (2006). The new global map of human brucellosis.*Lancet Infectious Diseases* 6: 91-99

10. Assana E, Van Wettere A, Chepnda V, Awah NJ and Tarbet B (2017). Prevention of emerging zoonoses in Central Africa: an overview from a veterinary perspective. *Tropical Medicine International Health* 22: 147-148

11. Stevens MG, Olsen SC and Cheville NF (1995). Comparative analysis of immune responses in cattle vaccinated with Brucellaabortus strain 19 or strain RB51. *Infection and Immunity*63 : 3199-3205.

12. Dorneles EMS, Sriranganathan N and Lage AP (2015) Recent advances in *Brucellaabortus* vaccines.*Veterinary Research* 46, 76 (2015). [doi: 10.1186/s13567-015-0199-7]

13. McDiarmid A (1957) The degree and duration of immunity in cattle resulting from vaccination with S19 *Brucellaabortus* vaccine and its implication in the future control and eventual eradication of brucellosis. *Veterinary Record*69:877–879
14. McDiarmid A (1960) The prophylaxis of brucellosis in cattle. *Veterinary Record* 72: 917–920.

15. Poester FP, Goncalves VS, Paixao TA, Santos RL, Olsen SC, Schuring GG and Lage AP (2006) Efficacy of strain RB51 vaccine in heifers against experimental brucellosis. *Vaccine* 24: 5327-5334. PMID: 16713034

16. Cheville NF, Olsen SC, Jensen AE, Stevens MG, Palmer MV, Florance AM (1996) Effects of age at vaccination on efficacy of *Brucellaabortus* strain RB51 to protect cattle against brucellosis. *American Journal of Veterinary Research* 57: 1153 - 1156

17. Miranda KL, Dorneles EM, Pauletti RB, Poester FP, Lage AP (2015) *Brucellaabortus* S19 and RB51 vaccine immunogenicity test: evaluation of three mice (BALB/c, Swiss and CD-1) and two challenge strains (544 and 2308). *Vaccine* 33: 507 -511

18. Schurig GG, Roop RM 2nd, Bagchi T, Boyle S, Buhrman D, Sriranganathan N (1991) Biological properties of RB51; a stable rough strain of *Brucellaabortus*. *Veterinary Microbiology* 28: 171-188.

19. Islam MA, Khatun MM, Were SR, Sriranganathan N and Boyle SM (2013). A review of Brucellaseroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities.*Veterinary Microbiology* 166: 317-326 [doi: 10.1016/j.vetmic.2013.06.014]

20. 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]

21. Rahman AKMA, Aegerman C, Berkvens D, Melzer F, Neubauer H, Fretin D, Abatih E, Dhand N and Ward MP (2017). *Brucellaabortus* is prevalent in both humans and animals in Bangladesh. *Zoonoses and Public Health* 64: 394-399 [doi: 10.1111/zph.12344]

22. Rahman MM, Rahman MS,RahmanAKMA, Hossain MM,Hasan MR,Rana MS, Melzer F and Neubauer H (2020). Sero-molecular epidemiology and rick factors analysis of brucellosis in human and lactating cows of military dairy farms in Bangladesh. *Journal of Veterinary Medical and One Health Research* 2: 81-114 [doi: 10.36111/jvmohr.2090.1(2).0018]

23. Islam MS, Islam MA, Khatun MM, Saha S, Basir MS and Hasan MM (2018). Molecular detection of *Brucella* spp. from milk of seronegative cows from some selected areas in Bangladesh. *Journal of Pathogens*. Article ID 9378976,7 [doi: 10.1155/2018/9378976]

24. Islam MS, Garofolo G, Sacchini L, Dainty AC, Khatun MM, Saha S and Islam MA (2019). First isolation, identification and genetic characterization of Brucellaabortusbiovar 3 from dairy cattle in Bangladesh.*Veterinary Medicine and Science* 5: 556-562 [doi: 10.1002/vms3.193]

25. IslamMS, El Zowalaty ME, van Vliet AHM, Thaku S, Khatun MM, Saha S, Rahman MT, Noreddin A and Islam MA (2019). First genome sequence of *Brucellaabortus*Biovar 3 strain BAU21/S4023, isolated from a dairy cow in Bangladesh.*Microbiology Resource Announcement* 8.24: e00446-00419 [doi: 10.1128/MRA.00446-19]

26. Maruf AA, Yasmin F, Yrasmin F, Alam MN, Rahman MM, Hasan MM, Alam M, Alam MR, Rahman AKMA and Rahman MS (2019). Assessment of haemato-biochemical and therapeutic responses of chronic brucellosis in crossbred dairy cows in Bangladesh.*Journal of Veterinary Medical and One Health Research* 1: 211-229 [doi: 10.36111/jvmohr.2090.1(2).0013]

27. Rahman MS (2019). Brucellaabortus killed vaccine: The achievement of 52 years (1967-2019) in Bangladesh. *EC Veterinary Science* ECO.02: 14-21

28. Maruf AA, Yasmin F, Yeasmin F, Rahman AKMA, Hossain MM, Neubauer H and Rahman MS (2019). Comparison of humoral immune responses between heat-inactivated *Brucellaabortus*biovar 3 and strain RB51 vaccines in indigenous cattle of Bangladesh.*Journal of Veterinary Medical and One Health Research* 1: 247-259 [doi: 10.36111/jvmohr.2090.1(2).0015]

29. Mohamud AI,Mohamud AA,Rahman MS,Ehsan MA,Maruf AA, Yasmin F, Karim F and Neubauer H (2020). Comparison of humoral immune responses between cattle and buffaloes immunized with commercial *Brucellaabortus* strain RB51 vaccine in Bangladesh. *Journal of Veterinary Medical and One Health Research* 2: 405-415 [doi: 10.36111/jvmohr.2090.2(2).0022]

30. Team RC (2020). R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria [https://www.R-project.org]

31. Pinheiro J, Bates D, DebRoy S, Sarkar D, Heisterkamp S, Van Willigen B and Maintainer R (2020). Package 'nlme'. *Linear and nonlinear mixed effects models, version*, *3*(1).

32. Lenth R and Lenth MR (2018). Package 'lsmeans'. *The American Statistician*, *34:*216-221.

33. Rahman AKMA (2015). Epidemiology of brucellosis in humans and domestic ruminants in Bangladesh.PhD thesis. [doi: 10.13140/2.1.3923.2800]

34. Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G, Cosivi O, Carrin G and Otte J (2003). Human health benefits from livestock vaccination for brucellosis: case study. *Bulletin of the World Health Organization* 81: 867-876

35. Olsen SC (2000). Immune responses and efficacy after administration of a commercial Brucella abortus strain RB51 vaccine in cattle. *Veterinary Therapeutics: Research in Applied Veterinary Medicine* 1: 183-191

36. Olsen SC, Kreeger TJ and Palmer MV (2002). Immune response of elk to vaccination with *Brucella abortus* strain RB51. *Journal of Wildlife Diseases* 38: 746-751

37. Fosgate GT, Adesiyun AA, Hird DW, Johnson WO, Hietala SK, Schurig GG and Ryan J (2002). Bayesian comparison of brucellosis serologic tests without a gold standard in cattle and water buffalo (*Bubalusbubalis*) of Trinidad.*American Journal of Veterinary Research* 63: 1598-1605

38. Baksi S, Dave H, Rao N, Oza R and Khan M (2016). Immune response of cattle to vaccination with *Brucellaabortus* strain S19 in India. *Bangladesh Veterinarian* 33: 28-32

39. Lalsiamthara J and Lee JH (2017). Development and trial of vaccines against Brucella.*Journal of Veterinary Science* 18: 281-290 [doi: 10.4142/jvs.2017.18.S1.281]