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SEROLOGICAL RESPONSE IN CROSS-BRED HEIFERS IMMUNIZED WITH BRUCELLA ABORTUS STRAIN RB51 VACCINE UNDER SMALLHOLDER DAIRY FARM MANAGEMENT SYSTEM IN BANGLADESH

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ABSTRACT

Background: *Brucella abortus* live vaccines (strains 19 and RB51) have successfully been used to control bovine brucellosis especially to protect cattle against infection and abortion worldwide. Most of the knowledge of the protective immune response of these vaccines against brucellosis induced by immunization derives from the studies in mice. Some studies on humoral immune response of these vaccines have been studied in bovine and buffaloes and an attempt is made further to evaluate the serological responses of RB51 vaccine in cross-bred heifers of smallholder dairy farms in Bangladesh.

Objective: This study was conducted to measure serological responses induced in cross-bred dairy heifers immunized with RB51 *Brucella abortus* vaccine by using indirect ELISA.

Materials and Methods: Five cross-bred (Holstein × Local) heifers were selected for this experiment which aged four months and sero-negative for Brucella infection in smallholder dairy farms in the district of Kushtia. Each of the selected heifer received 2.0 ml imported commercial *B. abortus* RB51 strain vaccine subcutaneously in the neck region at day 0 and then booster dose at 60 days after the first vaccination with similar dose and route during the period from January to July 2020. Each of the collected serum samples of five 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 serological responses (antibody level) was detected by commercial indirect ELISA OD values in the serum of cross-bred heifers induced by using *B. abortus* strain RB51 commercial live vaccine resulted 0.097 OD value at 0 day (pre-vaccination) and 0.108 at 7th day of post-immunization. It appears that the OD values in the immunized heifers was started to rise from the first week and it was gradually increased and reached the peak level at 60 days (OD value 0.223). Booster vaccination administered at 60 days was resulted peak antibody level at day 90 (OD value 0.313) but its level was started to decline from 120 days with a highest declined at day 150 (OD value 0.199).

Conclusions: Further studies to define the cellular immune response and protection against *B. abortus* infection are recommended before routine use of the vaccine in cattle in Bangladesh.

Keywords: Sero-response, *Brucella abortus* RB51 vaccine, i-ELISA, Cross-bred heifers, Smallholders

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INTRODUCTION

Smallholder livestock farmers in low-income and middle-income countries depend on animals for their livelihoods. Animals are critical because they provide food, income, status and are financial reserve for the family. Many of those animals die or do not achieve their productive potential due to disease and some of them are zoonotic diseases causing morbidity and mortality in human population.[1] Brucellosis is caused by Brucella genus has been recognized as one of the major zoonotic diseases in animals and public health importance that affects livestock and wild animal species including humans. Cattle are the preferred host of *Brucella abortus* and the economic importance of bovine brucellosis is associated with direct losses caused by late abortions, stillbirths, weight loss, decreased milk production and barriers to international trade of animals and their products.[2] Effective livestock vaccination has the potential to raise prosperity and food security for the rural poor in low and middle income countries. Existing vaccines could prevent and control some of these diseases but frequently the vaccines do not reach smallholder farmers, especially marginalized populations, making it necessary for specific vaccine adaptation strategies.[1] the strategies for the use of animal vaccines differed depending on whether the vaccines are aimed at diseases that cause economic losses, government-controlled diseases or neglected diseases. The adaptation of vaccines for neglected diseases prevents a major challenge because they are mostly for zoonotic diseases like brucellosis that produce few or no clinical signs in the animals, making it more difficult for the farmers to appreciate the value of the vaccines.[1] Live vaccines are widely accepted to be superior to inactivated vaccines for protection against brucellosis and suggesting that the localization and persistence of Brucella antigens are key factors in the development of protective immunity,[3] The S19 and RB51 are the *B. abortus* vaccine strains more commonly used to prevent brucellosis in cattle. The initially used S19 strain @ 10^{10} colony forming unit (CFU), followed by RB51 @ 10^{10} CFU and most commonly used challenge strain was *B. abortus* 2308 @ 10^{7} CFU by the intra-conjunctival route. A dose of 10^{9} CFU for S19 and 10^{10} CFU for RB51 are the most suitable for the prevention of abortion and infection caused by *B. abortus* in cattle.[4] Almost all the knowledge available on the protective response induced by both *B. abortus* vaccines strains comes from research using the mouse model.[1] Humoral and cell-mediated immune responses have been reported in non-pregnant heifers affected and vaccinated against *B. abortus*.[3,5] Serological prevalence of *B. abortus* infection in 40-49% humans[6] and 2.66% in cattle[7] against natural infection and humoral immune responses in cattle[8-10] and buffaloes[10] immunized against *B. abortus* vaccines have been reported from Bangladesh. This paper describes the serological responses induced in smallholder cross-bred heifers immunized with RB51 *B. abortus* vaccine in Bangladesh.

MATERIALS AND METHODS

This study was conducted on the Holstein-Frisian × Local cross-bred heifers maintained at the smallholder management system in Daulatpur upazila, Kushtia, Bangladesh during the period from January to June 2020. A smallholder dairy farm consisted of a total of 15 cross-bred cattle of which six were cross-bred heifer calves of four months age. Among these six heifer calves, five were randomly selected for this study.

The *B. abortus* strain RB51 vaccine was 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. The immunized heifers were observed for six months with especial emphasis to two to three hours post-vaccination for any immediate untoward reactions and boosting at the days of 60 with same dose and route. Then vaccinated heifer calves 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 as described.[9] 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.[11]

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.[11] 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. 200 µl 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 antibody reagent 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[12] for statistical analysis. Repeated measure ANOVA model was built using "nlme" package[13] 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.[14]

RESULTS

Table 1 and Fig.1 show the mean values of antibody level in terms of OD values in smallholder crossbred heifers immunized with commercial *B. abortus* strain RB 51 vaccine.

Table 1. Antibody OD values at pre- and post-vaccination with *Brucella abortus* strain RB51 vaccine in smallholder cross-bred heifers

SN Parameters	Days of post-vaccination (n = 5)								
	0	7	14	21	28	60	90	120	150
1. Vaccination	1st	-	-	-	-	Booster		-	-
2. Mean OD value	0.097	0.108	0.124	0.138	0.157	0.223	0.313	0.242	0.199

It appears from the Table 1 that the OD values of the serum of calves immediately before vaccination was 0.097 (at 0 day) and 0.108 at 7th day. After that, the OD value started to rise significantly. The booster dose of vaccine was inoculated on day of 60 followed by antibody titer reached to a peak level at 90th day (OD 0.313) and then started to decline gradually (Table 1).

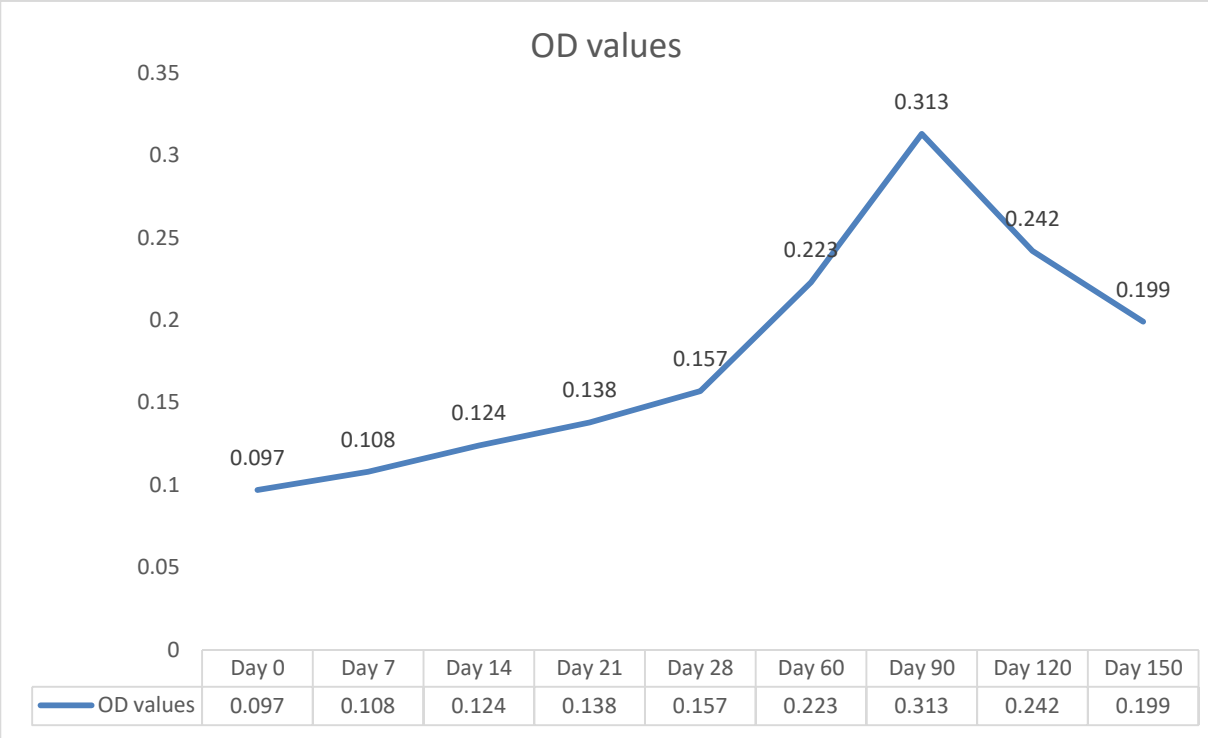

Fig. 1. A graph showing the mean OD values of i-ELISA of cross-bred heifer calves at different days of immunization with *Brucella abortus* strain RB51 vaccine.

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 90th day (0.313 ± 0.0032) and then started to decline the antibody titer gradually with a lowest level at day 150 of post-vaccination (Table I and Fig. I).

DISCUSSION

Brucellosis occurs worldwide in both animals and humans except in those high income countries where bovine brucellosis has been eradicated. Currently, it is an endemic zoonotic disease in most of the low to medium income countries being responsible for more than 500,000 new cases yearly[15] and also causes devastating losses to the livestock industry especially smallholder farmers. Bovine brucellosis is usually associated with reduced fertility, abortion, weak calves with poor weight gain and decreased of milk production but it remains a neglected disease in the developing world.[16] It appears from the research reports that this disease is not a serious problem either in animals or in humans in Bangladesh.[9,11] However, it is maintained at a low level in different livestock species and humans in Bangladesh[11,17] and possibility to exist chronic zoonotic infection in humans as *Brucella* sp. has been detected *B. abortus* biovar 3 from dairy cattle.[18,19] However, the geographical distribution of brucellosis is constantly changing with new foci emerging or re-emerging[20-22] or even wildlife ruminants may act as a silent reservoir of brucellosis.[23]

Recent reports showed re-emergence of bovine brucellosis with increased prevalence in smallholder dairy farms in low to medium income country like Tanzania.[24] Since 2007 many new Brucella species have been detected some of them are highly zoonotic and there are many reasons to believe about possible new comeback or emergence of brucellosis may occur in near future.[15] These findings suggest to evaluate feasible intervention for controlling bovine brucellosis in smallholder dairy farms that indirectly will safeguard public health in low to medium income countries. The internationally approved methods for eradication of brucellosis include vaccination, culling of infected animals, surveillance testing or a combination of any of these.[25] 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.

The antibody responses and efficacy of RB51 vaccine have been evaluated in cattle, buffaloes and elk elsewhere[26-28] and in indigenous adult cattle and buffaloes in Bangladesh[8,10] and also in cross-bred cattle in the military dairy farms[9] in Bangladesh but not in cross-bred heifers under smallholder dairy farming system. Understanding immune responses of immunized cross-bred heifers with SRB51 may be beneficial for the assessment of an efficacious brucellosis vaccine under local field conditions.

The result of the present study confirms the possibility of using commercial indirect 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, cross-bred dairy cattle in Bangladesh.[8-11]

This study has recorded the rise of antibody level from the second weeks of vaccine administration and highest OD (0.223) at 60 days after initial vaccination and the peak OD at 90 days (0.313) 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 and also in cross-bred cattle of Bangladesh.[8-10]

CONCLUSIONS

Results of this study showed that the *B. abortus* RB51 strain vaccine has induced satisfactory antibody response with initial dose and significantly higher 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 higher antibody titer 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 in *B. abortus* RB51 vaccinated animals with challenge studies to the detect the types of immunological responses induced by brucellosis with its protection efficacy in Bangladesh. Currently, very low level of Brucella infection exists in animals in Bangladesh and accordingly surveillance testing and culling of infected animals would be the choice to prevent and eradicate this disease under local conditions. When the incidence of brucellosis is controlled in the animal reservoirs, there is a corresponding and significant decline in the incidence in humans. As it is an important zoonotic disease, it requires 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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