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COMPARISON OF HUMORAL IMMUNE RESPONSES BETWEEN CATTLE AND BUFFALOES IMMUNIZED WITH COMMERCIAL *BRUCELLA ABORTUS* STRAIN RB51 VACCINE IN BANGLADESH

A. I. Mohamud,[1*] A. A. Mohamud,[1*] M. S. Rahman,[1**] M. A. Ehsan,[1] A. A. Maruf,[1]
F. Yasmin,[2] F. Karim[1] and H. Neubauer[3]

[1]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, [*1]Part of MS Thesis (Faculty of Veterinary Science, Benadir University, Mogadishu, Somalia), [2]Department of Animal Science, Faculty of Veterinary Science, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, [3]Friedrich Loeffler Institut, Germany [**]E-mail: prithul02@yahoo.co.uk

ABSTRACT

Background: The effective control and eradication of brucellosis can be achieved by rapid and accurate diagnosis and effective vaccination but both have limitations. Therefore, brucellosis research is currently focused on the improvement of the diagnosis and vaccine induced prophylaxis. Moreover, diagnostic tests and immunization have not been thoroughly studied in buffaloes and even not compared with cattle. Therefore, the comparative evaluation of the immunological responses of Brucella vaccinated cattle and buffaloes would be required for both the diagnosis and vaccine induced efficacy.

Objectives: The main objective of this study was to compare the humoral immune response (HIR) between cattle and buffalo cows immunized with *B. abortus* RB51 vaccine by using indirect ELISA

Materials and Methods: Each of the three randomly selected *B. abortus* sero-negative native cows and three buffaloes received 2.0 ml imported commercial *B. abortus* SRB51 vaccine subcutaneously in the neck region at day 0 and then booster dose at 60 days after first vaccination with similar dose and route. Each of the collected serum samples of both the cattle and buffaloes was tested to detect the antibody status by using commercial indirect ELISA kit.

Results: The results showed that the OD value of the serum of cows and buffalos before inoculation of RB51 *B. abortus* vaccine was 0.088 ± 0.009 and 0.096 ± 0.011 at 0 week and 0.124 ± 0.018 and 0.111 ± 0.010 at 1st week, near about the negative control OD value (0.106). After that, the OD value started to rise from the 2nd week (OD value (0.144 ± 0.023 and 0.1333 ± 0.007) and reached to a peak level at 90 days (OD value 0.376 ± 0.0080 and 0.316 ± 0.219) and then started to decline from 120 days (OD value 0.2963 ± 0.0416 and 0.2863 ± 0.070) to 180 days (OD value 0.1943± 0.073 and 0.176± 0.172) in cows and buffalos respectively.

Conclusion: This study suggests that the RB51 vaccination has induced satisfactory HIR with initial inoculation but significantly higher immune responses with booster immunization which enhancing immunity against both in the cattle and buffaloes. The CMI plays major role in protection against brucellosis needs further investigation in both cattle and buffaloes in Bangladesh.

Keywords: Brucellosis, SRB51 vaccine, Humoral immune response (HIR), I-ELISA, Cattle and Buffaloes

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INTRODUCTION

Brucella abortus can infect most of the mammalian species but bovine species are the preferred host, whereas humans are effectively dead-end-host not transmitted between people. The occurrence of brucellosis in humans is largely dependent on the animal reservoir. Infected animals are the main sources of human infection and accordingly many developed nations have invested heavily in brucellosis control programs in livestock due to public health benefits for the eradication of brucellosis from animals. Although many industrialized countries have eradicated brucellosis in livestock, still it persists in free-ranging and wild animals and it remains a concern for the reintroduction of Brucella organism to livestock.[1] However, brucellosis remains as the main problem in livestock and human health in developing nations of the world. *B. abortus* biovar 1 has been recognized as the etiological agent for both the cattle and water buffaloes[2,3] but their susceptibility has been reported to be varied with lower susceptibility in buffaloes to *B. abortus* infection than cattle.[4] There are 24.491 million cattle and 1.493 million buffaloes in Bangladesh[5] and buffaloes are an economically important livestock species in many Asian countries including Bangladesh.[6] Review reports showed that 3.7% cattle and 4.0% buffaloes found Brucella sero-positive in Bangladesh and most of the research reports on brucellosis in Bangladesh have been made on sero-prevalence and sero-epidemiology.[7,8] Moreover, research reports on brucellosis in buffaloes are very limited in Bangladesh.[6] Brucella control program has mostly based on vaccination with attenuated *Brucella abortus* strain 19 vaccine which provides good level of protection against *B. abortus* and prevents premature abortions in cattle. However, this vaccine has the drawback of inducing o-polysaccharide-specific antibodies that interfere with the discrimination between vaccinated and infected animals during serological screening.[9] In addition, they retain pathogenicity and sometimes cause abortion in vaccinated animals and cause the disease in humans.[10] To overcome the problem of serological cross reaction, RB51 a mutant vaccinal rough strain that is devoid of the LPS O-side chain has been developed.[11] Reports reveals that RB51 vaccine prevents abortion and infection in cattle under experimental and field conditions,[12] although there are few reports about abortion induced by RB51 vaccine[13] and shedding of the Brucella organism in both cows[14] and buffalo[15] milk. Due to some drawbacks shown by these two vaccines much effort has been undertaken for the development of new vaccines, safer and more effective that could be used in other susceptible species of animals.[16] Immunity against brucellosis involves both humoral and cell mediated immune (CMI) responses. Cattle vaccinated with *B. abortus* SRB51 exhibited high level of protection characterized by good CMI,[17] whereas it did not produce any protective efficacy against infection in buffaloes on standard dose recommended for cattle.[18] This has been explained as the SRB51 vaccine induced poor CMI response in buffaloes.[19] The different immunodiagnostic tests have been evaluated for the diagnosis of brucellosis in cattle but the reports on standardization and application of these tests in buffaloes are very limited and the results obtained from the cattle cannot be applied directly on the buffaloes due to of the inherent difference of the two species.[20,21] The differences in sensitivity and specificity of brucellosis serological tests between cattle and buffalo have been reported.[22] In addition, the *B. abortus* RB51 administered at the

recommended dose failed to protect buffaloes from infection following natural exposure to *B. abortus* biovar 1.[18] The immune responses of live attenuated *B. abortus* S19 vaccine in cattle and buffaloes have been evaluated in India.[23,24] Comparison of HIR between heat-inactivated *B. abortus* biovar 3 and strain RB51 in cattle have been studied in Bangladesh.[25] This paper describes the comparison of HIR of commercial *B. abortus* strain RB51 vaccine between cattle and buffaloes in Bangladesh.

MATERIALS AND METHODS

Three native cows and three native buffalo cows with same age were selected for this study and maintained at the animal experimental shed of the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh during the period from January to June 2020. Each of the animal examined thoroughly and appeared good general health. All animals were tested negative for brucellosis by using commercial indirect ELISA.

Preparation for *Brucella abortus* Strain RB51 Vaccines

B. abortus strain RB51vaccine imported from Spain which 1.0 ml contains 10×10^9 cfu organism, one vial contains 25 doses. This vaccine is usually in powder form and suspension is made by mixing with diluent supply by CZ Veterinaria (Spain). Each animal received 2.0 ml of vaccine contained 10-34 $\times$ 10^9 cfu subcutaneously in neck region at day 0 and booster dose at 60 days of first vaccination with similar dose according to the manufacturer's recommendations. The animals were observed for two to three hours post-vaccination for any immediate untoward reactions.

Collection of blood and sera samples from cows

Venous blood (approximately 10.0 ml) was collected from each of the experimental animal by jugular venipuncture before and at day 7, 14, 21, 28, 60, 90, 120, 150 and 180 days post-vaccinations. Then sera were separated from the clotted blood by centrifugation at 1500 rpm for 15 minutes. The sera samples were stored at -20°C till used for Enzyme-Linked Immunosorbent Assay (ELISA).

Enzyme-Linked Immunosorbent Assay (ELISA)

Level of antibody was detected by Antibody Test Kit (IDEXX Montpellier SAS, France) according to the protocol of the manufacturer and reading was performed by automated ELISA reader.

Procedure of ELISA test

All reagents were equilibrated at room temperature and the coated plate were removed from the foil sachet and inserted into the strip holder. According to the manufacturer instructions, four micro-wells were required for control (two positive controls and two negative controls). 190 µl of dilution buffer N.2 was dispensed into each well. 10 µl of undiluted positive and negative control solution were pipetted into the respective control wells. 10 µl of undiluted samples were dispensed into remaining wells and gently mixed after tapping. Then the plate was incubated for one hour at room temperature. Then each micro well was washed with wash

solution for three times. 100 µl of conjugate was added to each well and sealed the plate following incubation for 30 minutes at room temperature and then washed with the wash solution for three times. The 100 µl TMB substrate was added to each well and kept for 20 minutes at RT away from direct light. Finally, 100 of stop solution were added to each well and OD value was read at 450 nm within 5 minutes.

RESULTS

Table 1 shows the comparative used materials and methods with humoral immune response (antibody titer) in indigenous cows and native adult buffaloes immunized with commercial *Brucella abortus* SRB51 vaccine. Fig.1 shows the comparative antibody titer in cows and buffaloes vaccinated with commercial *B. abortus* RB51 vaccine.

Table 1. Comparison of humoral immune response (antibody titer) in indigenous cows and native buffalos immunized with commercially *Brucella abortus* SRB51 vaccine

SN Parameters	Test used	Cattle (n = 3)	Buffalo (n = 3)
01. Type of vaccine used	-	Attenuated live	Attenuated live
02. *B. abortus* strain	-	SRB51	SRB51
03. Dose of vaccine	-	2.0 ml (10-34 × 10^9)	2.0 ml (10-34 × 10^9)
04. Route of administration	-	Subcutaneously	Subcutaneously
05. Total observation period	-	180 days	180 days
A. Pre-immunization			
06. Ab titer / OD values	I-ELISA	0.088 ± 0.009	0.096 ± 0.011
B. Post-immunization			
a. 07 days (1st week)	I-ELISA	0.124 ± 0.018	0.111 ± 0.010
b. 14 days (2nd week)	I-ELISA	0.144 ± 0.023	0.1333 ± 0.007
c. 21 days (3rd week)	I-ELISA	0.148 ± 0.020	0.137 ± 0.162
d. 28 days (4th week)	I-ELISA	0.1833 ± 0.031	0.1733 ± 0.55
e. 60 days (-)	I-ELISA	0.346 ± 0.087	0.283 ± 0.347
f. 90 days (-)	I-ELISA	0.376 ± 0.0080	0.316 ± 0.219
g. 120 days (-)	I-ELISA	0.2963 ± 0.0416	0.2863 ± 0.070
h. 150 days (-)	I-ELISA	0.2763 ± 0.040	0.2593 ± .0531
i. 180 days (-)	I-ELISA	0.1943± 0.073	0.176± 0.172

n = Number of experimental animals

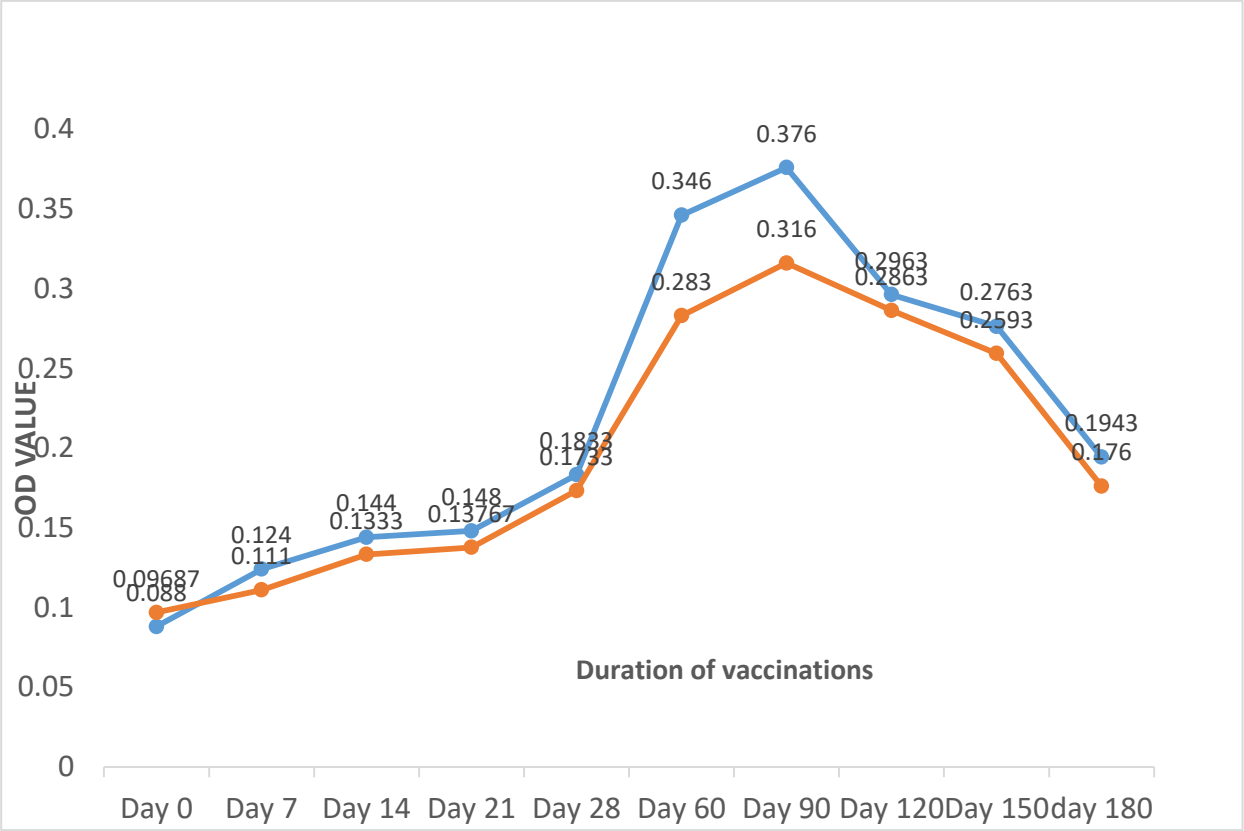

Fig. 1: Comparative antibody response in cows and buffaloes vaccinated with *Brucella abortus* RB51 Vaccines

It appears from the Fig.1 that the OD values of the serum of cows and buffalos before inoculation of RB51 *B. abortus* vaccine were 0.088 ± 0.009 and 0.096 ± 0.011 at 0 week and 0.124 ± 0.018 and 0.111 ± 0.010 at 1st week, near about the negative control OD value (0.106). After that, the OD values started to rise from the 2nd week (OD value 0.0.144 ± 0.023 and 0.1333 ± 0.007) and reached to a peak level at 90 days (OD values 0.0.376 ± 0.0080 and 0.316 ± 0.219) and then started to decline from 120 days (OD values 0.2963 ± 0.0416 and 0.2863 ± 0.070) to 180 days (OD values 0.1943 ± 0.073 and 0.176 ± 0.172) in cows and buffalos respectively.

DISCUSSION

Brucellosis is a zoonotic disease of socio-economic and public health importance with significant impact on the international trade of animals and animal products associated with abortions and reduced fertility in cattle and buffaloes.[26,27] Currently, there are many vaccines of brucellosis available including *Brucella melitensis* Rev.1, Mucosal vaccine subunit, RB51, S19

and DNA vaccines[28-30] but S19, RB51, 45/20, and SR82 are the only a few vaccines that have been used greatly in bovine immunization against *B. abortus* and mostly RB51 and S19 vaccines are widely used[31] and most commonly practiced vaccines in cattle are RB51 and S19 Vaccines.[32,33] An ideal *Brucella* vaccine for both humans and animals should be effective, a-virulent and induce long-lasting protection but the currently used and evaluated live attenuated and inactivated vaccines cannot full the characteristics of an ideal *Brucella* vaccine.[34,35] Although elsewhere have evaluated antibody responses and efficacy of RB51 vaccine in cattle,[36] buffaloes[22] and elk[37] but it seems not to be simultaneously evaluated in both cattle and buffaloes in Bangladesh. Understanding immune responses of buffaloes and cattle to 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 indigenous cattle and buffalos (booster at 90 days) of Bangladesh, monitor antibody responses to RB51 vaccine up to 180 days. This study has recorded the rise of antibody level from the second weeks of vaccine administration and peak levels at 90 days and then started to decline from 120 to 180 days in both the cattle and buffaloes. All animals were sero-negative for Brucella antibodies before vaccination which indicates that experimental animals were neither infected nor vaccinated, whereas satisfactory immune responses produced after vaccination indicates inoculation of vaccine produced satisfactory HIR in both the cattle and buffaloes. However, some variations on the first appearance of antibodies in cattle and buffaloes immunized with *B. abortus* vaccines have been reported elsewhere. These explanations substantiate with the findings that the heifers vaccinated with SRB51 acquired visible immune response four weeks post-immunization,[38] a peak in antibody response in adult cattle vaccinated with RB51 at day 30 by employing an I-ELISA.[39] 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.[23] The antibodies titer began and reached the maximum as early as first week of RB51 vaccination in buffalo calves, remain steady till two weeks post vaccination (WPV), fluctuating till the 6^{th} WPV, then dropped sharply when it disappeared at 11 WPV till the end of the experiment.[40] This is significantly different from the results of RB51 vaccination in cattle in which an IR that began with an increase at day 6 post-vaccination, the antibody level remained constant for two months, then progressively decreased. All vaccinated animals remained negative from day 162 post vaccination to the end of 300 days study period.[41] The IR of buffalo calves (six months age) and heifers (11-12 months age) to a full and half dose *Brucella abortus* S19 vaccine developed high SAT (serum agglutination test) titers with full dose and in heifers 11 to 12 months age than six months old calves, and the rate of decline and the rate of decline of SAT titers in heifers reported much slower than calves.[42] However, these findings contradict with the report that the adult cattle, pregnant or not vaccinated once or twice with 2×10^9 viable *B. abortus* strain RB51 did not seroconvert in the traditional brucellosis tests (Rose Bengal, Serum agglutination and Mercaptoethanol tests), whereas animals vaccinated while pregnant did not abort and no *B. abortus* was isolated from their vaginal mucus and milk. [39]

It has also been reported that the vaccination with RB51 alone is not enough to control brucellosis in endemic areas, and therefore it has suggested eliminating all positive animals at

the time of vaccination and all new positive animals after that for long periods of time.[43]

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.[44]

CONCLUSIONS

RB51 are appropriate *Brucella abortus* vaccine strains mostly used to protect bovine against brucellosis and abortion. Reports reveal that the immune responses and efficacy of Brucella vaccines in cattle and buffaloes somewhat varied and CMI has been suggested to play major role in protection. Comparative characterization of immune responses of both humoral and cellular between cattle and buffaloes to Brucella vaccines may be required for development of an efficacious brucellosis vaccine.

ETHICAL APPROVAL

All animal-related procedures and methods were carried out in accordance with the Animal Ethical Committee of the University.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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