

## COMPARISON OF HUMORAL IMMUNE RESPONSES BETWEEN HEAT-INACTIVATED *BRUCELLA ABORTUS* BIOVAR 3 AND STRAIN RB51 VACCINES IN INDIGENOUS CATTLE OF BANGLADESH

A. A. Maruf,<sup>1</sup> F. Yasmin,<sup>2</sup> F Yeasmin,<sup>1</sup> A. K. M. A. Rahman,<sup>1</sup> M. M. Hossain,<sup>3</sup> H. Neubauer,<sup>4</sup> and M. S. Rahman<sup>1\*</sup>

<sup>1</sup>Department of Medicine, <sup>3</sup>Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202; <sup>2</sup>Department of Animal Science, Faculty of Veterinary Science, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh, <sup>4</sup>Friedrich Loeffler Institut, Germany \*E-mail: prithul02@yahoo.co.uk

### ABSTRACT

**Background:** Live attenuated *Brucella abortus* strains 19 and RB51 vaccines have been used as a key method for the control and eradication of brucellosis in cattle worldwide for decades. Due to certain limitations of these live vaccines, research has been undertaken for the development of an ideal more effective and safer vaccine for animals and human brucellosis.

**Objective:** The main objective of this study was to compare the humoral immune responses (HIR) between the heat-inactivated *Brucella abortus* biovar 3 and attenuated live RB51 vaccines in native cattle of Bangladesh.

**Materials and Methods:** The methods of isolation, identification, preparation of inoculum dose ( $10 \times 10^{10}$  cfu/5 ml) and heat inactivation of *B. abortus* biovar 3 was followed as described earlier. Each of the three *B. abortus* sero-negative native cows was inoculated with heat-inactivated *B. abortus* vaccine @ 5.0 ml ( $10 \times 10^{10}$  cfu /5 ml)/ cow SC single injection. Similarly, each of five native calves of 6 to 9 months old was inoculated with live attenuated RB51 vaccine (CZ Veterinaria, SA, Spain) @ 2.0 ml ( $10\text{-}34 \times 10^9$ ) SC as single dose. The sera of cows were collected at 0, 7, 14, 21, 28, 40, 60 and 90 days post vaccination, whereas the sera of the calves were collected at 0, 7, 14, 21, 28, 60, 90, 120, 150 and 180 days post-vaccination. All the collected sera of both the groups were tested to evaluate antibody titer by RBT followed by ELISA with commercial tests kits.

**Results:** The HIR of the cows inoculated with heat-inactivated vaccine showed antibody (Ab) titer started to rise significantly ( $p < 0.05$ ) from the 14 days ( $OD 0.2116 \pm 0.0397$ , Ab titer 1:120) and reached a peak level at 28 days ( $OD 0.319 \pm 0.172$ , Ab titer 1:800) and then started to decline significantly ( $p < 0.05$ ) from 40 days ( $OD 0.234 \pm 0.0415$ , Ab titer 1:35) to 60 days ( $OD 0.094 \pm 0.0075$ , Ab titer 0). The mean Ab titer in calves inoculated with RB51 vaccine showed that Ab titer started to appear insignificantly ( $p > 0.05$ ) from day 7 ( $OD 0.094 \pm 0.01603$ ) and reached peak level at day 60 days ( $OD 0.592 \pm 0.398$ ), changes are very significant from day 0 ( $p < 0.05$ ), after 60 days Ab level start to decrease and reach at lowest level at day 150 ( $OD 0.112 \pm 0.0188$ ), Ab level found similar to day 0 ( $OD 0.0826 \pm 0.00517$ ) at 180 days ( $OD 0.0822 \pm 0.00249$ ).

**Conclusions:** The S19 and RB51 are the approved *B. abortus* vaccine strains have been widely and successfully used with some limitations to prevent bovine brucellosis worldwide. In addition to live attenuated and inactivated vaccines, recombinant genes, proteins, vectors, DNA and recombinant mutant vaccines have also been evaluated for the prevention of brucellosis but further research would be required to develop an ideal vaccine for both the humans and animals.

**Keywords:** *Brucella abortus* vaccines, Attenuated live vaccine, Inactivated vaccine, Humoral immune responses, Cattle, RBT and ELISA

Article Info: Article Code No. © LEP: JVMOHR/0015/2019

Received: 15 Nov. 2019 Revised: 10 Dec. 2019 Accepted: 22 Dec 2019 Published: 31 December 2019

**Citation:** Maruf AA, Yasmin F, Yeasmin F, Rahman AKMA, Hossain MM, Neubauer H and Rahman MS (2019). Comparison of humoral immune responses between heat-inactivated *Brucella abortus* biovar 3 and strain RB51 vaccines in indigenous cattle of Bangladesh. *J. Vet. Med. OH Res.* 1(2): 247-259 [ DOI: 6111/jvmohr.2019.1(2).0015]



**Copy right** © 2019. The Authors. Published by LEP. This is an open access article under the CC-BY-NC-ND License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

## INTRODUCTION

Vaccines and vaccinations are used for making the population immune (resistant) to natural infection but the success of any vaccination program depends mainly on the effectiveness of the vaccine used and its coverage in the target population.<sup>1</sup> Bovine brucellosis in some developed countries has been controlled by using vaccination and culling the affected cattle from the herd and vaccination has considered as the only key method to control brucellosis but it has some limitations to eradicate this disease. However, only four live attenuated *B. abortus* strains (S19, RB51, 45/20 and SR82) have been used in cattle immunization but S19 and RB51 are most widely used vaccines to prevent bovine brucellosis in the world.<sup>2-5</sup> The live attenuated *B. abortus* S19 vaccine is the first vaccine to be used extensively for the prevention of brucellosis in cattle and it still remains the reference vaccines to which other vaccines have been compared and evaluated.<sup>1</sup> The *B. abortus* S19 was isolated in 1923 from milk of a Jersey cow by Dr. John Buck.<sup>6</sup> This virulent culture was accidentally left out at room temperature for one year and when tested in guinea-pigs showed lower virulence compared with previous tests.<sup>7</sup> Subsequently, S19 showed to be highly successful in immunization of calves<sup>6</sup> and its efficacy was proved by experimental tests in cattle<sup>8</sup> and under field conditions.<sup>9</sup> This live vaccine is normally used in female calves aged between 3 and 6 months as a single SC dose of  $5-8 \times 10^{10}$  viable *B. abortus* organism.<sup>10</sup> However, it does not permit discrimination of antibodies between natural infection and vaccinated animals. In addition, low rate (1.5%) of abortion, passed through 10% milk samples and significant reduction in milk production has been reported with *B. abortus* S19 vaccination<sup>4,5,8</sup> and remains the source and pathogenic to humans.<sup>1</sup> The *B. abortus* strain RB51 vaccine is a rough attenuated organism which was originally derived from a rifampicin-resistant mutant of *B. abortus* strain 2308 and has replaced *B. abortus* S19 strain as a vaccine strain in some developed countries.<sup>10,11</sup> The RB51 is a very stable strain and it has no or highly reduced virulence to cause abortion in cattle.<sup>11</sup> The protective efficacy and immunity induced by RB51 strain has been reported to be similar to or better than induced by strain S19.<sup>12,13</sup> The recommended dosage for RB51 calf-hood vaccination is  $1.0-3.4 \times 10^{10}$  CFU.<sup>5</sup> However, this vaccine organism is still infectious to humans and even the vaccinated cattle with RB51 have been reported to be susceptible to brucellosis.<sup>14,15</sup> The *B. abortus* 45/20 is a rough strain which was isolated from smooth strain 45/0 following 20 passages in guinea-pigs and its vaccine has been used in guinea-pigs and cattle to prevent *Brucella* infection but the reversions to the wild smooth type has limited its use as a live vaccine.<sup>16,17</sup> Accordingly, this vaccine is prepared only as heat-inactivated *B. abortus* biovar 1 rough strain 45/20 combined with oil adjuvant to avoid reversion to a virulent strain for use in adult cattle.<sup>18</sup> It does not interfere with serological diagnosis and it is safer in pregnant animals but only has been tested in some countries.<sup>4</sup> The SR82 strain is a *B. abortus* biovar 6 live attenuated vaccine used since 1974 by the former USSR for the control of bovine brucellosis.<sup>19</sup> The SR82 induced protection level similar to S19 and reported to be effective under field conditions.<sup>19,20</sup> Currently, this vaccine is used in the Russian Federation, Azerbaijan, Tajikistan and other countries.<sup>19</sup> The inactivated vaccines of *B. abortus* strain 45/20 in cattle and sheep and *B. melitensis* H38 in mice and cows have been evaluated but lack of sufficient protection after challenge.<sup>21</sup> Recent developments to improve brucellosis vaccines include generation of

## Antibody responses in cattle immunized with *B. abortus* vaccines

knockout mutants by targeting genes involved in metabolism, virulence and the lipopolysaccharide synthesis pathway as well as generation of DNA vaccines, mucosal vaccines and live vector vaccines have all evaluated with varying degrees of success.<sup>1,10</sup> However, the isolation and characterization of *B. abortus* biovar 3,<sup>22</sup> therapeutic trial of chronically *B. abortus* infected cattle<sup>23</sup> and evaluation of inactivated *B. abortus* vaccine in guinea-pigs<sup>24</sup> have been studied in Bangladesh. This paper describes the comparison of humoral immune responses between the attenuated live RB51 and experimentally produced inactivated *B. abortus* vaccines in cattle of Bangladesh.

### **MATERIALS AND METHODS**

The *B. abortus* organism was isolated from the aborted fetal membranes of four cows aborted between 5<sup>th</sup> to 8<sup>th</sup> months of pregnancy at the Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka and the methods for isolation and identification of *B. abortus* have been described.<sup>24</sup> The *B. abortus* was confirmed by Polymerase chain reaction (PCR) using *B. abortus* specific IS711 primer.<sup>25</sup>

#### **Total viable count (TVC) from broth by using pours plate method**

The TVC was detected by pour plate technique using tenfold serial dilution for the counting cfu / ml of *B. abortus* from broth for dose calculation of heat killed vaccine.

#### **Centrifugation for bacterial pellet formation**

Centrifugation of 400 ml of the cultured broth were performed three times at 10,000 rpm for 15 minutes and bacterial pellets were washed with PBS every time after centrifugation.

#### **Preparation of required dose for killed vaccines**

The bacterial pellet was mixed with required amount of PBS to obtain  $10 \times 10^{10}$  cfu organisms in 5.0 ml dose for inoculation in each experimental cow and homogenization was performed using vortex machine.<sup>26</sup>

#### **Heat inactivation of the organism**

The prepared doses of the selected *B. abortus* organisms were inactivated by heating the suspension at 80°C for 90 minutes in water-bath for producing heat-inactivated vaccine.<sup>26</sup>

#### **Preparation of experimental cattle**

Three native cows with same age groups were selected for inoculation of inactivated *B. abortus* vaccine and five native calves with 6 to 8 months old reared in Veterinary Teaching Hospital, BAU, Mymensingh, Bangladesh for this purpose.

#### **Immunization of cows with heat-inactivated vaccine**

Each of the three cows was inoculated with the heat-inactivated *B. abortus* vaccine suspension @ 5.0 ml ( $10 \times 10^{10}$  cfu)/ cow subcutaneously.<sup>26</sup> The immunized cows were observed for 3 months.

### **Immunization of calves with live attenuated *B. abortus* strain RB51 vaccine**

The *B. abortus* strain RB51 vaccine was imported from Spain (CZ Veterinaria SA, Spain) and 2.0 ml of the vaccine contains  $10\text{-}34 \times 10^9$  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 calf was inoculated strain RB51 vaccine @ 2.0 ml subcutaneously in neck region. The immunized animals were observed for six months with especial emphasis to two to three hours post-vaccination for any immediate untoward reactions.

### **Collection of blood and sera samples from cattle**

Approximately 10 ml of blood samples were collected from the jugular vein of each of the experimental cow at 0, 7, 14, 21, 28, 60 and 90 days post-immunization with heat-inactivated vaccine. Similarly blood samples were collected from each of calf at 0, 7, 14, 21, 28, 60, 90, 120, 150 and 180 days post-immunization with live attenuated strain RB51 vaccine. Then sera were separated from all the collected blood samples by centrifugation at 1500 rpm for 10 minutes by using conventional method. All the collected sera were stored at  $-20^{\circ}\text{C}$  for reciprocal antibody titer by Rose Bengal Test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA).

### **Reciprocal antibody titer by RBT**

The RBT was performed to determine the reciprocal antibody titer based on the procedure described by the kit manufacturer.<sup>27</sup> Briefly, 30  $\mu\text{l}$  of antigen was placed on a fine plastic plate circled approximately 1.5 cm in diameter and two fold dilutions of 30  $\mu\text{l}$  of tested serum was performed (1:5, 1:10, 1:20, 1:40, 1:80 respectively up to the dilution where agglutination stop) with the use of PBS and was put beside each of the antigen respectively up to the disappearing of the agglutination. The antigen and serum were mixed on the plate with a stirrer and rotated for four minutes.

### **Application of ELISA**

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

### **Procedure of ELISA**

All reagents were equilibrated at room temperature and the coated plate were removed from the foil sachet and inserted into the strip holder. Four micro-wells were required for control (two positives and two negative controls). 190  $\mu\text{l}$  of dilution buffer N.2 was dispensed into each well. 10  $\mu\text{l}$  of undiluted positive and negative control solution were pipetted into the respective control wells. 10  $\mu\text{l}$  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 washing solution for three times. 100  $\mu\text{l}$  of conjugate was added to each well and sealed the plate following incubation for 30 minutes at room temperature and then washed with the washing solution for three times. 100  $\mu\text{l}$  TMB substrate was added to each well and kept for 20 minutes at RT away from direct light. Finally, 100  $\mu\text{l}$  of stop solution was added to each well and OD value was read at 450 nm within 5 minutes.

## Antibody responses in cattle immunized with *B. abortus* vaccines

### Statistical analysis

The t-test was used for statistical analyses by using SPSS program version 22 computer program to find the significant different antibody level detected by ELISA. P value < 0.05 was assumed for statistical significance.

### RESULTS

The humoral immune response (antibody titer) between experimentally produced heat inactivated *B. abortus* biovar 3 and the commercially available attenuated *B. abortus* strain RB51 vaccines were compared in guinea-pigs, cows and calves (Table 1). It appears from Table 1 and Graph 1 that the reciprocal antibodies was 0 with RBT and mean OD value was  $0.0868 \pm 0.0069$  with 0.106 as negative control. The cows immunized with inactivated vaccine showed reciprocal antibodies titers with RBT were found 1 : 50 at 7 days, 1 : 120 at 14 days, 1 : 400 at 21 days, 1 : 800 at 28 days, 1 : 35 at 40 days and 0 at 60 days.

The graph 1 shows that the reciprocal antibody titer was 0 at the pre-inoculation day of heat-inactivated *B. abortus* vaccine and started to rise from the 14 days and reach a peak level 28 days and then started to decline up to 60 days and at antibody level was similar to the day 0 of inoculation (Fig. 1).

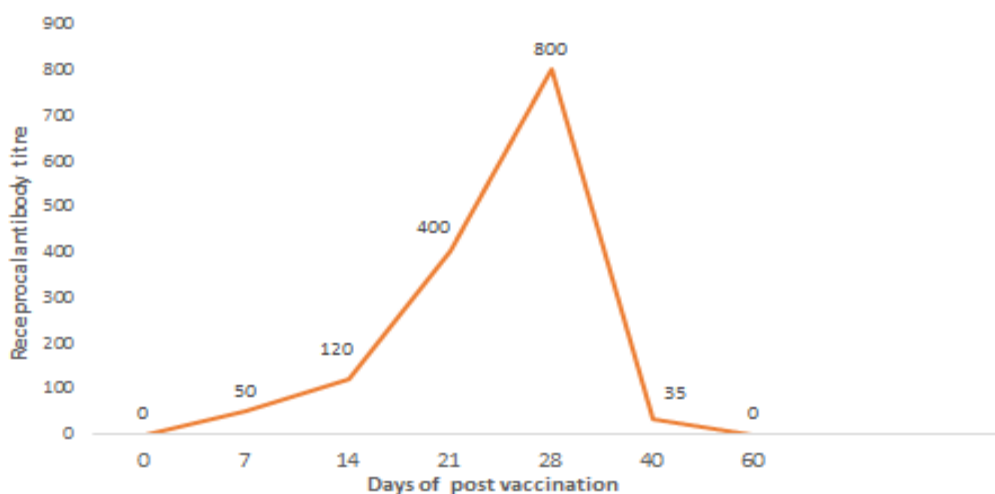


Fig. 1: Rose Bengal Test (RBT) antibody titer in native cows immunized with locally isolated *Brucella abortus* biovar 3 heat-inactivated vaccine.

It appears from Table 1 and Graph 2 that the mean ELISA antibody titer in case of RB51 vaccinated calves start to increase at 7 days of post-vaccination (OD value  $0.091 \pm 0.01603$ ) and then increased gradually and reached at peak at day 60 (OD value  $0.592 \pm 0.398$ ). After sixty days it start to decline and reach lower level at day 150 (OD value  $0.112 \pm 0.0188$ ) and at day 180 (OD value  $0.0822 \pm 0.00249$ ) antibody titer was found similar to day 0 ( $0.0826 \pm 0.0051$ ).

Table 1. Comparison of humoral immune response (antibody titer) in cattle and guinea-pig immunized with locally produced inactivated and commercially produced attenuated live *Brucella abortus* vaccines

SNParameters	Test used	Guinea-pigs <sup>24</sup> (n= 4)	Cows (n = 3)	Calves (n = 5)
1. Types of vaccine used	-	Inactivated	Inactivated	Attenuated live
2. <i>B. abortus</i> strain/biovar	-	Biovar 3	Biovar 3	SRB 51
3. Dose of vaccine (cfu)	-	2.0 ml ( $4 \times 10^{10}$ )	5.0 ml ( $10 \times 10^{10}$ )	2.0 ml ( $34 \times 10^9$ )
4. Route of administration	-	Subcutaneously	Subcutaneously	Subcutaneously
5. Total observation period	-	9 wks (63 days)	12.86 wks (90 days)	25.71 wks (180 days)
6. Ab titer / OD values				
Pre-immunization	RBT	0	0	-
(day 0)	ELISA	0.0945(0.106)	$0.0868 \pm 0.0069$	$0.0826 \pm 0.0051$
Post-immunization:				
07 days (1 <sup>st</sup> week)	RBT	1:5	1 : 50	-
	ELISA	0.1025	$0.1047 \pm 0.0112$	$0.091 \pm 0.01603$
14 days (2 <sup>nd</sup> week)	RBT	1 : 120	1 : 120	-
	ELISA	0.2287	$0.2116 \pm 0.0397$	$0.1012 \pm 0.0226$
21 days (3 <sup>rd</sup> week)	RBT	-	1 : 400	-
	ELISA	-	-	$0.3338 \pm 0.229$
28 days (4 <sup>th</sup> week)	RBT	1 : 800	1 : 800	-
	ELISA	0.2842	$0.3190 \pm 0.172$	$0.463 \pm 0.3326$
40 days (-)	RBT	-	1 : 35	-
	ELISA	-	$0.234 \pm 0.0415$	-
42 days (6 <sup>th</sup> week)	RBT	1 : 35	-	-
	ELISA	0.1832	-	-
60 days (-)	RBT	-	0	-
	ELISA	-	$0.094 \pm 0.0075$	$0.592 \pm 0.398$
63 days (9 <sup>th</sup> week)	RBT	0	-	-
	ELISA	0.1015	-	-
90 days (-)	RBT	-	-	-
	ELISA	-	$0.0821 \pm 0.00705$	$0.3202 \pm 0.1993$
120 days (-)	RBT	-	-	-
	ELISA	-	-	$0.24 \pm 0.1697$
150 days (-)	RBT	-	-	-
	ELISA	-	-	$0.112 \pm 0.0188$
180 days (-)	RBT	-	-	-
	ELISA	-	-	$0.0822 \pm 0.00249$
	Mean $\pm$ SD		- = Not tested	

## Antibody responses in cattle immunized with *B. abortus* vaccines

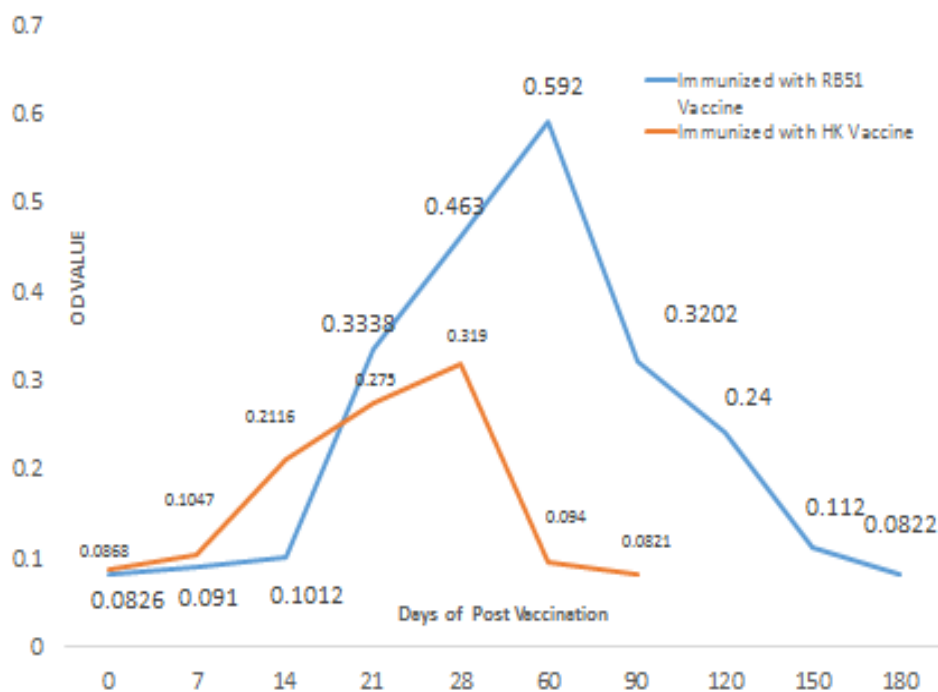


Fig. 2: Comparison of the ELISA antibody titer between the locally isolated *Brucella abortus* biovar 3 heat-inactivated and the commercial live attenuated *B. abortus* strain RB51 vaccines in cattle

## DISCUSSION

There is no licensed vaccine for prevention of human brucellosis and therefore, the vaccination in animal is a major factor for the control and eradication of brucellosis in both animals and humans. WHO has recommended the general strategies to eradicate brucellosis are: (a) prevention of spread between and monitoring of brucellosis-free herds and zones, (b) elimination of infected animals by test and slaughter programs to obtain brucellosis-free herds and regions and (c) vaccination to reduce the prevalence.<sup>28</sup>

The vaccination programs minimized the economic losses due to abortion, infertility, and weak offspring and decreased milk production.<sup>5</sup> The vaccine programs are currently based on control of brucellosis mainly due to *B. melitensis* and *B. abortus*.<sup>29</sup> The attenuated live and inactivated vaccines have been evaluated to prevent brucellosis but attenuated live vaccines have been shown to be superior protective immunogen against the facultative intracellular *Brucella* organism.<sup>30</sup> When live vaccine administered in the animals, the organism present in the live vaccine is allowed to multiply within the host cells allowing *in vivo* gene expression and the cell mediated and long lasting immunity developed.<sup>3</sup>

The use of live attenuated vaccines against brucellosis represents a risk due to its potential ability to revert to virulence, cause abortion in pregnant animals, shed in milk and infect humans come into contact with the vaccine like farmers, abattoir workers and veterinarians.<sup>30</sup>

A wide variety of killed vaccines have been evaluated for prevention of brucellosis but none have approached the protection levels afforded by the live attenuated vaccines.<sup>30</sup> The killed vaccines of *B. abortus* strain 45/20 was used in cattle and sheep and *B. melitensis* strain H38 vaccine was tested in mice and cows but lack of sufficient protection after challenge but induced persistent antibody titer. Brucella specific antibodies have important roles at the initial phase of a Brucella infection but they have limited roles following intracellular localization. The strong humoral immunity unaccompanied by cell-mediated immunity (CMI) cannot provide total protection against Brucella organism.<sup>10</sup>

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.<sup>31</sup> The recombinant genes, proteins, vectors, DNA and recombinant mutans have been evaluated as vaccines which are promising vaccine candidates because they are less bio-hazardous, well-defined, a-virulent, non-infectious and nonviable. Several antigenic fractions extracted from Brucella have been tested as a vaccines with adjuvants which included cell envelopes,<sup>32</sup> outer membrane proteins,<sup>32,33</sup> insoluble residues of hot sodium dodecyl sulfate (SDS) extracts of cell envelopes,<sup>33</sup> phenol insoluble (PI) fraction,<sup>34</sup> soluble SDS extract,<sup>2</sup> Brucella soluble antigens,<sup>35</sup> periplasmic proteins and salt extractable proteins,<sup>36,37</sup> chemically modified Brucella proteins,<sup>38</sup> smooth and rough LPS,<sup>36</sup> recombinant Cu-Zn superoxide dismutase and synthetic peptides<sup>39</sup> and whole killed cells.<sup>40</sup> The protection was only conferred by PI fraction vaccine which lasted around 18 to 24 months in laboratory workers.<sup>41</sup> However, these inactivated tried vaccines were not caused infection but poor protection, local reaction at the site of inoculation and interfere with sero-diagnosis ended their research activities at the laboratories stage. Moreover, these new vaccines are mainly evaluated in mice model and have not been properly tested and were not effective in the target cattle species.<sup>1</sup>

Recently, locally isolated *B. abortus* biovar 3 has been evaluated as an inactivated vaccine in guinea-pigs<sup>24</sup> and an attempt has been made to compare the humoral immune responses between the inactivated locally isolated *B. abortus* and live attenuated commercial RB51 vaccines in cattle. The antibody response induced by the immunization with heat killed isolate of *B. abortus* has been reported as reciprocal antibody titer by using ELISA test.<sup>27,42</sup> The antibody response was recorded from the first week and reached a peak level at fourth week of post-immunization with heat-inactivated *B. abortus* vaccine. This immune response was due to the use of a single dose vaccine without any booster or adjuvant. These findings support the earlier report in which the killed vaccine prepared from *B. abortus* smooth strain 544 with adjuvant (water-in-oil emulsion) induced 230-fold more protective immunity in guinea-pigs than the same without any adjuvant.<sup>43</sup>

The humoral immune responses recorded in calves immunized with the live attenuated RB51 vaccine in this study are in accord with the results in buffalo calves immunized with the same *B. abortus* vaccine.<sup>44</sup> The antibody was started to appear at day 6 post-vaccination and constantly persists the peak antibody level for two months and then progressively decreased. All vaccinated animals remained negative from day 162 post vaccination to the end of the study. The results of this study confirm the possibility of using I-ELISA to identify RB51



## Antibody responses in cattle immunized with *B. abortus* vaccines

vaccinated calves and moreover, monitor antibody responses to RB51 vaccination up to 180 days. These observations corroborate with the findings that the cattle vaccinated with RB51 acquired detectable immune responses four weeks post immunization,<sup>45</sup> a peak in antibody response in adult cattle vaccinated with RB51 at day 30 by employing an I-ELISA<sup>46</sup> and significant difference in dot blot antibody titers of vaccinated and control buffaloes four weeks post immunization.<sup>47</sup> Adult vaccinated buffaloes have reported to develop higher levels of serum antibodies followed by heifers and calves during the initial four months of experiment which may be attributed to increased immune-competence or delayed clearance of vaccinal organism from sexually mature animals. The CFT titers retained in more than 80% of 6 to 10 months old animals for more than four months whereas the overall antibody titers waned out earlier in calves than heifers and adults.<sup>47</sup>

When the data of individual animal was evaluated for persistence of positive titers, it was observed that all of the calf's maintained absorbance values above the cut off (threshold point) after 120 days whereas only one to two calves retained the titers for 150 to 180 days. These results are in consistence with the report in adult cattle<sup>48</sup> and the titers above the threshold values up to seven months in 6 to 10 months old buffaloes have also been reported.<sup>47</sup>

Cattle vaccinated with higher ( $1.8 \times 10^{10}$  CFU) dose of *B. abortus* RB5 produced significant antibody level earlier (seventh day) than those with lower ( $1 \times 10^{10}$  CFU) dose (21st day) and the antibody persisted longer (up to 150 days) with higher dose in compare to the lower dose (only up to 120 days). However, both the groups showed maximum immune responses on the same observation period on 60th day.<sup>49</sup>

The live attenuated *B. abortus* RB51 vaccine administered at recommended dose at calf-hood failed to protect water buffalo from natural exposure to *B. abortus* biovar 1.<sup>50</sup> The elk (*Cervus elaphus*) vaccinated with RB51 didn't protect from natural infection and abortion.<sup>51</sup> The poor cell-mediated immune response might be the reason for this vaccine inefficacy.<sup>52,53</sup>

The comparative results on the humoral immune responses between the inactivated *B. abortus* vaccine in cows and live attenuated RB51 vaccine in calves revealed higher antibody responses with RB51 vaccinated calves than the cows immunized with inactivated vaccine and even the antibody titer in cows ended earlier in cows immunized with inactivated vaccine.

## CONCLUSIONS

Most of the evaluated reports on immune responses and efficacy of inactivated vaccines of *B. abortus* in animals have shown discouraging results against natural infection in comparison to live attenuated vaccines elsewhere. An attempt was made for the first time in Bangladesh to compare the humoral immune responses between the locally isolated *B. abortus* heat-inactivated vaccine and the commercial live attenuated *B. abortus* RB51 vaccine in cattle. The peak antibody titer and its persistence duration were found lower in heat-inactivated vaccine in comparison to attenuated live vaccine. However, higher antibody responses with longer persistence time have been reported with inactivated vaccines added with adjuvant which has not been tried in this experiment. Therefore, research on the efficacy of inactivated *B. abortus* vaccine could be assessed with adjuvant added vaccine followed by challenged infection in cattle. Moreover, currently used and evaluated all types of brucellosis vaccines including

cellular vaccines are not without limitations and accordingly, the search for highly effective and safe ideal brucellosis vaccines remains active in the world. So, further research will be required to fully evaluate the benefits and risks of a-cellular vaccines for the prevention of brucellosis in animals and humans.

### **ETHICAL APPROVAL**

All animal-related procedures and methods were carried out in accordance with the Animal Ethical Committee of the University (AWEEC/BAU/2019/17).

### **CONFLICT OF INTEREST**

The authors declared no conflict of interest.

### **ACKNOWLEDGEMENTS**

This research work was supported by the Krishi Gobeshona Foundation, Farm Gate, Dhaka, Bangladesh under the Project No. TF-44L/17 and Project title, 'Livestock and human brucellosis: molecular diagnosis, treatment and control' during the financial years 2018-2021.

The authors are grateful to Prof. Dr. M. A. Samad, Editor, JVMOHR, Department of Medicine, BAU, Mymensingh, Bangladesh for writing assistance and editing this article.

### **REFERENCES**

- 01 Dorneles EMS, Sriranganathan N and Lage AP (2015). Recent advances in *Brucella abortus* vaccines. *Veterinary Research* 46: 76 [doi: 10.1186/s13567-015-0199-7].
- 02 Sterne M, Trim G and Broughton ES (1971). Immunization of laboratory animals and cattle with non-agglutinogenic extracts of *Brucella abortus* strain 45-20. *Journal of Medical Microbiology* 4: 185- 194 [doi: 10.1155/2013/743509]
- 03 Schurig GG, Sriranganathan N and Corbel MJ (2002). Brucellosis vaccines: past, present and future. *Veterinary Microbiology* 90: 479-496
- 04 Moriyon I, Grillo MJ, Monreal D, Gonzalez D, Marin C, Lopez-Goni I, Mainar-Jaime RC, Moreno E and Blasco JM (2004). Rough vaccines in animal brucellosis: structural and genetic basis and present status. *Veterinary Research* 35: 1-38 [doi: 10.1052/vetres:2003037]
- 05 Olsen SC and Stoffregen WS (2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert Review of Vaccines* 4: 915-928 [doi: 10.1586/14760584.4.6.915]
- 06 Buck JM (1930). Studies on vaccination during calf-hood to prevent bovine infectious abortion. *Journal of Agricultural Research* 41: 667-689
- 07 Graves RR (1943). The story of John M Buck's and Matilda's contribution to the cattle industry. *Journal of American Veterinary Medical Association* 102: 193-195 [doi: 10.1371/journal.pone.0002193]
- 08 McDiarmid A (1957). The degree and duration of immunity in cattle resulting from vaccination with S19 *Brucella abortus* vaccine and its implication in the future control and eventual eradication of brucellosis. *Veterinary Record* 69: 877-879 [doi: 10.1371/journal.pone.0136696]
- 09 Koh SH and Morley FH (1981). The effect of calf-hood vaccination with strain 19 on the serological diagnosis and eradication of bovine brucellosis. *Australian Veterinary Journal* 57: 551-553 [doi: 10.1111/j.1751-0813.1981.tb00432.x]

## Antibody responses in cattle immunized with *B. abortus* vaccines

- 10 Lalsiamthara J and Lee JH (2017). Development and trial of vaccines against Brucella. *Journal of Veterinary Science* 18 (suppl. 1): 281-290 [doi: 10.4142/jvs.2017.18.S1.281]
- 11 Schurig GG, Roop RM, Bagchi T, Boyle S, Buhrman D and Sriranganathan N (1991). Biological properties of RB51; a stable rough strain of *Brucella abortus*. *Veterinary Microbiology* 28: 171-188
- 12 Cheville NF, Olsen SC, Jensen AE, Stevens MG, Palmer MV and Florance AM (1996). Effects of age at vaccination on efficacy of *Brucella abortus* strain RB51 to protect cattle against brucellosis. *American Journal of Veterinary Research* 57: 1153-1156
- 13 Lord VR, Schuring GG, Cherwonogrodzky JW, Marcano MJ and Melendez GE (1998). Field study of vaccination of cattle with *Brucella abortus* strains RB51 and 19 under high and low disease prevalence. *American Journal of Veterinary Research* 59: 1016-1012
- 14 Olsen SC, Boyle SM, Schurig GG and Sriranganathan NN (2009). Immune responses and protection against experimental challenge after vaccination of bison with *Brucella abortus* strain RB51 or RB51 overexpressing superoxide dismutase and glycosyltransferase genes. *Clinical Vaccine and Immunology* 16: 535-540 [doi: 10.1128/cvi.00419-08]
- 15 Van Campen H and Rhyan J (2010). The role of wildlife in diseases of cattle. *Veterinary Clinics of North American Food Animal Practice* 26: 147-161
- 16 McEwen AD (1940). Experiments on contagious abortion. The immunity of cattle inoculated with vaccines of grades virulence. *Veterinary Record* 52: 815-819
- 17 Taylor AW and McDiamid A (1949). The stability of the a-virulent characters of *Brucella abortus* strain 19 and strain 45/20 in lactating and pregnant cows. *Veterinary Record* 61: 317-318 [doi: 10.1177/030098589603300607]
- 18 McEwen AD and Samuel JMD (1955). *Brucella abortus*: heat stable protective antigen revealed by adjuvant and present in a rough variant strain 45/20: immunization experiments on guinea-pigs. *Veterinary Record* 67: 546-548 [doi: 10.3389/fmicb.2018.02593]
- 19 Ivanov AV, Salmakov KM, Olsen SC and Plumb AGE (2011). A live vaccine from *Brucella abortus* strain 82 for control of cattle brucellosis in the Russian Federation. *Animal Health Research Reviews* 12: 113-121 [doi: 10.1017/s1466252311000028]
- 20 Salmakov KM, Fomin AM, Plotnikova EM, Safina GM, Galimov GM, Salmakova AV, Ivanov AV, Panin AN, Sklyarov OD, Shumilov KV and Klimanov AI (2010). Comparative study of the immunobiological properties of live brucellosis vaccines. *Vaccine* (28): F35-F40.
- 21 Plommet M, Renoux G, Philppon A, Lorentz C and Gestin J (1970). Brucellose experimentale. I. Comparaison de l'efficacite des vaccines B19 and H38 vaccine. *Annales de Recherches Veterinaires (Paris)* 1: 189-201 [doi: 10.1155/2013/743509]
- 22 Islam MS, Garofolo G, Sacchini L, Dainty AC, Khatun MM, Saha S and Islam MA (2019). First isolation, identification and genetic characterization of *Brucella abortus* biovar 3 from dairy cattle in Bangladesh. *Veterinary Medicine and Science* 5: 556-562 [doi: 10.1002/vms3.193]
- 23 Maruf AA, Yasmin F, Yeasmin 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.2019.1(2).0013]
- 24 Rahman MS (2019). *Brucella abortus* killed vaccine: The achievement of 52 years (1967-2019) in Bangladesh. *EC Veterinary Science ECO* 02: 14-21
- 25 Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R and Leunissen JAM (2007). Primer3 Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* 35.2: W71-74 [doi: 10.1093/nar/gkm306]

- 26 Alton G, Jones L, Angus R, Verger J (1988). Techniques for the Brucellosis Laboratory, Institute National de la Recherche Agronomies, Paris.
- 27 Baek BK, Rahman MS, Hur J (2002). Protective immunity of *Brucella abortus* strain RB51 in Sprague Dawley rat. *Korean Journal of Veterinary Public Health* 26: 235-242.
- 28 WHO (1998). Human and Animal Brucellosis. WHO/MZCP, Damascus, Syria.
- 29 Oliveira SC, Giambartolomei GH and Cassataro J (2011). Confronting the barriers to develop novel vaccines against brucellosis. *Expert Review of Vaccines* 10: 1291-1305 [doi: 10.1586/erv.11.110]
- 30 Avila-Calderon ED, Lopez-Merino A, Sriranganathan N, Boyle SM and Contreras-Rodriguez A (2013). A history of the development of Brucella vaccines. *BioMed Research International*. Article ID 743509 [doi: 10.1155/2013/743509]
- 31 Perkins SD, Smither SJ and Atkins HS (2010). Towards a Brucella vaccine for humans. *FFMS Microbiology Reviews* 34: 379-394 [doi: 10.1111/j.1574-6976.2010.00211.cx]
- 32 Montaraz JA and Winter AJ (1986). Comparison of living and non-living vaccines for *Brucella abortus* in BALB/c mice. *Infection and Immunity* 53: 245-251
- 33 Winter AJ and Rowe GE (1988). Comparative immune responses to native cell envelope antigens and the hot sodium dodecyl sulfate insoluble fraction (PG) of *Brucella abortus* in cattle and mice. *Veterinary Immunology and Immunopathology* 18: 149-163 [doi: 10.1016/0165-2427(88)90057-8]
- 34 Roux J and Serre A (1971). Prophylaxie individuelle et vaccination contre les brucellosis humaines par une fraction antigenique de *Brucella melitensis* (complex proteines-glycoproteines-glycosaminoproteptides). *Revue d'epidemiologie, Medecine Sociale et Sante Publique* 19: 503-516
- 35 Dzata GK, Confer AW and Wyckoff JH (1991). The effects of adjuvants on immune responses in cattle injected with a *Brucella abortus* soluble antigen. *Veterinary Microbiology* 29: 27-48 [doi: 10.1016/0378-1135(91)90108-R]
- 36 Tabatabai LB, Pugh GW, Stevens MG, Phillips M and McDonald TJ (1992). Monophosphoryl lipid A-induced immune enhancement of *Brucella abortus* salt-extractable protein and lipopolysaccharide vaccines in BALB/c mice. *American Journal of Veterinary Research* 53: 1900-1907
- 37 Pugh GW and Tabatabai LB (1996). Variation in *Brucella abortus* 2308 infection in BALA/c mice induced by prior vaccination with salt-extractable periplasmic proteins from *Brucella abortus* 19. *Infection and Immunity* 64: 548-556 [doi: 10.1016/0264-410x(94)90035-3]
- 38 Stevens MG, Tabatabai LB, Olsen SC and Cheville NF (1994). Immune responses to superoxide dismutase and synthetic peptides of superoxide dismutase in cattle vaccinated with *Brucella abortus* strain 19 or RB51. *Veterinary Microbiology* 41: 383-389
- 39 Tabatabai LB and Pugh GW (1994). Modulation of immune responses in Balb/c mice vaccinated with *Brucella abortus* Cu-Zn superoxide dismutase synthetic peptide vaccine. *Vaccine* 12: 919-924
- 40 Blasco JM, Gamazo C, Winter AJ, Jimenez de Bagues MP, Marin C, Barberan M, Moriyon I, Alonso-Urmeneta B and Diaz R (1993). Evaluation of whole cell and subcellular vaccines against *Brucella ovis* in rams. *Veterinary Immunology and Immunopathology* 37: 257-270 [doi: 10.1016/0165-2427(93)90198-D]
- 41 Roux J (1979). Epidemiology and prevention of brucellosis. *Bulletin of the World Health Organization* 57: 179-194
- 42 Patra, K. P., Saito, M., Atluri, V. L., Rolán, H. G., Young, B., Kerrinnes, T., Vinetz, J. M. (2014). A protein-conjugate approach to develop a monoclonal antibody-based antigen detection test for the diagnosis of human brucellosis. *PLoS Neglected Tropical Diseases*, 8(6), e2926. doi:10.1371/journal.pntd.0002926 [doi: 10.1371/journal.pnyd.0002926]

## Antibody responses in cattle immunized with *B. abortus* vaccines

- 43 Keppie J, Witt K and Smith H (1972). The immunization of guinea-pigs and mice with a whole-culture extract of a smooth and a rough strain of *Brucella abortus*. *British Journal of Experimental Pathology* 53: 518-528
- 44 Tittarelli M, Bonfini B, Massis FD, Giovannini A, Ventura MD, Nannini D and Caporale V (2008). *Brucella abortus* strain RB51 vaccine: immune response after calthood vaccination and field investigation in Italian cattle population. *Journal of Immunology Research*. Article ID 584624 [doi: 10.1155/2008/584624]
- 45 Stevens, M. G., S. C. Olsen and N. F. Cheville (1995). Comparative analysis of immune responses in cattle vaccinated with *Brucella abortus* strain 19 or strain RB51. *Infection and Immunity* 63(8): 3199-3205.
- 46 Poester, F. P., E. T. Ramos, M. J. P. Gomes, C. Chiminazzo and G. Schurig (2000). The serological response of adult cattle after vaccination with *Brucella abortus* strain 19 and RB51. *Brazilian Journal of Veterinary Research and Animal Sciences* 37: 61-64 [doi: 10.1590/51413-9596]
- 47 Diptee M. D., A. A. Adesiyun, Z. Asgarali, M. Campbell and R. Adone (2006). Serologic responses, biosafety and clearance of four dosages of *Brucella abortus* strain RB51 in 6-10 months old water buffalo (*Bubalus bubalis*). *Veterinary Immunology and Immunopathology* 109: 43-55 [doi: 10.1016/j.vetimm.2005.07.020]
- 48 Poester FP, Goncalves VSP, Paixão TA, Santos RL, Olsen SC, Schurig GG, Lage AP, Santos RL, Olsen SC, Schurig GG, Lage AP (2006). Efficacy of strain RB51 vaccine in heifers against experimental brucellosis. *Vaccine* 19: 5327-34 [doi: 10.1016/j.vascne.2006.04.020]
- 49 Nimisha KN, Saseendranath MR, Prasad V, Nair GK, Tresamol PV and Mercy KA (2019). Sero-conversion studies of *Brucella abortus* SR51 vaccine in cattle. *EC Veterinary Science* 4: 376-378
- 50 Fosgate GT, Adesiyun AA, Hird DW, Johnson WO, Hietala SK, Schurig GG, Ryan J (2002). Bayesian comparison of brucellosis serologic tests without a gold standard in cattle and water buffalo (*Bubalus bubalis*) of Trinidad. *American Journal of Veterinary Research* 63: 1598–1605.
- 51 Kreeger TJ, Cook WE, Edwards WH, Elzer PH, Olsen SC (2002). *Brucella abortus* strain RB51 vaccination in elk. II. Failure of high dosage to prevent abortion. *Journal of Wildlife Diseases* 38: 27–31 [doi: 10.7589/10090-3558-38.1.27]
- 52 Olsen SC (2000). Responses of adult cattle to vaccination with a reduced dose of *Brucella abortus* strain RB51. *Research in Veterinary Sciences* 69: 135–140.
- 53 Olsen SC (2000). Immune responses and efficacy after administration of a commercial *Brucella abortus* strain vaccine to cattle. *Veterinary Therapeutic* 1: 183–191.