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ORIGINAL ARTICLE

Immune response of a heat killed *Brucella abortus* vaccine in guinea pig

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Abstract

Background: Brucellosis is an endemic disease in Bangladesh which has economic impacts attributable to humans and animals. To control bovine brucellosis two types of vaccines are available- vaccine S19 and vaccine SRB51 but they have some adverse effects. On the other hand the heat killed vaccine produces less immunity but no adverse effect. Vaccination against brucellosis in Bangladesh has not yet been initiated and not recommended in subsistence management systems due to very low level of prevalence. But in commercial management systems the prevalence is reported to be higher and vaccination may be initiated. Before importing live vaccine which have some adverse effects locally prepared killed vaccine can be tested for its immune response. Hence this study was undertaken to evaluate the immune response of heat killed vaccine prepared from local isolate in guinea pig.

Methods: *Brucella abortus* recently isolated from aborted fetal membranes (unpublished data) was used for vaccine production. Pour plate technique was used by tenfold serial dilution of the isolate to count cfu (colony forming unit)/ml of *Brucella abortus* for dose calculation of heat killed vaccine. Bacterial pellet was prepared by centrifugation of 200ml of the cultured broth at 10,000 rpm for 10 mins. The bacterial pellet was mixed with required amount of PBS (phosphate buffer saline) to obtain 4×10^{10} cfu organisms in 2ml dose for guinea pig inoculation. Then heat killed vaccine was prepared by heating the organism at 80˚C for 90 minutes and the prepared vaccine was inoculated subcutaneously 2ml (4×10^{10}cfu) in each of the guinea pig. The sera of guinea pigs were collected at 1st, 2nd, 4th, 6th and 9th week after inoculation to determine the reciprocal antibody (Ab) titre by Rose Bengal test (RBT) and to examine the rise of antibody level by Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The antibody level started to rise significantly ($p<0.01$) from the 2nd week (OD value 0.2287, Reciprocal Ab titre 1:120) and reached a peak level at 4th week (OD value 0.2842, Reciprocal Ab titre 1:800) and then started to decline significantly ($p<0.01$) from 6th week (OD value 0.1832, Reciprocal Ab titre 1:35) to 9th week (OD value 0.1015, Reciprocal Ab titre 0).

Conclusions: Heat killed vaccine without adjuvant induces immune response in guinea pigs which persists for a maximum period of 6 weeks. A further study to investigate the immune response of killed vaccine with adjuvant is recommended.

Key words: Brucellosis, Heat killed vaccine, ELISA, RBT, Reciprocal antibody titre.

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Introduction

Brucellosis is a zoonotic disease with economic impacts attributable to human, livestock and wildlife disease. In animals, brucellosis mainly affects reproduction and fertility, with abortion and reduced milk yield. In man, the clinical picture resembles many other febrile diseases, but sacroiliitis and hepato-splenomegaly are the most

prominent (Franco *et al.*, 2007). Brucellosis was first investigated in 1967 (Mia and Islam, 1967) in Bangladesh (former East Pakistan). The overall animal-level sero prevalence of bovine brucellosis in Bangladesh varied from 2.4%-18.4% (Rahman *et al.*, 2006; Rahman *et al.*, 2014b). Variable prevalence of bovine brucellosis in the subsistence and commercial management systems in Bangladesh were also reported which ranged from 0.6% and 20.4% (Sikder *et al.*, 2012; Belal and Ansari, 2013; Rahman *et al.*, 2019). Brucellosis in humans and animals in Bangladesh were reported to be caused by *Brucella abortus* (Rahman *et al.*, 2014a; Rahman *et al.*, 2016a; Rahman *et al.*, 2017). The knowledge of available *Brucella* species and their biovars are essential in disease control decisions. As a zoonosis, the control of animal brucellosis will lead to the control of human brucellosis (Islam *et al.*, 2013; Rahman *et al.*, 2016b; Sarker *et al.*, 2016; Ahasan *et al.*, 2017; Rahman *et al.*, 2017). To control bovine brucellosis two types of vaccines are available. In bovines, the most successful vaccine (S19) is only used in calves, as adult vaccination results in orchitis in male, prolonged infection, and possible abortion complications in pregnant female cattle (Schurig *et al.*, 2002). S19 vaccine also creates problem in serological diagnosis of brucellosis. Another vaccine produced from, *Brucella abortus* strain RB51 (SRB51), does not interfere serological diagnosis of brucellosis but may cause abortion when administered in pregnant animal (Arellano *et al.*, 2004). On the other hand the heat killed vaccine produces less immunity but there is no adverse effect. Vaccination against brucellosis in Bangladesh has not yet been initiated. Brucellosis is endemic in Bangladesh (Rahman *et al.*, 2017) and the prevalence varies greatly in different management systems. In low prevalence subsistence management systems, vaccination is not recommended due (Rahman *et al.*, 2019). However, in intensive management system the prevalence of bovine brucellosis is reported to be higher and vaccination may be initiated. Before importing live vaccine which have some adverse effects locally prepared vaccine can be tested for its immune response. Hence, the objective of the study was to evaluate the immune response in the guinea pig with the killed vaccine prepared from the local isolate.

Materials and Methods

Ethical approval

The study protocol was approved by Bangladesh Agricultural University Animal Welfare and Experimentation Ethics Committee (AWEEC/BAU/2019/17).

Isolation

Isolation was performed by maintaining the standard protocol (Alton et al., 1988) and confirmed by Polymerase Chain Reaction assay (PCR) using *Brucella abortus* specific IS711 primer (Unpublished data).

Total viable count (TVC) from broth by using pour plate method

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

Centrifugation for bacterial pellet formation

Centrifugation of 200ml of the cultured broth were done three times at 10,000 rpm for 10 mins were performed and bacterial pellet were washed with PBS every time after centrifugation.

Preparation of required dose

The bacterial pellet were mixed with required amount of PBS to obtain 4×10^{10} cfu organisms in 2ml dose for guinea pig inoculation (Alton *et al.*, 1988) and homogenization was performed using vortex machine.

Heat inactivation of the organism

Organisms were killed by heating the suspension at 80˚C for 90 minutes in hot water bath for producing heat killed vaccine (Alton *et al.*, 1988).

Guinea pig inoculation

Two ml of inoculum (4×10^{10}cfu) of heat killed bacterial suspension were injected to each of four guinea pigs subcutaneously (Alton *et al.*, 1988). The guinea pigs were observed for nine weeks.

Collection of blood and sera samples from guinea pig

1.5 ml of blood were collected from each of the guinea pig directly from the heart at 1st,2nd,4th,6th and 9th week after inoculation of heat inactivated *Brucella* organism. Then sera were prepared from the blood. The sera samples were stored at -20° C for reciprocal antibody titre by Rose Bengal Test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA).

Reciprocal antibody titre by Rose Bengal Test (RBT)

The RBT were performed to determine the reciprocal antibody titer based on the procedure described by Baek *et al.* (2002). 30 µl of antigen were placed on a fine plastic plate circled approximately 1.5 cm in diameter and two fold dilutions of 30 µl of tested serum were done (1:5, 1:10, 1:20, 1:40, 1:80 respectively up to the dilution where agglutination stop) with the use of PBS and were put beside each of the antigen respectively upto the disappearing of the agglutination. The antigen and serum were mixed on the plate with a stirrer and rotated for 4 mins.

Enzyme-Linked Immunosorbent Assay (ELISA)

Rise of antibody level were detected after vaccination by IDEXX Brucellosis Antibody Test Kit according to the protocol of manufacturer and reading was performed by automated ELISA reader.

Results

Table 1 shows the antibody titer of heat killed vaccine in guinea pigs.

Table 1. Reciprocal antibody (Ab) titer from Rose Bengal Test (RBT) and OD values of ELISA test

Week post vaccination	Antibody titer	
	Mean of Reciprocal Ab titer by RBT	Mean OD value of ELISA
0 week	0	0.0945
1st week	1:5	0.1025
2nd week	1:120	0.2287
4th week	1:800	0.2842
6th week	1:35	0.1832
9th week	0	0.1015

The graph showed that the reciprocal antibody titre was 0 at the day of inoculation of heat killed *Brucella abortus* vaccine and started to rise from the 2nd week and reach a peak level 4th week and then started to decline up to 9th week and at 9th week antibody level was similar to the 0th week of inoculation (Figure 1).

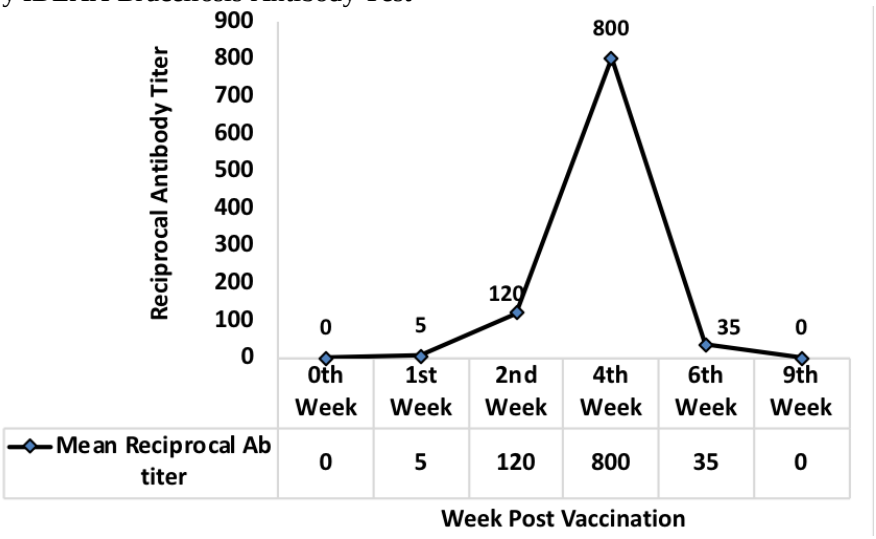

Figure 1. Reciprocal antibody titre

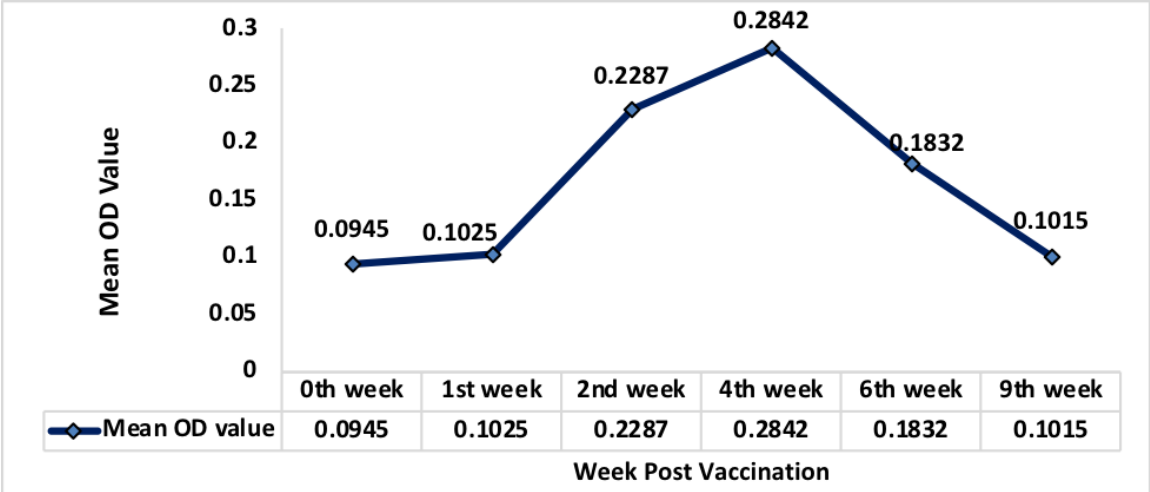

Figure 2. OD value of ELISA

The graph showed that the OD value of the serum of guinea pig after inoculation of heat killed *Brucella abortus* vaccine was 0.0945 at 0 week and 0.1025 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 0.2287) and reached to a peak level at 4th week (OD value 0.2842) and then started to decline from 6th week (OD value 0.1832) to 9th week (OD value 0.1015). The OD value at 9th week post vaccination was also similar to the negative control OD value (0.106) (Figure 2).

Discussion

We have evaluated the immune response of a heat killed *Brucella abortus* vaccine in guineapig. The vaccine was prepared from local isolate. The protective immunity from the heat killed isolate of *Brucella abortus* can be measured by reciprocal antibody titer and ELISA test (Baek et al., 2002; Patra et al., 2014). We observed that the antibody level was increased after vaccination from second week and reached a peak level at fourth week post-vaccination. This immune response was due to the use of single dose vaccine without any booster dose. The role of booster dose and addition of adjuvant will supposed to rise both the level of antibody and duration of the immune response. It was reported that the heat killed vaccine prepared from *Brucella abortus* smooth strain 544 with adjuvant (water-in-oil emulsion) induced 230-fold more protective immunity in guinea-pigs than the same without the adjuvant (Keppie et al., 1972). Vaccine against brucellosis is usually given to prepubescent heifers (4-12 month) as live *Brucella* vaccine may cause abortion in pregnant animals (Oslen and Tatum, 2010). However, heat killed *Brucella abortus* vaccine will not induce abortion if administered in pregnant animals (Moriyon et al., 2004). The major clinical manifestation of brucellosis in cattle is abortion. The bacterial concentrations in fetal fluids or placenta after abortion can be very high (10^9 to 10^{10} cfu/g) and only 10^3 to 10^4 cfu can cause infection in a cattle (Olsen and Tatum, 2010). So, the use of killed vaccine in pregnant cow will reduce the rate of abortion and thereby reduce the transmission of this disease in the cattle population. Before applying this vaccine in pregnant cattle further studies are required to know the role of booster dose and adjuvant in the immune response of this vaccine.

Conclusion

Heat killed brucellosis vaccine without adjuvant induces immune response in guinea pigs. The peak immune response was observed at fourth week post vaccination. The immune response persists for a maximum period of 6 weeks. A further study to investigate the immune response of killed vaccine with adjuvant is recommended.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgement

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