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Immune response of cattle to vaccination with *Brucella abortus* strain 19 in India

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Abstract

Brucellosis is an important contagious bacterial disease of livestock, which can be transmitted to humans. It is prevalent in many countries including India. Vaccination and biosecurity can reduce the prevalence of brucellosis. Evaluation of immune response of live *Brucella abortus* vaccine containing S19 strain in 10 calves was studied, with 10 unvaccinated controls. The percentage inhibition (PI) values (78.0 ± 4.9) were positive from one month after vaccination and the protection was sustained up to six months after vaccination. Rose Bengal Test (RBT) values were positive in 9/10 calves until 10 months after vaccination. (*Bangl. vet*. 2016. Vol. 33, No. 1, 28 – 32)

Introduction

Brucellosis is an infectious bacterial disease caused by *Brucella abortus*. The disease affects cattle and buffalo, andsometimes horses, dogs, swine, and humans (Seyed *et al*., 2012; Singh *et al*., 2012).

In cattle, brucellosis is primarily a disease of cows. Bulls can be infected: they do not readily spread the disease, but show signs of seminal vesiculitis and orchitis. In the female the organism localizes in the udder, uterus, and lymph nodes adjacent to the uterus. The effects include abortion in the last third of pregnancy, retained afterbirth, and weak calves at birth (OIE, 2009).

Brucella abortus are Gram-negative coccobacilli measuring about 0.6 to 1.5 µm by 0.5-0.7 µm. The outer cell membrane consists of dominant lipopolysacharide (LPS) component and three main groups of proteins. They show little action on carbohydrates in conventional media. The World Health Organization (WHO) laboratory biosafety manual classifies *Brucella* in Risk group III.

Brucellosis is a major zoonotic disease of global importance. The disease is common in Middle East, Asia, Africa, South and Central America. It causes heavy economic through abortion, delayed conception and infertility. The disease also infects humans. About 50,000 cases of human brucellosis are reported every year globally (Mohan *et al*., 2016).

Surveillance consists of conducting serological tests to detect *Brucella abortus* antibodies (Cardena *et al*., 2009; Khaled *et al*., 2011). Animals that test negative to the initial screening test are classified as "negative" and are considered not to be infected.

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Animals that are positive on the initial screening test are classified as "positive," and further testing is required. In recent years, the enzyme-linked immunosorbent assay (ELISA) using well characterized smooth lipopolysacharide of *Brucella abortus* in an indirect ELISA or the O-polysaccharide in a Competitive ELISA has been shown to be a very sensitive and specific measure of antibody. Rose Bengal Test (RBT) can be used as a serological method to indicate antibodies against infection or vaccination (Nielsen and Yu, 2010).

Brucellosis can be controlled by the use of S19 strain by subcutaneous or conjunctival routes in young or adult females (Elaine *et al.*, 2015). In controlled conditions, these resulted in good protection against brucellosis. This study was undertaken to investigate efficacy of Brucella vaccine to produce specific immune response in 12 months period.

Materials and Methods

Grouping and rearing of calves

Twenty healthy, 3 - 6 months old female calves of Gir breed were grouped as follows:

Group 1: Vaccinated (n = 10, Vaccinated with live *Brucella abortus* vaccine, from Hester Biosciences Limited, India)

Group 2: Unvaccinated control (n = 10)

Vaccinated calves were kept in Teniwada village, Gujarat, India. Unvaccinated calves were kept separately in Rajosana village, Gujarat, India. Calves of both groups were allowed free access to food and water and were found healthy throughout the study under close observation.

Vaccination, blood collection and testing

Blood was collected at day 0 from calves of both groups to check prevaccination titre. After collection, Group 1 was vaccinated with one dose of $5\text{-}8\times10^{10}$ viable organisms subcutaneously in neck region. The vaccine was Brucella abortus vaccine, Live, S19 strain, from Hester Biosciences Limited, India. After vaccination, blood was collected from both groups every month up to 12 months. The serum was separated and stored at -20^{o}C.

Serological evaluation

C-ELISA

Serological titres of animals to Brucella were determined by competitive enzyme linked immunosorbent assay (C-ELISA). The micro plate was pre-coated with non infectious Brucella antigen on the well. The BIONOTE Brucella Ab C-Elisa kit (Bionote inc, Republic of Korea) was used for detection of brucella antibodies in serum as prescribed by the manufacturer. All reagents and samples were allowed to reach room temperature (18~25^{o}C) before use. Results were recorded as Percentage

Inhibition (PI). The serum was considered as negative, if PI <30 and positive for PI ≥30.

Rose Bengal Test (RBT)

Rose Bengal Test was performed with all serum samples as described by OIE (Rose Bengal, IDvet, France). After four minutes of mixing of samples, agglutination was observed. The samples were considered as positive for brucella antibodies if agglutination was observed.

Data analysis

All data were entered into Microsoft Office Excel sheet (Microsoft Corporation, 2013) and expressed as mean ± SEM Data were analyzed with one way ANOVA with individual groups. P<0.05 was considered significant difference.

Results and Discussion

PI>30 was considered as positive for presence of Brucella antibodies. In all samples PI was <30 before the study. After vaccination, the PI value significantly increased in the vaccinated calves after one month (78.0 ± 4.9). Thereafter the titre declined every month up to six months. The average PI value remained positive in the vaccinated calves up to six months, and then was negative throughout the study period of 12 months. The titre of unvaccinated calves was negative throughout the study period (Fig. 1).

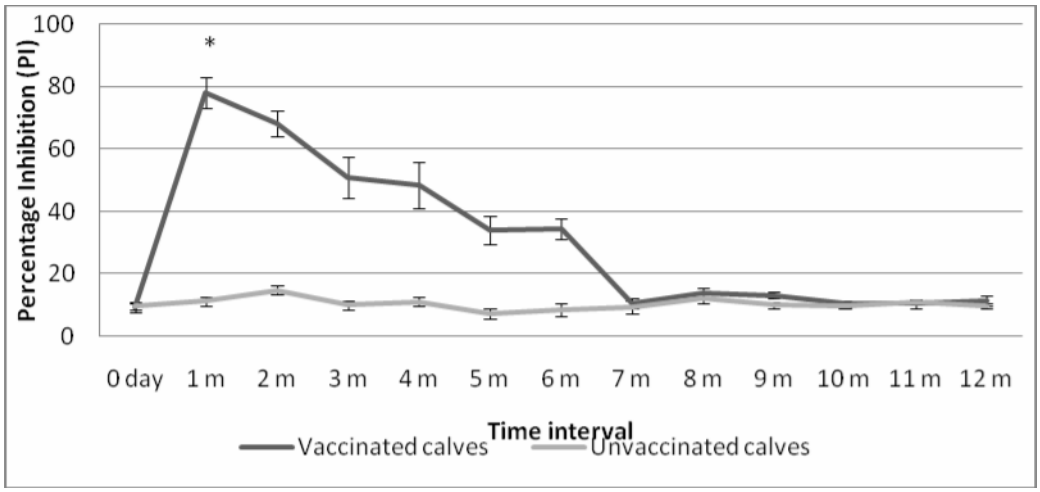

Fig. 1. Pecentage Inhibition (Vaccinated and unvaccinated calves)

The data are experssed as mean ± SEM; *P<0.05, significantly different as compared to 0 day

Rose Bengal Test (RBT) was negative in all the unvaccinated calves. Out of ten vaccinated calves, nine samples were positive (90%) with RBT one month after

vaccination. As the study period went on, number of positive samples decreased and reached zero 11 months after vaccination (Fig. 2).

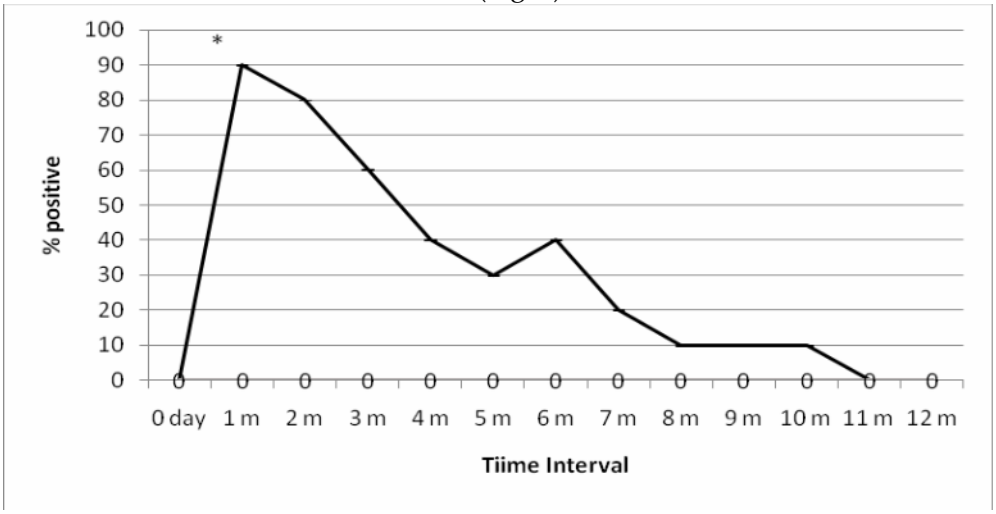

Fig. 2. % Positive values with RBT (Vaccinated and unvaccinated calves)
The data are experssed as mean ± SEM; *P<0.05, significantly different as compared to 0 day

The efficacy of Brucella vaccine has been investigated by many workers who have used c-ELISA and RBT as satisfactory methods (Carrasco *et al.,* 1998; Fiorentino *et al.,* 2008; Waleed *et al.,* 2014). In present study, all animals were seronegative for Brucella antibodies before vaccination, which indicates that animals were neither infected, nor vaccinated. After vaccination, the PI value increased to 78.01 ± 4.99, which indicates satisfactory immune response produced by *Brucella abortus* vaccine. Similar observations were found by Carrasco *et al.* (1998). After one month, titre decreased gradually up to six months, after which it was negative up to 12 months. RBT showed no antibody found before vaccination, as no agglutination was observed. Vaccinated calves showed positive RBT, similar to the results of other workers (Afzal *et al.,* 2000; Mohan *et al.,* 2016; Poester*et al.,* 1998).

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