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Titers of Anti-Brucella Antibodies by Enzyme Linked Immunosorbant Assay in Vaccinated and Unvaccinated Brucellosis Infected Cattle

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Abstract

Brucellosis is an important re-emerging zoonotic disease caused by *Brucella* organisms. In the absence of a Differentiation of Infected from Vaccinated Animal (DIVA) assay for bovine Brucellosis, it becomes difficult to assess whether the anti-*Brucella* antibody response in an animal is due to vaccination or infection. We compared the anti-*Brucella* antibody titers of naturally Brucellosis affected unvaccinated cows, previously vaccinated infected cows, normal healthy vaccinated cows and healthy unvaccinated calves. The titers of anti-*Brucella* antibodies were estimated by indirect ELISA. The mean titer ($\log_{10}$) was found to be 1.518 ± 0.005 in case of naturally Brucellosis affected cattle which had been vaccinated during calf hood. The mean titer in case of naturally infected cattle which had never been vaccinated was 1.5441 ± 0.005. The mean titer in healthy unaffected cattle vaccinated during calf hood was 1.504 ± 0.002 and that of unvaccinated healthy calves was 0.560 ± 0.016. It was interesting to find that the antibody titers in naturally affected cattle which had never been vaccinated were very significantly ($p < 0.01$) higher than those of Brucellosis affected cows which had been vaccinated during calf hood. The titer in vaccinated infected cattle was very significantly ($p < 0.01$) higher than that of uninfected vaccinated cows.

Keywords

Antibody Titer, Brucella, Brucellosis, Bovine, ELISA

1. Introduction

Brucellosis is endemic in India and is prevalent in all parts of the country. It

causes heavy economic losses to the industry through delayed conception, late-term abortions, and retention of placenta [1] in females and orchitis and epididymitis in males. The organisms are excreted in semen, uterine discharges, and milk [2]. The occurrence of the disease varies from 10% in marginal herds to 50% in organized farms.

The *Brucella abortus* strain 19 vaccine against bovine Brucellosis has been found to be very useful under most conditions but has some undesirable traits also [3]. Although vaccination with S19 is effective in preventing Brucellosis in cattle and buffaloes, it is not uncommon to find adult cattle vaccinated in calf hood with S19 vaccine, getting naturally infected with Brucellosis [4]. Although there is vast information on various aspects of bovine Brucellosis in the available literature, there is hardly any systematic study on the comparison of antibody levels between infected and vaccinated animals. We therefore compared the antibody titers of different categories of animals by ELISA to investigate if titers alone could indicate whether the animal is infected or vaccinated. ELISA has been claimed to be more sensitive followed by RBPT and STAT when applied to cattle sera. The present study offers valuable insight which could help in devising appropriate control strategies for this dreaded zoonosis.

2. Materials and Methods

Permission of the Institutional Animal Ethics Committee (IAEC) was obtained and IAEC guidelines were followed throughout the study.

2.1. Collection of Serum

Blood samples were collected from naturally infected clinical cases of Brucellosis in cattle which had never been vaccinated (6) identified in villages in and around Ludhiana district, from naturally infected animals vaccinated during calfhood (21), normal healthy (uninfected) vaccinated cattle (6) and healthy unvaccinated calves (6) of a dairy farm in Haibowal area, Ludhiana. Sera were separated from clotted blood and stored at −20˚C till further use for studying the antibody response of the animals.

2.2. Rose Bengal Plate Test (RBPT)

RBPT was carried out on sera as per the standard method [5]. Equal volumes (25 μl each) of RBPT colored antigen (Punjab Veterinary Vaccine Institute, Ludhiana) and test serum were mixed on a clean glass slide. The slide was observed till 3 min. for formation of clumps. Formation of clumps (agglutinate) indicated a positive reaction while the absence of clear clumps was considered as a negative reaction.

2.3. Enzyme Linked Immunosorbant Assay (ELISA)

Serum samples of cattle were tested by ELISA [6] using Ingezim *Brucella* Bovina 2.0 Brucellosis serum ELISA test kit (Ingenasa). ELISA was carried out as per the

manufacturer's instructions accompanying the kit described below.

a) 90 µl of diluent was added in each well of the plate except the ones destined as controls. 10 µl of diluted individual sample (1/10) was added to the remainder wells of the plate.

b) 100 µl of the controls provided in the kit were added. Plate was incubated for 1 hour at room temperature (RT, 20˚C - 25˚C).

c) The plate was washed three times with 300 µl of washing solution.

d) 100 µl of the conjugate was added to each well and plate was incubated for 30 minutes at room temperature. The plate was washed three times with 300 µl washing solution.

e) 100 µl of substrate was added to each well and plate was kept in dark for 10 min at RT.

f) 100 µl of the stop solution was added to each well.

g) Optical density (O.D.) of each well with spectrophotometer was done at 450 nm within 5 minutes after the addition of stop solution.

The test was valid when: O.D. value for positive control serum was ≥ 1.0, and was considered negative when the OD of the control serum was ≤ 0.2.

Cut off = $OD_{450\,nm}$ positive Control × 0.4 = 40% positivity

2.4. Statistical Analysis of Data

Data pertaining to serum antibody titers was statistically analyzed by ANOVA.

3. Results and Discussion

The antibody titers estimated by ELISA are presented in Tables 1-4; Figure 1. The mean antibody titer (log_{10}) in case of Brucellosis affected cattle which had been vaccinated during calf hood was found to be 1.518 ± 0.005 (Table 1). The mean titer in case of naturally infected cattle which had never been vaccinated was 1.5441 ± 0.005 (Table 2). In case of healthy unaffected cattle vaccinated during calf hood the mean titer was 1.504 ± 0.002 (Table 3) and in unvaccinated healthy calves it was 0.560 ± 0.016 (Table 4). It was interesting to find that in naturally affected cattle which had never been vaccinated previously the antibody titers were very significantly ($p < 0.01$) higher than those of Brucellosis affected cattle which had been vaccinated during calf hood and normal healthy vaccinated cattle unaffected by the disease. The mean titer in Brucellosis affected cattle which had been vaccinated during calf hood was very significantly ($p < 0.01$) higher than that of normal healthy vaccinated cattle unaffected by the disease.

The differences between mean titers of vaccinated infected and unvaccinated infected, vaccinated infected versus vaccinated healthy, vaccinated infected versus unvaccinated healthy, unvaccinated infected versus vaccinated healthy, unvaccinated infected versus unvaccinated healthy and vaccinated healthy versus unvaccinated healthy were very significant ($p < 0.01$).

The titers in unvaccinated infected cattle were very significantly ($p < 0.01$)

S. Raj *et al.*

Table 1. Titers of anti-*Brucella* antibody by ELISA in infected cattle vaccinated in calf-hood.

Animal number	Titer
B1	1.5221
B2	1.5167
B3	1.5133
B4	1.5126
B5	1.5199
B6	1.5169
B7	1.5133
B8	1.5134
B9	1.5267
B10	1.5187
B11	1.5154
B12	1.5253
B13	1.5169
B14	1.5157
B15	1.5139
B16	1.5139
B17	1.5135
B18	1.5335
B19	1.5148
B20	1.5226
B21	1.5209
Mean ± SD	**1.518 ± 0.005**

Table 2. Anti-*Brucella* antibody titers by ELISA in unvaccinated infected cattle.

Animal no.	Titer
I	1.5467
II	1.5387
III	1.5481
IV	1.5469
V	1.5361
VI	1.5481
Mean ± SD	**1.5441 ± 0.005**

Table 3. Anti-*Brucella* antibody titers by ELISA in healthy vaccinated cattle.

Animal no.	Titer
1303	1.5027
1431	1.5076
1468	1.5045
1378	1.5032
1470	1.5034
1374	1.5075
Mean ± SD	1.504 ± 0.002

Table 4. Anti-*Brucella* antibody titers by ELISA in unvaccinated healthy calves.

Animal no.	Titer
1632	0.5671
1634	0.5589
1638	0.5847
1577	0.5334
1575	0.5564
1581	0.5634
Mean ± SD	0.560 ± 0.016
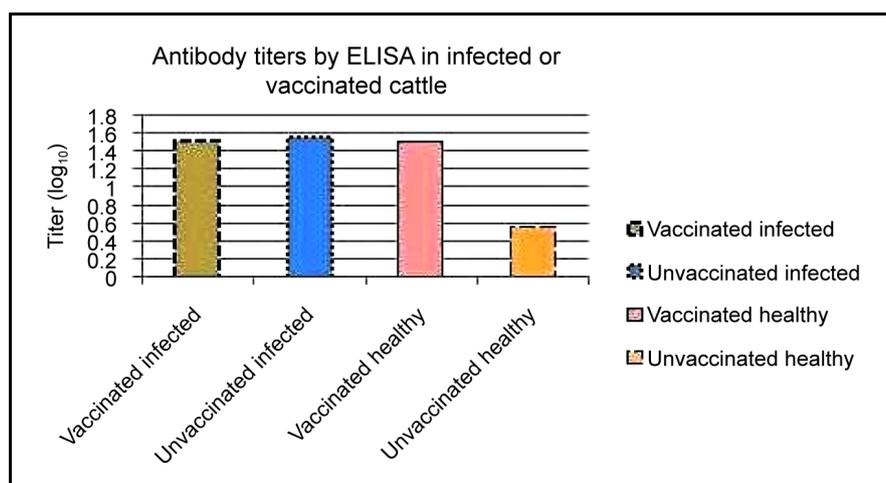
Figure 1. Antibody titers in infected and vaccinated cattle by ELISA.

higher than those of infected vaccinated, healthy vaccinated and healthy unvaccinated animals. The titers in vaccinated infected cattle were very significantly ($p < 0.01$) higher than those of healthy vaccinated and healthy unvaccinated animals.

In a study [7] the performance of an ELISA for detection of total antibodies to *Brucella* spp. was compared with that of the Rose Bengal Plate Test, standard tube agglutination test and Coombs test in the diagnosis of Brucellosis. ELISA

was the most sensitive test (97%), showing greater specificity (96%) and good predictive positive and negative values (98% and 94% respectively). ELISA was the only positive test in 6% of patients in whom Brucellosis had been confirmed by culture. ELISA has been claimed to be more sensitive followed by RBPT and STAT when applied to cattle sera [8].

Assessment of immunological data from *Brucella* infected cattle can be helpful in charting the disease process, diagnosis and prognosis and may help in understanding the pathophysiology of disease. It was reported [4] that naturally affected animals have very high titers of agglutinating antibodies than the vaccinated animals.

Numerous outer and inner membrane, cytoplasmic, and periplasmic protein antigens of *Brucella* have been characterized, yet the antigen that dominates the antibody response is Lipopolysaccharide (LPS). Some antigens are recognized by the immune system during infection and are potentially useful in diagnostic tests.

The role of humoral immunity against intracellular bacterial infections is limited and may not be protective. Antibody mediated opsonization by immunoglobulins (IgM, IgG1, IgG2a and IgG3) enhances phagocytic uptake of bacteria, limiting the level of initial infection with *Brucella* but has little effect on intracellular course of infection [9] [10]. The L7/L12 ribosomal proteins are important in stimulating cell-mediated responses [11].

Since the infected animals in the first group were the ones who had already been vaccinated during calf hood, the infection in these animals may suggest that the vaccine was unable to induce adequate protective levels of antibody. Secondly, the heightened antibody response after infection in vaccinated animals may indicate a secondary immune response to *Brucella* antigens against which the lymphocytes were primed by calf hood vaccination.

4. Conclusion

The anti-*Brucella* antibody titers by ELISA in naturally affected unvaccinated cattle were very significantly higher than those of Brucellosis affected vaccinated cattle and normal healthy vaccinated cattle unaffected by the disease.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgement

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