REDUCED-DOSE WHOLE HERD VACCINATION AGAINST BRUCELLOSIS

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ABSTRACT

Brucella abortus, S-19 vaccine was inoculated subcutaneously in three groups, each comprising of 10 crossbred cows, using full (2.5 mL), half and one quarter of the recommended dose. The sero analysis revealed non-significant difference in the protection afforded by different dose levels of *Brucella abortus*, S-19 vaccine. More rapid sero conversion resulted from various reduced doses of vaccine compared with the standard full dose.

INTRODUCTION

The usefulness of adult vaccination as a tool in the effort to eliminate brucellosis from cattle/buffalo herds has greatly increased because of the inability to practice test and slaughter or Bang's method for economic implications/constraints. Strain-19 vaccine has widely been used for calfhood vaccination in many parts of the world. Its efficacy in adult cattle also is well documented (Barton and Lomme, 1980). But the use of S-19 in adults was discouraged because of the resulting agglutinin titre problem that complicate the diagnosis of brucellosis. These problems have been reduced to minimal levels through the use of reduced doses of S-19 vaccine and more specific serological tests (Barton and Lomme, 1980). The present study was carried out to determine the relationship between vaccine dosage and level of resistance and residual titres in crossbred cows.

MATERIALS AND METHODS

The experiment was conducted at Livestock Experiment Station, Qadirabad on crossbred animals. The cows selected for trials were between 5-7 years of age. A total of 50 cows selected for the study were equally divided into 5 groups (Group A, B, C, D & E). Cows in group A were injected with recommended full dose (2.5 mL) of Brucella vaccine S-19 vaccine (Abresec). Group B was injected with 1/2 while group C with 1/4th of recommended dose of S-19 vaccine. Group D cows were served as un-inoculated control while group E was taken as naturally infected control.

Before inoculation, serum from all animals was collected and tested by serum agglutination test and ELISA for brucellosis, animals sero negative to brucellosis were included in group A, B, C and D. After inoculation of vaccine, serum was collected at monthly intervals and analysed for titres.

Serum Agglutination Test

Each serum sample was tested in duplicate. Serial two fold dilutions of the test serum starting from 1:10 upto 1:640 (volume 0.5 mL) were prepared in phenol saline (0.85 % NaCl solution containing 0.5 % phenol). The antigen was diluted (as per instructions of VRI) and an equal amount was added to each tube. Contents of the tubes were mixed thoroughly and incubated at 37°C for 24 hours. The degree of agglutination was determined by the degree of clearing without shaking the tubes. Known negative and positive sera were used as controls.

Complete agglutination and sedimentation with 100 per cent clear supernatant was marked as four plus (++++), similarly 75, 50 and 25 per cent were marked as three, two and one plus, respectively. No agglutination and no clearing was considered as negative.

The highest serum dilution showing 50 per cent clearing (++) was considered as titre of that serum. A titre of 1:40 or higher was considered as positive as per recommendations of FAO/WHO Expert Committee on Brucellosis (Alton and Jones, 1967).

ELISA Method (Magee, 1980)

After prewetting the antigen-coated wells with PBS-Tween-20, serial two-fold dilutions of the test serum (starting from 1:40) were made in PBS-Tween-20. Beginning with column 1 of the plate, 100 uL of each dilution was delivered to each of the 8 wells (from A-H). The series of eight wells thus contained 0.1 mL each of serial two fold dilution from 1 in 40 to 1 in 5120. This procedure was repeated until 9 different test

Table 1: Agglutination titres of the animals vaccinated with different doses of S-19

Group	Title	Months post inoculation							
		1	2-5	6	7	8-12	13-18	19	20-24
A	40	6	1	-	-	2	4	4	8
(Full	80	2	2	2	2	1	2	2	1
Dose)	160	2	2	2	2	1	2	2	1
	320	-	2	2	3	4	2	1	-
	640	-	3	4	3	1	-	-	-
	GMT	60.6	211.12	278.57	259.92	160.0	91.89	85.74	49.24
B	40	2	1	1	4	7	-	-	-
(Half	80	4	4	3	4	3	-	-	-
Dose)	160	4	2	4	1	-	-	-	-
	320	1	2	2	1	-	-	-	-
	640	1	1	-	-	-	-	-	-
	GMT	119.86	139.28	129.96	74.64	49.24	-	-	-
C	40	2	2	1	2	4	-	-	-
(One	80	3	2	2	1	5	-	-	-
Quarter	160	5	4	5	6	1	-	-	-
Dose)	320	-	2	1	1	-	-	-	-
	640	-	-	-	-	-	-	-	-
	GMT	98.49	121.25	149.28	121.25	64.98	-	-	-
D	40	1	1	-	-	-	-	-	-
Naturally	80	4	3	3	3	2	2	1	-
Infected)	160	3	2	2	2	2	2	2	4
	320	1	2	2	2	2	1	3	2
	640	1	1	2	2	2	2	1	1
	1280	-	1	1	2	2	2	1	1
	GMT	129.96	183.79	242.51	259.57	278.57	198.57	342.9	342.96

sera had been diluted and dispensed. Column 10, 11 and 12 were used for the negative control serum, positive control serum and for PBS-Tween-20, respectively. Unbound antibody was removed by the standard washing procedure (Magee, 1980) after incubation for one hour at 37°C. Anti- species IgG (H & L Chains) antibody conjugated with peroxidase (Gamma Lab., USA) was diluted 5000-fold in PBS-Tween-20 and 100 μL of the dilution was added to the appropriate wells and incubated for one hour at 37°C. Unbound conjugated antibody was removed by standard washing procedure. Retained enzyme activity was estimated by the addition of 100 μL of a solution of O-phenylenediamine dihydrochloride in phosphate citrate buffer (containing H_2O_2) to each well and incubated at room temperature in the dark for 30 minutes. The enzyme reaction was stopped by the addition of 100 μL of 0.2 N Sulphuric acid (H_2SO_3) to each well. The results were recorded visually. A positive reaction at a dilution of 1:160 was taken as positive for brucellosis (Arshad, 1989).

RESULTS AND DISCUSSION

The antibody titre in group A (inoculated full vaccine dose) persisted upto two years, whereas, in Group B (half dose) and group C (Quarter dose), the anti-body titres started decreasing after 6 months whereas, increasing antibody titres were recorded in Group E (Natually infected), however, the un-inoculated control (group D) remained free from any antibody titres against brucellosis during the course of the study (Table 1).

The ELISA detected antibody titres in groups A, B and C till 22 months and the titre dropped after 22 months to doubtfull level. The findings of the present study partially support the results of Ahmad (1993) who observed antibody titres in 87.0 percent animals for a period of 18-months, vaccinated by full dose of vaccine S-19. The findings do not agree with the observations of Beckett and Diarmid (1987) who observed persistent antibody titres in 15.10 per cent animals upto 4 years post-vaccination with reduced dose of vaccine S-19.

The possible explanation could be that the animals included in the previous study were vaccinated during calf hood, but in the present study the animals were neither pregnant when inoculated vaccine nor they were vaccinated during calf hood. Present finding support the results of Heck *et al.* (1981) who found ELISA the best test for detecting antibodies against brucellosis compared to other conventional tests. They further observed antibody titres in experimental animals upto 52 weeks. Present findings fully agree with the observation of Barton and Lomme (1980) and Ahmad (1995). They recorded that after reduced dose S-19 inoculation, the number of reactors decline after 120 days and the antibody titres go below detectable level within 18-24 months. In group C, the antibody titres declined after 180 days. This difference in the two groups inoculated with reduced dose might be due to variation in individual response of the animals.

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