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Antigenic Response of a Live *Staphylococcus aureus* Vaccine in Rabbits

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Abstract

The study was undertaken to prepare and evaluate the antigenic response of the experimental live attenuated *Staphylococcus aureus* vaccine in rabbits. *Staphylococcus aureus* isolates were selected on the basis of morphological and biochemical characteristics. One isolate of *Staphylococcus aureus* used for the preparation of vaccine was Gram +ve cocci, non motile, catalase and coagulase positive and gave complete haemolysis on 5% sheep blood agar. A total of 30 rabbits were divided into 3 groups, A, B and C each having 10 animals. Group A was given single injection of vaccine, group B was vaccinated twice at two weeks interval while group C was kept as control. Serum samples were collected from 3 rabbits selected randomly from each group at weekly interval. From day 0 to 6 weeks post vaccination, antigenic response was evaluated trough anticoagulase, antihaemolysin and bacterial agglutination tests which showed that antigenic response was highly positive at day 21 in group A and B while same was in group B at 28 day of post vaccination. The anticoagulase and antihaemolysin tests results were positive.

Key words: *Staphylococcus aureus*, Live attenuated vaccine, Antigenic response

Introduction

Mastitis is an inflammation of udder regardless of the cause. It is the most important and costly disease of dairy cattle (Lightener et al., 1988). Field surveys of major livestock diseases in Pakistan (Cady et al., 1983; Ajmal et al., 1990). It also poses the risk for transmission of some major zoonotic diseases like tuberculosis, leptospirosis and brucellosis (Rodostitis et al., 1994).

The intramammary infection caused by *Staph. aureus* is responsible for considerable economic loss from reduced milk production (Fox and Hancock, 1989). The strains of *Staphylococcus aureus* that produce coagulase are considered to be pathogenic (Cristie and North, 1941).

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Poor management and sanitary conditions, failure of therapeutics and control measures like pre and post milking udder infectious are the factors that motivate to develop effective vaccine against staphylococcal mastitis. This trial was therefore conducted with the following objectives.

To isolate and identify *Staph. aureus* from mastitic milk of buffaloes, to develop a live attenuated *Staph. areus* experimental vaccine and to check the antigenic response against *Staph. aureus* vaccine in rabbits.

Materials and Methods

A total of 30 milk samples from buffaloes affected with mastitis were collected following the procedure described by National Mastitis Council Inc. (1990). About 20 ml of milk from affected teats were taken to conduct Surf Field Mastitis test (Muhammad et al., 1994). For microbiological examination about 2 ml of milk sample was taken.

The positive milk samples were centrifuged at 2000 g for 10 minutes. The supernatant was discarded. Milk sediment was used for direct demonstration (Cruickshank et al., 1975).

Primary isolation was made on Staph-110 medium. The morphology of the organism was studied with gram's stain. The pigmentation (colony color) and haemolytic pattern by staphylococcus were observed on sheep blood agar.

Catalase, coagulase and haemolytic tests were performed for further identification of the organisms (Quinn et al., 1994).

Staphylococcus aureus isolated from mastitic milk was serially passaged on 5% sheep blood agar until it lost its hemolytic activity and then maintained on mannitol-salt agar slope at 4°C. the organism was cultured in nutrient broth for 18 hour at 37°C in an orbital shaker.

The bacteria were harvested by centrifugation (3000g, 10 minutes) washed once with sterile phosphate-buffered saline (PBS) (pH 7.2) and then resuspended in sterile PBS. The concentration of bacterial cells in suspension was determined by viable count and adjusted 10^9 cells/ml (Watson, 1984).

A total of 30 rabbits were kept and divided into 3 groups A, B and C each having 10 animals. Group A was inoculated with single dose of vaccine @ 0.2 ml (2×10^8 cells) per animal subcutaneously, while vaccine was administered twice to the group B animals at two weeks interval using the same dose and route. The groups C served as non-vaccinated control receiving no vaccine.

Blood samples were collected from 3 rabbits of each group at weekly interval from day 0 to 6 weeks post vaccination. Serum was obtained and stored at -4°C until required (Watson, 1984).

Bacterial agglutination test was performed using spot agglutination for the detection of antibodies described by Cruichshank (1975). The antihaemolysin and anticoagulase test for each serum sample against the bacterial culture suspension containing haemotoxin and coagulase was performed.

Results and Discussion

The positive milk samples (sub clinical and clinical mastitis) were streaked on the staphylococcus 100 medium (Oxide) (Chapman, 1946; Rottgardt and Florey, 1946 and Cruickshank *et al.*, 1975).

A total of 8 isolates of staphylococcus were studied for their morphology (shape, size arrangement and pigmentation and staining reaction). The isolates when subjected to morphological and cultural examination showed that all were Gram positive cocci, non-motile and produced yellow white and light yellow colored colonies on blood agar. These findings are in line with those described by Smith and Conant (1957), Cruickshank *et al.* (1975) Merchant and packer (1983) and Quinn et al. (1994).

All the isolates were positive for catalase and coagulase tests. The isolates number 1,2,3,5 and 5 were haemolysed completely while isolates no. 4, 7 and 8 showed partial haemolysis on 5% sheep blood agar.

One of the above isolates (catalase, coagulase positive and shows complete hemolytic activity) was selected randomly and used to make live attenuated vaccine. The suspension was inoculated @ 0.2 ml (2x108 cells) per animal subcutaneously to group A and B. same boosting dose was given on 14th day to second group (B). The thirds group (C) was kept as un-inoculated control.

The sera were separated from blood samples and collected at weekly interval from day 0 to 6 weeks post inoculation. The sera were used to determine the anticoagulase and antihaemolysing activities against the selected isolate of *Staphylococcus aureus*. In response to the inoculum, the animals developed proteins like anticoagulase and antihaemolysin along with antibodies against the antigen located on bacterial cell surface (Table 1 and 2).

The sera samples of the experimental animals were also examined through bacterial agglutination test to determine the antigenic response of the attenuated inoculum. The agglutination results were positive at day 21 post vaccination in group A and B and highly positive at day 28 post vaccination in group B (Table 3). These findings are in line with those of Buxton and Fraser (1977).

Table 1: Results of antihaemolytic test at 0, 7, 14, 21, 28, 35 and 42 days post vaccination in sera of experimental animals.

Groups	Sample No.	Results						
		0 day	7 day	14 day	21 day	28 day	35 day	42 day
A	1	-	+	+	+	+	+	+
	2	-	+	+	+	+	+	+
	3	-	+	+	+	+	+	+
B	1	-	+	+	+	+	+	+
	2	-	+	+	+	+	+	+
	3	-	+	+	+	+	+	+
C	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-

– = negative; + = positive

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Table 2: Results of anticoagulase test at 0, 7, 14, 21, 28, 35 and 42 days post vaccination in sera of experimental animals.

Groups	Sample No.	Results						
		0 day	7 day	14 day	21 day	28 day	35 day	42 day
A	1	-	+	+	+	+	+	+
	2	-	+	+	+	+	+	+
	3	-	+	+	+	+	+	+
B	1	-	+	+	+	+	+	+
	2	-	+	+	+	+	+	+
	3	-	+	+	+	+	+	+
C	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-

– = negative; + = positive

Table 3: Results of slide agglutination test at 0, 7, 14, 21, 28, 35 and 42 days post vaccination in sera of experimental animals.

Groups	Sample No.	Results						
		0 day	7 day	14 day	21 day	28 day	35 day	42 day
A	1	-	+	++	+++	++	++	+
	2	-	±	++	+++	+++	++	++
	3	-	±	+	+++	++	+	+
B	1	-	±	++	+++	+++	+++	+++
	2	-	±	++	+++	+++	+++	+++
	3	-	+	-	+++	+++	+++	+++
C	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-

– = negative; ± = doubtful; + = mildly positive; ++ = moderately positive; +++ = highly positive

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