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Short communication

PRIMARY AND SECONDARY ANTIBODY RESPONSE TO COMPOSITE FORMALIN-INACTIVATED *STAPHYLOCOCCUS AUREUS, STREPTOCOCCUS AGALACTIAE* AND *ESCHERICHIA COLI* IN RABBITS

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ABSTRACT

Twenty buffaloes clinically positive for mastitis were selected for the collection of milk samples. *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* were isolated from milk of mastitic buffaloes according to the procedures recommended by National Mastitis Council Inc., USA. The composite antigen containing *S. aureus*, *Str. Agalactiae* and *E. coli* was then prepared and the concentration of each isolate was adjusted to 1×10^9 cells / ml. To evaluate the antibody response, nine adult healthy rabbits were divided randomly into three groups (A, B and C) consisting of 3 rabbits each. Composite antigen preparation was injected subcutaneously to the rabbits of groups A and B @ 0.2 ml / rabbit while each rabbit of group C inoculated with 0.2 ml normal saline and served as control. The rabbits of group B were given a booster dose at day 15 of the primary injection to see the secondary antibody response. The level of antibodies specific to *S. aureus*, *Str. agalactiae* and *E. coli* were assayed by indirect haemagglutination test (HA) and finally geometric mean titre (GMT) was calculated. At day 0 to 15, it was observed that the antibody titre was almost same in both groups. At day 30 significant difference in antibody titres was observed between group A and B with GMT of 9.8 against *S. aureus*, and *Str. agalactiae* in group A whereas in group B GMT was 97.0 against *S. aureus* and 39.4 against *Str. agalactiae* which was higher than group A. The GMT against *E. coli* was 12.1 and 24.3 in group A and B, respectively. At day 45 and 60 there was progressive decrease in antibody titre against *Str. agalactiae* in group B. The antibody titre against *S. aureus* first increased at day 45 and then decreased at day 60 whereas the antibody titre against *E. coli* remained persistent till day 60 in rabbits of group B. Furthermore, the primary antibody response to *E. coli* of composite antigen was higher (GMT, 12.1 at day 15) compared with *S. aureus* and *Str. agalactiae* (GMT, 9.8 at day 15) whereas the secondary antibody response to *S. aureus* was higher (GMT, 128) compared with *Str. agalactiae* and *E. coli* (GMT, 24.3 at day 45).

Key words: Buffaloes, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, antibody

INTRODUCTION

Mastitis is the most common disease in adult dairy cows, accounting for 38% of morbidity. On an annual basis three of every ten dairy cows have clinical apparent inflammation of the mammary glands, 7% of effected cattle are culled and 1% die as a consequence of the disease (Smith, 1990). Mastitis has been recognized as one of the most expensive diseases affecting dairy animals worldwide. According to Ratafia (1987), annual losses due to this disease were nearly $ 35 billion at world level. In Pakistan, statistics of current losses due to this disease are not available although it was estimated about two decades ago that in Punjab alone, the total losses caused by clinical mastitis amounted to Rs. 240 million per annum (Chaudhry and Khan, 1978). It is pertinent to mention that this survey did not take into account the losses caused by sub-clinical mastitis, which is 15-40 times more prevalent than the clinical one (Nickerson, 1990). The disease is caused by a wide variety of microorganisms including bacteria, fungi, yeast and mycoplasma. However, bacteria are by far the most frequent pathogens associated with mastitis. Among bacteria, mastitis is mainly caused by *Staphylococcus aureus, Streptococcus agalactiae,*and *Escherichia coli.*

The role of vaccination in the control of mastitis has been reviewed by many workers (Tomita *et al.*, 2000). A considerable wealth of literature is available on the efficacy of mastitis vaccines in cows and ewes but reports regarding the efficacy of any kind of mastitis vaccine in buffaloes, the main stay of dairy industries in Pakistan and India are limited to a solitary report for a Staphylococcal vaccine (Pal and Pathak, 1977). In view of the polymicrobial etiological nature of mastitis, polyvalent vaccines comprising the most common mastitis pathogens (*S. aureus, Str. agalactiae, E. coli*) seem to have a wider application as compared to monovalent mastitis vaccines. In order to evolve an effective vaccine to minimize the incidence of mastitis in the target species i.e., buffaloes, it is mandatory to evaluate the antibody responses to important mastitis pathogens in laboratory animals. Therefore, the present study has been designed to evaluate the primary and secondary antibody responses to formalin-inactivated composite antigen preparation containing *S. aureus, Str. agalactiae,* and *E. coli.*

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MATERIALS AND METHODS

The milk samples from 20 buffaloes clinically positive for mastitis were collected for isolation of *S. aureus*, *Str. agalactiae*, and *E. coli*. The isolation and biocharacterization of *S. aureus*, *Str. agalactiae*, and *E. coli* were made according to the procedures recommended by Anon. (1990). The purified field isolates were preserved in trypticase soya broth containing 20% glycerol at -20°C (Muhammad, 1992). The selected field isolates were grown separately in modified nutrient broth. Expression of pseudocapsule of *S. aureus* was confirmed by autoagglutination (Watson and Watson, 1989). Bacterial isolates were inactivated with formalin (0.4%) and harvested by centrifugation.

The composite antigen containing *S. aureus, Str. agalactiae* and *E. coli* was prepared (Opdebeeck and Norcross, 1982). The concentration of each of *S. aureus, Str. agalactiae* and *E. coli* was adjusted to 1×10^9 cells / ml by Breed and Smear method (Awan and Rehman, 2002) and with the help of spectrophotometer (Hirsch and Strauss, 1964). The prepared composite antigen was stored at 4°C until used. The sterility of prepared composite antigen was checked by streaking a loopful of composite antigen onto blood agar, MacConkey's agar and thioglycolate broth. To evaluate the safety of the composite antigenic preparation, 0.2 ml and 1 ml of prepared antigen was injected subcutaneously into three rabbits each.

Antibody response

Nine adult healthy rabbits were divided randomly into 3 groups (A, B, and C) comprising of 3 rabbits each. To the rabbits of groups A and B, 0.2 ml of composite antigenic preparation was injected subcutaneously whereas the rabbits of group C were inoculated with 0.2 ml normal saline and served as control. The rabbits of group B were given a booster dose at day 15 of the primary injection to see the secondary antibody response.

Serum samples were collected from all the rabbits before inoculation and subsequently at 15 days interval till day 60. The level of antibodies in serum specific to *S. aureus, Str. agalactiae* and *E. coli* were assayed by indirect haemagglutination test (Rehman *et al.*, 2003). Finally the geometric mean titre was calculated (Brugh, 1978).

RESULTS AND DISCUSSION

At day 0 the antibody titre in sera of rabbits of all the groups was almost same. At day 15, the antibody titre (GMT) in sera of rabbits of group A was 9.8 against each of. In sera of rabbits of group B, GMT was observed as 12.1 against each of *S. aureus*, and *Str. agalactiae* whereas it was 9.1 against *E. coli*. At day 30, significant difference in antibody titres was observed between groups A and B with GMT of 9.8 against each of *S. aureus*, and *Str. agalactiae* in group A whereas in group B GMT was 97.0 against *S. aureus* and 39.4 against *Str. agalactiae* which was higher than group A. The GMT against *E. coli* was 12.1 and 24.3 in groups A and B respectively. There was marked decrease in antibody titres in sera of rabbits of group A at day 45 (GMT 6.1 and 6.1 against *S. aureus*, and *Str. agalactiae* respectively) and day 60 (GMT 6.1, 2.0 and 4.0 against *S. aureus, Str. agalactiae* and *E. coli*, respectively). Progressive decrease in antibody titre was observed in sera of rabbits of group B at day 45 and 60 against *Str. agalactiae* and *E. coli* (Table 1) whereas antibody titre against *S. aureus* was first increased (GMT 128) at day 45 and then decreased (GMT 64) at day 60.

Table 1. Results of indirect haemagglutination (IHA) test in rabbits

Group (n = 3)	Organism	Geometric mean titre (GMT) at days				
		0	15	30	45	60
A	*S. aureus*	1.5	9.8	9.8	9.8	6.1
	Str. agalactiae	1.5	9.8	9.8	6.1	2.0
	E. coli	2.0	12.1	12.1	6.1	4.0
B	*S. aureus*	2.5	12.1	97	128	64
	Str. agalactiae	2.5	12.1	39.4	24.3	16.0
	E. coli	2.0	9.8	24.3	24.3	24.3
C	*S. aureus*	0.6	1.5	0	0.6	0
	Str. agalactiae	1.5	0	0.6	1.5	0
	E. coli	1.5	0.6	0	0.6	0.6

n = Number of rabbits in each group.

Furthermore, the secondary antibody response to *S. aureus* was higher (GMT 128) compared with *Str. agalactiae* and *E. coli* (GMT, 24.3 at day 45). From this study, it was concluded that rabbits receiving booster dose of composite antigen showed prolonged high antibody response compared to rabbits receiving single dose of composite antigen. These results were found in alignment with the findings of Opdebeeck and Norcross (1982) and Tamura *et al.* (1985).

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