Short communication

PRIMARY AND SECONDARY ANTIBODY RESPONSE TO COMPOSITE FORMalin-INACTIVATED STEPHANOLOCoccus AUREUS, STREPTOCoccus AGALACTiae AND ESCHERICHIA coli IN RABBITS

M. Avaz1, G. Muhammad2, A. Shehata1, M. Q. Bari2 and S. Hameed2

1Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
2University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Twenty buffaloes clinically normal for mastitis were selected for the collection of milk samples. Staphylococcus aureus, Streptococcus agalactiae and Escherichia coli were isolated from milk of 30% of buffaloes according to the procedures recommended by National Mastitis Council Inc. USA. The composite antigen containing S. aureus, S. agalactiae and E. coli was then prepared and the vaccination of each isolate was adjusted to 1 x 10^6 / ml / 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 intramuscularly in the rabbits of group A and B at 0.3 ml / rabbit which each isolate of group C inoculated with 0.3 ml normal saline not used as control. The rabbits of group B were given booster dose at day 15 of the primary injection to see the secondary antibody response. The level of antibodies specific to S. aureus, S. agalactiae and E. coli were estimated by indirect haemagglutination test (IHA) and finally mean titre (GMT) was calculated. At day 0, it was observed that the antibody titre was almost same in both groups. At day 30 significant difference in antibody titre was observed between group A and B with GMT of 9.8 against S. aureus, and 9.1 against S. agalactiae in group A whereas in group B GMT was 9.7 against E. coli and 9.4 against S. agalactiae which was higher than group A. The GMT against E. coli was 12,1 mean 24.3 in group A and B, respectively. At day 45 and 60 there was progressive decrease in antibody titre against S. agalactiae is group B. The antibody titre against S. aureus first increased on day 45 and then decreased at day 60 whereas the antibody titre against E. coli remained constant till day 60 in rabbits of group B. Consequently, the primary antibody response to S. aureus occurred first. Of composite antigen was higher (GMT 12.1) at day 15 compared with S. aureus and S. agalactiae (GMT 9.8 at day 15) whereas the secondary antibody response to S. aureus was higher (GMT 12.1) compared with S. 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, more than 75% of dairy cows have clinical or subclinical signs of mastitis, 7% of affected cattle are culled and 19% 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 Rajala (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. 250 million per annum (Chaudhry and Khan, 1987). It is pertinent to mention that this study did not take into account the losses caused by sub-clinical mastitis, which is 15-40 times more prevalent than the clinical one (Nicholson, 1990). The disease is caused by a wide variety of pathogens 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 vaccine in the control of mastitis has been reviewed by many workers (Torinna 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 major source of dairy industries in Pakistan and India are limited to a solitary report for a Staphylococcal vaccine (Pal and Pothak, 1977). In view of the polymicrobial etiology nature of mastitis, polyspecific vaccines comprising the most common mastitis pathogens (S. aureus, S. agalactiae, E. coli) need to have a wider application as compared to monovalent mastitis vaccines. It is evident 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, S. 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, Staphylococcus aureus, Staphylococcus aureus, and E. coli. The isolation and biochemical characterization of S. aureus, Staphylococcus aureus, and Staphylococcus aureus were performed by Dr. Park. The isolates were identified according to the procedures recommended by Anon. (1989). The purified field isolates were preserved in trypticase soy broth containing 20% glycerol at -20°C (Muhammad, 1992). The selected field isolates were grown separately in modified nutrient broth. Expression of plasmid associated with S. aureus was confirmed by agar overlay technique (Ward and Watson, 1989). Bacterial isolates were inactivated with formalin (0.4%) and harvested by centrifugation.

The composite antigen containing S. aureus, Staphylococcus aureus, and E. coli was prepared (Djerrabek and Nomv, 1982). The concentration of each of S. aureus, Staphylococcus aureus, and E. coli was adjusted to 1 x 10⁶ cells/ml by Bred and Starin method (Awan and Rehman, 2002) and with the help of spectrophotometer (Hirsch and Strauss, 1964). The prepared composite antigens were stored at 4°C until used. The identity of prepared composite antigens was checked by streaking a loopful of composite antigen onto blood agar, MacConkey's agar and tryptosephosphate broth. To evaluate the safety of the composite antigenic preparation, 0.2 ml and 1 ml of prepared antigens was injected subcutaneously into three rabbits each.

Antibody response

Vene 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 all day 60. The level of antibodies in serum specific to S. aureus, Staphylococcus aureus, and E. coli was 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 same. At day 15, the antibody titre (GMT) in sera of rabbits of group A was 9.8 against each of S. aureus, group B GMT was observed as 12.1 against S. aureus, and Staphylococcus aureus whereas it was 9.1 against E. coli. At day 30, significant difference in antibody titre was observed between groups A and B with GMT of 9.8 against each of S. aureus, and Staphylococcus aureus in group A whereas in group B GMT was 9.7 against S. aureus and 39.4 against Staphylococcus aureus 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 titre in sera of rabbits of group A at day 45 (GMT 6.1 and 6.1 against S. aureus, and Staphylococcus aureus respectively) and day 60 (GMT 6.1, 2.0 and 4.0 against S. aureus, Staphylococcus aureus, and E. coli respectively). Progressive decrease in antibody titre was observed in sera of rabbits of group B at day 45 and 60 against Staphylococcus aureus 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>
<thead>
<tr>
<th>Group</th>
<th>Organism</th>
<th>Geometric mean titre (GMT) at days</th>
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<tbody>
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<td>0</td>
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<tr>
<td>A</td>
<td>S. aureus</td>
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<td></td>
<td>Staphylococcus aureus</td>
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<tr>
<td>C</td>
<td>S. aureus</td>
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<td></td>
<td>Staphylococcus aureus</td>
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n = Number of rabbits in each group.

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Antibody response to composite antigen in rabbits

Furthermore, the secondary antibody response to S. aureus was higher (GMT 128) compared with Stj. agardhiae and E. coli (GMT: 24.2 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 Ophebeek and Noraees (1982) and Tamaura et al. (1985).

REFERENCES