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DOSE DEPENDENT IMMUNE RESPONSE TO FORMALIN INACTIVATED *ESCHERICHIA COLI* MASTITIC ISOLATE IN RABBITS

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ABSTRACT

The present study was conducted to evaluate dose-dependant immune response of *Escherichia coli* isolate in rabbits. *Escherichia coli* was isolated from mastitic buffaloes and characterized on the basis of morphological, cultural and biochemical tests. A total of 12 adult healthy rabbits, randomly divided into four equal groups (A, B, C and D) were used to evaluate the dose-dependent immune response to *Escherichia coli* antigen. Inocula containing 10^6 cells/ml of *Escherichia coli* were injected subcutaneously with the increasing doses of 0.20, 0.40 and 0.80 ml in groups A, B and C, respectively. The rabbits of group D were kept as un-inoculated control. Blood samples were collected at weekly intervals for four consecutive weeks from rabbits of all groups and analyzed for serum antibody titers. An increased geomean antibody titer (GMT), reaching 78.8, was observed in rabbits of Group C. The other two groups (Groups A and B) showed progressive increase in GMT up to day 21 which declined later, while the control group presented no response.

Key words: Immune response, *Escherichia coli*, mastitis, rabbits.

INTRODUCTION

Mastitis is recognized world wide as the most important and costly disease of dairy animals. Field surveys of major livestock diseases in Pakistan have indicated that mastitis is one of the most important health hazards in the country (Ajmal, 1990). This disease is caused by interaction of various factors associated with host, pathogens and the environment. Infectious agents like bacteria, viruses, fungi and algae are mostly the primary causes of this disease but coliform mastitis is one of the most difficult diseases to treat in the modern dairy industry. Curative therapy with antibiotics remains only moderately effective and depends on the stage at which the disease is treated. The most successful strategies for combating coliform mastitis appear to be prevention by hygienic management or prophylactic immunization (Dosogne *et al.,* 2002)

The incidence of clinical mastitis caused by environmental pathogens such as *Escherichia coli* is a concern of the dairy industry. The control of sub clinical mastitis with a subsequent reduction in milk somatic cell count (SCC) does not appear to decrease the incidence of clinical mastitis and may increase the susceptibility of cows to clinical mastitis caused by coliforms (Scott *et al.,* 1998).

Because of extremely small herd size (more than 80% animals are kept in herds of 3-4 animals/family; Teufel, 1998), widely rampant poverty, illiteracy and lack of any milk quality premium, standard mastitis control practices are conceivably difficult to be adopted in a country like Pakistan. In fact, these practices are totally non-existent even on well-organized private dairy farms and those in the public sector. Therefore, vaccination holds the promise of a suitable alternative mastitis control strategy in Pakistan.

In order to evolve an effective vaccine to minimize the incidence of mastitis in buffaloes and cows, it is mandatory to evaluate the antigenic responses to important mastitis pathogens in laboratory animals so that optimum antigenic dose of these organisms could be determined. Since *E. coli* is among the most common mastitic pathogens, the present study was conducted to compare the immune response to various doses of formalin inactivated *E. coli* isolate in rabbits.

MATERIALS AND METHODS

Isolation and bio-characterization of field isolates

Isolation and bio-characterization of bacterial isolates from 20 mastitic buffaloes was performed following the procedure described by National Mastitis Council (1990). The purified *Escherichia coli* isolate was preserved in trypticase soy broth (Difco Labs. Michigan, USA) containing 20% glycerol and was kept at -20°C.

Preparation of *Escherichia coli* antigen

Selected *Escherichia coli* isolate was inoculated in a 500 ml flask containing nutrient broth enriched with sterile bubaline whey (10%), obtained from the rennet precipitation of fresh defatted bubaline milk (Watson and Watson, 1989). It was kept on an orbital shaker at 60 rpm for 48 hours. Then formalin (0.4%) was added to kill the *Escherichia coli* isolate. The formalized isolate was kept for 24 h for the proper action of the formalin. The killed organisms were harvested by centrifugation at 6000 G for 1 hour at 4°C and then two washings with sterile PBS (pH 7.2) were given. The pellet thus obtained was re-suspended in PBS. The concentration of *Escherichia coli* was adjusted at $1x10^6$/ml by spectrophotometer. The preparation was stored at 4°C until utilized. Sterility was checked by streaking a loopful of the antigen onto blood agar, MacConkey agar plates and thioglycolate broth and incubating for 24-48 hours at 37°C.

Antigenic response in rabbits

A total of 12 adult healthy rabbits, randomly divided into four equal groups (A, B, C and D) were utilized to evaluate the dose-dependent immune response to *Escherichia coli* antigen. Inocula containing 10^6 cells/ml were injected subcutaneously with the increasing doses of 0.20, 0.40 and 0.80 ml in groups A, B and C, respectively. The rabbits of group D were kept as un-inoculated control. Blood samples were collected at weekly intervals for four consecutive weeks from rabbits of all groups and analyzed for serum antibody titers. The geomeom antibody titer (GMT) was determined by indirect haem-agglutination (IHA) method (Sawada *et al.,* 1981) and analyzed statistically using single factor analysis of variance and LSD.

RESULTS AND DISCUSSION

The study was undertaken to evaluate the dose dependent immune response to formalin inactivated *Escherichia coli* isolate in rabbits. The *Escherichia coli* isolate was selected on the basis of its morphological and biochemical characteristics. Morphologically, *Escherichia coli* isolate was gram negative rods and colony colour was transparent (pinkish) on MacConkey agar. Biochemically, *Escherichia coli* isolate was catalase positive, lactose fermentation test positive, triple sugar iron test positive (Butt formation), potassium hydroxide test positive but Simmon's citrate test negative.

Dose-dependent immune response

Sera samples of animals of groups A and B indicated progressive increase in GMT, reaching 24.3 and 39.4 respectively, at day 21. Group C showed increase in GMT with maximum value of 78.8 at day 28. Rabbits of control group D showed no increase in titers (Table 1). The statistical analysis of these titers showed that there was a significant difference among the groups ($P<0.0001$). Further analysis showed significant differences among A & C, A & D, B & C, B & D, C & D groups (Table 2). This indicated a positive dose dependent response of *Escherichia coli* isolate, which is in agreement to the findings of Tamura *et al.* (1985). The reason could be that the higher dose at some specific level may give the higher and prolong titer as compared to other dose levels.

Table 1: Geomean antibody titers (GMT) of rabbits inoculated with different doses of formalin inactivated *Escherichia coli*

Group	Dose rate of *E. coli* 10^6/mL	GMT at post inoculation day				
		0	7	14	21	28
A	0.2 ml	2.0	12.1	19.7	24.3	16.0
B	0.4 ml	2.5	16.0	32.0	39.4	24.3
C	0.8 ml	2.5	24.3	39.4	64.0	78.8
D	Control	1.5	1.5	0.5	2.0	0.5

Table 2: Results of multiple means comparison

Groups	Mean difference	Probability
A and B	-7.60	0.284
A and C	-28.40	0.000
A and D	15.20	0.035
B and C	-20.80	0.004
B and D	22.80	0.002
C and D	43.60	0.000

A similar study was conducted by Vangroenweghe *et al.* (2004) to evaluate the dynamics of infection and the immunological response to varying numbers of *Escherichia coli* injected into the mammary glands of primiparous cows during the periparturient period. Primiparous cows were observed to be more resistant to intramammary *E. coli* challenge, and an increase of the inoculum dose by 2 log10 units induced a more rapid clinical response and clearance of the organisms.

This study shows that antigenicity of *Escherichia coli* isolate is dose dependent. So there would be a direct relationship between antigen dose and its immune response. Thus, the *Escherichia coli* antigen isolated in the present study may be used to evaluate the immune response against mastitis buffaloes and can be an initial step for the preparation of an effective mastitis vaccine.

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