

DEVELOPMENT OF ROUGH MUTANT *E. COLI* VACCINE AGAINST COLIFORM MASTITIS

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INTRODUCTION

Mastitis caused by Gram-negative lactose fermenters which includes *Escherichia coli*, *Klebsiella* and *Enterobacter species (aerogenes and cloacae)* is called as coliform mastitis (Sharma *et al.*, 2012). The incidence of coliform mastitis peaks at post-parturition period soon after the cows enter the lactation cycle (Hogan and Smith, 2003). Since sanitizing by teat dipping, dry cow therapy and treatment have failed to control coliform infections; immunization of dairy cows against coliform mastitis may be a highly valuable procedure to bring these infections under control (Bushnell, 1984). The present study has been undertaken to develop vaccine against coliform mastitis by using local rough *E. coli* isolate.

MATERIALS AND METHODS

Isolate used

Escherichia coli isolate number 38 isolated from mastitis milk and maintained at Department of Veterinary Microbiology was

used for vaccine preparation against coliform mastitis. This *E. coli* isolate was confirmed as *E. coli* by biochemical tests viz., Catalase, Oxidase and ENTERO-Rapid 24 kit and was subjected to sugar fermentation test as per Quinn *et al.*(1994). Further this isolate was serotyped at National Escherichia and Salmonella Centre, Kausali, Himachal Pradesh as rough (R) *E. coli* strain. This R mutant was screened for virulent genes viz., *stx1*, *stx2* and *eaeA*. The rough mutant strain was subjected to virulent gene multiplex PCR. The DNA was isolated by snap chill method and stored in – 20°C for use as template DNA (Bean *et al.*, 2004) and plasmids isolated as per Sambrook and Russel,(2001).

Vaccine preparation

Two types of formalin inactivated vaccines were prepared using this R mutant *E.coli* stain with two types of adjuvants. First, the R mutant *E. coli* was cultured in tryptic soy broth for 18 h to achieve 4×10⁹ colony forming units and was inactivated by using 0.5% formalin and incubated at 37°C for 48 h. One

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part of Al(OH)₃ adjuvant was added to three parts of culture broth and mixed. For Montanide (SEPPIC) adjuvant, equal parts of broth and adjuvant were added and mixed (Vidhya, 2005). The vaccines were tested for sterility in various medium and for safety in both rabbits and calves.

Immunization

Six pregnant cows were kept as untreated control and 27 pregnant cows were used for testing each type of vaccine. Five ml of the vaccine was given subcutaneously in the neck region to 7 to 8 month pregnant cows at the beginning of drying off period and 30 days after the drying off period (Hogan *et al.*, 1995). Blood samples for serum were collected from these animals at drying off, 30 days after drying off, one month post calving and two months post calving to assess the immune response by Indirect ELISA. Milk smears were taken to estimate the somatic cell count at one month post calving and two months post calving. Milk samples were collected from mastitis affected cows, if any, from both vaccinated and unvaccinated groups and were subjected to etiological agent identification.

Results and Discussion

The isolate used in the present study did not ferment galactose. It may be due to lack of the enzyme uridine diphosphate galactose 4-epimerase as suggested by Elbein and Heath (1965). This R mutant *E. coli* did not contain any virulent genes (Fig 2) (*stx 1*, *stx 2* and *eaeA*)

and plasmids when screened by multiplex PCR(Fig 3).

Mean ELISA OD values at drying off, 30 days after drying off, one month post calving and two months post calving are presented in Table 1. There was 3.1 and 3.3 fold increase in antibody titer at 30 days after drying off compared to initial drying off period, in Al(OH)₃ and Montanide adjuvant added rough mutant *E. coli* vaccines respectively (Fig. 1). These results are in accordance with Hogan *et al.*, (1992a), Hogan *et al.*, (1992b), Aslam *et al.*, (1995), Hogan *et al.*, (1997) and Smith *et al.*, (1999). There was no significant difference in performance as assessed by antibody titer between Al(OH)₃ and Montanide adjuvant rough mutant *E. coli* vaccine groups.

The immune response to the vaccine was measured by indirect ELISA in serum samples. There was a significant increase in the antibodies against rough mutant *E. coli* at 30th day, which was maintained till two months post calving in vaccinated group of animals compared with zero day. There was significant increase in the antibody titer in vaccinated animals compared with control group. There was no significant difference observed in antibody titer between two vaccine groups.

Mean SCC values at one month post calving and two months post calving are given in Table. 2. There was no significant difference observed in somatic cell count value between vaccinated animals and unvaccinated control

animals. These results are in accordance with the results of Giarudo *et al.* (1997).

There was no incidence of clinical coliform mastitis in vaccinated group of animals and effective in controlling the coliform mastitis during the first two months of the lactation which is considered to be the susceptible period. But there was one incidence of clinical coliform mastitis in unvaccinated control group of animals. There was incidence of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis* bacterial mastitis in both vaccinated and control group of animals. The present vaccine prepared was able to protect the animals against mastitis caused by gram-negative bacteria in addition to coliforms based on incidence of mastitis recorded. These results are in accordance with Gonzalez *et al.* (1989), Hogan *et al.* (1992a), Hogan *et al.* (1992b), Hogan *et al.* (1992c).

Summary

This study has shown that the prepared coli mastitis vaccines were effective in controlling the coliform mastitis in vaccinated animals during the first two months of the lactation when it was injected during drying off and 30 days after drying off.

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Table 1.

Mean ELISA OD values at drying off, 30 days after drying off, one month post calving and two months post calving

Treatment		Drying off	30 days after drying off	1 month post calving	2 month post calving
Unvaccinated Control		0.4166 ±0.014	0.43416 ±0.028 ^{NS}	0.485 ±0.028 ^{NS}	0.413 ±0.017 ^{NS}
Vaccinated	Al(OH) ₃ adjuvanated vaccine	0.3648±0.011	1.139 ±0.032 ^{**}	1.136 ±0.014 ^{**}	1.0619 ±0.026 ^{**}
	Montanide adjuvanated vaccine	0.3773±0.013	1.2463 ±0.023 ^{**}	1.156 ±0.019 ^{**}	1.1228 ^b ±0.026 ^{**}

NS – Non-significant, ** - Significant at 1 per cent level, Means bearing different superscripts in a column differ significantly

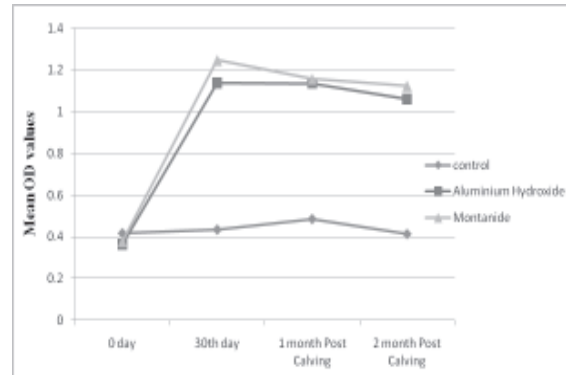
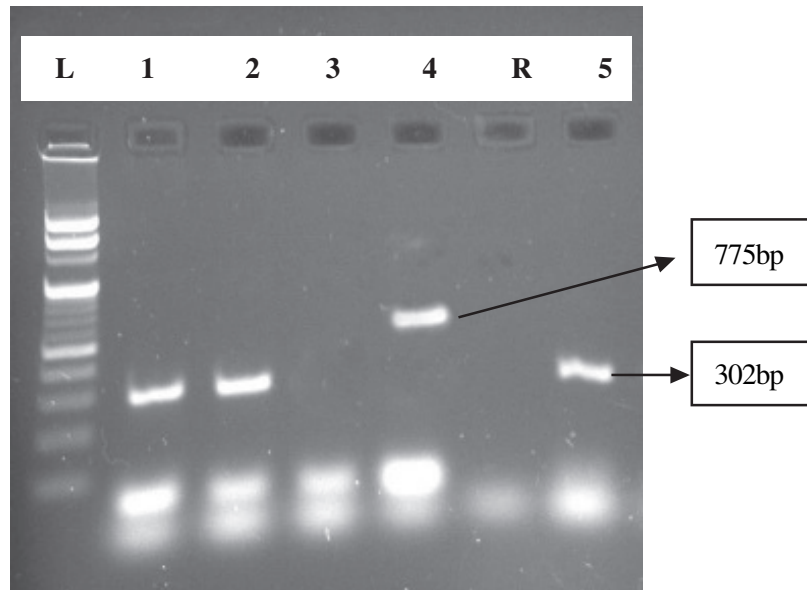


Fig. 1 Antibody response to coliform mastitis vaccine

Table 2.
Mean Somatic cell count values at one month post calving and two months post calving

	Treatment	1 month Post Calving	2 month Post Calving
	Unvaccinated Control	127142 ± 5527	127142 ± 5527
Vaccinated	Al(OH)₃ adjuvanated vaccine	125299 ± 3355	124685 ± 3892
	Montanide adjuvanated vaccine	125299 ± 2984	124070 ± 3223

Fig 2 Identification of virulent gene by multiplex PCR



L - 100bp marker

1 - Isolate No.1

2 - Isolate No.2

3 - Isolate No.3

4 - Isolate No.4

R - Isolate No.38 (Rough mutant *E. coli*)

5 - Isolate No.5

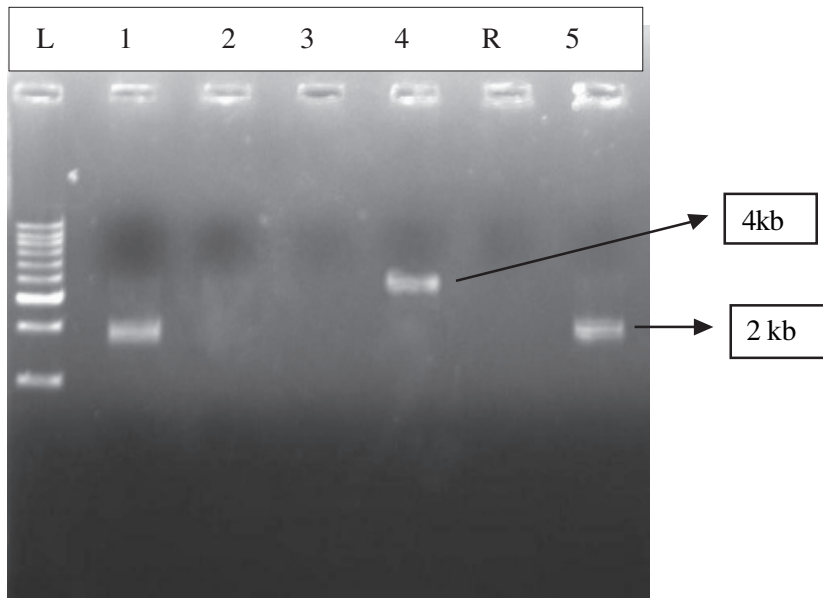


Fig.3 Plasmid Screening

L - 1 kb marker

1 - Isolate No.1

2 - Isolate No.2

3 - Isolate No.3