Control of Coliform Mastitis with J5 Vaccine: Special Reference to J5 Vaccination in the Saudi Arabian Dairy Herds

A. M. Alluweimi
Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, P.O. Box-1767, AL-Ahsa 31982, Saudi Arabia

Abstract: Coliform mastitis is widely incriminated as an environmental disease in modern dairy herds. J5 vaccine. Rec mutant with exposed common core antigen, was introduced to control the coliform mastitis. Experimental infection and field trial studies indicated the failure of the vaccine in preventing the infection. Nevertheless, it had significantly reduced the incidents of infection and/or the severity of the disease. In contrast, in Saudi Arabian dairy herds, J5 vaccine failed to accomplish, what was reported elsewhere. Further studies are advised to scrutinize the feasibility of the vaccine in control of the disease in Saudi Arabian dairy herds.

Key words: Cytokines, coliform, J5, E. coli, Saudi Arabia, mastitis.

Introduction
Mastitis is inflammation of the mammary gland mainly due to bacterial infection (National Mastitis Council, 1996). Mastitis causes extensive economic losses to the dairy industry. It was found that mastitis caused 70-80% of the estimated $140 to $200 per cow/year loss due to the reduction in milk production (Gill et al., 1990). The importance of bovine mastitis and assessment of effective methods in control of bovine mastitis was first brought to attention in 1938. In that year, Munch-Petersen published a comprehensive review of the literature on mastitis (Dodd, 1983). This review shed the light on different forms of mastitis and organisms involved in the mammary gland infection. The review also elaborated the useful procedure in control of certain mastitis like that caused by Streptococcus agalactia (Dodd, 1985).

It is well established today that bovine mastitis is caused by two forms of bacteria, contagious and environmental. Pathogenic contagious bacteria, mainly Staphylococcus aureus and Streptococcus agalactia are incriminated in wide range of mastitis incidents in modern dairy farms. On the other hand, environmental mastitis is caused by pathogens whose primary reservoir is the cow's environment. Bacteria involved in environmental mastitis are of diverse and heterogeneous forms. Coliform bacteria and Streptococci species represent the major groups of organisms that are involved in environmental mastitis (Smith et al., 1985). The most frequently encountered coliform bacteria in environmental mastitis are Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter aerogenes and species of Citrobacter, Serratia and Proteus (Smith et al., 1985).

The frequency and severity of the environmental mastitis was studied in dairy herds (Smith et al., 1986). About 81% of coliform bacteria and 83% of Streptococcal produced clinical infection during lactation. It was shown that the incidents of clinical cases elevated dramatically at the first 76 days of lactation and during summer. Coliform mastitis was seen more persistent than Streptococcal infection, 69% of coliform mastitis which persisted for 30 days of lactation in comparison with 59% of Streptococcal infection for the same period (Smith et al., 1986).

The main purpose of this study is to review the literature on the efficacy of the only available vaccine, J5, in control of coliform mastitis and to evaluate the Saudi dairy farms experience in use of this vaccine.

Coliform mastitis: "Coliform" is a term applied to a group of gram-negative Enterobacteriaceae with lactose-fermenting property (Eberhart, 1984). Escherichia coli, Klebsiella and Enterobacter species are the most heavily incriminated coliforms in bovine mastitis. Coliform mastitis is a well-known form of environmental infection (Eberhart et al., 1979; Eberhart, 1984). The non-contagious nature of the disease stem from the wide spread of the coliforms in the cow's environment like bedding, poorly sanitized teat cup liners, damp walk-ways, manure-covered yards and heavily contaminated water (Eberhart et al., 1979; Eberhart, 1984). The etiological agents of coliform mastitis gain access to the mammary gland through the teat duct into the teat cistern. It appears that the access of the coliform agents to the teat canal is not completely related to the milking time. This was indicated by the failure of germicidal teat dip in restricting the incidence of the infection (Eberhart et al., 1979; Eberhart, 1984).

The incidence of the coliform mastitis peaks at post-parturition period soon after cow enters the lactation cycle. Dry period is the most likely period when the organism establishes itself in the mammary gland. Stress factors like parturition and commencement of milk production were found strong enough to unmask the dormant infection (Eberhart et al., 1979; Eberhart, 1984). Coliform mastitis is complicated by the involvement of several species of coliform bacteria particularly the involvement of the wide range of E. coli strains. Coliform mastitis could be peracute, acute, or chronic (Green & Bradely, 1998). The clinical picture of the infection is related to the level of endotoxin dissemination. Intact or released endotoxin attracts a high number of leukocytes to the mammary gland. The common signs of peracute infection are anorexia and depression. The body temperature may reach 40-42 °C. Toxemia develops and systemic signs may be observed before any visible changes in the mammary gland. Peracute coliform mastitis is common among lactating cows. In acute form, signs are milder and affected quarters are slightly swollen with watery secretions containing flakes. Chronic coliform mastitis however appears as repeated episodes of subacute mastitis, which is indistinguishable from mastitis caused by other microorganisms. Subclinical coliform mastitis may occur and it is characterized by the presence of coliform bacilli in milk with no obvious clinical signs (Jones, 1990).

Treatment of coliform mastitis is based on supportive antimicrobial therapy. Supportive therapy is aimed to hasten the inflammatory responses and the consequences of the endotoxic shock. Effective antimicrobial therapy is warranted by the penetrative capacity of antimicrobial agent to inflamed mammary gland (Eberhart et al., 1979; Eberhart, 1984). Intr
mammary infusion of mammary gland with antibiotic should be facilitated by milk drainage to enhance its transmision in inflamed udder. On the other hand, successful systemic antibiotic therapy depends on the ability of antibiotic to transposes the blood barrier to mammary tissues. In general, effective and safe parenteral antimicrobial therapy is restricted by availability of antibiotics with high microbicidal effects on gram-negative bacteria and penetration of blood barriers to the mammary gland with minimum residues in milk and meat (Ebehart et al., 1975; Ebehart, 1984).

![Diagram of Gram-negative cell wall](image)

**Fig. 1:** Schematic illustration of the three layers of gram-negative cell wall bacteria.

![Diagram of lipopolysaccharide layer of gram-negative bacteria](image)

**Fig. 2:** Schematic illustration of lipopolysaccharide layer of gram-negative bacteria. The arrows indicate the sites of mutation in the lipopolysaccharide and type of rough mutants.

**The general properties of J6 vaccine:** Gram-negative bacteria have highly complex and sophisticated cell wall. The outer cell wall is made of three layers from inside to outside: mucopolysaccharide - peptidoglycan - phospholipid - protein and lipopolysaccharide (LPS). (Fig 1). LPS is made of a variable oligosaccharide region linked to a conserved core polysaccharide and lipid A regions. Lipid A is responsible for the endotoxic activity of gram-negative bacteria. Oligosaccharide or "O" antigen (somatic antigen) represent the variable region that determine the bacterial serotype. In contrast, to somatic antigens, core antigens of gram-negative bacteria are highly conserved antigens, shared by major species, genera and groups of gram-negative bacteria (Tyler et al., 1990). Mutation in the outer layer of the cell wall may rise due to the lack of specific enzymes necessary for the synthesis of the somatic side chain. Bacteria with this type of mutation are called rough mutant (R-mutant). Different types of R-mutants are identified by subscript a, b, c, d or e according to the level of mutation in the outer layer. For instance, Ra mutants have nearly complete LPS and only back side chains due to the deficiency of enzyme that links the side chains to the core antigen. While Re mutants are deficient in core oligosaccharide due to the lack of enzyme required for the assembly of somatic side chain to lipid A components (Fig. 2) (Tyler et al., 1990; 1991). Exposure of core antigen in R-mutant represents a suitable model to test the efficacy of core antigen in providing the protective immunity against wide range of gram-negative bacterial infection. Among the wide range of R-mutant organisms, E. coli 0111:B4 (J6) is one of the most widely used Re-mutant in this aspect. J6 is a uridine diphosphate galactose epimerase deficient mutant strain. Absence of the linkage between galactose and glucose in the core antigen is the consequence of this enzyme deficiency (Tyler et al., 1990).

**Immune responses to J6 vaccine:** J6 vaccine was a subject of comprehensive studies on the efficiency of core antigen in providing protective immunity against gram-negative bacterial infection in animals and human (Baumgartner et al., 1988; Culloc, 1991; Lachman et al., 1984; Law & Melvin, 1986; Morris et al., 1986; Tyler et al., 1989; 1991; Vance, 1989; Ziegler et al., 1982). Common core antigen provides a suitable solution for the etiologic diversity of gram-negative bacteria. Nevertheless, generated antibodies to heterologous gram-negative bacteria are limited by the stage of the bacterial cell wall development (Tyler et al., 1991). Immunoglobulins with good affinity to common core antigen were seen restricted to the logarithmic stage of cellular growth (Tyler et al., 1990). However, the role of such immunoglobulins was seen efficient in activation of the complement cascade, enhancement of optimization and blocking the free LPS (Tyler et al., 1991). Trials of passive immunization in animals and human were conducted to determine the protective merit of the heterologous antibodies to core antigen (Morris et al., 1986; Ziegler et al., 1982). In human, administration of anti-J5 antiserum had dramatic effect in amelioration of the clinical signs of endotoxaemia and consequently a significant reduction in mortality rate (Ziegler et al., 1982). On the other hand, anti-J6 antiseraum failed to exert a significant protection in horses inoculated with E. coli endotoxin (Morris et al., 1986). Passive immunity trials faced serious obstacles in providing the clear cut information on efficacy of heterologous antiserum in reducing the severity of gram-negative infection. In addition, to the logistics difficulties in successful transfer of immunoglobulins to mucosal surfaces, gram-negative flora represents an important barrier in masking the antibodies from providing the effective immunization. In general, anti-J5 antibodies encounter a serious difficulty in recognizing the heterologous common core antigen. This is either due to the rapid multiplication and development of gram-negative organisms that limit the exposure time of the immune system to the core antigen or the capability of certain bacteria like Salmonella dublin to take phagocytes as a harbor from the neutralizing
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| Vaccinated | 1211 | 610 | 46 | 47 | 0.47 |
| Nonvaccinated | 1137 | 626 | 42 | 48 |       |

The application of J5 vaccine in the Saudi Arabian dairy herds:
Since 1976 Saudi Arabia has embarked an ambitious, strong and highly ranked dairy plant projects. The dairy herds are in hundreds thousands which are divided into multi groups of 500-90000 cattle. The herd populations mainly comprised of the Holstein breed and are distributed on the giant dairy farms. The cattle are reared in highly intensive system. Each animal is milked four times daily and the average milk production of each cow per year was well above 12000 liters (Edacherli, 2000). Companies like Al-Marai, Al-Safi, Nada and Nadec represents the highly developed and extraordinary organized dairy companies in the country. In such highly intensive rearing system mastitis remained a great challenge for Saudi dairy industry. Coliform mastitis among the most serious form of mastitis, which persists despite the scrutinized hygienic measures. Cattle of second and above lactation with high milk yield were seen more susceptible to coliform mastitis mainly after calving.

Certain farmers introduced J5 vaccine (Pharmacia Upjohn, USA) to combat the coliform mastitis. Cattle were vaccinated twice, 60 days prior to calving and right after calving. Both doses were given subcutaneously at the neck region.

The potency and efficacy of J5 vaccination in control of coliform mastitis in Saudi Arabia was examined by reviewing the records of several years of different farms. The effect of J5 vaccination on the incidence of E. coli and other coliform infection was compared with the non-vaccination period. Reviewing the record of 1137 cattle at pre vaccination period revealed that 526 cattle contracted E. coli infection, whereas 42 cattle were infected with other coliform bacteria. The percentage of the infection in non-vaccinated cattle was 48% (Table 1). Introduction of J5 vaccine did not show any drastic changes in the level of infection. The percentage of infection was 47% (510 cattle out of 1211 vaccinated cattle) (Table 1).

The t-test analysis revealed no significant difference between vaccinated and non vaccinated cattle (P > 0.471). In addition to the non-significant reduction in the level of infection, the severity of the clinical signs of coliform mastitis was not mitigated.

Inefficacy of the J5 vaccine in Saudi herds can not be explained due to the lack of controlled studies on the vaccination program. However, the low potency of the vaccine might be due to the environmental factors, handling procedure especially transport of the vaccine and its storage during vaccination. Identification of the circulating E. coli strains in the dairy herds might also add an important insight in elucidating the factors behind the failure of the vaccine in Saudi Arabia.

Although the results indicated the low profile of J5 vaccine in protection and/or decreasing the coliform mastitis in certain Saudi dairy farms, further studies on the vaccine is vital to...
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provide a better insight on its efficacy in Saudi dairy herds. Experimental infection and field studies are necessary to evaluate the J6 vaccine efficiency under the Saudi climate. The decision makers in Saudi dairy companies should not discontinue J5 vaccination program, as it had happened in certain dairy farms, or continue merely on the basis of sporadic observations. The merits of the evidence that will be generated by the above proposed studies are the only valid reference that will aid in making a suitable decision about the efficacy of J5 vaccine.

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References