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

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Abstract: Coliform mastitis is widely incriminated as an environmental disease in modern dairy herds. J5 vaccine, Rc 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 $300 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, 1983).

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 pneumonae*, *Klebsiella oxytocia*, *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.*, 1985). About 81% of coliform bacteria and 53% 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.*, 1985).

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 (Ebehart *et al.*, 1979; Ebehart, 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 (Ebehart *et al.*, 1979; Ebehart, 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 (Ebehart *et al.*, 1979; Ebehart, 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 (Ebehart *et al.*, 1979; Ebehart, 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 undistinguishable 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 (Ebehart *et al.*, 1979; Ebehart, 1984). Intra

mammary infusion of mammary gland with antibiotic should be facilitated by milk drainage to enhance its transfusion 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 micro biocidal effects on gram-negative bacteria and penetration of blood barriers to the mammary gland with minimum residues in milk and meat (Ebehart *et al.*, 1979; Ebehart, 1984).

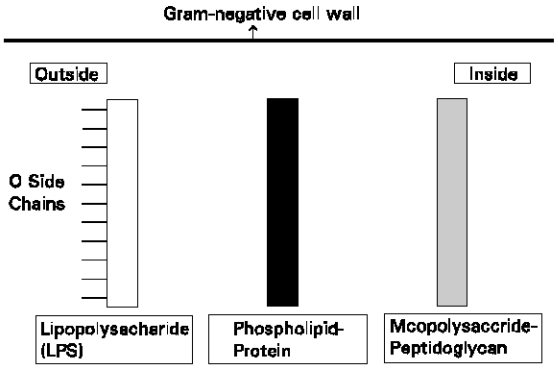

Fig. 1: Schematic illustration of the three layers of gram-negative cell wall bacteria.

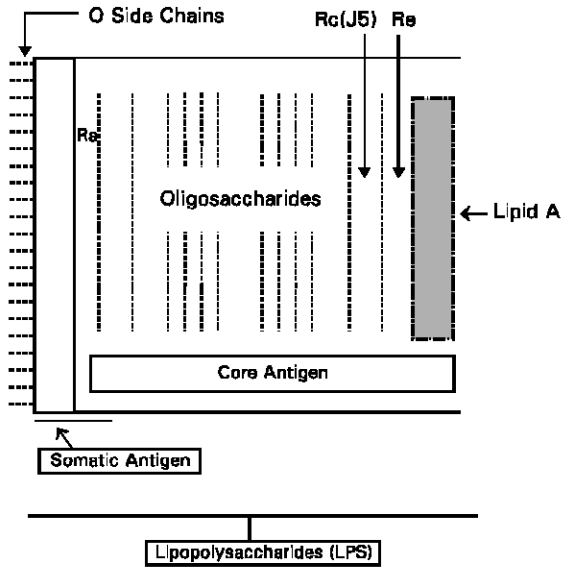

Fig. 2: Schematic illustration of lipopolysaccharide layer of gram-negative bacteria. The arrows indicate the sites of mutation in the lipopolysaccharide and type of rough mutants.

The general properties of J5 vaccine: Gram-negative bacteria have highly complex and sophisticated cell wall. The outer cell wall is made of three layers from inside to outside: mucopolysacchride - 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 (J5) is one of the most widely used Rc-mutant in this aspect. J5 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 J5 vaccine: J5 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.*, 1985; Cullor, 1991; Lachman *et al.*, 1984; Law & Melvin, 1985; Morris *et al.*, 1986; Tyler *et al.*, 1990; 1991; Yancey, 1999; 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 cultural 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 endotoxemia and consequently a significant reduction in mortality rate (Ziegler *et al.*, 1982). On the other hand, anti-J5 antiserum 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 antisera 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 their common core antigen or the capability of certain bacteria likes *Salmonella dublin* to take phagocytes as a harbor from the neutralizing

Table 1: Comparison of incidence of *E. coli* and other coliform infection in J5 vaccinated and nonvaccinated cattle.

	No. of Cattle	*E. coli* infection	Other coliform infection	% infection	t-test
Vaccinated	1211	510	45	47	
					0.471
Nonvaccinated	1137	526	42	48	

antibodies (Tyler *et al.*, 1990).

J5 vaccine and coliform mastitis: Coliform mastitis is an environmental infectious disease caused by a wide range of coliform bacteria. Gram-negative R-mutants with exposed common core antigen were shown to be convenient solution in stimulating a protective immunity. Hence, *E. coli* Rc mutant 0111:B4 (J5) was introduced to control the prevailing coliform mastitis in dairy cattle (Cullor, 1991; Hogan *et al.*, 1992a; b; 1995; Tomita *et al.*, 1998; 2000).

The efficacy of J5 vaccine in preventing coliform mastitis was a subject of a wide range of studies (Hogan *et al.*, 1992a; b; 1995; Tomita *et al.*, 1998; 2000). Several studies have tested the potency of J5 through Intra mammary challenge (Hogan *et al.*, 1992a; Tomita *et al.*, 1998; 2000). J5 vaccine was given at drying off, after drying off and a shortly before calving. All studies concluded that anti-common core antibodies were not adequately strong to overcome the Intra mammary challenge. Vaccinated cattle succumbed to Intra mammary infection, though vaccination exquisitely decreased the severity of the infection (Hogan *et al.*, 1992a; Tomita *et al.*, 1998; 2000). Similar outcome was also observed in vaccinated cattle challenged with mild smooth heterologous strains of *E. coli* (Hogan *et al.*, 1995).

The efficacy of J5 vaccine was also scrutinized in a wide scale of field trials (Hogan *et al.*, 1992b; Gonzales *et al.*, 1989; Cullor, 1991). Taking into consideration different types of J5 preparations and vaccination schedules, all field trials reached to a conclusion similar to that observed with experimental Intra mammary infection. The scale and severity of coliform mastitis was significantly reduced in vaccinated cattle in comparison with unvaccinated cattle. For instance, Cullor (1991) showed that only 2.5% of 246 vaccinated cows developed clinical mastitis, whereas 12.77% of 240 unvaccinated cows showed coliform mastitis.

Mild infection in vaccinated cattle was mainly due to the rapid clearance of free LPS and enhancement of bacterial removal from challenged quarters (Hogan *et al.*, 1992a; b; 1995; Tomita *et al.*, 1998; 2000). Immune response to J5 vaccine was dominated by high titer of IgG. Hogan *et al.* (1992c) has investigated the role of the opsonic activity of immunoglobulin-G (IgG) and immunoglobulin-M (IgM) in clearance of heterologous bacteria in vaccinated cattle. IgM level was correlated with high level of phagocytic index. However, IgM opsonic activity in milk and serum was reduced as the lactation progressed. It appears that IgM in milk has a natural opsonic activity (Hill *et al.*, 1983). Hill *et al.* (1983) showed that whey pooled from cattle at early lactation was enriched with IgM and expressed high opsonic activity to encapsulated and noncapsulated *E. coli*. The level of IgM and its opsonic activity was dramatically reduced in whey pooled from milk at mid-lactation. IgG, on the other hand was related to the clearance of free LPS and decrease of endotoxic shock.

The role of cell mediated immunity (CMI) in J5 immunity was not fully studied. The scale of CMI in J5 immunity is important in defining its role in activation and recruitment of lymphocytes and other mononuclear cells in the mammary gland. Study of cytokine and type and subtype of mononuclear cells that are stimulated by J5 vaccine could provide a better insight of CMI in J5 immunity.

In general, the overall impression that could be rewarded from the overwhelming studies on J5 vaccine is the limitation of J5 vaccine in generating the effective immunity, particularly antibodies that clear heterologous bacteria. Nevertheless, the stimulated immunity was sufficient to provide protection against the deleterious effect of coliform infection. Hence, reduction in coliform mastitis incidence and/or its severity was one of the major reasons behind J5 celebrity in light of complete absence of alternative efficient treatment and protective vaccine.

The application of J5 vaccine in the Saudi Arabian dairy herds: Since 1975 Saudi Arabia has embarked an ambitious, strong and highly ranked dairy plant projects. The dairy herds are in hundred thousands which are divided into multi groups of 500-30000 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 (Edacheril, 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 farms 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 efficiency 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 records 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

provide a better insight on its efficacy in Saudi dairy herds. Experimental infection and field studies are necessary to evaluate the J5 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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