

Rethinking Coliform Vaccine Programs

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Abstract

Core-antigen vaccine technology has been available for the prevention of coliform mastitis for over ten years. However, unresolved issues regarding the most effective method to incorporate this technology into a dairy herd health program remain. These include number of vaccinations, effect of vaccinations on milk production of cows, and if risk factors associated with the incidence of clinical coliform mastitis correlate to vaccination schedules. This paper will discuss the immunological justification and limitations of vaccination protocols for coliform mastitis, and propose applications of this information.

Introduction

Exposure to coliform bacteria cannot be avoided, even in the best managed herds, because they are always present in the cow's bedding, manure, water and feed. Clinical coliform mastitis occurs when intramammary gram-negative bacteria elicit strong, acute inflammatory responses in the udder, which must occur to control infection. Mammary inflammation is associated with rapid proliferation of gram-negative bacteria in milk, which triggers acute edema, activation of the milk complement system, and pronounced increases in the somatic cell count of milk³⁵. While causing pain, swelling, and abnormal milk, the edema of coliform mastitis is critical because it allows transudation of immune antibodies, complement and conglutinin from the blood into the udder for facilitated clearance of opsonized pathogens by milk neutrophils.³² The dramatic increase in milk somatic cells that accompanies coliform mastitis reflects massive recruitment of blood neutrophils into milk under the influence of proinflammatory cytokines (mainly TNF- α , IL-1 β , IL-8) secreted by pathogen-activated mammary macrophages, epithelial cells and blood vessel endothelial cells.^{35,36} These cytokines induce expression of adhesion molecules on the endothelium (E- and P-selectins, and ICAMs) and blood neutrophils (b2-integrins, IL-8 receptors), providing a molecular scaffolding along which fast

flowing blood neutrophils can tether, slow down and migrate into the infected mammary gland. Once in the gland, migrated neutrophils phagocytose antibody-, complement-, and conglutinin-coated bacteria to clear the infection. This acute inflammatory response must occur within hours of infection and possess significant magnitude for spontaneous cure of coliform mastitis to result.^{17,36} Spontaneous cure is the normal outcome in the majority of coliform mastitis cases, but comes at a significant cost in terms of discarded milk and decreased milk production from infected quarters.^{11,20} Additionally, cases of clinical mastitis caused by coliform organisms are far more likely to result in the loss of the cow from death or culling than cases caused by other bacteria.^{1,12}

However, the acute inflammatory response to intramammary coliforms is not functional in many cows around the time of calving.^{13,17} This is likely due to the fact that key neutrophil genes normally expressed by the cells for proper migration, phagocytic and killing functions are inhibited around parturition.² Not surprisingly, the occurrence of severe coliform mastitis is significantly increased at calving and in early lactation.^{10,17,36,39} The mediator of the severe form of coliform mastitis is lipopolysaccharide (LPS), or endotoxin, a major component of the outer membranes of coliform bacteria. It is now recognized that certain lipoproteins of the outer membranes are as potent as LPS in mediating systemic inflammation that leads to total organ failure and death.³¹ Recent evidence suggests that bacteremia may also play a role in more severe cases.⁵⁰

The challenge in development of effective coliform mastitis vaccines has been to find an effective immunogen that elicits cross protective antibodies against the wide variety of coliforms that proliferate well in milk and shed LPS and lipoprotein into the blood stream of periparturient cows. In the late 1980's a breakthrough in coliform mastitis vaccine development occurred with the introduction of bacterins containing rough mutant strains of gram-negative bacteria.^{52,53} Since then, these vaccines have been widely used by producers to help them control coliform mastitis in parturient and early lactation cows. However, it is becoming increasingly apparent that present-day vaccinated cows still succumb

to severe coliform mastitis and that clinical disease can occur much later in lactation than previously observed.¹² It is our belief that an updated understanding of the biology of bovine B lymphocytes and the antibodies produced by these cells could lead to novel applications of currently available vaccines for better protection against coliform mastitis and its associated systemic disease. The goals of this presentation are to update readers about the biology of bovine B lymphocytes and antibodies in the context of immune defense against intramammary infections, and to introduce some novel immunization strategies we are exploring that are based on currently available coliform mastitis vaccines.

Coliform Mastitis and The Biology of B Lymphocytes and Antibodies

While the teat canal is the first defense against intramammary pathogens in cows, milk neutrophils are the main immunological defense once pathogens breach the teat canal.³² Neutrophils clear bacteria best when the pathogens are coated (opsonized) by certain antibodies (Figure 1), complement and (or) conglutinin. These proteins are produced by antigen-activated B lymphocytes (antibodies) and various other tissues of the body (complement and conglutinin). When intramammary coliforms are not rapidly controlled by milk neutrophils, allowing LPS and lipoproteins to escape into blood, serum antibodies capable of blocking and neutralizing these potent toxins also must be available. Therefore, a major goal in coliform mastitis prevention through vaccination is to achieve high levels of effective anti-coliform and anti-toxin antibodies in blood, milk and around the teat canal.³²

Certain classes (isotypes) of antibody work better than others in facilitating neutrophil clearance of coliforms in the udder and neutralizing the LPS and lipoproteins that escape from the milk into blood. These antibodies are expected to be particularly important in parturient cows because neutrophils of these animals do not function optimally.²⁸ Thus, milk neutrophils of parturient animals need extra support from antibodies, not only of the correct isotype, but also with high affinity and cross-specificity for the wide variety of coliforms which cause mastitis. *In designing coliform mastitis vaccines and immunization protocols, isotype, crossreactivity, and affinity for antigens of the elicited antibodies should be at least as important as the ability of the vaccine to induce high antibody concentrations in blood and milk.* In thinking about such vaccines, it is also important to recognize that the ruminant mammary gland is unique in that lymphocyte trafficking, which is essential to antibody-mediated immunity, is shared with the peripheral immune system rather than the common mucosal immune system.²⁸ Thus, B lymphocytes stimu-

lated by immunogens in coliform mastitis vaccines home to local lymph nodes to divide and differentiate into anti-coliform antibody secreting cells, rather than to the mammary gland. The majority of protective anti-coliform antibodies thus originate in blood. These antibodies gain entry to the milk via receptor-mediated transport across mammary epithelial cells¹⁵, or via passive transudation during the edema that follows.^{5,16}

Antibody Isotypes and the Major Role of IgG₂ in Defense Against Intramammary Pathogens

In cattle, there are four isotypes of immunoglobulins IgM, IgG₁, IgG₂ and IgA.³ IgM is a pentamer, a

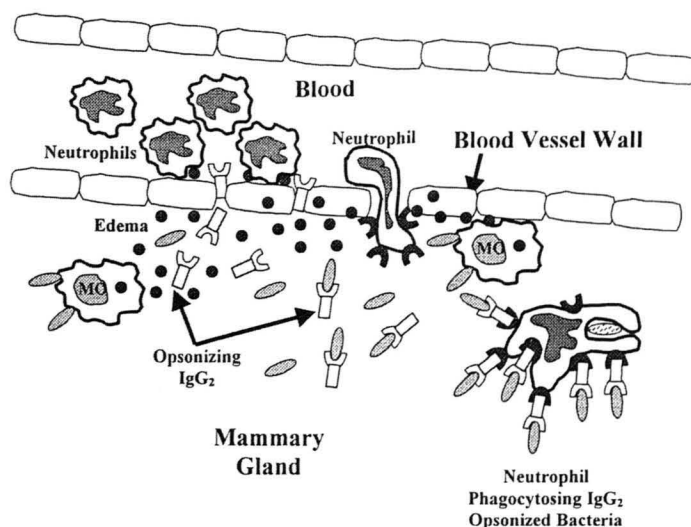


Figure 1. Blood neutrophils are recruited into infected mammary quarters when tissue macrophages (MO) secrete cytokines (black dots) after phagocytosing the infecting bacteria (gray rods). On the way into the gland, neutrophils become highly activated for phagocytosis and bacterial killing. For example, migrating neutrophils up-regulate IgG₂ Fc receptors (black disks) and complement receptors (not shown). When opsonizing IgG₂ antibodies (white Y) leak into the inflamed quarter from the blood fluid. During edema they bind with specific antigens on the bacteria to target these intruders for efficient phagocytosis by migrated neutrophils, a process called opsonization. Thus, bacterial opsonization by IgG₂ antibodies and subsequent phagocytosis of the opsonized bacteria by milk neutrophils can be considered key immune defense mechanisms against intramammary infections in dairy cows. Antigen-specific IgG₁ antibodies are not good opsonins for bovine neutrophils because the leukocytes do not possess IgG₁ Fc receptors. However, IgG₁ antibodies can activate the complement cascade and complement is an efficient opsonin for bovine neutrophils. The relative contributions of IgG₂ versus complement opsonization to neutrophil-mediated defense against coliform mastitis is not known, but complement concentrations in bovine milk are low. Also, *in vitro* studies have shown that high levels of IgG₁ can inhibit IgG₂-mediated phagocytosis by successfully competing with IgG₂ for antigen binding sites on bacteria.

Table 1. Normal serum and milk (> 2 weeks postpartum) concentrations of the major antibody classes (isotypes) in dairy cows (adapted from Butler, 1983; Tizzard, 1982).

Antibody isotype	~Size (kDa)	Serum concentration (and range) in mg/100 ml	Milk concentration (and range) in mg/100 ml	Main biological functions (mediated via Fc portion of the molecule)
IgM	900	305 (60 – 430)	8.6 (3.7 – 15.0)	Agglutination Complement fixation Opsonization Blocking/neutralization
IgG ₁	160	1120 (600 – 1510)	58 (33 – 120)	Complement fixation Blocking/neutralization
IgG ₂	150	920 (500 – 1350)	5.5 (3.7 – 6.0)	Opsonization Blocking/neutralization
IgA	360	37 (6 – 100)	8.1 (5.0 – 11.0)	Pathogen agglutination Blocking/neutralization

large molecule present in moderate concentrations in serum but relatively low concentrations in milk (Table 1). However, because of its ability to bind with 5 to 10 antigens and to fix complement, serum IgM is a highly effective in neutralizing blood-borne toxins and agglutinating and opsonizing blood-borne pathogens for efficient clearance by spleen and liver macrophages (which express surface complement receptors). There is some evidence that milk IgM may also serve as an opsonin for bovine neutrophils.³⁰ Therefore, coliform vaccines that elicit high levels of serum IgM antibodies should protect cows from systemic shock and lessen the severity of local symptoms of clinical coliform mastitis.

IgA antibodies are usually dimerized and, like IgM, are present in relatively low concentration in bovine milk (Table 1). This is in contrast to non-ruminant species for which IgA constitutes the majority of milk antibodies, and reflects our new understanding of the bovine mammary gland as part of the peripheral immune system rather than the mucosal immune system.²⁸ As in other species, high levels of IgA are found in mucosal secretions of cattle where the molecules effectively prevent pathogen colonization and tissue damage via agglutination and toxin.^{3,4}

IgG₁ and IgG₂ antibodies are much smaller than IgA or IgM antibodies and are found at relatively high concentrations in bovine serum (Table 1). These, along with IgM, are the main antibodies of the peripheral immune system. Due to active receptor-mediated transport across mammary epithelial cells, IgG₁ makes up the most significant antibody isotype in bovine milk.^{4,15} On the other hand, normal milk concentrations of IgG₂

are extremely low in dairy cows (Table 1). Both IgG isotypes effectively block pathogens from interacting with host cells and neutralize toxins in blood and tissues. *However, it is IgG₂ antibodies that possess the main opsonizing activity for bovine neutrophils* (Figure 1). Because neutrophils are the main form of host immunological defense against intramammary bacteria that cause mastitis in dairy cows, blood-derived IgG₂ antibodies play the most significant role of all antibody isotypes in local resistance to, and clearance of intramammary infections. This is because freshly migrated bovine neutrophils express high levels of surface Fc receptors that are specific for pathogen-bound IgG₂ antibodies.^{26,41,51} Therefore, bovine IgG₂ serves not only as a classical opsonin but also as a potent cytophilic antibody for bovine milk neutrophils.²⁹

Given the importance of IgG₂ in mammary defense against intramammary pathogens, it makes perfect sense that the acute phase of the mammary inflammatory response leads to substantial increases in blood-derived IgG₂ in milk. This passive transudation of IgG₂ is highly associated with neutrophil recruitment into the gland and significantly increases the opsonic activity of milk.^{5,16,18,33} Interestingly, the selective transfer of blood IgG₁ into milk appears to be suppressed during the acute phase of the mammary inflammatory response.²⁹ The return to active IgG₁ transport post acute inflammation is accompanied by the formation of immune complexes in milk, which inhibit neutrophil phagocytosis by blocking Fc receptors and competing with IgG₂ for binding sites on.^{41,42,43} *Thus, coliform mastitis vaccine programs should elicit high serum levels of anti-*

coliform IgG₂ antibodies so these are available for transudation into milk during the acute phase of the inflammatory response to intramammary infection.

The ability of IgG₁ to fix complement (Table 1) may be important to neutrophil phagocytosis in non-mammary tissues but its role in mammary defense against mastitis-causing pathogens is not clear. Endotoxin-induced inflammation has been shown to increase the levels of complement detected in bovine milk, but milk itself has also been shown to mask the inflammatory activity of activated complement.^{6,34} Therefore, although IgG₁ antibodies may feasibly participate in the neutralization of LPS and lipoproteins in the mammary gland, a role for these antibodies in neutrophil clearance of intramammary coliforms is not at all clear.

Rough Mutant Strains of Gram-Negative Bacteria Elicit Crossreactive Anti-Coliform Antibodies

In wild type gram-negative bacteria, LPS in the outer membrane contains variable O polysaccharide chains attached to core sugars, which in turn are linked to a highly conserved lipid A moiety. Antibodies elicited against O polysaccharides do well to protect animals against homologous gram-negative bacterial strains, but are not useful in protection against heterologous bacterial strains. Instead, since the lipid A component of LPS is so well conserved across gram-negative bacteria, antibodies elicited against this core antigen should confer cross-reactive protection against most gram-negative bacteria. Rough mutants of gram-negative bacteria, which lack O polysaccharide chains and have exposed lipid A, have been used successfully as immunogens for coliform mastitis vaccines that elicit crossreactive serum antibodies in immunized dairy cows.^{46,48} The isotype, opsonizing capacity, and affinity of these antibodies for heterologous bacteria have not been established. Each of the commercially available vaccines are bacterins of rough mutants of either *E. coli* J5 or *Salmonella* spp. The J5 (Rc) mutant of *E. coli* 0111:B4 has been studied intensively as a coliform mastitis vaccine, including in our research group. Thus, the remainder of this paper focuses on traditional and novel applications of the J5 vaccine in prevention of coliform mastitis.

Typically, *E. coli* J5 vaccines are administered three times, once at dry off, once at 30 days into the dry period, and again within 14 days after calving, subcutaneously in the neck region. The theory behind this immunization schedule is that the two booster shots will increase blood levels of crossreactive antibodies for mammary protection from heterologous coliforms during colostrum formation and early lactation. Indeed, the first J5 vaccination field trials^{7,14}, and experimental challenge trials^{23,24} demonstrated that this 3-shot protocol could

significantly reduce the incidence and the severity of clinical mastitis in the first 90 days of lactation, including reduced fever and duration of intramammary infections, faster return to normal milk production, and less depression of dry matter intake. Cows with naturally occurring serum anti-J5 *E. coli* antibody titers < 1:240 had 5.33 times the risk of developing clinical coliform mastitis than cows with titers > 1:240⁴⁹, and herd vaccination programs would be profitable when > 1% of cow lactations resulted in clinical coliform mastitis.⁹ J5 vaccination also reduces the severity and duration of coliform mastitis in primigravid heifers experimentally challenged with heterologous strains of *E. coli*.^{25,40} However, vaccination in the area of the supramammary lymph node was no better than subcutaneous vaccination in the neck area in terms of severity of clinical coliform mastitis following experimental challenge.⁴⁷

The antibody responses elicited by J5 *E. coli* vaccination have not been well characterized in terms of isotypes elicited, persistency in serum and milk throughout lactation, biological activity, or affinity for antigen. One study did report that the typical 3-shot vaccination schedule increased milk and serum total IgG to whole cell J5 *E. coli* through 30 and 37 days post calving, respectively.²³ The cows in that study were experimentally challenged with a heterologous strain of *E. coli* at 30 days post calving. The same research group then showed that phagocytosis of a smooth heterologous strain of *E. coli* was enhanced when the bacteria were opsonized with serum from cows vaccinated three times with J5 *E. coli* compared to serum from unvaccinated control cows.²¹ However, it was the IgM content of the immune serum that correlated with enhanced phagocytosis in that study, not the IgG content as the group's previous study may have predicted.²³ Increased serum and milk IgG anti-J5 *E. coli* antibody titers were reported at calving and up to 30 days in milk in J5 vaccinated heifers and may have contributed to the reduced severity of clinical coliform mastitis in that study.²⁵ Similarly, serum and milk IgG₁ and IgG₂ anti-J5 *E. coli* antibody titers were higher in vaccinated Jersey cows that recovered more quickly from experimental coliform mastitis than in unvaccinated controls.⁴⁵ In yet another study that tested two commercial J5 bacterins, serum and mammary IgG₁ and IgG₂ anti-J5 *E. coli* antibody titers were increased over those for unvaccinated controls, while IgM was not different across treatment groups.⁴⁷ Unfortunately, neither vaccine influenced the severity of clinical coliform mastitis in that study even though milk of vaccinated cows tended to have lower numbers of the challenge strain of *E. coli*. In fact, no studies to date have shown that vaccinating cows with J5 *E. coli* reduces the rate of gram-negative intramammary infections. One study even showed that immunization of cows with the J5 or Rc mutant K-12

strains of *E. coli* completely failed to protect cows against clinical coliform mastitis.¹⁹

Given the bovine antibody isotypes available for coliform mastitis prevention (Table 1), the best scenario for any coliform mastitis vaccine would be to elicit high levels of crossreactive serum IgM and IgG₂ antibodies. Naïve B lymphocytes developing in the ileal Peyer's patches (CD5⁻) depend heavily on gene conversion and point mutation to form the antibody repertoire of the cow.⁴ CD5⁺ B lymphocytes are thought to be a self-regenerating pool of B cells and primarily synthesize low affinity IgM antibodies (i.e., do not undergo isotype switching or affinity maturation) when stimulated directly by highly repetitive epitopes.²⁷ These are the types of epitopes found in LPS of gram-negative bacteria. It is possible, therefore, that the currently used 3-shot J5 vaccination schedule stimulates predominantly CD5⁺ B cells, eliciting IgM antibodies of low affinity. This would do little to push the isotype switching capacity of naïve CD5⁻ B cells from IgM to IgG₁ to IgG₂. With this in mind, we began to seek novel ways to apply the J5 vaccine for improved antibody protection against coliform mastitis.

Can More J5 Immunizations Elicit Better Antibody Responses in Cattle?

Given our proposition that bovine B lymphocytes may require more time and antigen stimulation for isotype switching and affinity maturation, we have carried out three experiments to test the effect of J5 hyperimmunization in cattle. In the first experiment, six Holstein steers were vaccinated with a commercial J5 bacterin at 5 months of age, one month later, and then ten subsequent times two weeks apart.³⁷ Data in Figure 2 show that the steers had relatively high levels of naturally occurring anti-J5 *E. coli* IgM and IgG₁ antibodies before vaccination (vaccination number 0), but only low naturally occurring anti-J5 *E. coli* IgG₂ antibodies. Hyperimmunization caused a gradual but steady increase in the levels of each antibody isotype through vaccination number 9, with a linear increase in IgG₂. Beyond vaccination 9, IgM antibody levels decreased, IgG₁ antibody levels leveled off, and IgG₂ antibody levels continued to increase. Statistically, anti-J5 *E. coli* IgM antibody levels were significantly ($P < 0.05$) higher than background from the second vaccination on. However, five vaccinations were required to elevate ($P < 0.02$) anti-J5 *E. coli* IgG₁ antibody levels above background and to effect significant isotype switching to IgG₂. The kinetics of these antibody responses following J5 hyperimmunization may be important biologically because IgG₂ is the main opsonin for bovine neutrophils.

We were curious to know if the serum antibodies elicited from J5 hyperimmunization were qualitatively

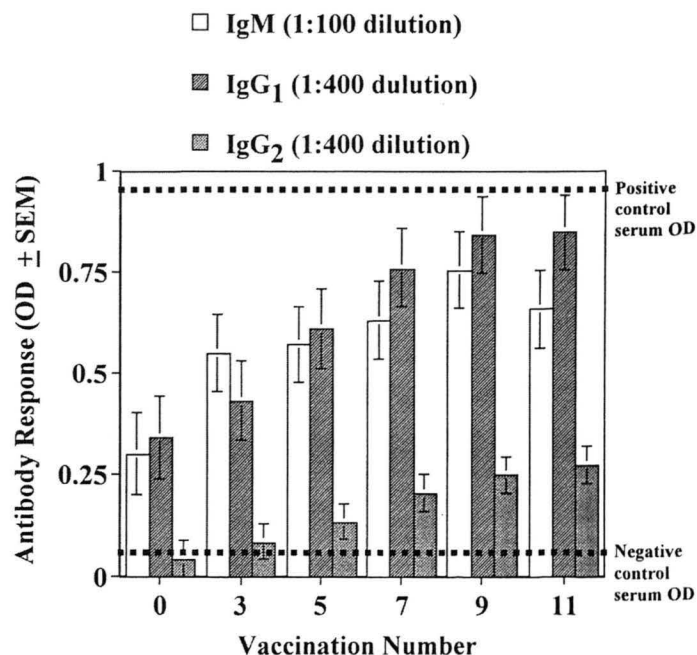


Figure 2. Serum anti-J5 *Escherichia coli* antibody responses for five Holstein steers hyperimmunized with a commercial J5 Bacterin. Antibody responses are given as mean optical densities (OD ± SEM) from four samples collected on days 4, 6, 11, and 13 after each vaccination. Steers were vaccinated twice over a one-month period and then every other week for vaccination numbers 3 through 12. Shown are anti-J5 *E. coli* IgM, IgG₁, and IgG₂ antibody response data from vaccination numbers 0 (preimmunization), 3, 5, 7, 9, and 11. Sera were diluted 1:100 for IgM data collection and 1:400 for IgG₁ and IgG₂ data collections, which were done by ELISA using whole J5 *E. coli* as antigen. Mean OD values for the positive control serum (J5 hyperimmune serum donated by Dr. James Cullor, UC Davis) and negative control serum (fetal bovine serum) are shown as black dotted lines.

different than antibodies elicited from three injections of the vaccine. To address this question we developed a flow cytometric phagocytosis assay to test the opsonic activity of various sera for bovine neutrophils.⁸ In a preliminary experiment, hyperimmune serum of one steer that contained an extremely high level of anti-J5 *E. coli* IgG₂ antibody was heat inactivated and used to opsonize J5 *E. coli* during log phase of growth. Data in the top panel of Figure 3 show that the hyperimmune serum was highly opsonic, with 30% of neutrophils phagocytosing opsonized J5 *E. coli* at 60 minutes of incubation (stippled bars). Little phagocytosis occurred in wells that containing unopsonized J5 *E. coli* (white bars). In a second experiment, opsonic activities of three sources of heat inactivated serum were tested using our neutrophil phagocytosis assay. Fetal bovine serum served as the negative control and sera from the third and eleventh vaccinations were used as the test sera. As shown in Figure 2, these sera contained very differ-

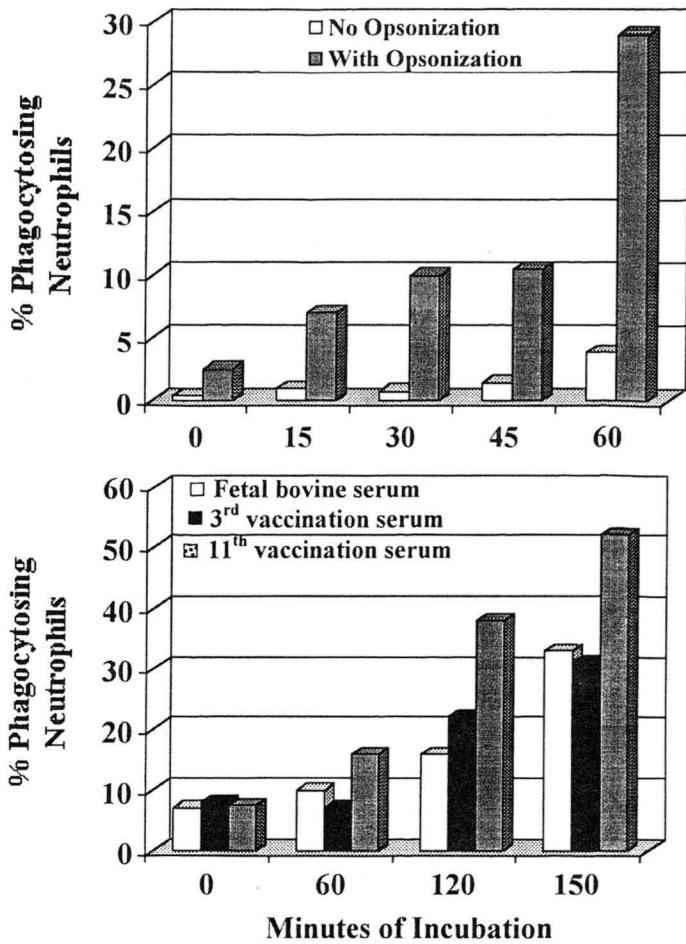


Figure 3. *J5 Escherichia coli* opsonized with serum from Holstein steers vaccinated 11 times with a commercial *J5* Bacterin are more readily phagocytosed by bovine blood neutrophils than bacteria left unopsonized (upper panel) or bacteria opsonized with fetal bovine serum or serum from steers vaccinated 3 times with the *J5* bacterin (bottom panel). Bacteria used for this assay were transformed with a GFP-containing plasmid so they fluoresced green. Percentages of phagocytosing neutrophils were determined using flow cytometric analysis as proportion of total neutrophils that were fluorescing green after various periods of incubation at 39°C (leukocyte:bacteria ratio = 1:100). All test sera for opsonization were used at 1:50 dilutions (in RPMI-160 medium) and opsonization was allowed to occur for 30 minutes at 37°C.

ent levels of anti-*J5 E. coli* IgM, IgG₁ and IgG₂ antibodies. Data in the bottom panel of Figure 3 show that the hyperimmune serum (stippled bars) contained higher opsonic activity for bovine neutrophils than fetal bovine serum (white bars) or serum collected after the third *J5* vaccination (black bars). Thus, data in Figure 3 suggest that hyperimmunization elicited antibodies with high opsonic activity that outperformed the antibodies elicited by three *J5* vaccinations.

We were also curious to know if *J5* hyperimmunization could elicit the same high anti-*J5 E. coli* antibody responses in dairy cows as it did in dairy

steers.³⁸ Control cows were vaccinated three times; at 60 days pre-calving/dry off, 30 days later and within 14 days after calving. Test cows were given the same three vaccinations as control cows plus two additional vaccinations 30 and 60 days after the third vaccination. Blood for ELISA of serum anti-*J5 E. coli* antibodies was collected from all animals immediately prior to the first vaccination and 7 days after each vaccination. We continued to collect control cow blood serum beyond the third vaccination to use as comparative samples against the test cow samples. Data in the top panel of Figure 4 show that there was little stimulation of IgM antibodies in control and test cows following three vaccinations, and that the fourth and fifth vaccinations in the test

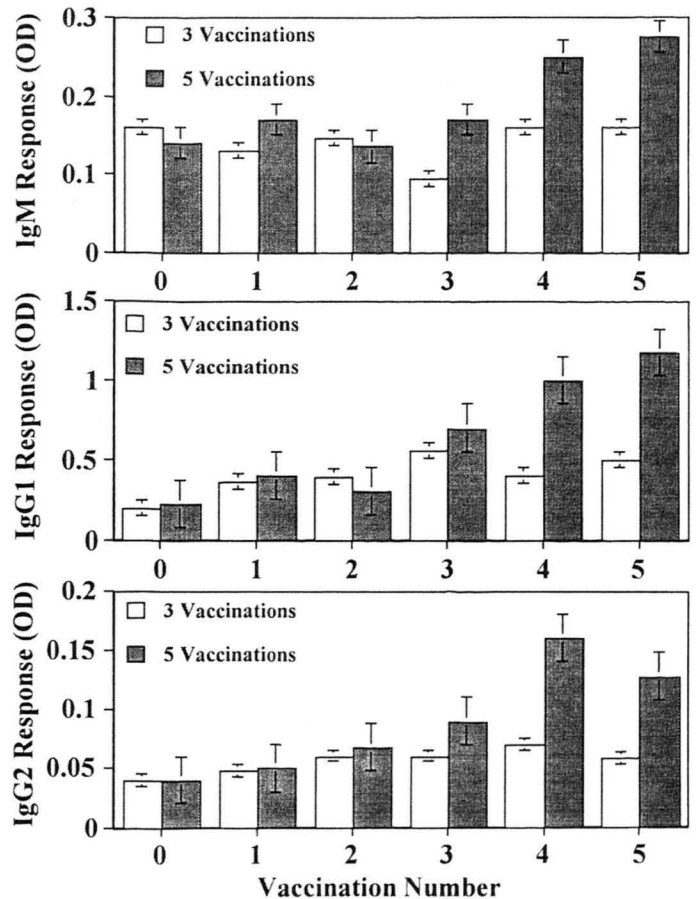


Figure 4. Serum anti-*J5 Escherichia coli* antibody responses for ten Holstein cows immunized with *J5* bacterin. Antibody responses are given as mean optical densities (OD ± SEM) from samples collected on the seventh day after each vaccination. The 5 control cows received the recommended 3 vaccinations and the 5 test cows received the same 3 vaccinations plus two additional vaccinations one month apart (see text for details). Blood from control cows was sampled with the same timing as that for the test cows, even after they received no further vaccinations. Sera were diluted 1:100 for IgM (top panel) and 1:400 for IgG₁ (middle panel) and IgG₂ (bottom panel) for ELISA.

cows caused only slight increases in the IgM response. The overall effect of treatment tended towards significance ($P = 0.10$) for the IgM antibody response. This was in contrast to the IgG₁ (middle panel of Figure 4) and IgG₂ (bottom panel of Figure 4) antibody responses, for which treatment was highly significant ($P < 0.01$). In both cases antibody levels increased significantly following the fourth and fifth vaccinations but were still close to background levels following the third vaccination. Therefore, in this group of relatively young cows, at least four J5 vaccinations were required to elicit significant serum anti-J5 *E. coli* antibody responses.

Conclusions

Failure of vaccines is difficult to define.⁵² Because of the relative ease in collecting massive amounts of immune response and health data from milk and blood samples of cows, we may ask more of coliform mastitis vaccines than we do of other successful vaccines, which simply prevent clinical disease. Nonetheless, some important questions about the efficacy of current J5 vaccination schemes in contemporary dairy cows have surfaced. Also, the isotype-specific antibody responses elicited by J5 bacterins have not been characterized well in dairy cows. Given that bovine neutrophils, B lymphocytes, antibodies and the mammary gland function differently than those in humans and rodents, the time seems right to define just what J5 vaccination is capable of doing to control coliform mastitis. Preliminary research work from our group sheds light on two novel applications of this decade-old core antigen technology, including the hope for total prevention of coliform mastitis via monthly immunizations of lactating cows and passive immunization of mammary glands with highly purified polyclonal anti-coliform antibodies for dry and early lactation cows.

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