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Managing Mastitis in Heifers: 
An Initial Step in Improving Dairy Herd Health

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1. Introduction

Replacement heifers are critical to dairy herd productivity because they represent the future milking and breeding stock of all dairy operations. The goal should be to provide an environment for heifers to develop full lactation potential at the desired age with minimal expense. Animal health and well-being play vital roles in achieving this potential, and one disease that can influence future productivity is heifer mastitis caused by Staphylococcus aureus, the coagulase-negative staphylococci (CNS), and the environmental streptococci (Streptococcus uberis, Streptococcus dysgalactiae).

Unfortunately, most producers regard young dairy heifers as uninfected, and the presence of mastitis is not observed until freshening or until the first clinical flare-up in early lactation. Thus, animals may carry intramammary infections (IMI) for a year or more before they are diagnosed with mastitis (Boddie et al., 1987). The greatest development of milk-producing tissue in the udder occurs during the first pregnancy, so it is important to protect the heifer mammary gland from pathogenic microorganisms to ensure maximum milk production during the first and future lactations. In the USA, Louisiana researchers found that if bred heifers infected with Staph. aureus were left untreated, they produced 10% less milk in early lactation than those receiving therapy (Owens et al., 1991; Trinidad et al., 1990c). Likewise, research in New Zealand demonstrated that Staph. aureus mastitis in heifers resulted in significant production losses during the first lactation, which carried over into the subsequent lactation because of damage to milk-producing tissues of the udder (Woolford et al., 1983).

2. Prevalence of mastitis in unbred and primigravid heifers

A greater focus on heifer mastitis began in the mid 1980s after several dairy producers in Louisiana complained to university researchers that a large percentage of their heifers were...
freshening with clinical mastitis. Subsequent study of breeding age animals in a research herd revealed that IMI may be diagnosed as early as 6 months of age, and infections persisted throughout pregnancy and into lactation (Boddie et al., 1987). Other studies demonstrated that greater than 90% of breeding age and bred heifers (12 - 24 months of age) may be infected (Trinidad et al., 1990b). Most of the infections were shown to be caused by the CNS (Staphylococcus chromogenes and Staphylococcus hyicus) followed by Staph. aureus (20%). Mixed isolates of CNS and Streptococcus species were also found.

The prevalence of mastitis in 10 unbred Jersey heifers (10 - 12 months of age) in Louisiana was initially evaluated in 1985 and monitored over a 1-year period, which covered breeding age and gestation (Boddie et al., 1987). Samplings from the teat skin, teat canal, and mammary secretion were performed bimonthly and continued through time of calving. For bacteriologic analysis, a total of 388 samples from teat skin, 388 from teat canal keratin, and 216 from secretions were examined; not all quarters contained sufficient volumes of secretions.

For teat skin, staphylococci were isolated on mannitol salt agar, and the predominant colony type of a particular isolate based on color, rough/smooth, size, hemolytic pattern, and appearance was collected, sub-plated for culture on blood agar, and identified to the species level (Boddie et al., 1987). Staphylococcus xylosus (20.9%) and Staph. chromogenes (14.9%) were the predominant flora isolated from teat skin, followed by Staph. warneri (6.7%), Staph. sciuri (6.2%), Staph. aureus (2.8%), Staph. hyicus (1.3%), and Staph. simulans (1.3%). The most prevalent bacteria found in teat canals were Staph. chromogenes (41.0%), followed by Staph. hyicus (16.8%), Staph. aureus (10.0%), Staph. xylosus (1.0%), Staph. warneri (0.8%), and Staph. sciuri (0.5%).

In mammary secretions, Staph. chromogenes (49.5%) was the predominant organism, followed by Staph. hyicus (21.3%), Staph. aureus (13.0%), Streptococcus uberis (1.4), and Staph. xylosus (0.9%). The CNS species in teat canals and in secretions from a particular mammary quarter were present at the first sampling, and the same CNS species were isolated from teat canals and secretions from the same individual quarters with each successive sampling of the trial. Also, there appeared to be a correlation between colonization of the teat canal and IMI. For example, the major CNS species colonizing the teat canal (Staph. chromogenes (41.0%), Staph. hyicus (16.8%), Staph. aureus (10.0%), and Staph. xylosus (1.0%)) were also the predominant organisms causing IMI (Staph. chromogenes (49.5%), Staph. hyicus (21.3%), Staph. aureus (13.0%), and Staph. xylosus (0.9%)). Thus, teat canals infected with Staph. chromogenes (41.0%) were positively correlated with secretions (49.5%) infected with these bacteria. The overall correlation between teat canal colonization and IMI was 82.2% across these 4 CNS species.

This initial study (Boddie et al., 1987) indicated that teat skin, teat canals, and mammary secretions of heifers are colonized with CNS as well as Staph. aureus at an early age, and that infections may persist for up to 1 year. Species identification demonstrated that nearly all isolates from the same quarter throughout the study were the same biovariant based on the API Staph-Ident System as previously modified (Watts et al., 1984), which supports the
contention that isolates from each quarter over time were from persistent infections and not from new IMI occurring between sampling periods. Although the percentage of *Staph. aureus* isolates was lower than CNS, presence of this major pathogen demonstrated that it colonized teat canals at a much earlier age than documented previously (Rendel & Sundberg, 1962).

In a subsequent herd survey (Trinidad et al., 1990b), the prevalence of mastitis in breeding age and pregnant heifers was determined in 4 commercial dairies. Teat canal keratin and secretion samples were collected from 116 Jersey heifers, and results revealed that teat canal colonizations were present in 93.1% of heifers and 70.7% of quarters. *Staph. aureus* was isolated from teat canal keratin samples of 31% of heifers and 12.3% of quarters. Other organisms isolated from keratin and percentage frequencies were *Staph. chromogenes* (42.9%), *Staph. hyicus* (25.2%), other staphylococcal species (5.7%), *Strep. dysgalactiae* (0.6%), *Strep. species* (3.1%), and mixed isolates containing staphylococci and streptococci (5.7%). In this 4- herd survey, IMI were found in 96.9% of heifers and in 74.6% of quarters. Twenty-nine percent of heifers and 15.1% of quarters showed clinical symptoms of mastitis as evidenced by clots, flakes, and blood. *Staph. aureus* was isolated from 14.7% of quarters. This microorganism was also isolated from 25% of quarters with clinical symptoms. *Staph. aureus* causes severe damage to mammary tissue (Trinidad et al., 1990a), and infections are very difficult to eliminate in lactating cows. Other organisms isolated from secretions and percentage frequencies were *Staph. chromogenes* (43.1%), *Staph. hyicus* (24.3%), other staphylococcal spp. (3.6%), *Strep. dysgalactiae* (0.4%), *Strep. spp.* (3.3%), *Nocardia* species (0.4%), and mixed isolates containing staphylococci and streptococci (5.1%).

These initial studies on heifer mastitis were performed in Louisiana, USA, where a warm and humid climate with a long fly season may be conducive to IMI in these young dairy animals. Thus, a subsequent national trial was carried out using 1583 breeding age heifers from 28 herds to determine the prevalence of heifer mastitis in the states of California, Washington, and Vermont as well as in Louisiana (Fox et al., 1995). The majority of quarter infections were caused by the CNS (mean = 27.1%). The mean prevalence of *Staph. aureus* among the 4 sites was 2.9%, but was highest in Louisiana at 10.1% during the spring season. The overall IMI prevalence was greatest in Louisiana, and the highest frequency was during the 3rd trimester of gestation just prior to parturition, which was due to an increase in IMI caused by *Staph. aureus*, CNS, and environmental streptococci. Likewise, following parturition, the greatest prevalence of IMI was in Louisiana, which had the greatest percentage of heifers with IMI caused by the environmental streptococci and *Staph. aureus*, and the 2nd greatest prevalence of CNS among the 4 sites. For breeding age and bred heifers, the stage of pregnancy significantly affected IMI prevalence, which was highest for heifers in the last trimester of pregnancy, especially CNS IMI. This study by Fox et al. (1995) showed that site location had a significant effect on prevalence of mastitis, and that Louisiana had the greatest prevalence of IMI. This was postulated to be due to the warm and humid climate, as well as to a prolonged horn fly season in Louisiana that exposed udders and teats to an increased bacterial load that management practices failed to control.
3. Mammary leukocyte response to intramammary infection

In lactating cows, the milk somatic cell count (SCC) composed of leukocytes (macrophages, lymphocytes, neutrophils) and a small percentage of mammary epithelial cells, is considered an important parameter for assessing mammary health status (e.g. inflammation); milk yield decreases as SCC and incidence of mastitis increase. Thus, SCC in breeding age and pregnant heifer mammary gland secretions have been analyzed to measure the degree of inflammation and potential reductions in future milk yield. In a study by Boddie et al. (1987), the mean arithmetic SCC of quarters from unbred heifers infected with *Staph. chromogenes*, *Staph. hyicus*, and *Staph. aureus* were 7.8, 8.5, and 9.2 x 10^6/ml, respectively, whereas the mean SCC of uninfected quarters was 3.5 x 10^6. The mean SCC of heifer secretions collected on the day of freshening were 3.2 x 10^6/ml and 1.6 x 10^6/ml for quarters infected by staphylococci and uninfected quarters, respectively. The mean SCC during the first 3 months of lactation in quarters infected with *Staph. chromogenes*, *Staph. hyicus*, and *Staph. aureus* were 168, 193, and 578 x 10^3/ml, respectively, while the SCC of uninfected quarters was 39 x 10^3/ml. Thus, this study found SCC approaching 200 x 10^3 for quarters infected with CNS during the first 3 months of lactation, and, based on previous studies (Jones et al., 1984; Kirk, 1984), SCC in this range are associated with milk loss. Approximately 13% of quarter secretions sampled prepartum contained *Staph. aureus*, and after freshening, the SCC of these quarters averaged 578 x 10^3/ml, a cell count that has been associated with a loss of >4.4 lb (2.0 kg) of milk/day (Kirk, 1984).

Other studies have also demonstrated elevated SCC in heifer mammary glands infected with mastitis-causing bacteria. For example, in a study by Trinidad et al. (1990b), average arithmetic SCC in secretions from 325 quarter samples regardless of infection status was 11.7 x 10^6/ml. Infected (n = 240) and uninfected (n = 85) quarters had secretion SCC of 13.6 x 10^6/ml and 5.7 x 10^6/ml, respectively. Of the staphylococci, *Staph. aureus*-infected quarters had the highest secretion SCC/ml (17.3 x 10^6), followed by *Staph. chromogenes* (12.8 x 10^6), and *Staph. hyicus* (12.4 x 10^6). The mean SCC for non-agalactiae streptococci was 15.5 x 10^6/ml.

The volume of mammary secretion is very low in breeding-age animals; thus, SCC become concentrated, resulting in high SCC even in uninfected quarters. However, SCC approach 20 x 10^6/ml in quarters infected with *Staph. aureus*, and over 13.6 x 10^6/ml in those infected with the CNS and *Strep.* species. Such elevated SCC over a long period of time suggests that mammary tissue in affected quarters are in a state of chronic inflammation, which could adversely affect development of milk-producing tissues and negatively affect future milk yield. In response to infection, neutrophils become the major leukocyte type that infiltrates mammary tissue from the vascular system. The function of the neutrophil influx via chemotaxis is to phagocytose and kill mastitis-causing bacteria. But there is also evidence that this defense response can impair and disrupt mammary function (Akers & Thompson 1987; Capuco et al., 1986; Zhao & Lacasse, 2008). For example, in addition to the damage caused by bacterial toxins, the migration of leukocytes, namely neutrophils, across the mammary epithelial surfaces is thought to cause mechanical damage and/or chemical
damage (via release of reactive oxygen species) to the milk secretory cells as well as to the ductal cells of the mammary gland.

4. Histological response of the mammary gland to IMI

Initially, the mammary glands of dairy heifers were studied to determine histological responses to teat canal colonization with *Staph. chromogenes* and *Staph. hyicus* (Boddie et al. 1987). Two unbred heifers were slaughtered, one at 8 months and the other at 18 months of age, and examination of mammary tissues from both heifers demonstrated a leukocyte reaction to the colonization of the teat canal. Cross-sections through the mid-teat canal demonstrated cocci colonizing keratinized cells of the canal lumen, and sections of distal teat cisternal tissues demonstrated heavy leukocyte infiltration with lymphocytes and plasma cells at Fürstenberg’s rosette compared with uninfected tissues.

Subsequently, 7 unbred heifers, 14 - 26 months of age, were studied to evaluate the effects of IMI on leukocyte infiltration and characteristics of secretory tissue in developing mammary glands (Trinidad et al., 1990a). Histologic observations of tissue samples from lobes of mammary parenchyma of uninfected quarters showed that alveoli were small; the epithelial lining was composed of a single layer of cuboidal cells surrounding a small luminal space with little or no stained secretory product (See Figures 1 through 3). Interalveolar connective tissue area composed approximately half of the observed lobes, and a few infiltrating leukocytes, mainly lymphocytes, were observed.

Infected tissues, particularly those with *Staph. aureus* IMI, exhibited large amounts of interalveolar connective tissues and reductions in epithelial and luminal areas (See Figures 4-8). Such areas also exhibited leukocytic infiltration, particularly lymphocytes and neutrophils, into stromal and luminal areas. Hyperplasia of ducts and cisterns as a result of infection was also observed, and macro- and microscopic abscesses were found in the parenchyma of one quarter infected with *Staph. aureus*. Abscesses were tubercule-like with a circular, stratified fibrosis containing numerous lymphocytes, neutrophils, plasma cells, and multinucleated giant cells.

Results of morphometric analysis on parenchymal tissue components showed that percentages of each component in uninfected quarters were very similar to percentages from quarters infected with CNS, although quarters infected with CNS exhibited significantly more stromal area. Percentages of alveolar epithelium and lumen in quarters infected with *Staph. aureus* were significantly lower (P < .05) than those in uninfected quarters and in quarters infected with CNS. Quarters infected with *Staph. aureus* also showed a greater percentage (P < .05) of interalveolar stroma than did uninfected quarters and quarters infected with CNS. Thus, *Staph. aureus*-infected tissue demonstrated reduced secretory activity. The greatest development of secretory tissue in young heifers occurs during the first pregnancy, and developing secretory tissues may be affected adversely by bacterial infection and inflammation, leading to deposition of connective tissue stroma instead of milk secretory tissue and a subsequent deleterious effect on future milk yield.
Figure 1. General view of a cross-section of a lobe of mammary tissue from an uninfected quarter exhibiting large ducts (D) and undeveloped lobules of parenchyma (P) among adipose tissue stroma. (A). x18.

Figure 2. Portion of mammary parenchymal tissue typical of that obtained from uninfected quarters and those infected with coagulase-negative staphylococci exhibiting small alveoli with empty, ovoid lumens (1) and those with some secretions (2). x180.
Figure 3. Portion of uninfected parenchymal tissue revealing limited stroma (S), flattened epithelium (E), and distended luminal areas (L) engorged with flocculent matter suggested active secretion. x180.

Figure 4. Parenchymal tissue from a quarter infected with *Staph. aureus* exhibiting a large interalveolar connective tissue stroma (S) and limited alveolar luminal areas (L). D = Duct. x180. Trinidad et al., 1990a.
Figure 5. Parenchymal tissue from a quarter infected with *Staphylococcus aureus* showing numerous neutrophils (arrows) infiltrating a luminal area (L) of one alveolus. x500.

Figure 6. Extensive epithelial hyperplasia (H) was observed in ductal linings in the parenchyma from one quarter infected with *Staphylococcus aureus*. Lymphoid cells (arrowheads) were numerous in the epithelium as well as in the underlining connective tissue. D = duct. x180.
Figure 7. Abscess (A) from one quarter infected with *Staphylococcus aureus* exhibiting tubercle-like morphology with circular stratified fibrosis (arrows) and marked cellular infiltration. E = Portion of enlarged in Figure 8. P = Parenchyma. x18.

Figure 8. Magnification of an edge of the abscess shown in Figure 7. Neutrophils (arrows), macrophages (arrowheads), and multinucleated giant cells (MGC) were present in this area of the abscess. x500. Trinidad et al., 1990a.
Leukocyte infiltration into cisternal and parenchymal mammary tissues was also evaluated. Quarters infected with *Staph. aureus* exhibited the greatest tissue leukocytosis, followed by quarters infected with CNS and uninfected quarters. Leukocyte infiltration in gland cistern and secretory tissue for infected quarters was significantly higher (P < .05) than that for uninfected quarters. Leukocytosis into teat cistern tissue was similar for uninfected quarters and those infected with CNS, but significantly lower (P < .05) than quarters with *Staph. aureus* IMI. None of the uninfected quarters or quarters infected with CNS demonstrated marked leukocyte infiltration. However, marked leukocyte infiltration, particularly lymphocytes, into cisternal and parenchymal areas was commonly observed in quarters that were infected with *Staph. aureus*. The majority of leukocytes observed within alveolar lumina were neutrophils.

5. Efficacy of nonlactating cow antibiotic therapy

Because of the high level of infection commonly found in breeding age and pregnant heifers, especially mastitis caused by *Staph. aureus*, as well as the associated elevated SCC, antimicrobial therapy should be considered. The testing of various staphylococcal isolates obtained from heifers for susceptibility to antibiotics commonly incorporated into mastitis infusion tubes has shown that antibiotic resistance is usually low (Watts et al., 1995). Greater than 90% of mastitis-causing staphylococci are generally killed by the drug preparations used, based on in vitro sensitivity testing using zone diffusion analysis (Watts et al., 1995). From a practical standpoint, the administration of antibiotics by a parenteral route would be preferred; however, in the author’s experience, neither subcutaneous nor intramuscular injections of drugs have been found to cure IMI in heifers because sufficient antibiotic does not pass into the mammary gland to be bactericidal. Thus, intramammary infusion is the route of choice.

In an initial study to evaluate the effectiveness of treatment, several heifers from each of the 4 commercial herds studied by Trinidad et al. (1990c) were randomly selected to receive a single intramammary treatment of a penicillin and dihydrostreptomycin product. Antimicrobial susceptibility testing demonstrated that 97% of the *Staph. aureus* isolates were sensitive to 12 antibiotics, including the product selected for treatment (Trinidad et al., 1990d). Teat ends were sanitized, and a dry cow antibiotic containing 1,000,000 units of penicillin and 1 gram of dihydrostreptomycin was infused into all quarters of 35 heifers using the partial insertion technique; 38 heifers served as untreated controls. Treatments were made at approximately 60 days prior to the calculated calving date.

Results showed that 97.1% of treated heifers (73.2% of quarters) were infected at the time of treatment, but, at calving, infected heifers and quarters in the treatment group were reduced to 40 and 34%, respectively. Antibiotic residues were limited to two heifers that were treated within 3 weeks of calving because estimated dates of parturition were miscalculated, but all quarters were free of antibiotic residue after 5 days; 2.9% of treated quarters had antibiotic residues at time of calving. Of the 38 untreated control heifers, 100% (71.2% of quarters) were infected at initial sampling, and at calving, mastitis in control heifers was reduced only
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Slightly to 97.4%; percentage of infected quarters increased slightly to 77.8%. The mean SCC at calving was also reduced as a result of therapy. For treated heifers, SCC decreased significantly (P < .001) from 11,825 x 10^3/ml at time of treatment to 3,439 x 10^3/ml at calving. In the control group, SCC decreased from 11,047 x 10^3/ml to 5,594 x 10^3/ml (P > .05, not significant).

In the same study, Staph. aureus was isolated from 11 quarters of 6 treated heifers before antibiotic infusion (45.8%), but, at calving, this organism was isolated from only 1 quarter of 1 heifer (4.2%). In the control group, 18 quarters of 10 heifers were infected with Staph. aureus at time of treatment (45%). At calving, 6 of the control heifers still had Staph. aureus mastitis in 11 quarters (55%). Thus, the overall incidence of IMI was reduced 60% and that caused by Staph. aureus was reduced over 90%. From 20 to 26% of quarters with subclinical and clinical Staph. aureus mastitis are typically cured after antibiotic therapy (Dodd, 1992). In this study, 90.9% of quarters were cured; thus, therapy in heifers was highly effective in eliminating S. aureus mastitis compared with that for lactating cows.

In this study, an economic analysis was performed to justify use of the heifer treatment program (Trinidad et al., 1990c). Production data collected over the first 2 months of lactation demonstrated that Staph. aureus-infected heifers receiving nonlactating cow therapy during pregnancy produced an average of 5.5 pounds (2.5 kg) more milk per day than Staph. aureus-infected herd mates that did not receive treatment. At the milk price received at that time, the greater milk yield translated to a $42.00 increase for treated heifers, which was well worth the $5.00 cost of treatment. Other advantages include a longer productive life and higher income due to quality milk premiums.

Subsequent studies (Owens et al., 1991, 1994) with pregnant and nonpregnant heifers using a cephalosporin-based product formulated for nonlactating cows was also successful. Heifers that were either experimentally or naturally infected with Staph. aureus were infused 8-12 weeks prepartum with one dose of 300 mg of a cepahpirin benzathine product and were compared with untreated controls infected with Staph. aureus. Results demonstrated that 100% of experimentally induced IMI and 87% of naturally occurring Staph. aureus IMI were eliminated in treated heifers at calving, and cured quarters remained negative at biweekly samples collected 2 months into lactation. Quarters remaining infected at calving with Staph. aureus were treated with a lactating cow product containing 200 mg of cepahpirin benzathine, but the cure rate was lower (50-56%). Thus, cure rates were greater when a nonlactating product was administered 8-12 weeks prepartum than when a lactating cow product was given at time of calving.

After antibiotic infusion, SCC in infected quarters that cured decreased from 15 x 10^6/ml to 4 x 10^6/ml 1 week later, and to 700 x 10^3/ml at calving. In contrast, none of the untreated control quarters infected with Staph. aureus cured spontaneously by the time heifers calved, and SCC at calving were 5 x 10^6/ml. Treated heifers in which Staph. aureus IMI were cured yielded a mean of 36.1 lb (16.4 kg) milk/day, and untreated controls that retained Staph. aureus IMI yielded 31.9 lb (14.5 kg)/day or 11% less during the first 2 months of lactation.
Generally, spontaneous cure rates for major mastitis pathogens are low. For example, in a subsequent study on heifer mastitis (Owens et al., 1994), spontaneous cure rates for Staph. aureus and the environmental streptococci were 9 and 6%, respectively. Thus, treatment is required to cure such infected quarters in these young dairy heifers. New IMI rates in uninfected quarters receiving no therapy over the period from 8 - 10 weeks prepartum were very low for most species of staphylococci. However, new environmental streptococcal IMI were common in previously uninfected, untreated heifers. So, as part of this study (Owens et al., 1994), a trial was initiated to treat quarters found to be negative at 8 – 10 weeks prepartum with cephalirin benzathine to determine if establishment of new environmental streptococcal IMI could be prevented during this 8 – 12 week prepartum period. Results showed that prophylactic treatment of such quarters prepartum reduced new environmental streptococcal IMI at calving by 93%. Thus, in this trial, use of nonlactating cow therapy was effective in preventing new IMI as well as curing existing infections. Reasons for this high cure rate are unclear, but the relatively small secretory tissue area of heifer mammary glands compared with mature cows might allow for greater drug concentrations in the udder of the heifer. Similarly, histological studies have demonstrated less scar tissue and abscess formation in the mammary glands of heifers compared with older cows (Trinidad et al., 1990a), a condition which would allow for better drug distribution and greater contact with colonized bacteria.

6. The optimum treatment schedule for maximizing efficacy of nonlactating cow therapy

The question arises as to when is the best time to treat bred heifers for optimizing cures against Staph. aureus mastitis. A 2-year study involving 233 Jersey heifers was designed to answer this question (Owens et al., 2001). In this trial, heifers were quarter sampled shortly after they were confirmed pregnant and at 4-week intervals thereafter. At the initial sampling, 56.5% of quarters were infected with some type of organism, and 15.4% of quarters were infected with Staph. aureus. After the initial sampling, animals were treated with a one-time infusion of 1 of 5 nonlactating cow infusion products during the first (0 - 90 days), second (91 - 180 days), or third (181 - 270 days) trimester of pregnancy. Products evaluated were: 1) a combination of 1 million units of penicillin and 1 gm dihydrostreptomycin; 2) 300 mg cephalirin benzathine; 3) a combination of 400 mg novobiocin and 200,000 units of penicillin G; 4) 300 mg tilmicosin; and 5) 250 mg cephalonium (this last product was only infused during the first trimester of gestation). Results showed that cure rates for the 5 products were high, ranging from 67 to 100%, and significantly higher than the spontaneous cure rate (25%) observed in untreated control quarters. No differences were observed among the three treatment time periods during gestation. However, fewer new Staph. aureus infections occurred after treatment in the group infused during the 3rd trimester, indicating that treatment during this time will reduce incidence of new IMI after infusion and continuing to calving. Because therapy during the first, second, or third trimester of gestation had no effect on treatment efficacy, the timing of treatment is best determined by what is most convenient for the management practices of a
particular dairy. For example, heifers could be treated: 1) at time of artificial insemination; 2) during routine rectal palpation to determine pregnancy status; or 3) when moved to a new location in preparation for calving. Treatment should be administered no less than 45 days prior to expected calving date to prevent antibiotic residues at calving.

Sampimon & Sol (2005) studied the efficacy of nonlactating cow therapy (600 mg cloxacillin) administered intramammarily 8-10 weeks prepartum in 1) 5 low prevalence farms in which less than 15% of heifers had SCC of >150,000/ml at the beginning of the trial, and 2) 8 high prevalence farms in which greater than 15% of heifers had SCC of >150,000/ml at the beginning of the trial. Results showed that in high prevalence farms, treated heifers produced significantly more milk during the first 100 days of lactation compared with untreated controls; however, in low prevalence farms, treatment had no effect on production. In both groups of farms, SCC and incidence of clinical mastitis were significantly lower in heifers receiving antibiotic therapy. Authors concluded that nonlactating cow therapy was beneficial in high prevalence farms, but not in low prevalence farms.

The treatment of heifers during pregnancy with a nonlactating cow product is advantageous because: 1) the cure rate is higher than during lactation, especially against Staph. aureus; 2) there are no milk losses during therapy; 3) the risk of antibiotic residues is minimal; 4) SCC at calving is reduced; 5) new IMI with environmental streptococci is prevented; and 6) milk production is increased by approximately 10% in successfully treated animals in some herds.

7. Efficacy of parenteral antibiotic treatment and an infusible teat seal in curing and preventing IMI

In one study involving 1,067 pregnant heifers in 30 New Zealand herds (McDougal et al., 2005), mammary quarters were treated 1 month prepartum with 1) an infusible teat seal composed of bismuth subnitrate; 2) parenteral administration of antibiotic via injection of 5 gm of tylosin i.m. daily for 3 days; 3) teat seal plus tylosin; or 4) no treatment in order to determine if treatment reduced the prevalence of IMI and incidence of clinical mastitis postpartum. Results demonstrated that heifers treated prepartum with teat seal exhibited a reduced prevalence of IMI as well as reduced incidence of clinical mastitis. However, treatment with tylosin prepartum did not reduce prevalence of mastitis or incidence of clinical mastitis postpartum.

8. Efficacy of lactating cow products in curing IMI

Lactating cow products have been used successfully in heifers when treating infections caused by the environmental streptococci and CNS immediately prior to calving. Studies on this subject are typically performed on heifers in late gestation 2 - 3 weeks before calving. In an initial study conducted at the University of Tennessee, quarters of 115 pregnant Jersey heifers were infused one time at approximately 1 week prepartum with either 200 mg
sodium cloxacillin, 200 mg cephapirin sodium, or left untreated (Oliver et al., 1992). At the
time of infusion, approximately 90% of heifers were infected in one or more quarters. For
heifers left untreated, 78% of animals (44.5% of quarters) remained infected at time of
calving. However, only 17.6% of the heifers (4.5% of quarters) remained infected at calving if
they were treated prepartum, regardless of the product used. Results demonstrated that
significantly fewer antibiotic treated heifers and quarters were infected at calving compared
with untreated controls. This study also examined the influence of prepartum antibiotic
treatment on subsequent lactational performance, and demonstrated that heifers receiving
treatment produced approximately 1,000 lb (455 kg) more milk per lactation than untreated
controls.

Mastitis pathogens were isolated from 76% of untreated control quarters at 7 days before
calving, from 47% of samples at 3 days after calving, and from 29% of samples at 10 days
postpartum. Throughout the remainder of lactation, pathogens were isolated from 30% of
control quarters. A similar percentage of samples (70%) was positive for mastitis pathogens
at 7 days before calving in antibiotic-treated quarters; however, only 8% of samples obtained
at 3 days after calving and 4% of samples obtained 10 days postpartum contained
pathogens; throughout the remainder of lactation, mastitis pathogens were isolated from
only 11% of quarters. Strep. uberis, Strep. dysgalactiae, and CNS species were isolated most
frequently in both untreated controls and antibiotic-treated heifer mammary glands.

In a subsequent study reported by Oliver et al. (1997b), mastitis pathogens were isolated
from 67% of samples obtained from control mammary glands (quarters to be left untreated)
14 days prior to expected calving, from 56% of samples obtained 3 days after calving, and
from 36% of samples obtained 30 days postpartum; throughout the remainder of lactation,
mastitis pathogens were isolated from 45% of quarters. In quarters to be treated with 200 mg
cephapirin sodium, 64% were positive for mastitis pathogens prior to antibiotic treatment;
however, only 16% of samples obtained at 3 days after calving and 8% of samples obtained
30 days postpartum contained pathogens. Throughout the remainder of lactation, mastitis
pathogens were isolated from an average of 12% of quarters. Coagulase-negative
staphylococci were isolated most frequently followed by environmental mastitis pathogens.

A follow-up study was conducted to determine if prepartum therapy with penicillin-

novobiocin or pirlimycin hydrochloride was effective in reducing the percentage of infection
with mastitis pathogens during early lactation (Oliver et al., 2004). Almost 73% of Holstein
heifers (34.3% of quarters) were infected 14 days before expected calving. Of the quarters
infected 14 days before parturition, 76% were uninected following treatment with
penicillin-novobiocin, 59% were uninected following treatment with pirlimycin, and 26%
were uninected in the untreated control group. The majority of IMI were due to CNS (44%)
and Staph. aureus (30%).

Among the Jersey heifers, 96% of animals and 71.3% of quarters were infected 14 days
before calving. Of the quarters infected at 14 days before parturition, 75% were uninected
following treatment with penicillin-novobiocin, 87% were uninected following treatment
with pirlimycin, and 56% were uninected in the untreated control group. The majority of
IMI were due to CNS (61%), *Strep. spp.* (19%), and *Staph. aureus* (8%). Thus, prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was effective in reducing the percentage of heifers and quarters infected with mastitis pathogens during early lactation.

As a part of the University of Tennessee studies, milk production and somatic cell score data from 82 control heifers and 111 heifers treated with antibiotics before calving were evaluated (Oliver et al., 2003). Milk production (actual and 305-day) was significantly higher in heifers treated prepartum with antibiotics. Additionally, treated heifers had a significantly lower somatic cell scores than control heifers (2.04 vs. 2.63). Thus, prepartum antibiotic treatment to reduce the rate of mastitis in heifers during early lactation was economically beneficial. Treated heifers produced 1,168 lb (531kg) more actual milk than the untreated controls. Based on a milk price of $18.50/cwt this resulted in $216.24 per heifer increase in gross revenue. Considering treatment costs of $15.60 per heifer including teat hygiene ($0.10), antibiotic ($10.00), labor ($2.50), and residue testing ($3.00), the net revenue amounted to $200.64 per heifer. The researchers concluded that it would be profitable to treat heifers before calving as long as the milk price was above $0.013 per pound and as long as the increase in milk production was greater than 84 pounds (Oliver et al., 2003).

Middleton et al. (2005) also evaluated the prepartum treatment of heifers with pirlimycin hydrochloride at 10-14 days before parturition on the prevalence of IMI at calving in 2 dairy farms. Postpartum sampling revealed that heifers receiving treatment in herd A had a higher overall cure rate and higher cure rates for IMI caused by CNS and *Staph. aureus* as well as lower SCC and lower prevalence of chronic IMI compared with untreated controls. The cure rate for *Staph. aureus* of 78% was significantly greater than that for untreated controls (8%). Treated heifers in herd B had a higher overall cure rate and higher cure rate for IMI caused by CNS compared with untreated controls, but CMT (SCC) scores and prevalence of chronic IMI were not different. In contrast to previous results of using lactating cow therapy, (Oliver et al., 2003), milk production did not differ between treatment groups. Authors concluded that routine prepartum therapy of heifers may not benefit all herds.

Subsequently, Borm et al. (2006) evaluated the efficacy of lactating cow intramammary therapy (cephapirin) administered to heifers 10-21 days prior to expected calving date in a 9-herd study involving 561 animals. Results demonstrated a 59.5% cure rate among mammary quarters that were infected prepartum and treated with antibiotic vs. untreated controls (31.7%). However, treatment did not significantly affect SCC or milk yield during the first 200 days of lactation. This observation is similar to that found by Middleton et al. (2005) who observed that although successful, treatment did not necessary reduce SCC or result in greater milk production. Authors concluded that prepartum treatment of heifers with lactating cow antibiotics may not be warranted as a universal strategy for mastitis management.

The studies on prepartum treatment with lactating cow therapy administered 7 - 21 days before calving (Borm et al., 2006; Oliver et al., 1992, 1997b, 2003, 2004) have shown treatment
to be effective for quarters infected with CNS but waiting until this time to treat chronic Staph. aureus mastitis might be too late. A mammary gland that has been infected with Staph. aureus for several months to a year will not develop normally, and treatment during the immediate prepartum period would most likely be of little benefit in curing infections or salvaging mammary tissue. At this point, the tissue damage would have already been done, and affected quarters should have been treated earlier in gestation to: 1) cure existing infections; 2) reduce chronic inflammation; and 3) allow mammary tissue to develop normally during the later stages of pregnancy.

Results of these trials demonstrated that nonlactating and lactating cow antimicrobial treatment of heifers known to be at risk for developing IMI is advantageous because the cure rate is much higher than that obtained when treating infections during lactation. In addition, most studies showed that SCC are lower, there is no milk loss due to therapy, risk of antibiotic residue at calving is minimal, and future milk production is increased in heifers cured of IMI.

9. Antibiotic residues in milk following prepartum lactating cow treatment

A disadvantage of prepartum lactating cow antibiotic therapy for controlling mastitis in heifers is the potential for antibiotic residues, especially if heifers calve sooner than expected. In one study (Oliver et al., 1992), it was shown that 17% of colostrum samples from heifer mammary glands infused with cloxacillin were positive for antibiotic residues by the Bacillus stearothermophilus disc assay, the majority of which were from heifers that calved within 5 days of treatment. Only 4.5% of samples obtained at the first milking after parturition were positive for antibiotic residues if intramammary infusion of cloxacillin occurred ≥ 7 days before parturition. All samples obtained 3 days after parturition, the time when milk would likely be marketed for human consumption, were negative for antibiotic residues. In contrast, 85% of colostrum samples and 28.2% of samples obtained 3 days after parturition were positive for cephapirin residues, and marked variability between time of antibiotic treatment and parturition with persistence of antibiotic residues was observed. Thus, antibiotic treatment of heifer mammary glands earlier in gestation may be advantageous from a residue standpoint, but the timing of antibiotic treatment and subsequent persistence in mammary secretions could impact efficacy.

Another study was conducted to determine if antibiotic treatment of heifer mammary glands earlier in the prepartum period reduced occurrence of residues in milk (Oliver et al., 1997a). A total of 82 Jersey heifers was used. Approximately half served as negative controls (n = 42) and half received an intramammary infusion of 200 mg cephapirin sodium (n = 40) 14 days before calving. Forty percent of samples from cephapirin-treated quarters were positive at the first milking after calving, but only 3.1% of samples obtained from antibiotic-treated quarters at the sixth milking (3 days) after calving were positive; 3 of the 4 positive samples were from a heifer that calved early and within 3 days of treatment.
Thus, the interval between prepartum antibiotic treatment and calving was related to persistence of residues during early lactation, and infusion of antibiotics 14 days prepartum (Oliver et al., 1992) compared with 7 days prepartum (Oliver et al., 1997a) reduced occurrence of residues in milk during early lactation. Similarly, Middleton et al. (2005) found that after heifers were treated 10-14 days prior to expected calving date with a pirlimycin lactating cow product, prevalence of IMI in early lactation was decreased without causing pirlimycin residues in milk at 3 days postpartum, even when a heifer was treated 1 day before calving (heifer calved early).

10. Role of vaccination in mastitis control

Although antimicrobial therapy is successful, the goal from a herd management perspective is to prevent new infections from occurring, and vaccination has been attempted as a prophylactic measure. Recent research has demonstrated that several experimental *Staph. aureus* vaccines, as well as one commercial vaccine, can increase antistaphylococcal antibody titers and reduce the new infection rate in heifers. A *Staph. aureus* vaccine formulated to stimulate pseudocapsule and alpha toxin antibodies was evaluated in heifers in New York (Sears et al., 1990). At 4 and 2 weeks prior to calving, heifers were given subcutaneous injections into the supramammary lymph node, and after calving, heifers were challenged with *Staph. aureus*. Vaccinates demonstrated a 52% reduction in new IMI. In addition, 64% of intramammary infections in control cows became chronic compared with only 12% in vaccinates.

A field study in Norway evaluated a *Staph. aureus* vaccine that contained two strains of whole, formalin-inactivated bacteria with pseudocapsule, alpha and beta toxoids, and mineral oil as an adjuvant (Nordhaug et al., 1994). A total of 108 pregnant heifers on 16 farms with an average *Staph. aureus* prevalence of 19.2% was used. Vaccinates were injected subcutaneously in the area of the supramammary lymph node with a dose of 2.5 ml at 8 and 2 weeks before calving. Results showed a 46% reduction in new IMI during the subsequent lactation. Antibody titers to *Staph. aureus* pseudocapsule and alpha toxin were markedly elevated in the serum of vaccinates, and these titers remained significantly higher in serum and milk during the entire lactation compared with those of unvaccinated controls.

In Argentina, a vaccine was developed based on an inactivated, encapsulated *Staph. aureus* strain, a crude extract of *Staph. aureus* exopolysaccharides, and inactivated, unencapsulated *Staph. aureus* and *Streptococcus* species in an aluminum hydroxide adjuvant (Giraudo et al., 1997). This formulation was evaluated in three groups of ten 24- to 26-month-old heifers each in a 7-month trial. The first group received an intramuscular injection of the vaccine in the neck at 8 and 4 weeks prepartum, the second group was vaccinated similarly at 1 and 5 weeks postpartum, and a third group (control) received placebo injections at 8 and 4 weeks prepartum. The research herd from which the heifers were selected had a bulk tank SCC ranging from 480,000 to 730,000, and 19% of quarters were infected with *Staphylococcus aureus*. This immunization program showed that the frequency of new *Staph. aureus* infection was reduced from 18.8% in controls to 6.7 and 6.0% for heifers vaccinated
In view of more recent studies showing success of vaccines in heifers, researchers in Louisiana evaluated a commercially available *Staph. aureus* vaccine in young dairy animals (Nickerson et al., 1999). The vaccine was a lysed culture of polyvalent *Staph. aureus* somatic antigens representing 5 phage types in an aluminum hydroxide adjuvant base, including serotypes 5, 8, and 336, the most common *Staph. aureus* serotypes associated with clinical mastitis (Lysigin®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA). At 6 months of age, 35 Jersey heifers were vaccinated using a 5-ml dose intramuscularly in the semimembranosus muscle of the rear leg, and 14 days later, vaccinates received a booster dose, which was repeated at 6-month intervals. Another 35 heifers served as unvaccinated controls. Results demonstrated that: (1) the number of quarters exhibiting chronic intramammary infection during pregnancy was reduced 43.1% in vaccinates compared with controls; (2) rate of new intramammary infection during pregnancy was reduced 44.8%; (3) rate of new intramammary infection at freshening was reduced 44.7%; and (4) the SCC was reduced by 50% in vaccinates compared with controls.

In a subsequent, more in depth study using the same vaccine (Lysigin®), 106 Holstein heifers from the James River Correctional Center dairy herd Goochland, VA, USA were evaluated (Nickerson et al., 2009). This herd had a 9,979-kg rolling herd average milk production with an average SCC of ~200,000/ml. Previous microbiological culture of heifer mammary secretions indicated that approximately 35% of animals were infected with *Staph. aureus*. At approximately 6 to 18 months of age, heifers were processed through a restraining chute to collect aseptic quarter mammary secretion samples for microbiological. Fifty-three heifers were vaccinated using a dose of 5 ml intramuscularly that was administered as above, and the other 53 heifers served as unvaccinated controls. Fourteen days after the initial processing, the vaccinated group was again processed through the chute and boosted with Lysigin®. All animals were maintained on pasture and rotated by age group through calving. At 6-month intervals after the initiation of the trial and through time of calving, the vaccinated group was again processed through the chute for boosting.

At 2-month intervals after the trial initiation and through calving, mammary secretion samples were collected for bacteriological culture and for the determination of electronic SCC (A/SN Foss, Hillerod, Denmark). Microbiological examination of quarter samples collected from bred heifers over gestation demonstrated that 19.8% of heifers (9.4% of quarters) were infected with *Staph. aureus*, 68.9% of heifers (34.3% of quarters) were infected with CNS, 6.6% of heifers (2.3% of quarters) were infected with environmental streptococci, and 1% of heifers (0.3% of quarters) were infected with coliforms.

At time of calving, heifers were enrolled in the Dairy Herd Improvement Program (DHIA) and data were recorded for milk yield, percentages and actual pounds fat and protein, days in milk, and SCC. Data on vaccine efficacy were examined in terms of mean percentage reduction in rate of new *Staph. aureus* or CNS intramammary infections achieved among immunized heifers compared with the rate among unimmunized controls at the time of...
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Immunization with Lysigin® did not cause any adverse reactions at the injection site or systemically. Minimal swelling (<2.5 cm) was occasionally observed, which disappeared within 48 hours of administration. Vaccine efficacy data showed that the percentage of heifers with *Staph. aureus* intramammary infections at freshening was lower in vaccinates (13.3%) compared with controls (34.0%); a reduction of 60.9% (P < 0.01). Likewise, an examination of health records showed that the percentage of heifers that were culled or died during the trial was reduced by approximately one-third by vaccination: 16.9% in vaccinates and 24.5% in controls (P > 0.05). The vaccinated group also experienced a slight, insignificant reduction in mastitis caused by CNS. At freshening, intramammary infections with CNS were lower in vaccinates (64.2%) compared with controls (69.8%); a reduction of 8.1%.

Somatic cell counts in samples collected during first week of lactation irrespective of infection status were 45% lower in vaccinates compared with controls (287,317 vs. 522,345/ml). Somatic cell counts in samples collected during first week of lactation from uninfected heifers for vaccinates and controls were 66,095 and 132,754/ml, respectively; a 50.2% reduction; and for infected heifers, SCC were 441,764 and 892,176/ml, respectively; a 50.5% reduction. Somatic cell counts in samples collected during the prepartum period were highest for *Staph. aureus* (6,730 x 10^3), followed by the environmental streptococci (3,850 x 10^3), and CNS (3,510 x 10^3).

An examination of the 305-day lactation milk yield for the 1st lactation of both vaccinated and unvaccinated control heifers demonstrated an approximate 8.6% increase in vaccinates vs. controls (11,217 vs. 10,332 kg, respectively) or a difference of 886 kg. On a complete lactation basis, vaccinated animals produced 839 kg more milk than controls (12,537 vs. 11,698 kg, respectively); an increase of 7.3%.

The percentage of 305-day lactation fat was higher in vaccinates than controls (3.64 vs. 3.27%, respectively); however, the percentage of 305-day lactation protein was slightly higher in controls than vaccinates (3.06 vs. 2.95, respectively). Actual 305-day kilograms of both fat and protein were higher in vaccinates than controls (fat: 408 vs. 339 kg, respectively; protein: 330 vs. 315 kg, respectively). Likewise, on a complete lactation basis, actual kilograms of both fat and protein were higher in vaccinates than controls (fat: 460 vs. 393, respectively; protein: 370 vs. 353, respectively).

An examination of the number of days in milk for the first lactation demonstrated that vaccinates persisted 13 days longer than the unvaccinated controls (349 vs. 336 days). In addition, average first lactation SCC were 11,000 cells/ml lower in vaccinates compared with controls (49,000 vs. 60,000/ml).

Results of this Virginia investigation demonstrated that vaccinating dairy heifers according to the prescribed protocol with a commercial USDA licensed *Staph. aureus* bacterin, Lysigin®, reduced the number of new *Staph. aureus* intramammary infections at time of calving by 60.9%, lowered the SCC by 50%, and decreased the culling rate by approximately one-third.
In addition, overall milk yield, production of fat and protein, and number of days in milk were greater in vaccinated heifers compared with controls.

The decrease in frequency of new *Staph. aureus* intramammary infections at calving (60.9%) in vaccinates using Holstein heifers is higher than the 44.7% reduction observed in a Louisiana trial using the same vaccine in Jersey heifers (Nickerson et al., 1999). In both trials, SCC at calving were reduced by approximately 50%. The 60.9% efficacy found in the present trial is also higher than the 40.2% efficacy observed by Giraudo et al. (1989), the 46% efficacy observed by Nordhaug et al. (1994), and the 52% efficacy observed by Sears et al. (1990). However, it is difficult to compare among the latter three trials as the vaccine formulations were all quite different.

The question becomes: Is it economically feasible to use this commercial vaccination protocol on young dairy heifers? Based on an average of 1,864 more lb milk per vaccinated heifer, which translates to 18.64 hundredweights (cwt) of milk (1,864/100), at the current (2008) price of $25.00/cwt, an increased income of $466.00/heifer would be realized (18.64 cwt x $25.00/cwt = $466.00). If each heifer was vaccinated beginning at 6 months of age until calving, this would entail vaccinations at 1) 6 months, 2) a booster 2-weeks later, and subsequently at 3) 12 months, 4)18 months, and 5) 24 months, or a total of 5 immunizations through calving. At $1.50/dose, this cost would total $7.50, which when subtracted from the increased income from milk production, would yield a net income of $458.50 per heifer ($466.00 - $7.50). This figure does not include the costs of labor involved in the immunization process; however, it is evident that vaccination is well worth the cost of the vaccine. Not only does it reduce new infections in first calf heifers at parturition, it may also reduce the introduction of *Staph. aureus* to the milking herd.

It is obvious that use of experimental and commercially available *Staph. aureus* vaccines can be used to prevent new infections, especially when used in heifers. Efficacy has been shown to range between 44 to 61% with reductions in SCC of 50%. This prevention strategy may represent a major control mechanism for managing *Staph. aureus* in the future, especially as new antigens and adjuvants are added to vaccine preparations.

11. Use of fly control to manage heifer mastitis

Historically, the major association between flies and intramammary infections has been with the development of summer mastitis, in which the biting fly, *Hydrotoea irritans*, is the proven vector (Chirico et al., 1997). Summer mastitis is an isolated seasonal problem primarily in July, August, and September in heifers and dry cows of northern Europe, and may be controlled by insecticidal sprays. In the US, fly control is used to reduce these insect pests on farm premises, and subsequently reduce animal stress, but its application as an adjunct management practice for preventing new cases of mastitis and reducing SCC has not really been considered or embraced by producers.

Surprisingly, very little research has been conducted on the relationship between mastitis in adult cows and fly control; most studies in this regard have been carried out in dairy heifers.
An initial survey performed at Louisiana State University showed that prevalence of mastitis in bred heifers was significantly lower in dairy herds that used some form of fly control for their lactating cows, dry cows, and heifers compared with herds applying no fly control (Figure 9) (Nickerson et al., 1995). The greatest reductions were in numbers of *Staph. aureus* and the environmental streptococci, both major mastitis pathogens in adult cows associated with elevations in SCC.

Results of this survey also demonstrated that bred heifers having teats with bite lesions and scabs caused by the blood-sucking horn fly (*Haematobia irritans*), exhibited a 70% frequency of intramammary infection compared with a 40% frequency in heifers with normal teats. Such infections are always associated with elevated SCC in excess of 5 million/ml in these young animals. See Figure 10 below illustrating horn flies and lesions on heifer teats.

![Figure 9. Prevalence of mastitis in Louisiana dairy herds with and without a fly control program.](image-url)

Since that first survey, researchers have proven through DNA studies that the horn fly is not only responsible for teat lesions on heifers, but is indeed a vector in the transmission of mastitis-causing bacteria, such as *Staph. aureus*, from heifer to heifer (Owens et al., 1998). Such mastitic heifers serve as sources of IMI for transmission to the entire lactating and nonlactating herds.

Once it was established that the horn fly was a vector in the transmission of mastitis-causing bacteria, the next step was to develop management practices to reduce flies and lower the prevalence of intramammary infections. Insecticide-impregnated tags placed on the tail switch in close proximity to the udder during the spring and summer months were successful in reducing horn fly populations by 60% as well as the incidence of mastitis during the first 2 months after placement (Nickerson et al, 1997). In heifers with tail tags, mastitis incidence increased from 8.6 to 15% (1.7-fold increase), while in controls, incidence
increased from 17.1 to 52.4% (3.1-fold increase). As observed above, infections were associated with marked elevations in SCC. However, after 2 months, tags fell off and replacing them was impractical from a management standpoint.

Figure 10. Udder of a 10-month-old heifer illustrating horn flies and lesions on teat ends.

In a subsequent trial, the daily dietary supplementation of an insect growth regulator helped to suppress fly populations but not enough to prevent new cases of mastitis in dairy heifers (Owens et al., 2000).

Lastly, the use of an insecticidal pour-on every 2 wk for 6 wk followed by treatment with insecticidal ear tags reduced fly populations and decreased the incidence of new *Staph. aureus* by 83% during a 6-mo trial in heifers during the warm season in Louisiana (Owens et al., 2002). Mastitis in heifers caused by *Staph. aureus* was associated with SCC in excess of 10 million/ml.

These studies demonstrate that, during the warm and humid months of the year, horn flies do serve as vectors in the transmission of heifer mastitis, which is associated with elevated SCC in these young dairy animals. Although research has not been conducted to show this same association in lactating and dry adult cows, it is assumed that fly populations play some role in the elevation of mastitis and SCC observed in the hot summer months. And, with the proposed reduction in the SCC legal limit to 400,000/ml in the USA, and in light of the fact that milk buyers are imposing their own limits, some as low as 250,000/ml, it is imperative that dairymen utilize all possible means to prevent new cases of mastitis and their associated SCC. A simple fly control program can serve as an important adjunct to the basic 5-point plan of mastitis control and assist dairymen in lowering their bulk tank SCC and earn quality premiums for their product.
12. Influence of dietary supplementation on mammary health

Another management tool to reduce the level of infection and SCC when heifers calve as well as throughout lactation is through dietary supplementation. Diet plays a role in udder resistance to infection because certain nutrients affect various mammary resistance mechanisms, namely: (1) leukocyte function, (2) antibody transport, and (3) mammary tissue integrity. In one study, heifers received selenium (0.3 ppm/day) and vitamin E (50 to 100 ppm/day) supplementation starting 60 days prepartum (Hogan et al., 1993). A selenium booster injection (50 mg) was administered 21 days prior to freshening, and the dietary supplementation was continued throughout lactation. Dietary supplementation reduced staphylococcal and coliform infections at calving by 42%. Although rate of new infection during lactation did not differ from unsupplemented controls, the duration of infection caused by organisms other than Corynebacterium bovis was reduced 40 to 50% in supplemented heifers. Clinical mastitis in supplemented heifers was reduced 57% in early lactation and 3.2% throughout lactation, and the mean SCC was lower. Thus, vitamin E and selenium improved udder health of heifers, and the effect of dietary supplementation was most evident at calving and in early lactation.

In a more recent study, dairy heifers were fed a daily supplement beginning at 5 months of age containing an immunostimulant composed of B-complex vitamins and yeast extract (Eubanks et al., 2011). Compared with unsupplemented control animals, those supplemented with the immunostimulant exhibited greater leukocyte expression of L-selectin and interleukin-8 cell surface receptors, suggesting the capability for a greater immune response to bacterial infection. Preliminary results also showed a lower incidence of Staph. aureus mastitis in supplemented heifers.

13. Conclusions

Prevalence of mastitis in unbred, breeding age, and pregnant dairy heifers is higher than formerly realized. Infected mammary quarters, especially those with Staph. aureus IMI exhibit reduced secretory potential and marked leukocyte infiltration and accompanying inflammation. Both nonlactating and lactating commercial antibiotic infusion products have been used successfully to cure existing infections and reduce SCC, and nonlactating therapy prevents new IMI with environmental streptococci. However, the goal is to prevent new infections from occurring in these young dairy animals through management strategies aimed at vaccination, fly control, and dietary supplementation. Results of experimental and commercial vaccine trials illustrate that immunization will reduce Staph. aureus mastitis in heifers at calving between approximately 45 and 60%, with reductions in SCC of 50%. Likewise, a fly control program for heifers will decrease incidence of Staph. aureus mastitis by up to 83%. Lastly, dietary supplementation to boost the immune systems of heifers has been shown to reduce incidence of mastitis at calving as well as to lower SCC. As global milk quality standards become more stringent, management practices based on curing existing infections and preventing new IMI in heifers will ensure that these young dairy animals enter the milking herd free of mastitis with low SCC. Such practices should be considered for incorporation into dairy herd health programs in herds suffering from a high prevalence of heifer mastitis, especially that caused by Staph. aureus.
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