

# Prevalence, Risk Factors, and Strategies for Controlling Mastitis in Heifers During the Periparturient Period

Stephen Paul Oliver, PhD  
Barbara Erin Gillespie, MSc  
Susan Juanita Headrick, BSc  
Mark James Lewis, BSc  
Henry Hamilton Dowlen, MSc

*Department of Animal Science and the Food Safety Center of Excellence  
The University of Tennessee  
Knoxville, TN*

**KEY WORDS:** heifer mastitis, prevalence, intramammary infection, mastitis control, somatic cell counts, periparturient period

## ABSTRACT

Intramammary infections (IMIs) in unbred and pregnant dairy heifers were once thought to be very infrequent. However, during the last 2 decades, several studies have shown that IMIs in heifers occur frequently during the prepartum and peripartum periods. Many of these infections can persist for long periods of time, may be associated with elevated somatic cell counts (SCC), and may impair mammary development and affect milk production after calving. The purpose of this communication is to review literature published on the prevalence of mastitis in heifers, potential risk factors associated with heifer mastitis, and to describe results of different approaches that have been taken to control mastitis in heifers.

## INTRODUCTION

Mastitis in heifers was first recognized over 60 years ago.<sup>1,2</sup> However, it was generally believed that IMIs in unbred and pregnant

heifers occurred infrequently. Over 20 years ago, Oliver and Mitchell<sup>3</sup> showed that a high percentage of pregnant heifers' mammary glands were infected during late gestation, at calving, and during early lactation. During the last two decades, several additional studies on the prevalence of mastitis in heifers have been published. All of these studies suggest that IMIs in heifers during the prepartum period occur frequently. However, marked herd variation in the rate of IMI and types of pathogens causing IMI have been reported.<sup>4-16</sup>

## Prevalence of IMI in Heifers

Trinidad et al.<sup>15</sup> demonstrated that the prevalence of IMIs in unbred heifers and heifers during different stages of pregnancy was very high. Unbred heifers had a higher percentage (86.7%) of infected mammary quarters compared with the overall mean for pregnant heifers (70%). *Staphylococcus* species were observed most frequently and 8 different species were isolated. The 3 most common species isolated from unbred and pregnant heifer mammary glands were *Staphylococcus chromogenes*, *Staphylococcus hyicus*, and *Staphylococcus*

*aureus*. Coagulase-negative *Staphylococcus* species (CNS) accounted for 67.4% of bacteria isolated. Mammary secretions from infected mammary glands had significantly higher somatic cell counts (SCC) than secretions from uninfected mammary quarters. In addition, tissue from mammary glands of unbred heifers infected with CNS exhibited greater leukocyte infiltration and increased connective tissue compared with tissue from uninfected mammary glands.<sup>17</sup> Thus, infection of heifer mammary glands by mastitis pathogens can occur at a very early age and some of these infections may impair mammary growth and development and influence future milk production.

Pankey et al.<sup>13</sup> reported that approximately 46% of heifers and 19% of quarters were infected during early lactation based on duplicate samples obtained from 382 heifers within 3 days after calving. CNS species were the most prevalent bacteria isolated and were found in 22.8% of heifers and 11.4% of quarters. Matthews et al.<sup>7</sup> indicated that 35.5% of colostrum samples were positive for 7 different *Staphylococcus* species. Species isolated most frequently were *S chromogenes*, *S aureus*, and *S simulans*. *Staphylococcus* species were isolated from about 18% of heifer mammary glands weekly for the first 5 weeks of lactation. Oliver and Sordillo<sup>11</sup> showed that 19.7% of heifer mammary glands (59 of 300) were infected at calving and CNS species caused 71.2% of these IMIs. During early lactation, 15.7% of heifer mammary glands (47 of 300) were infected and 48.9% were due to CNS. Thus, the number of mammary quarters infected with CNS species decreased significantly from calving to early lactation, suggesting that some CNS species isolated from heifer mammary glands were either colonizing the teat duct and subsequently eliminated as a result of the milking procedure, or that a high rate of spontaneous elimination occurred. Similar findings were reported in multiparous cows.<sup>10,18</sup>

Oliver et al.<sup>12</sup> conducted a study to determine the prevalence of mastitis and types of

pathogens causing IMI in pregnant Jersey heifers prior to calving and during early lactation. This study was conducted in a herd that was *Streptococcus agalactiae*-negative and had a low prevalence of *S aureus*. This pattern of infection would be typical of many dairy herds that practice postmilking teat disinfection and antibiotic dry cow therapy. About 90% of 115 heifers and 61% of quarters were infected during the prepartum period. The majority of IMIs (243 of 279) were due to CNS species. This is higher than what was observed previously in another herd,<sup>3,11</sup> but types of mastitis pathogens isolated were similar. Trinidad et al.<sup>15</sup> also observed considerable herd-to-herd variation both in prevalence of IMI and mastitis pathogens causing IMI in unbred and pregnant heifers. For example, in one herd, 44.3% of quarters were uninfected; 12.3% were infected with *S aureus*, 41.5% were infected with CNS species, and 1.9% were infected with streptococci other than *S agalactiae*. In another herd, 17.6% of quarters were uninfected; 23.1% were infected with *S aureus*, 49.5% were infected with CNS species, and 9.9% were infected with *Streptococcus* species. In other studies,<sup>3,12,19,20</sup> CNS species were isolated most frequently followed by environmental mastitis pathogens, primarily *Streptococcus* species.

Fox et al.<sup>5</sup> reported on a survey of 28 dairies in 4 states to determine the prevalence of IMI in unbred and pregnant dairy heifers and to determine potential factors that influenced herd variation. Most IMIs were due to CNS species and *S aureus*. Location, herd, season, and trimester of pregnancy significantly influenced prevalence of IMI in heifers. Heifers in the third trimester of pregnancy had the highest prevalence of IMIs.

Myllys<sup>8</sup> indicated that CNS species were the most frequently (57.8%) isolated bacteria from mammary secretions obtained from 200 heifers with mastitis and from 65 non-mastitic control heifers, followed by *S aureus* (20.1%) and streptococci (11.3%). *S simulans*, *S hyicus*, *Staphylococcus xylosum*,

and *S chromogenes* were frequently found in milk from heifers with clinical mastitis after calving, whereas other CNS species were equally or more often found in non-mastitic control heifers.

Aaerstrup and Jensen<sup>4</sup> reported that *S chromogenes* was isolated from 15% of all mammary quarters and was the most commonly found bacterial species in heifer mammary secretions obtained before parturition in Denmark. However, *S chromogenes* IMIs decreased shortly after parturition to around 1% of mammary quarters. Infections caused by *S simulans* and *Staphylococcus epidermidis* occurred in 1% to 3% of mammary quarters both before and after parturition. *S simulans* IMIs persisted for several weeks, while *S epidermidis* IMIs tended to be more transient. *Streptococcus dysgalactiae* subspecies *dysgalactiae* (*S dysgalactiae*) was isolated from 4% to 6% of mammary quarters before and immediately after calving, and the prevalence of *S dysgalactiae* decreased during early lactation. Infections due to *S aureus* were rarely observed before calving, but the rate of *S aureus* IMIs increased greatly the first week after calving. The presence of an IMI in a mammary quarter before parturition increased the risk of IMI for the lactating cow.<sup>4</sup>

One common denominator of all studies on heifer mastitis is the high prevalence of CNS-caused IMI. Thus, CNS will likely cause the majority of IMIs in unbred and pregnant heifers and variation in the prevalence of CNS IMIs in heifers should be expected among herds. The designation CNS is used to include all staphylococci and micrococci isolated from milk samples that are not *S aureus*.<sup>21</sup> As a rule, they are coagulase-negative; however, there are exceptions. The commonly isolated CNS species are part of the normal skin flora and include the species *S simulans*, *S hyicus*, and *S epidermidis*. In contrast, novobiocin-resistant species (*S xylosum*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, and *Staphylococcus cohnii*) are found free-living in the environment. The CNS species appear

to be opportunists and infect the teat canal and gland from skin sources. Infections by novobiocin-resistant species may originate from the environment. *S chromogenes* and *S hyicus* appear to readily colonize the teat canal and may persist for longer periods of time than the other CNS species. Many CNS infections are transient and cow-to-cow spread is thought to be a low-risk cause for infection.<sup>21</sup> *S chromogenes* was isolated most frequently in 3 separate studies.<sup>4,7,15</sup> However, isolation of other CNS species varied considerably. For example, *S simulans* was isolated frequently in studies by Aaerstrup and Jensen<sup>4</sup> and Matthews et al.,<sup>7</sup> while *S hyicus* was isolated frequently by Trinidad et al.<sup>15</sup> Thus, while CNS species are often grouped together, considerable variation in the frequency of CNS isolation between herds has been reported and it is possible that some CNS species may be more problematic than others.

The prevalence of mastitis pathogens other than CNS also varies considerably. In studies by Oliver et al.,<sup>12,19,20</sup> 8% to 10% of heifer mammary glands were infected by environmental mastitis pathogens, primarily *Streptococcus* species, which was consistent with the pattern of IMI in lactating cows in these herds. Conversely, other studies<sup>5,15</sup> reported that *S aureus* was the most prevalent major mastitis pathogen isolated from unbred and pregnant heifer mammary glands. Differences in the incidence of IMI and types of bacteria causing IMI in pregnant heifers is likely due to the prevalence of mastitis pathogens in the herds evaluated. Thus, a reasonable hypothesis is that heifers from herds with a high prevalence of contagious mastitis will likely be infected predominantly by contagious mastitis pathogens. Similarly, environmental mastitis pathogens will likely be the predominant major pathogens isolated from heifer mammary glands from herds with an environmental mastitis problem.

Some IMI in heifers result in clinical mastitis during the prepartum period and during early lactation. Nickerson et al.<sup>9</sup> indi-

cated that 29% of heifers and 15% of mammary quarters exhibited clinical mastitis at breeding age as evidenced by clots or flakes in mammary secretions. *S dysgalactiae* and *Streptococcus uberis* were isolated from 34.4% and 19.5%, respectively, of heifers with clinical mastitis occurring from puberty up to 14 days after calving in a large study involving bacterial analyses of 2,069 udder secretions isolated from 1,481 heifers with clinical mastitis in Sweden.<sup>6</sup> Bacterial species generally regarded as important pathogens in the summer mastitis complex including *Actinomyces (Arcanobacterium) pyogenes*, Stuart-Schwan coccus, and strictly anaerobic bacteria such as *Peptostreptococcus indolicus*, *Fusobacterium necrophorum*, and *Bacteroides melaninogenicus*, were isolated at low frequencies (13.25%, 6.3%, 9.4%, 3.8%, and 1.3%, respectively). When cases of clinical mastitis were restricted to those appearing in heifers prepartum during the summer mastitis season (May 15 to October 14), these bacterial species were isolated at higher percentages (27.1%, 14.4%, 21.4%, 13.5%, and 5.2%, respectively). There were no significant differences in the frequency of *A pyogenes* isolated during different seasons of the year. There were geographical differences in bacterial incidence; for example, *S aureus* was isolated significantly more often in northern regions whereas *S dysgalactiae* was more common in the south. These data support the theory that *A pyogenes* and strictly anaerobic bacteria are “secondary invaders” that depend on *S dysgalactiae* to cause a primary infection. Jonsson et al.<sup>6</sup> stressed that udders of all heifers should be examined daily so that cases of mastitis can be treated immediately.

More recently, Waage et al.<sup>16</sup> reported results of a 1-year field investigation of clinical mastitis in heifers in Norway. The study included 1,361 cases of clinical mastitis in 1,040 heifers that occurred prepartum or within 14 days after calving. Mastitis pathogens isolated most frequently from mammary quarters with clinical mastitis

were *S aureus* (44.3%), *S dysgalactiae* (18.2%), *S aureus* together with *S dysgalactiae* (1.2%), CNS species (12.8%), *A pyogenes* (3.5%), *A pyogenes* together with *S dysgalactiae* (0.5%) or *S aureus* (0.4%), and *Escherichia coli* (6.4%). Of the CNS species isolated, *S simulans* (53.7%), *S hyicus* (14.8%), and *S chromogenes* (14.8%) were the most prevalent species. Except for a higher relative percentage of *A pyogenes* in cases that occurred before parturition (8.2%) than in cases that occurred after parturition (2.7%), no significant differences were observed in the distribution of the various organisms among prepartum and postpartum cases of mastitis. Regional variations were observed in the distribution of organisms. *S aureus* and *A pyogenes* clinical mastitis were highest in late autumn and early winter, CNS-caused clinical mastitis was lowest in late autumn and early winter, and *E coli* clinical mastitis was highest in the summer.

### Heifer Mastitis Risk Factors

Several potential heifer mastitis risk factors have been identified. In an epidemiological survey of 171 dairy farms from 5 regions of Spain, Martin-Richard et al.<sup>22</sup> found that risk factors for heifer mastitis were calving in summer, high herd SCC, presence of *S aureus* and *Mycoplasma* species, absence of fly control, feeding calves mastitic milk, contact among calves, absence of antibiotic therapy to heifers, contact with adult cows, inadequate milking practices, and poor housing conditions. Other heifer mastitis risk factors identified include an increase in the incidence of clinical mastitis in a herd; a decrease in the bulk tank SCC; an increase in herd mean milk yield; calving in late spring or early summer; increased age at first calving; milk leakage;<sup>23</sup> blood in the milk; udder and teat edema;<sup>24</sup> and presence of pathogens on heifer body sites.<sup>25</sup> Presence of IMI before calving increased the risk of infection during lactation;<sup>4</sup> IMI at calving increased the risk of clinical mastitis within the first week after calving, and mastitis

prior to parturition and mastitis within the first week after calving increased the risk of further cases of mastitis and culling during the first 45 days of lactation.<sup>26</sup>

Studies have provided convincing evidence that the horn fly (*Hameotobia irritans*) is an important vector in the transmission of *S aureus* mastitis in heifers. *H irritans* can be colonized with *S aureus* during feeding activities and can remain colonized for several days with substantial numbers of organisms present. When *S aureus*-colonized horn flies were allowed to feed on teats of uninfected dairy heifers, IMI with the same *S aureus* DNA fingerprint subtype resulted.<sup>27</sup> This indicates that the horn fly can transmit *S aureus* to heifer teats if a sufficient source of organisms is present. That source was shown to be present in existing scabs on teat ends of heifers.<sup>28</sup> High concentrations of *S aureus* ( $>10^7$  colony forming units/mg) were found in scab material present on heifer's teats. When uncolonized flies were allowed to feed on this material they became colonized with *S aureus* just as readily as flies that had fed on experimentally infected blood. Thus, a vector shown capable of transmitting infection is readily present. When a source of *S aureus* exists such as scabs on heifer teats, the potential for passage of IMI from heifer to heifer via horn flies exists. The threshold number of flies needed to transmit IMI is unknown. However, since fly populations can rapidly increase to several thousand per animal under favorable conditions, the need for early fly control on dairy heifers is apparent. Once scabs are obvious and fly populations are high, spread of new infections is likely. Prevention of initial high populations of flies on heifers is important to help reduce new infections.

### **Susceptibility of Pathogens Causing Mastitis**

Considerable evidence suggests that IMI in pregnant heifers occurs frequently and that some infections may be detrimental to mammary gland development and influence

subsequent lactational performance. Methods of controlling mastitis in heifers may eliminate or markedly reduce the deleterious effects of prepartum infections. One common denominator of all studies on heifer mastitis is the high prevalence of CNS-caused IMI. Trinidad et al.<sup>29</sup> demonstrated that 90% of 311 staphylococcal isolates (primarily CNS species) from heifer mammary glands were susceptible to antibiotics in vitro. Some variability in antimicrobial susceptibility of bacteria obtained within and among herds was noted; however, in general, bacteria were highly susceptible to all antibiotics evaluated. Watts et al.<sup>30</sup> determined minimum inhibitory concentrations of penicillin, cloxacillin, cephalosporin, ceftiofur, novobiocin, enrofloxacin, erythromycin, and pirlimycin against 1,494 microorganisms isolated from heifer mammary glands. The majority of *Staphylococcus* species were susceptible to the antimicrobial agents evaluated. However, antimicrobial susceptibility was variable for *Streptococcus* species and poor against Gram-negative enteric organisms. These data suggest that antibiotic therapy may be an effective means of eliminating *Staphylococcus* species-caused IMIs that have been shown to cause the majority of IMIs of heifer mammary glands.

### **Use of Dry Cow Antibiotic Preparations for Controlling Mastitis**

A strategy that has received considerable research attention is based on intramammary treatment of heifer mammary glands with a dry cow antibiotic formulation during different trimesters of pregnancy.<sup>31-35</sup> Mammary quarters of 35 breeding-age and primigravid Jersey heifers were infused with a nonlactating cow antibiotic formulation containing penicillin/streptomycin. Thirty-eight breeding-age and primigravid Jersey heifers served as untreated controls.<sup>35</sup> Of the 35 treated heifers, 34 (97.1%) were infected at the time of treatment. In the untreated control group, all 38 heifers (100%) were infected at treatment time. At parturition,

prevalence of IMI in treated heifers decreased to 40%, whereas prevalence in the control group remained about the same (97.4% of heifers). Prevalence of *S aureus*-caused mastitis in treated heifers was reduced from 17.1% to 2.9% after treatment. In the control group, prevalence of *S aureus* mastitis decreased from 26.3% to 15.8%. Heifers treated during the second trimester of pregnancy had the greatest reduction in prevalence of mastitis. Results of this study<sup>35</sup> suggested that intramammary treatment of primigravid heifers during pregnancy was effective in reducing prevalence of mastitis and SCC at parturition.

In another study taking a somewhat different experimental approach, a nonlactating cow antibiotic formulation containing cephapirin benzathine was evaluated in pregnant and nonpregnant Jersey heifers for its effect on experimentally induced *S aureus* mastitis.<sup>31</sup> Cephapirin was detectable in mammary secretion of nonpregnant heifers for up to 5 weeks and in tissue for 1 week after intramammary infusion. *S aureus* was not detectable in tissue and secretion of treated quarters at 1 and 3 weeks but was not eliminated from 2 quarters of one heifer tested at 6 weeks after treatment. Histologic evaluation of mammary tissue from nonpregnant heifers revealed significant differences in leukocytosis between uninfected and *S aureus*-infected mammary quarters but no differences in epithelium, lumen, and stroma, indicating no difference in secretion potential or glandular development. Pregnant Jersey heifers (n = 25) were experimentally infected in 2 mammary quarters with *S aureus* 12 to 14 weeks prepartum. After 1 to 3 weeks, 13 heifers were infused in 21 *S aureus*-infected mammary quarters with a commercial cephapirin formulation approved for use in nonlactating cows. Nine infected mammary quarters were left untreated. All treated mammary quarters were bacteriologically negative both at calving and through 2 months after calving. Of the 9 infected mammary quarters not treated prepartum, 1 spontaneously cured and 2

became nonfunctional. The remaining quarters were treated at calving with a commercial cephapirin formulation approved for use in lactating cows. Of these, 3 were cured and 3 failed to resolve. Heifers with cured *S aureus*-caused IMI produced 16.4 kg of milk per day while heifers that remained infected with *S aureus* produced 14.5 kg of milk per day (11% less milk).<sup>31</sup>

In a subsequent study Owens et al.<sup>32</sup> showed that intramammary infusion of a nonlactating cow formulation containing cephapirin into mammary quarters of 18 Jersey heifers 10 to 12 weeks prepartum resulted in cure rates of existing IMIs of 96% (24/25), 100% (4/4), and 90% (28/31) for *S aureus*, *Streptococcus* species, and *Staphylococcus* species, respectively. Cure rates of IMI that had been treated with a commercial cephapirin formulation approved for use in lactating cows at parturition were 62.5% (15/24), 100% (22/22), and 100% (3/3) for *S aureus*, *Streptococcus* species, and *Staphylococcus* species, respectively. Initial SCC of secretions from infected mammary quarters were greater than from uninfected mammary quarters. At 2 months postpartum, the SCC of milk from treated and cured mammary quarters were reduced in comparison with mammary quarters that remained infected. Cephapirin was present at detectable concentrations in 94%, 80%, 68%, and 61% of treated mammary quarters at 1, 2, 3, and 4 weeks, respectively, after infusion of the commercial cephapirin formulation approved for use in nonlactating cows. At parturition, 24% of treated mammary quarters were positive for inhibitors; however, no mammary quarters remained positive for inhibitors at 5 days postpartum. An additional 40 heifers from a commercial herd were sampled and infused in all mammary quarters with the commercial cephapirin formulation approved for use in lactating cows at 16 to 20 weeks prepartum. Cure rates for the commercial herd were 94% (29/31), 94% (16/17), 100% (44/44), and 100% (3/3), respectively, for mammary quarters infected by *S aureus*,

*Streptococcus* species, *Staphylococcus* species, and coliforms.

In another study<sup>34</sup> conducted in 42 dairy heifers, a total of 24 *S aureus*-infected mammary quarters, 53 *Staphylococcus* species-infected mammary quarters, and 20 *Streptococcus* species infected mammary quarters were observed 12–14 weeks prepartum. Intramammary therapy of primigravid dairy heifers 12–14 weeks prepartum with 2 commercially available antibiotic formulations approved for use in nonlactating cows (penicillin-novobiocin or cephalixin) resulted in cure rates of 94%, 97%, and 100% for *S aureus*, *Staphylococcus* species, and *Streptococcus* species, respectively. No protective effect was observed for dry cow treatment of uninfected mammary quarters of heifers for either of the commercially available antibiotic formulations approved for use in nonlactating cows. No antibiotic was detectable in heifer secretions collected at parturition, indicating that antibiotic concentrations may have fallen below protective levels prior to parturition.

In a much larger study, 233 dairy heifers were treated 0 to 90 days, 90 to 180 days, or 180 to 270 days prepartum with 1 of 5 different antibiotic formulations for use in nonlactating cows to determine the best time to treat and the most effective product to use.<sup>33</sup> At the initial sampling, 56.5% of mammary quarters were infected; 15.4% of mammary quarters were infected with *S aureus*. Treatments included a commercially available cephalixin dry cow product, a commercially available penicillin-novobiocin dry cow product, a commercially available penicillin-streptomycin dry cow product, an experimental dry cow product containing tilmicosin, and a cephalonium dry cow product not available in the United States. Cure rates for the 5 antibiotic products were equally effective against *S aureus* and all were significantly more effective than the spontaneous cure rate observed in untreated control mammary quarters. Furthermore, no differences in efficacy were observed due to the different treatment times prepartum.

However, fewer new *S aureus* infections occurred after treatment in the third trimester of pregnancy. Fox et al.<sup>5</sup> indicated that the prevalence of heifer IMIs was highest during the last trimester of pregnancy. Thus, methods of controlling mastitis in heifers would likely be more effective if administered during the last trimester of pregnancy as opposed to early gestation.

### **Lactating Cow Antibiotic Preparations Before Expected Calving**

Another strategy that has received considerable research attention is based on intramammary treatment of heifer mammary glands with antibiotic formulations approved for use in lactating cows during the periparturient period.<sup>12,19,20</sup> A study by Oliver et al.<sup>12</sup> was conducted to determine if prepartum infusion of lactating cow antibiotic preparations containing cloxacillin or cephalixin into heifer mammary glands influenced rates of IMI during early lactation. Results of that study demonstrated that almost 90% of heifers were infected 7 days prior to expected calving. During early lactation, 78% of control heifers and 44.5% of mammary quarters were infected. In contrast, 17.6% of antibiotic-treated heifers and 5.4% of antibiotic-treated quarters were infected during early lactation. Fewer ( $P < 0.001$ ) antibiotic-treated heifers and quarters were infected during early lactation than in controls. Intramammary antibiotic therapy before calving was highly effective ( $P < 0.001$ ) against CNS. It should be noted, however, that 27% of CNS IMIs in control heifers were not detected during early lactation, suggesting a high rate of spontaneous elimination. Nine of 14 major pathogen IMIs in control heifers and 3 of 22 major pathogen IMIs in antibiotic-treated mammary glands of heifers persisted into early lactation. Differences in major pathogen IMIs between antibiotic-treated and control animals during early lactation were significant ( $P < 0.025$ ).<sup>12</sup>

Mastitis pathogens were isolated from 76% of samples obtained from untreated control mammary quarters 7 days before

expected calving, 47% of samples obtained 3 days after calving, and 29% of samples obtained 10 days postpartum. Throughout the remainder of lactation, mastitis pathogens were isolated in milk from about 30% of control mammary quarters. A similar percentage of samples (70%) was positive for mastitis pathogens at 7 days before expected calving prior to antibiotic treatment. However, only 8% of samples obtained 3 days after calving and 4% of samples obtained 10 days postpartum from quarters of antibiotic-treated heifers contained mastitis pathogens. Throughout the remainder of lactation, mastitis pathogens were isolated from an average of about 11% of mammary quarters. The percentage of samples with mastitis pathogens was higher in untreated controls than in antibiotic-treated quarters at most sampling intervals during lactation. *S. uberis*, *S. dysgalactiae*, and CNS were isolated most frequently in both untreated controls and antibiotic-treated heifer mammary glands.<sup>12</sup>

### **Inhibitors in Milk Following Prepartum Treatment**

One disadvantage of prepartum antibiotic administration for controlling mastitis in heifers is the potential for antibiotic residues in milk. This is especially important if heifers calve sooner than expected. To address this concern, samples of mammary secretion from all mammary quarters of 98 heifers were collected at the first and sixth milking after calving and at 10 days after calving for antibiotic residue analysis.<sup>12</sup> Samples were analyzed qualitatively by the *Bacillus stearothermophilus* disc assay. Zones of inhibition greater than 16 mm in diameter were interpreted as positive for inhibitors in milk. Sensitivity of the *B. stearothermophilus* disc assay for cephalosporins and cloxacillin has been reported to be 0.025 µg/mL and 0.031 µg/mL, respectively.<sup>36,37</sup>

About 17% of colostrum samples from heifer mammary glands infused with cloxacillin were positive for inhibitors by the *B. stearothermophilus* disc assay.<sup>12</sup> The

majority of positive samples were from heifers that calved within 5 days of treatment. Only 4 of 88 samples obtained at the first milking after parturition were positive for inhibitors if intramammary infusion of cloxacillin occurred  $\geq 7$  days before parturition. All samples obtained 3 days after parturition, the time when milk would likely be marketed for human consumption, were negative for inhibitors.

In contrast, inhibitors were detected frequently during early lactation in samples from heifer mammary glands infused with cephalosporins.<sup>12</sup> Almost 85% of colostrum samples and 28.2% of samples obtained 3 days after parturition were positive for inhibitors. Marked variability between time of antibiotic treatment and parturition with persistence of antibiotic residues was observed. For example, 2 heifers calved 8 days after treatment and all samples obtained 3 days after parturition were negative for inhibitors. Conversely, 4 heifers calved 10 days after cephalosporin treatment and 6 of 16 samples were positive for inhibitors. All samples ( $n = 24$ ) from 6 heifers obtained 3 days after calving were negative for inhibitors if intramammary infusion of cephalosporins occurred  $\geq 11$  days before calving. Thus, it would appear that antibiotic treatment of heifer mammary glands earlier in gestation may be advantageous from an antibiotic residue standpoint. However, the timing of antibiotic treatment and subsequent persistence of antibiotics in mammary secretions following treatment could affect efficacy.

Another study was conducted to determine if treatment of heifer mammary glands with cephalosporin sodium earlier in the prepartum period at 14 days prior to expected calving reduced the occurrence of inhibitors in milk without influencing efficacy.<sup>19</sup> That study demonstrated that only 4 of 127 samples (3.1%) obtained from cephalosporin-treated mammary quarters at the sixth milking after calving were positive and 3 of 4 positive samples were from a heifer that calved within 3 days of treatment. Thus, as observed in an earlier study,<sup>12</sup> the interval between



prepartum antibiotic treatment and calving was related to the presence of inhibitors in milk during early lactation. Intramammary infusion of cephapirin earlier in the prepartum period reduced the occurrence of inhibitors in milk during early lactation without affecting treatment efficacy.<sup>19</sup>

Recently, a study was conducted in 2 herds to determine if prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was effective for reducing the percentage of heifers and mammary quarters infected with mastitis pathogens during early lactation.<sup>38</sup> Almost 96% of Jersey heifers (67 of 70) and 71.3% of quarters (199 of 279) were infected 14 days before expected calving. Of the quarters infected 14 days before expected parturition, 75% (54 of 72) were uninfected following treatment with penicillin-novobiocin; 87% (61 of 70) were uninfected following treatment with pirlimycin, and 56% (32 of 57) were uninfected in the untreated negative control group. The majority of IMIs in Jersey heifers were due to CNS (61%), *Streptococcus* species, primarily *S uberis* (19%), and *S aureus* (8%). Almost 73% of Holstein heifers (40 of 55) and 34.3% of mammary quarters (73 of 213) were infected 14 days before expected calving. Of the mammary quarters infected at 14 days before expected parturition, 76% (19 of 25) were uninfected following treatment with penicillin-novobiocin; 59% (17 of 29) were uninfected following treatment with pirlimycin, and 26% (5 of 19) were uninfected in the untreated negative control group. The majority of IMIs in Holstein heifers were due to CNS (44%) and *S aureus* (30%). In both herds, the bacteriological cure rate was significantly higher in heifer mammary glands treated with penicillin-novobiocin or pirlimycin than in untreated controls. Prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin was an effective procedure for significantly reducing the percentage of heifers and quarters infected with mastitis pathogens during early lactation.

## **Influence of Heifer Prepartum Antibiotic Treatment on Lactation**

Oliver et al.<sup>20</sup> determined the influence of prepartum antibiotic treatment on subsequent lactational performance of Jersey heifers. Milk production and SCC score data from 82 control heifers and 111 heifers treated with antibiotics before calving were evaluated. Milk production (actual and 305-day) was significantly higher in heifers treated with antibiotics. Milk from heifers treated with antibiotics before calving also had a significantly lower SCC score than milk from untreated control heifers (2.63 vs. 2.04).

Prepartum antibiotic treatment to reduce mastitis in Jersey heifers during early lactation was economically beneficial.<sup>20</sup> Actual milk production averaged 5,195 kg (11,429 pounds) for untreated heifers and 5,726 kg (12,597 pounds) for antibiotic-treated heifers. Multiplying the increase in actual milk production [531 kg (1168 pounds)] in prepartum antibiotic-treated heifers by a milk price of \$18.50/hundredweight (cwt) (\$0.407/kg) yielded a \$216.24 per-heifer increase in gross revenue. Treatment costs of \$15.60 were as follows: teat hygiene (\$0.10), which included the cost of a premilking teat disinfectant, barrier postmilking teat disinfectant and disposable paper towel; antibiotics (\$10.00); and labor (\$2.50). Another cost that may arise is the cost of testing for antibiotic residues in milk of heifers that calve too soon after treatment, which we estimated to be \$3.00. Subtracting the cost of treatment (\$15.60/heifer) from gross revenue resulted in a net revenue increase of \$200.64 per heifer. These net revenue figures included the cost of testing for antibiotic residues for all antibiotic-treated heifers.<sup>20</sup>

Break-even analysis indicated that it would be profitable to treat heifers before calving as long as the milk price was above \$0.013 per pound or \$1.30/cwt (\$0.029/kg). Milk price would not likely fall low enough to make treatment of prepartum heifers unprofitable. A similar relationship between the increase in net revenue and the hourly wage rate of labor was determined. Given a

milk price of 0.407/kg (\$0.185/pound), net revenue is equal to zero where the hourly wage rate of labor equals \$812.56/h. This suggests that treating heifers with antibiotics before calving would be profitable for wage rates below \$812.56/h. The relationship between net revenue increases and the increase in kilograms (pounds) of milk produced due to treatment, given a wage rate of \$10.00/h and a milk price of \$0.407/kg (\$0.185/pound) was determined also. Treatment would be profitable as long as the increase in milk production is greater than 38.2 kg (84 pounds).<sup>20</sup>

### **Other Strategies for Controlling Mastitis**

Vaccination as a method to control mastitis in heifers has also been conducted; however, results of those studies are equivocal. Nordhaug et al.<sup>39</sup> used 108 heifers in a placebo-controlled multicenter study to evaluate an experimental *S aureus* mastitis vaccine containing whole, inactivated bacteria with pseudocapsule, alpha and beta toxoids, and a mineral oil as adjuvant. Heifers were injected in the area of the supramammary lymph nodes twice before calving. None of the vaccinated animals developed clinical *S aureus* mastitis, and 8.6% developed subclinical *S aureus* mastitis. Conversely, 16.0% of control heifers developed clinical or subclinical *S aureus* mastitis. Mean SCC in vaccinated and control heifers were the same throughout lactation. In the statistical analyses, when cow was used as the unit of concern, no significant differences occurred between groups. However, when all parameters on udder health were considered together, results indicated a potential protective effect of this vaccine during the entire lactation. More recently, Nickerson et al.<sup>40</sup> suggested a positive effect of vaccination with a polyvalent *S aureus* vaccine by increasing anti-staphylococcal antibody titers and in preventing new *S aureus* infections when the program was initiated at an early age in heifers from a herd with a high exposure to *S aureus*.

A placebo-controlled field study was performed to evaluate the effect of a herd-specific vaccine against *S aureus* on IMI,

SCC, and clinical mastitis.<sup>41</sup> Heifers in the vaccination group (n = 164) were vaccinated twice at 5 weeks and 2 weeks before expected calving. Heifers in the control group (n = 157) received the same treatment with a placebo containing no bacterial antigen. The prevalence of *S aureus* in quarter milk samples taken at calving and 3 to 4 weeks after calving did not differ significantly between the vaccine and control group. Incidence of clinical mastitis during the first 3 months after calving and the prevalence of *S aureus* in quarter milk samples taken before the onset of treatment did not differ significantly between groups. The SCC was lower in vaccinated than in control heifers. However, the difference was only significant on the third milk test day. Use of a herd-specific vaccine against *S aureus* did not prove to be effective on this farm.

Thus, based on the few studies that have been reported, data are equivocal regarding efficacy of vaccination for the prevention of mastitis in heifers. One significant advantage of strategies based on vaccination is that this is a non-antibiotic approach for controlling mastitis, and potential problems associated with antibiotic residues and antibiotic resistance are avoided. One important disadvantage, however, is that vaccination is pathogen specific. Since mastitis in heifers is caused by many different pathogens, vaccination against a single pathogen will not eliminate IMI caused by pathogens not targeted in the vaccine.

Another approach for controlling mastitis in heifers was based on prepartum teat disinfection with a germicide barrier teat disinfectant.<sup>42-43</sup> The effect of teat dipping with a barrier teat dip prior to parturition on IMI and clinical mastitis during the first 5 days postpartum was investigated in a split udder trial in 149 Holstein-Frisian heifers. Two mammary quarters were dipped 3 times weekly with a barrier teat disinfectant containing 0.1% polyvidon iodine from day 260 of gestation until parturition, and the remaining mammary quarters served as untreated controls. Bacteria were isolated

from 52.2% of quarter milk samples collected immediately after parturition prior to first machine milking. *S aureus* and CNS were isolated most frequently (29.2% and 35.6% of the positive samples, respectively). At parturition, 6.7% of heifers showed signs of clinical mastitis and another 27.5% developed signs of clinical mastitis during the first 5 days of lactation. No significant differences in IMI and clinical mastitis were found between mammary quarters dipped in the barrier teat dip prior to parturition and undipped control quarters. The authors concluded that teat disinfection prior to parturition in primigravid dairy heifers did not improve udder health in this trial.<sup>42</sup>

## CONCLUSIONS

Intramammary infections in breeding-age and pregnant heifers occur at a much higher rate than previously thought. Many of these infections can persist for long periods of time, may be associated with elevated SCC, and may impair mammary development during gestation and affect milk production after calving.

Several potential heifer mastitis risk factors have been identified. The presence of IMI before calving increased risk of infection during lactation, IMI at calving increased the risk of clinical mastitis within the first week after calving, and mastitis prior to parturition and mastitis within the first week after calving increased the risk of further cases of mastitis and culling during the first 45 days of lactation. Prepartum intramammary antibiotic infusion of heifer mammary glands is an effective procedure for eliminating many infections in heifers during late gestation and for reducing the prevalence of mastitis in heifers both during early lactation and throughout lactation. Two studies reported that prepartum antibiotic-treated heifers produced significantly more milk than control heifers and had significantly lower SCC scores than untreated control heifers. These observations are likely associated with or due to the lower prevalence of mastitis pathogen isolation in prepartum antibiotic-treated heifers throughout lactation.

One disadvantage of this strategy for controlling heifer mastitis is the potential for antibiotic residues in marketable milk. If heifers are treated with antibiotics before calving, dairy producers must make sure that milk is free of inhibitors, since milk contaminated with antibiotics is unfit for human consumption.

## ACKNOWLEDGMENT

This work was supported by The University of Tennessee Food Safety Center of Excellence, the Tennessee Agricultural Experiment Station, and The University of Tennessee College of Veterinary Medicine Center of Excellence Research Program in Livestock Diseases and Human Health. The authors express their appreciation to personnel in the Lactation/Mastitis/Food Safety Research Program at The University of Tennessee and to personnel at the Dairy Experiment Station and Middle Tennessee Experiment Station for excellent technical assistance.

## REFERENCES

1. Palmer CC, Kakavas JC, Hay J. Studies on bovine mastitis in heifers. *Am J Vet Res.* 1941; 2:18–34.
2. Schalm OW. *Streptococcus agalactiae* in the udder of heifers at parturition traced to suckling among calves. *Cornell Vet.* 1942;32:49–60.
3. Oliver SP, Mitchell BA. Intramammary infections in primigravid heifers near parturition. *J Dairy Sci.* 1983; 66:1180–1183.
4. Aaerstrup FM, Jensen NE. Prevalence and duration of intramammary infection in Danish heifers during the peripartum period. *J Dairy Sci.* 1997; 80:307–312.
5. Fox LK, Chester ST, Hallberg JW, Nickerson SC, Pankey JW, Weaver LD. Survey of intramammary infections in dairy heifers at breeding age and first parturition. *J Dairy Sci.* 1995; 78:1619–1628.
6. Jonsson P, Olsson SO, Olofson AS, Faith C, Holmeberg O, Funke H. Bacteriological investigations of clinical mastitis in heifers in Sweden. *J Dairy Res.* 1991;58:179–185.
7. Matthews KR, Harmon RJ, Langlois BE. Prevalence of *Staphylococcus* species during the periparturient period in primiparous and multiparous cows. *J Dairy Sci.* 1992; 75:1835–1839.
8. Myllys V. Staphylococci in heifer mastitis before and after parturition. *J Dairy Res.* 1995;62:51–60.

9. Nickerson SC, Owen WE, Boddie RL. Mastitis in dairy heifers: initial studies on prevalence and control. *J Dairy Sci.* 1995;78:1607–1618.
10. Oliver SP. Frequency of isolation of environmental mastitis causing pathogens and incidence of new intramammary infection during the nonlactating period. *Am J Vet Res.* 1988;48:1789–1793.
11. Oliver SP, Sordillo LM. Udder health in periparturient period. *J Dairy Sci.* 1988;71:2584–2606.
12. Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH. Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. *J Dairy Sci.* 1992; 75:406–414.
13. Pankey JW, Dreschsler PA, Wildman EE. Mastitis prevalence in primigravid heifers at parturition. *J Dairy Sci.* 1991;74:1550–1552.
14. Smith KL, Hogan JS, Todhunter DA, Weiss WP, Schoenberger PS. Intramammary infection and clinical mastitis in heifers at calving and dynamics over a 14 year period in a dairy herd [abstract]. *J Dairy Sci.* 1994;77(suppl. 1):197.
15. Trinidad P, Nickerson SC, Alley TK. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. *J Dairy Sci.* 1990;73:107–114.
16. Waage S, Mork T, Roros A, Aasland D, Hunshamur A, Odegaard SA. Bacteria associated with clinical mastitis in dairy heifers. *J Dairy Sci.* 1999;82:712–719.
17. Trinidad P, Nickerson SC, Adkinson RW. Histopathology of staphylococcal mastitis in unbred heifers. *J Dairy Sci.* 1990;73:639–647.
18. Harmon RJ, Crist WL, Hemken RW, Langlois BE. Prevalence of minor udder pathogens after intramammary dry treatment. *J Dairy Sci.* 1986;69:843–849.
19. Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH. Antibiotic residues and prevalence of mastitis pathogen isolation in heifers during early lactation following prepartum antibiotic therapy. *J Vet Med B.* 1997;44:213–220.
20. Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH, Jaenicke EC, Roberts RK. Milk production, milk quality and economic benefit associated with prepartum antibiotic treatment of heifers. *J Dairy Sci.* 2003;86:1187–1193.
21. Oliver SP, Gonzalez RN, Hogan JS, Jayarao BM, Owens WE. *Microbiological procedures for the diagnosis of bovine udder infection.* 4<sup>th</sup> ed. Madison, WI: National Mastitis Council, Inc., 2004.
22. Martin-Richard M. Prevalence of mastitis in heifers and associated risk factors. In: *Proceedings of the 3<sup>rd</sup> Symposium de Calidad de Leche y Seguridad Alimentaria.* Leon, Spain, 2001;59–61.
23. Waage S, Sviland S, Odegaard SA. Identification of risk factors for clinical mastitis in dairy heifers. *J Dairy Sci.* 1998;81:1275–1284.
24. Waage S, Odegaard SA, Lund A, Brattgierd S, Rothe T. Case-control study of risk factors for clinical mastitis in postpartum dairy heifers. *J Dairy Sci.* 2001;84:392–399.
25. Roberson JR, Fox LK, Hancock DD, Gay JM, Besser TE. Sources of intramammary infections from *Staphylococcus aureus* in dairy heifers at first parturition. *J Dairy Sci.* 1998;81:687–693.
26. Edinger D, Tenhagen BA, Heuwieser W, et al. Effect of puerperal mastitis in primiparous cows on milk production, cell count and culling. *Dtsch Tierarztl Wochenschr* 1999; 106:470–474.
27. Gillespie BE, Owens WE, Nickerson SC, Oliver SP. Deoxyribonucleic acid fingerprinting of *Staphylococcus aureus* from heifer mammary secretions and from horn flies. *J Dairy Sci.* 1999;82:1581–1585.
28. Owens, WE, Oliver SP, Gillespie BE, Ray CH, Nickerson SC. The role of horn flies (*Haemotobia irritans*) in *Staphylococcus aureus* mastitis in dairy heifers. *Am J Vet Res.* 1998;59:1122–1124.
29. Trinidad, P, Nickerson SC, Luther DG. Antimicrobial susceptibilities of staphylococcal species isolated from mammary glands of unbred and primigravid dairy heifers. *J Dairy Sci.* 1990;73:357–362.
30. Watts JL, Salmon SA, Yancey RJ, et al. Antimicrobial susceptibility of microorganisms isolated from the mammary gland of dairy heifers. *J Dairy Sci.* 1995;78:1637–1648.
31. Owens WE, Nickerson SC, Washburn PJ, Ray CH. Efficacy of a cephalirin dry cow product for treatment of experimentally induced *Staphylococcus aureus* mastitis in heifers. *J Dairy Sci.* 1991;10:3376–3382.
32. Owens WE, Nickerson SC, Washburn PJ, Ray CH. Prepartum antibiotic therapy with a cephalirin dry-cow product against naturally occurring intramammary infections in heifers. *Zentralbl Veterinarmed B.* 1994;41:90–100.
33. Owens WE, Nickerson SC, Boddie RL, Tomita GM, Ray CH. Prevalence of mastitis in dairy heifers and effectiveness of antibiotic therapy. *J Dairy Sci.* 2001; 84:814–817.
34. Owens WE, Ray CH. Therapeutic and prophylactic effect of prepartum antibiotic infusion of heifers. *Zentralbl Veterinarmed B.* 1996; 43:455–459.
35. Trinidad P, Nickerson SC, Alley TK, Adkinson RW. Efficacy of intramammary treatment in unbred and primigravid dairy heifers. *J Am Vet Med Assoc.* 1990;197:465–470.
36. Bishop JR, White CH. Antibiotic residue detection in milk: a review. *J Food Prot.* 1984; 47:647–665.
37. Ginn RE, Case R, Packard VS, Titini S. Quantitative assay of beta lactam residues in raw milk using a disc assay method. *J Food Prot.* 1982; 45:571–577.

38. Oliver SP, Ivey SJ, Gillespie BE, et al. Influence of prepartum intramammary infusion of pirimycin hydrochloride or penicillin-novobiocin on mastitis in heifers during early lactation. *J Dairy Sci.* 2004;87:1727–1731.
39. Nordhaug ML, Nesse LL, Norcross NL, Gudding R. A field trial with an experimental vaccine against *Staphylococcus aureus* mastitis in cattle. 1: Clinical parameters. *J Dairy Sci.* 1994;77:1267–1275.
40. Nickerson SC, Owens WE, Boddie RL. Efficacy of a *Staphylococcus aureus* mastitis vaccine in dairy heifers. In: *Proceedings of the Symposium on Immunology and the Ruminant Mammary Gland*, Stresa, Italy, 2000:426–431.
41. Tenhagen, BA, Edinger D, Baumgartner B, Kalbe P, Klunder G, Heuwieser W. Efficacy of a herd-specific vaccine against *Staphylococcus aureus* to prevent post-partum mastitis in dairy heifers. *J Vet Med Assoc.* 2001; 48:601–607.
42. Edinger D, Tenhagen BA, Kalbe P, Klunder G, Baumgartner B, Heuwieser W. Effect of teat dipping with a germicide barrier teat dip in late gestation on intramammary infection and clinical mastitis during the first 5 days postpartum in primiparous cows. *J Vet Med Assoc.* 2000;47:463–468.