

Heifer Mastitis: How to Help Heifers Calve Clean

Pamela Ruegg, DVM, MPVM
University of Wisconsin, Madison

Introduction

The profitable production of high quality milk from healthy adult cows is the ultimate goal of most heifer management programs. The successful calving of a healthy heifer is the result of investments in effective animal health management over the 2 year period beginning at birth and culminating in the first calving. Deficiencies in health management during this period can result in the occurrence of diseases that will severely decrease the productivity of the heifer as she enters the lactating herd.

Mastitis remains one of the most significant diseases of adult dairy cows and results in considerably reduced profitability for the dairy industry (USDA, 2008a). Mastitis is defined as inflammation of the mammary gland and is almost always caused by bacterial infection of the udder. Subclinical and clinical mastitis are well recognized as occurring in lactating dairy cows and in recent years, mastitis has been recognized as a concern for some heifers. While it is unusual to observe clinical mastitis in heifers prior to calving, it is now understood that heifers can develop subclinical infections with mastitis organisms before calving (Oliver and Mitchell, 1983). The occurrence of mastitis may not be recognized until the heifer begins milking and abnormal milk is noted or the somatic cell count (SCC) of the milk is indicative of subclinical mastitis. Heifers that develop subclinical or clinical mastitis may be less productive and at greater risk for future mastitis cases and thus prevention of the initial infection should be one focus of heifer management programs. The aim of this paper is to review important concepts and research related to heifer mastitis.

Milk Quality Terminology and Background Information. Mastitis caused by a variety of bacterial pathogens and can occur in either a clinical or subclinical state. Clinical mastitis occurs when the bacteria that infect the udder have caused sufficient disruption of udder function so that milk, udder or cow appears visibly abnormal. Clinical mastitis is defined as the production of abnormal milk with or without the occurrence of secondary symptoms such as swollen quarters or an elevated body temperature. Clinical mastitis can be recognized in colostrum or milk by the occurrence of garget, abnormal texture or discoloration. Additional symptoms may include swollen mammary quarters, greatly reduced milk production or an obviously ill cow but fortunately this level of illness is not common. For about 80-90% of the clinical cases, the most severe symptom is simply the occurrence of abnormal appearing milk. Subclinical disease is defined as abnormalities of function that are detectable only by diagnostic or laboratory tests. Subclinical mastitis is the most common mastitis problem on most farms and is the source of somatic cells in the bulk tank. Detection of subclinical mastitis is possible only by use of indirect tests such as enumeration of somatic cells or bacteriological analysis of milk samples (to detect the mastitis pathogen). Cows are usually considered to have subclinical mastitis when the SCC of a quarter exceeds about 200,000 cells/ml but lower thresholds (such as 100,000cells/ml) may indicate the occurrence of mastitis

in heifers. Subclinical mastitis is often undetected and has the greatest economic consequence because the udder infections result in long term reductions of milk yield. The occurrence of subclinical intramammary infections (IMI) in non-lactating heifers was once considered rare but in recent years, studies have indicated that the prevalence of IMI in heifers can be as high as 97% in some herds (Trinidad et al., 1990b; Trinidad et al., 1990c). These infections are important because infections acquired during the prepartum period may damage developing secretory tissue, increase the SCC (Trinidad et al., 1990a; Trinidad et al., 1990b; Nickerson et al., 1995; Oliver et al., 2003) and reduce lifetime milk production (Oliver et al., 2003, De Vliegher, et al., 2004). Therefore, the control of IMI in heifers is receiving increased attention.

Mastitis is caused by a variety of bacteria that usually enter the udder when the teats become exposed to large numbers of the pathogens. Bacteria that cause mastitis are often classified as “contagious” or “environmental” based on their primary reservoir and mode of transmission. The udder of cows with subclinical infections serves as the primary reservoir for contagious pathogens and transmission occurs when teats of healthy animals are exposed to organisms in milk that originated from infected udders. Calves can be exposed to mastitis bacteria when they are fed raw milk that came from infected cows. Droplets of infected milk left on milking equipment, towels used to dry teats of several cows, hands of milking technicians or infected milk left on bedding surfaces are common mechanisms for spread of contagious mastitis. In the U.S., the most common contagious mastitis pathogens are *Staphylococcus aureus*, and *Mycoplasma bovis* but a few herds may still experience problems with *Streptococcus agalactiae* (USDA, 2008). Successful control programs for contagious mastitis focus on reducing exposure of teats to pathogens found in milk that came from infected cows.

The term “environmental pathogen” refers to mastitis caused by opportunistic bacteria that reside in the environment. Common environmental mastitis pathogens include Gram negative bacteria (such as *E. coli* and *Klebsiella* spp.) and Gram positive bacteria (such as *Streptococcus uberis* and *Streptococcus dysgalactia*). Some environmental pathogens are more likely to cause clinical mastitis while others may result in long term subclinical infections. Springing heifers often become exposed to these pathogens when they are in contact with moisture, mud, and manure in precalving areas. Successful control of environmental pathogens is based on reducing exposure of teats to pathogens present in the environment. Heifers that are exposed to wet and muddy environments during the precalving period often calve with subclinical infections caused by environmental bacteria, have high SCC at their first DHIA test and may be at greater risk for clinical mastitis during the early lactation period.

Diagnosis of Mastitis in Heifers

Mastitis in heifers is rarely apparent prior to calving but few farms actively look for mastitis before animals calve. Subclinical mastitis is difficult to detect because the milk appears normal but the SCC is elevated and bacteria may be present. There is no absolute threshold of SCC that defines the occurrence of subclinical mastitis but DHIA centers use a threshold of >200,000 cells/ml (>linear score 4.0) to indicate the likely presence of subclinical IMI.

Review of SCC values of the first monthly DHIA test is a good method to monitor udder health of heifers. More than 90% of heifers should have SCC <200,000 cells/ml (<linear somatic cell score of 4.0) at the first test. It is important to recognize that SCC values obtained from Dairy Herd Improvement tests are from milk that is co-mingled from all four quarters. Single quarter infections in heifers may not be apparent until the SCC value of the infected quarter is very high. An indirect cowside test such as the California Mastitis Test (CMT), the PortaSCC (from PortaCheck) or use of the Direct Cell counter (Delaval) can be used to identify quarters that are likely to be infected. These tests can be used on fresh heifers to detect differences between quarters and are most accurate for use at least 5 days post-calving. While the CMT is the least expensive option, it should be interpreted cautiously because the slightest amount of thickening indicates the presence of a SCC that exceeds 200,000 cells/ml. After day 5, all quarters with CMT reactions of trace or greater should be suspected of subclinical mastitis.

Microbiologic exam of milk samples obtained from suspect quarters or cows is a standard diagnostic procedure and it is important to determine the pathogen in order to determine the best control strategy. If quarters are sampled before calving, it is extremely important that samples are obtained using strict hygienic conditions so that infections aren't inadvertently introduced during sampling. Differences in sample collection technique, in shedding patterns of bacteria and in laboratory procedures make the interpretation of microbiologic results difficult. The isolation of mastitis causing bacteria from milk samples or secretions obtained from the udder prior to calving is highly suggestive that a quarter is infected with mastitis. However, the lack of bacterial growth from a single milk sample is not always diagnostically useful and about 50% of milk samples obtained from quarters with high SCC will be microbiologically negative when examined in the laboratory (Makovec and Ruegg, 2003). The absence of bacteria in a milk sample obtained from a high SCC quarter does not always indicate that the bacteria are gone, as this outcome can be a result of intermittent shedding.

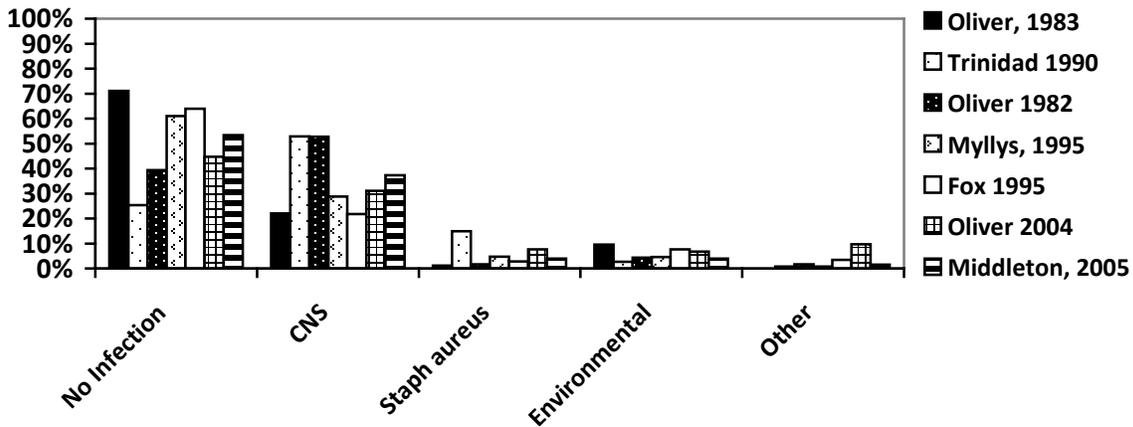
Prevalence & Pathogens That Cause Mastitis in Dairy Heifers

While many farms raise mastitis free dairy heifers, in some studies, bacteria have been isolated from more than 50% of quarters of prepartum heifers (Oliver and Mitchell, 1983, Fox et al., 1995; Trinidad et al., 1990). In a review of heifer mastitis studies, the prevalence of mastitis pathogens recovered from mammary secretions obtained before the first parturition ranged from 29% to 74% (Figure 1) while the prevalence of infection at first calving ranged from 12% to 57% (Fox, 2009)). Recovery of *Mycoplasma* organisms was not included in these studies.

Staphylococci (primarily coagulase-negative Staphylococci but also *Staphylococcus aureus*) are the most frequent isolates recovered from heifers but environmental pathogens such as Streptococci are also frequently recovered (De Vliegher, 2004). The CNS are comprised of >40 species of staphylococci. The name of this group of bacteria is derived from a common laboratory test that is used to differentiate *Staphylococcus aureus* from other staphylococci. CNS are the most common pathogens recovered

from heifers and a variety of CNS species have been recovered from teat skin, the streak canal and pre-calving udder secretion obtained from heifers (Borm et al., 2006; Nickerson et al., 1995). While CNS are the most common group of bacteria recovered from post-partum heifers, many of the apparent infections disappear within the first week after calving. One recent study, indicated that 41% of mammary quarters had CNS infections between days 1 and 4 post-calving but 46% of those infections had spontaneously disappeared by days 5-8 post-calving (Piepers et al., 2007). However, some herds may experience persistent infections with CNS in heifers resulting in increased SCC values and potentially decreased milk production. These herds require interventions to limit long-term effects on milk quality and production.

Figure 1. Recovery of mastitis pathogens from secretion of dairy heifers before calving



Staphylococcus aureus is one of the most important and costly mastitis pathogens because it deeply invades the udder tissue to cause chronic infections that greatly reduce milk quality and quantity. *Staph aureus* can be difficult to control in lactating herds and infection of dairy heifers usually indicates that a significant number of lactating cows are infected. While many herds have controlled *Staph aureus*, regional differences in the prevalence of infected dairy heifers have been reported (Fox, 2009). The exact mechanism of how non-lactating heifers develop *Staph aureus* infections is not known but studies have demonstrated that teat canals of heifer calves can become colonized at very young ages (Nickerson, 2009). Contagious mastitis organisms such as *Staph aureus* live primarily in the udders of infected cows and are spread to heifers when they are fed non-pasteurized milk or colostrum or when calves come in contact with objects contaminated with infected milk (Roberson, et al., 1998). While, ingested milk does not spread from the digestive tract to the udder, it is possible that calves may lick their udder or legs and colonize the teat skin, eventually leading to infections. Contagious mastitis caused by *Staph aureus* can also spread to heifers during the immediate pre or postpartum period. The use of fresh pens to house sick cattle can result in contamination of the bedding with secretions (milk, blood, feces etc.) from sick animals. These secretions may remain infectious for variable periods of time (depending upon the characteristics of the organism, the type of bedding and the environmental conditions) and serve as a point of exposure. Milking fresh heifers with equipment that has been previously used on cows that are shedding contagious mastitis organisms is also a common route of exposure. Several studies have demonstrated

that biting flies can play a role in the transmission of *Staph aureus* between infected and uninfected heifers (Nickerson et al., 1995). It is thought that flies congregating on teat ends of infected cows can become contaminated with bacteria present in milk droplets and mechanically transfer the bacteria when they land on the teats of uninfected animals. Co-mingling of preweaned calves is another risk factor for the development of contagious mastitis in heifers. Preweaned heifers that are grouped together may suckle on teats of other calves and transfer mastitis causing bacteria among the group.

Environmental mastitis pathogens such as *Streptococci spp.*, *E. coli* and others live primarily in moisture, mud and manure in the heifers housing environment. It is thought that heifers can become infected with these organism through continuous or overwhelming exposure in their environment. It is likely that the greatest risk for infection by these pathogens is during the precalving period when udder development is proceeding rapidly.

Mycoplasma bovis is an important disease causing organism of cattle. This organism is associated with respiratory disease, ear infections, joint infections, mastitis and other disease of dairy calves and cows (Maunsell and Donovan, 2009). Mastitis caused by *M. bovis* often causes serious long term infections with both subclinical and clinical symptoms and there are no treatments or vaccines that are considered effective. The mycoplasma organism is very tiny and lacks a cell wall which makes it resistant to many antibiotics (such as β -lactams) that work by destroying cell walls. *M. bovis* dwells primarily in the respiratory tract of cattle without causing clinical disease in the host. It is also unique in that respiratory infections can spread through the blood stream to infect other mucosal tissue such as the udder. Animals colonized with *M. bovis* can develop long term infections and infect other animals. *M. bovis* is usually introduced into herds by clinically healthy cattle that are carriers of the organism (Maunsell and Donovan, 2009). The organisms can be present in the respiratory tract or in the udders of cows with clinical or subclinical infections. *M. bovis* can colonize the developing udders of very young prepubertal heifers and eventually cause mastitis (Fox et al., 2008). The feeding of waste milk from infected cows has long been considered a primary route of infection with *M. bovis*. One study demonstrated that 100% of 50 calves fed milk experimentally contaminated with *M. bovis* became colonized in their respiratory tracts (Brown et al., 1998). Comingling of calves from different sources has consistently been identified as a risk factor for infection with this organism. Control of *M. bovis* is based on reducing the potential for contact with potentially infected shedders of the organism and ensuring that calves never receive unpasteurized milk. The organism's ability to colonize the respiratory tract of cattle also requires strict attention to limiting the potential for airborne transmission, thus adequate ventilation, isolation of calves from older animals or ill calves and reduced stocking densities are common sense precautions.

Risk Factors for Mastitis in Heifers

Most risk factors that contribute to the development of mastitis in heifers are related to exposure of heifers to mastitis causing organisms. The exception to this is the occurrence of udder edema. Udder edema occurs primarily in heifers and is a well

recognized risk factor for mastitis. A combination of genetics, diet and housing generally contribute to the development of udder edema in heifers. When udder edema occurs, the circulation of blood and lymph fluid through the udder is impaired and the function of the milk secreting cells is disrupted. Mastitis is a common consequence of this scenario. Prepartum milking of affected heifers has been shown to reduce the risk of developing mastitis but care should be taken to manage energy balance to prevent the development of ketosis (McDougall, et al., 2009).

Feeding of waste milk to calves is a well-recognized risk factor for mastitis in heifers and this practice should be discouraged unless the milk can be pasteurized prior to feeding. The exact mechanism that the organism is transferred to the udder is unknown but it is likely related to colonization of the teat skin and inner thighs with mastitis causing organisms. The prevalence of subclinical and clinical mastitis in the lactating cows is another important risk factor for mastitis in heifers (Fox, 2009). Herds that have a large number of cows infected with contagious mastitis pathogens are likely to have more heifers infected at their first calving. Housing decisions may affect the prevalence of mastitis. Calves that are group housed have the opportunity for cross suckling thus resulting in increased risk for transmission of contagious pathogens (McDougall, et al., 2009). Several studies have shown that contact of heifers with older cows before calving (even housing in the same barn) increases the risk of clinical mastitis after calving and separation of heifers from older cows is generally recommended (Barkema et al., 1999).

The hygiene of the environment is also an important determinant of the risk of developing mastitis in heifers, and this is true for heifers raised on pasture as well as heifers raised in confinement (Compton et al., 2007). Heifers with dirty udders postcalving and with teats that are closer to the ground have been shown to be at greater risk of mastitis. This is especially true for infections with environmental pathogens such as *Streptococci* and *Klebsiella* spp.

Prevention of Mastitis in Heifers

Mastitis occurs when teats are exposed to mastitis pathogens in sufficient quantities to defeat the response of the cows' immune system. Prevention of mastitis in heifers is based upon reducing exposure to mastitis pathogens and enhancing the ability of the heifers' immune system to respond. Prevention of mastitis in heifers include the following strategies: 1) Controlling the prevalence of mastitis in the existing adult herd - exposure to contagious mastitis is more likely to occur when many animals are infected as compared to herds with few animals infected. 2) The use of individual stalls for preweaned calves. 3) Culling of calves that persist in suckling other calves. 4) Feed milk replacer or pasteurized milk from healthy uninfected cows rather than waste milk. 5) Control flies – especially important in the control of *Staph aureus*. 6) Milk fresh heifers first using clean milking equipment. 7) Calve heifers in clean pens that have not been used to house sick cows. 8) House growing and prepartum heifers in an adequately bedded area that is clean and dry and provides sufficient space for all animals. 9) Feed a well-balanced diet that enhances the immune systems of the heifers. Recommended values include 1000 IU per day vitamin E during the prefresh

period and adequate selenium. 10) Boost the immunity of heifers by using gram negative J-5 vaccines – these vaccines work to reduce the severity of infections caused by gram negative bacteria such as E. coli. 11) problem herds may consider prepartum teat dipping several times a week (Lopez-Benavides et al., 2009) or the use of internal teat sealants administered about 1 month pre-calving (Parker et al., 2008).

Prepartum Intramammary Treatment of Heifers

Intramammary therapy with antibiotics is one of the tools used to control mastitis in heifers (Nickerson, 2009). Intramammary treatment of prepartum heifers is generally considered to be efficacious in reducing the prevalence of infection, can result in a reduction of SCC and in some cases has been shown to increase milk production in the subsequent lactation (Trinidad et al., 1990b; Nickerson et al., 1995; Owens et al., 2001; Oliver et al., 2003, Sampimon, et al., 2009). However, research shows that the production response is highly variable among herds and this strategy should be only routinely recommended for herds that have demonstrated problems with heifer mastitis (Borm et al., 2006). Oliver et al. (1992) reported cure rates of 84% and 97%, respectively for heifers infected with CNS that received intramammary treatment using 200 mg of either sodium cloxacillin or cephapirin sodium, the spontaneous cure rate was 27% for heifers in an untreated control group. Research has shown that administration of intramammary antimicrobial treatments during late gestation is generally highly effective and cure rates for Staphylococcal infections frequently exceed 90% (Oliver et al., 2003). Commercial intramammary products designed for both lactating and non-lactating cows (dry cow products) have been evaluated for use in pre-calving heifers (Nickerson, 2009). In virtually all instances, high bacteriological cure rates have been reported for both types of products and for treatments administered at different times throughout the gestation period. No significant different in treatment efficacy was found based on treatments given during the first, second or third trimester of pregnancy but fewer new Staph aureus infections were reported for animals treated in the 3rd trimester (Owens et al., 2001; Middleton et al., 2005). However, to minimize the potential for residues after calving, treatments should be administered at least 60 days before the expected due date and milk should be checked for residues after calving. Unlike intramammary treatments, systemic antibiotics administered to heifers have not been shown to be effective in reducing the prevalence of mastitis (McDougal et al., 2005).

The effect of precalving treatment on production of heifers appears to be variable among herds (Fox et al., 1995) and is not performed without introducing the risk of infections caused by improper administration of the intramammary tubes. The administration of internal teat sealants or intramammary antibiotics should only occur under hygienic conditions and following effective sanitation of teat ends. This practice is generally only recommended for herds that have demonstrated problems with heifer mastitis.

References

- Barkema, H. W., Y. H. Schukken, T.J. Lam, M.L. Beiboer, G. Benedictus and A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. *J Dairy Sci* 82:1643-1654.
- Borm, A.A., L.K.Fox, K. E. Leslie, J.S. Hogan, S.M. Andrew, K.M. Moyes, S.P. Oliver, Y.H. Schukken, D.D. Hancock, C.T. Gaskins, W.E. Owens, and C. Norman, C., 2006. Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers. *J. Dairy Sci.*, 89:2090-2098.
- Brown, M. B. D. M. Dechant, and G. A. Donovan. 1998. Association of *Mycoplasma bovis* with otitis media in dairy calves. *IOM Lett* 12:104-105.
- Compton, C. W. R., C. Heurer, K. Parker, and S. McDougall. 2007. Risk factors for peripartum mastitis in pasture-grazed heifers. *J Dairy Sci.* 90:4171-4180.
- De Vlieghe, S. 2004. Udder Health in Dairy Heifers - some epidemiological and microbiological aspects. PhD Thesis, Dept. of Repro, Obstetrics and Herd Health, Fac. Vet. Med. Ghent, University, Belgium.
- Fox, L. K., 2009. Prevalence, incidence and risk factors of heifer mastitis. *Vet Micro.* 134:82-88.
- Fox, L. K., S. T. Chester, J. W. Hallberg, S. C. Nickerson, J. W. Pankey, and L. D. Weaver. 1995. Survey of intramammary infections in dairy heifers at breeding age and first parturition. *J. Dairy Sci.* 78:1619-28.
- Fox, L.K., F. J. Muller, M. L. Wedam, C. S. Schneider, and M. K Biddle. 2008. Clinical *Mycoplasma bovis* mastitis in prepubertal heifers on 2 dairy herds. *Can Vet J* 49:1110-1112.
- Lopez-Benavides, M. G. , J. H. Williamson, S. J. Lacy-Hulbert, R. T. Cursons. 2009. Heifer teats sprayed in the dry period with an iodine teat sanitizer have reduced *Streptococcus uberis* teat end contamination and less *Streptococcus uberis* intramammary infections at calving. *Vet Micro* 134:186-191.
- Makovec JA and P.L Rugg. 2003. Characteristics of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. *J Dairy Sci* 86:3466-3472.
- Maunsell, F.P., G. A. Donovan. 2009. *Mycoplasma bovis* infections in young calves. *Vet Clin. Food Anim* 25:139-177.
- McDougall, S. K. Parker, C. Compton, and C. Heuer. 2005. Reducing subclinical and clinical mastitis in dairy heifers by precalving infusion of a teat sealant and/or parenteral antibiotic therapy. Proc. 4th Intl. Dairy Fed. Intl. Mastitis Conf. Maastricht, The Netherlands. Pp 269-273.
- McDougall, S., K. J. Parker, C. Heuer, and C. W. R. Compton. 2009. A Review of prevention and control of heifer mastitis via non-antibiotic strategies. *Vet Micro.*, 134:177-185.
- Middleton, J.R., L. L. Timms, R. Bader, J. Lakritz, C. D. Luby and B. J. Steevens. 2005. Effect of prepartum intramammary treatment with pirlimycin hydrochloride on prevalence of early first-lactation mastitis in dairy heifers. *J Am Vet Med Assoc* 27:1969-1974.
- Nickerson, S. C., 2009. Control of heifer mastitis: antimicrobial treatment – An overview. *Vet Micro.* 134:128-135.

- Nickerson, S.C., W. E. Owens, and R. L. Boddie., 1995. Mastitis in dairy heifers: initial studies on prevalence and control. *J. Dairy Sci.* 78, 1607-1618.
- Nickerson, S. C., 2009. Control of heifer mastitis: antimicrobial treatment – an overview. *Vet Micro* 134-:128-135.
- Oliver, S.P. and B.A. Mitchell. 1983. Susceptibility of bovine mammary gland to infections during the dry period. *J Dairy Sci* 66:1162-1166.
- Oliver, S.P., M.J. Lewis, B.E. Gillespie, and H.H. Dowlen. 1992. Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. *J Dairy Sci* 75:406-414.
- Oliver, S. P. M. J. Lewis, B.E. Gillespie, H. H. Dowlen, E. c. Janicke, and R.K. Robers. 2003. Milk production, milk quality and economics benefit associated with prepartum antibiotic treatment of heifers. *J Dairy Sci* 86:1187-1193.
- Owens, W. E., S. C. Nickerson, R. L. Boddie, G. M. Tomita, and C. H. Ray. 2001. Prevalence of mastitis in dairy heifers and effectiveness of antibiotic therapy. *J. Dairy Sci.* 84:814-817.
- Pankey, J. W. 1989. Premilking udder hygiene. *J Dairy Sci* 72:1308-1312.
- Parker, K. I., C.W.R. Compton, F. M. Annis, C. Heuer, and S. McDougall. 2008. Quarter-level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant or an injectable antibiotic or both precalving. *J Dairy Sci* 91:169-181.
- Piepers, S., S. De Vlieghe, A. de Kruif, C. Opsomer, 2007. Evaluation of quarter-milk somatic cell counts of dairy heifers in early lactation. In Proc. NMC 46th Ann. Meeting San Antonio TX, pp 250-251.
- Pol M, and P. L. Ruegg. 2007. Treatment practices and quantification of antimicrobial usage in conventional and organic dairy farms in Wisconsin. *J Dairy Sci* 90:249-261.
- Roberson, J. R., L. K. Fox, D. D. Hancock, J. M. Gay, and T. E. Besser. 1998. Sources of intramammary infections from *Staphylococcus aureus* in dairy heifers at first parturition. *J Dairy Sci* 81:687-693.
- Sampimon, O. C., S. de Vlieghe, H. W. Barkema, J. Sol, and T.J. G.M. Lam. 2009. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. *J Dairy Sci* 92:4395-4403.
- Trinidad, P., S. C. Nickerson, and T. K. Alley. 1990a. Prevalence of intramammary infection and
- Trinidad, P. S. C. Nickerson, and T. K. Alley. 1990b. Efficacy of intramammary treatment in unbred and primigravid dairy heifers. *J Am Vet Med Assoc* 197:107-114.
- teat canal colonization in unbred and primigravid dairy heifers. *J. Dairy Sci.* 73:107-14.
- USDA, 2008. Prevalence of contagious mastitis pathogens on U.S. dairy operations, 2007. USDA_APHIS_VS, http://nahms.aphis.usda.gov/dairy/dairy07/Dairy07_is_ContMastitis.pdf.