



Evaluation of the California Mastitis Test as a precalving treatment selection tool for Holstein heifers

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ABSTRACT

The objective of this study was to evaluate the California Mastitis Test (CMT) and a portable electrical conductivity meter for diagnosing precalving intramammary infection (IMI) in Holstein heifers. A total of 428 dairy heifers from 23 dairy herds were enrolled between 6 and 12 days before the expected calving date from June 2002 to June 2003. Mammary secretions were tested by both diagnostic methods and by bacterial culture for evidence of IMI. California Mastitis Test was considered negative if the score was negative, trace or 1 and was considered positive otherwise. Two cut-off points were evaluated for milk electrical conductivity (>5 and >6.5 mS/cm). From this study, an overall proportion of 69% of heifers had precalving IMI and the overall heifer prevalence of major pathogen IMI was 16.8%. At the quarter level, sensitivity and specificity of CMT (68.9% and 68.4%, respectively) and milk conductivity >5 mS/cm (41.0% and 65.2%, respectively) or >6.5 mS/cm (25.2% and 83.3%, respectively) to identify all IMI were low. However, the heifer level sensitivity and specificity of CMT for major pathogens were 91.0% (81.5–96.6) and 27.5% (22.8–32.6), respectively. Using a cut-off point of 5 mS/cm, the heifer level sensitivity and specificity for major pathogens was 68.7% (56.2–79.4) and 44.1% (38.7–49.6), respectively. A conductivity cut-off value of 6.5 mS/cm decreased the sensitivity and increased the specificity to 53.7% (41.1–66.0) and 59.5% (54.0–64.8), respectively. California Mastitis Test and milk electrical conductivity are not good predictors of major pathogen IMI in heifers during the last 2 weeks before calving. However, the negative predictive values at quarter or heifer level were high and the heifer false negative rate was 6–14% using CMT or conductivity, respectively. Therefore, these measures could be useful for screening out heifers or quarters that are unlikely to have a major pathogen IMI.

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Abbreviations: CMT, California Mastitis Test; IMI, intramammary infection; CNS, coagulase-negative staphylococci; SCC, somatic cell count; DIM, days in milk; NMC, National Mastitis Council; TSA, trypticase soy agar; BHI, brain heart infusion; cfu, colony forming unit; mS, millisie-men; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

1. Introduction

The importance of mastitis in heifers has been well recognized for 15 years (Oliver and Mitchell, 1983; Trinidad et al., 1990a; Pankey et al., 1991; Oliver et al., 1992; Fox et al., 1995; Owens et al., 2001). During this period, many studies on heifer intramammary infection (IMI) prevalence have been conducted, demonstrating up to 97% of heifers infected (Oliver and Mitchell, 1983; Trinidad et al., 1990a; Pankey et al., 1991; Oliver et al., 1992; Fox et al., 1995; Nickerson

et al., 1995; Owens et al., 2001). Those studies have reported that 24–75% of quarters and 25–97% of heifers are infected precalving; most IMI are caused by coagulase-negative staphylococci (CNS). Heifer IMI occurs at a critical moment in the mammary development of heifers, causing a decrease in the quantity of glandular tissue with the potential to reduce milk production during subsequent lactations (Nickerson et al., 1995). Infected heifers may produce less milk during their first lactation (Boddie et al., 1987; Myllys and Rautala, 1995). Furthermore, somatic cell count (SCC) is increased in infected heifers (Boddie et al., 1987; Trinidad et al., 1990a; Hallberg et al., 1995), and many IMI may persist for an extended period (Boddie et al., 1987; Oliver et al., 1997b).

Various methods to prevent and control heifer IMI have been considered (Schultze, 1985; Matthews et al., 1988; Nordhaug et al., 1994; Edinger et al., 2000; Tenhagen et al., 2001). The most promising approach is intramammary infusion of short or long acting antibiotic formulations during the precalving period. The proportions of precalving heifer IMI cases that are resolved at calving are greater for treated heifers than those for untreated heifers, even in the case of *Staphylococcus aureus* infection (Oliver et al., 1997a; Owens et al., 2001; Oliver et al., 2004). Using antibiotics in the precalving period may increase milk production (Trinidad et al., 1990b; Owens et al., 1991; Oliver et al., 2003), and decreases SCC in treated heifers (Trinidad et al., 1990b; Oliver et al., 2003).

However, the efficacy of precalving treatment can be variable among herds (Borm et al., 2006). Also, there are some concerns expressed by veterinarians, producers and consumers about risks of antibiotic residue in milk and about rational antibiotic use. An approach targeting only infected quarters or infected heifers would decrease the risk of antibiotic residue, theoretically decrease the risk of antibiotic resistance and decrease the total cost of precalving heifer treatment.

An ideal diagnostic tool to select quarters or heifers for precalving treatment should be quick, easy to perform on-farm, reliable and inexpensive. Milk bacteriology does not correspond to those criteria. It would be desirable that the appearance of mammary secretion could identify IMI, but it is not accurate enough (Hallberg et al., 1995; Owens et al., 2001). The CMT and hand-held milk electrical conductivity meter are already used on-farm to diagnose indirectly IMI on lactating cows. The sensitivity and specificity of CMT reported in the literature is variable (Pyörälä, 2003). For example, at 3 days in milk (DIM), Sargeant et al. (2001) found that sensitivity and specificity to identify any IMI at a quarter level were 57% and 56%, respectively. On the other hand, Vijaya Reddy et al. (1998) reported a sensitivity of 71% and a specificity of 75%. Sensitivity and specificity are improved when only major pathogens are considered (Sargeant et al., 2001; Dingwell et al., 2003). Several portable milk electrical conductivity meters were tested in the past (Okigbo et al., 1984; Hillerton and Walton, 1991; Musser et al., 1998). The sensitivity and specificity of electrical conductivity as a mastitis detection tool are variable as demonstrated by Nielsen et al. (1992) who reported 66% and 94%, respectively based on meta-analysis which is a statistical

synthesis of the data from a comparable set of existing studies on electrical conductivity. No data are currently available using CMT or milk conductivity on precalving heifers.

The objective of this study was to evaluate the CMT and a portable conductivity meter as diagnostic tools for precalving IMI in Holstein heifers.

2. Materials and methods

2.1. Herds and animal selection

A total of 428 Holstein heifers from 23 dairy herds in the region of Saint-Hyacinthe, Quebec, Canada were enrolled in the study. The participating herds were a convenience sample selected for willingness of the dairy producer to participate in the study, facilities to restrain heifers for sampling and geographical proximity of the farms to the researchers. A minimum of 8 gravid heifers per herd were enrolled between 6 and 12 days before the expected calving date over a 12 months period from June 2002 to June 2003. Heifers could not have received an antibiotic treatment during the previous 3 months and could not have been treated for a previous episode of mastitis. This study was part of another one (Roy et al., 2007) which evaluated the effect of precalving antibiotic treatment of pirlimycin hydrochloride (Pirsue, Pfizer Animal Health, Kirkland, QC) on Holstein heifers. In the initial study, 219 heifers were treated and 209 heifers were untreated controls. The precalving data from all heifers were used for this study.

2.2. California Mastitis Test

California Mastitis Test (CMT) was done on the farm before taking milk samples for bacteriology. CMT results were scored accordingly to the manufacturers guidelines (Dairy Research Products, Ancaster, ON, Canada) and results were noted as negative (N), trace (T), 1, 2 or 3. An equal volume of milk and reagent were mixed together (approximately 5 mL each) and an evaluation of the degree of gel formation was done by gently rotating CMT paddle and the results were noted. Then, duplicate milk samples were taken using aseptic technique from each quarter of all heifers. Teats were disinfected with gauze soaked in a 70% alcohol solution before sampling. The teat disinfectant used on the farm was applied daily for 3 days afterward to all heifers. Only one milk sample per quarter was taken when the quantity of secretion was insufficient. Milk samples were kept cool for transport and frozen within 12 h for later bacteriological analysis.

2.3. Bacteriological analysis

The first milk sample was sent to the clinical bacteriology laboratory at the Faculté de Médecine Vétérinaire of the Université de Montréal where the bacteriological analyses were performed according to NMC guidelines (NMC, 2004). Samples were thawed overnight in a refrigerator or for 30 min in a water bath. Samples were inoculated onto one quadrant of a trypticase soy agar plate enriched with 5%

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