

The association between the somatic cell count and isolated microorganisms during subclinical mastitis in heifers in Jordan

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ABSTRACT: Mastitis is an important cause of early culling of dairy heifers. The aims of this study were to identify microorganisms that cause subclinical mastitis (SCM) in Holstein heifers in northern Jordan, to estimate the relative importance of various microorganisms, and to examine the correlation between the somatic cell count (SCC) and the isolated microorganisms. Composite milk samples were collected aseptically from 133 Holstein heifers between Days 5 and 14 postpartum for bacterial culture and somatic cell counts. The prevalence of subclinical mastitis in this study was 57.1%. Seventy six (76) of the 133 cows tested had SCC of > 250 000 cells/ml. Bacteria were isolated from 36 (27.1%) cows. About sixty six percent (66.7%) of these isolates were cultured from cows with subclinical mastitis. Coagulase-negative Staphylococci (CNS) were the most prevalent bacteria among isolates (50%), followed by *Staphylococcus aureus* (22.2%), *Corynebacterium bovis* and coliforms (*E. coli* and *Klebsiella pneumoniae*) (5.6% each), and *Streptococcus uberis* (2.8%). Interestingly, the majority of the negative-culture samples (53.6%) had elevated SCC. All isolates except for the CNS group exhibited strongly elevated SCC (> 1×10^6 cells/ml). However, the coagulase-negative Staphylococci did not have a distinct pattern with respect to SCCs. There was a strong correlation ($P \leq 0.05$, correlation coefficient 0.213) between the results of bacterial culture (culture-positive vs. culture-negative) and SCC class but not between SCC class and the type of isolated bacteria. It is concluded that subclinical mastitis appears to be a significant health issue for dairy heifers in Jordan.

Keywords: subclinical mastitis (SCM); somatic cell count (SCC); heifers; aetiology; prevalence; Jordan

Mastitis, in general, is one of the most widespread and damaging diseases in dairy cows (Erskine 2011). Heifer mastitis is considered as a condition which is discrete from mastitis in older cows and has a different presentation (Valde et al. 2004) and distinctive features (Waage et al. 2001; Alekish and Kenyon 2006; Compton and McDougall 2008; Godden et al. 2008; De Vliegher et al. 2012).

Intramammary infections in heifers in early lactation potentially cause severe economic losses (Seegers et al. 2003; De Vliegher 2005a) because of detrimental effects on udder development and future production leading to an increased risk of premature removal from the herd (Rupp et al. 2000; Waage et al. 2000; Oliver et al. 2003; De Vliegher et al. 2005a; De Vliegher et al. 2005b; Santman-Berends et al. 2012; Archer et al. 2013). The prevalence of subclinical mastitis in heifers varies widely among

studies and ranges from 12% to over 57% of quarters infected (Barkema et al. 1999b; De Vliegher et al. 2001; Bowers et al. 2006; Andersen et al. 2010; Santman-Berends et al. 2012). Coagulase-negative Staphylococci (CNS) are the most prevalent cause of subclinical mastitis in heifers. In some studies, Coagulase-positive Staphylococci (CPS) were the second most prevalent pathogens, while environmental Streptococci found in soil, manure, and bedding ranked third on the list (Nickerson et al. 1995; Fox 2009; Nickerson 2009; De Vliegher et al. 2012).

Somatic cell count (SCC) in milk is an important indicator of the health status of the udder and remains the most widely used tool to diagnose subclinical mastitis (Nickerson 2009). Most studies suggest that cows with SCC of less than 200 000 cells/ml are not likely to be infected with major mastitis pathogens, while cows with SCC

of 300 000 cells/ml or greater are very likely to be infected (NMC 1997).

In Jordan, several studies have been conducted in order to investigate bovine mastitis (Lafi et al. 1994; Lafi and Hailat 1998a; Lafi and Hailat 1998b; Hawari and Al-Dabbas 2008), but only one study focused on mastitis in heifers (Al-Tarazi et al. 2011). In Jordan, research aimed at improving control measures for infectious diseases, especially mastitis, is still in its infancy at best. Therefore, the objectives of this study were to identify microorganisms that cause subclinical mastitis (SCM) in Holstein heifers in northern Jordan, to estimate the relative importance of various microorganisms, and to examine the correlation between the somatic cell count (SCC) and the isolated microorganisms.

MATERIAL AND METHODS

Study area and animal population. One hundred and thirty three (133) primiparous dairy cows were used in the study. This investigation was mainly conducted in the northern part of Jordan, where the major population of cows is Friesian-Holstein. This is also the major dairy breed in the county as a whole, with the rest being local breeds (DOS 2014). The animals involved in this study were randomly selected from 50 different medium-sized (50–200 cows) dairy farms. The traditional housing system for this size farm in Jordan is the confinement system, which comprise either padded packs or stanchions barns. The feeding system consists of component-fed rations, in which concentrates are fed separately from forages. The weight of the heifers ranged from 520–590 kg. All heifers were selected by the farm veterinarian after complete physical examination. The selected animals were agreed upon by the author after a telephone discussion with every farm veterinarian. The heifers were clinically healthy, with no apparent local or systemic signs of inflammation or any alterations in milk appearance or contents.

Milk sample collection. Composite milk samples were collected aseptically by the farm veterinarians following standard milk sampling techniques (Sears et al. 1993; Quinn et al. 1994; Oliver et al. 2004). Milk was collected from heifers between Days 5 and 14 postpartum. The teats were cleaned and wiped with 70% alcohol solution before taking the samples. The samples were collected from all four quarters as follows: near front, near rear, far rear and then far front

while holding the cap with the same hand as that holding the tube so as to minimize contamination as much as possible. The first three to five squirts of milk were discarded and approximately 10 milliliters of milk were collected in sterile tubes. Samples were kept on ice during transport to the laboratory. Somatic cell counting and bacteriological examination were always performed between 2 and 4 h after the sample collection.

Somatic cell count. Somatic cell count was determined by spreading 0.01 ml of thoroughly mixed milk from each sample over a 1 cm² area on a glass slide. The slides were left to air-dry on a flat surface and were stained by Newman-Lampert stain and examined microscopically using the procedure outlined by Embert (1986). Twenty to forty fields were counted for each sample to ensure reproducibility and accuracy. Grouping of SCCs was chosen arbitrarily by the author. A threshold of 250 000 cells/ml was considered a cutoff point for classification of subclinical mastitis in individual samples. Somatic cell counts were classified into three groups as follow: SCC1: SCC < 250 × 10³ cells/ml, SCC2: SCC = 250 × 10³ to 1 × 10⁶ cells/ml, SCC3 > 1 × 10⁶ cells/ml.

Bacteriological examination. All samples were prepared and cultured according to standard methods (Hogan et al. 1999). Separate samples were cultured on blood and MacConkey agar then incubated aerobically at 37 °C for up to 48 h. Bacteria on culture-positive plates were characterised mainly according to their gram-stain reaction, haemolytic features and colony morphology. The staphylococcal isolates were identified as *Staphylococcus aureus* and coagulase-negative Staphylococci (CNS) using catalase, tube coagulase, and fermentation tests for the production of acid from glucose, mannitol, and maltose. CNS strains were identified to the species level using a microtube identification system (Microbact, Staph 12S Staph-Ident System MB1561, Oxiod, UK). *Escherichia coli* and other Enterobacteriaceae were identified by catalase, oxidase, and IMVIC tests as well as growth on MacConkey agar and metallic sheen on EMB agar. A gram negative identification system was used for strain identification (Microbacter GNB 24E, Oxiod UK). Streptococci spp were identified using the CAMP test, esculin hydrolysis, hemolysis, and by the absence of growth on MacConkey agar. The Strepto system 9R (Lio Filchem S.KI, Italy) was used for speciation of Streptococci.

Statistical analysis. Statistical analysis of means and frequencies was performed to determine the

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prevalence of subclinical mastitis in heifers, as well as to show the distribution of the isolated microorganisms among all samples. The correlation between different somatic cell count classes, bacterial growth (culture-positive vs. culture-negative) and type of isolated bacteria was estimated using Spearman's correlation. All statistical analyses were carried out using SPSS v.17.0 software.

RESULTS

Prevalence of subclinical mastitis

The prevalence of subclinical mastitis in this study was 57.1%. Of the 133 cows sampled, 76 had a SCC of > 250 000 cells/ml (Figure 1).

Bacterial isolates

The number of bacterial species isolated and their distribution are shown in Table 1. Of all the milk samples examined, bacteria were isolated from 36 (27.1%) cows. About sixty six percent (66.7%) of these isolates were cultured from cows with subclinical mastitis, defined as those with SCC > 250 000 cells/ml. Only half as many isolates were from heifers with SCCs within the normal range. Coagulase-negative Staphylococci (CNS) were the most prevalent bacteria among isolates (50%), followed by *Staphylococcus aureus* (22.2%), *Corynebacterium* spp. and coliforms (*E. coli* and *Klebsiella pneumoniae*) (5.6% each), and *Streptococcus uberis* (2.8%). *Staphylococcus chromogenes* was cultured most frequently from the CNS-positive milk samples (19.4%), followed by *Staphylococcus epidermidis* (16.7%).

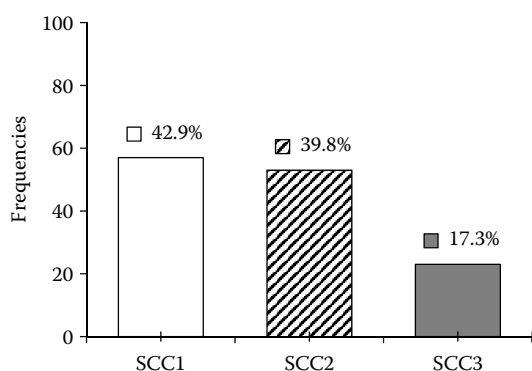


Figure 1. The frequency and the percentage of positive SCC

Table 1. Bacterial isolation rate and microorganism distribution among the positive culture samples

Bacterial isolates	Frequency	Percentage
<i>Staphylococcus aureus</i>	8	22.2
<i>Staphylococcus hyicus</i>	1	2.8
<i>Staphylococcus simulans</i>	2	5.6
<i>Staphylococcus cohnii</i>	2	5.6
<i>Staphylococcus chromogenes</i>	7	19.4
<i>Staphylococcus epidermidis</i>	6	16.7
<i>E. coli</i>	1	2.8
<i>Klebsiella pneumoniae</i>	1	2.8
<i>Streptococcus uberis</i>	1	2.8
<i>Corynebacterium bovis</i>	2	5.6
Contamination	5	13.9
Total	36	100

Somatic cell counts and bacterial cultures

The median of the somatic cell count among positive-culture samples was 460 000 cells/ml, while among the negative-culture samples the median was 280 000 cells/ml. Most of the cows that were positive by culture had subclinical mastitis (*Staphylococcus aureus*: 75%, CNS: 72.1%, *Corynebacterium*: 50%, *Streptococcus uberis*: 100% and coliforms: 100%). All isolates except for the CNS group were associated with highly elevated SCCs (> 1×10^6 cells/ml) (Figure 2). However, the coagulase-negative *Staphylococci* isolates did not have a distinct pattern with respect to elevated SCCs. Among all coagulase-negative *Staphylococci* isolates, 33.3% were associated with a highly elevated SCC (> 1×10^6 cells/ml), 38.8% were associated with a modestly elevated SCC (250×10^3 to 1×10^6 cells/ml) and 27.7% did not affect the SCC (< 250×10^3 cells/ml). A majority of the negative-culture samples (53.6%) had elevated SCCs; 43.3% of which were associated with a modest increase in the SCC (250×10^3 to 1×10^6 cells/ml), while 10.3% were associated with a highly increased SCC (> 1×10^6 cells/ml). Correlation analyses revealed a strong correlation ($P \leq 0.05$, correlation coefficient 0.213) between bacterial culture results (culture-positive vs. culture-negative) and SCC class. However, no correlation was detected between SCC class and the type of isolated bacteria.

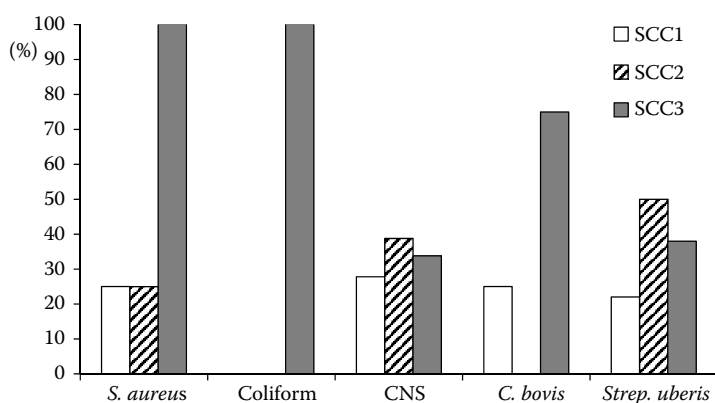


Figure 2. The SCC distribution among the most prevalent bacterial isolates

DISCUSSION

A study performed by Dr. Stephen Nickerson in 1995 was the first to remove the veil on the high prevalence of mastitis in the heifer population. Subsequent studies worldwide have investigated this important issue (Barkema et al. 1999a; Compton and McDougall 2008; De Vliegher et al. 2012), since heifers represent the genetic future and productivity of the dairy herd. However, the emphasis in these studies was on the prevalence of clinical mastitis rather than the subclinical form of the disease. In this study I have tried to draw attention to the prevalence of subclinical mastitis because of its massive effect on milk production as well as on future udder development.

In this study, the prevalence of subclinical mastitis in heifers in northern Jordan was 57.1%. This finding is similar with the rate reported in the Netherlands, United States and Belgium (Barkema et al. 1999b; Bowers et al. 2006; De Vliegher et al. 2001), where the rate ranges between 12 to over 57%. However, in an earlier study conducted in Jordan (Al-Tarazi et al. 2011), the rate of subclinical mastitis in heifers was reported to be 94%. However, these authors used the California mastitis test (CMT) as a diagnostic method for subclinical mastitis, while I used somatic cell counting, which is a more sensitive method for detection of subclinical mastitis (NMC 1997; Rupp et al. 2000; Lafi 2006). In addition, the geographical area of sample collection might have contributed to differences in the observed prevalence rate.

The prevalence rate (64%) of environmental pathogens (CNS 50%, *Corynebacterium bovis* 5.6%, coliform 5.6% and *Streptococcus uberis* 2.8%) as the major cause of subclinical mastitis in the present study is in agreement with other studies conducted regionally and worldwide (Oliver et al. 2004; Bowers et al.

2006; Compton and McDougall 2008; Andersen et al. 2010). A high prevalence of environmental pathogens is suggestive of poor cleaning and preparation of the udder before milking. Contaminated bedding, dirty environments, and colonisation of teat skin and teat injuries are potential sources of environmental pathogens (Santman-Berends et al. 2012).

Evidence for the presence of contagious mastitis was strengthened by the isolation of *Staphylococcus aureus* from 22.2% of the culture-positive samples. This finding runs counter to those of other studies that have been conducted nationally and internationally. Studies that have targeted the prevalence rate of contagious mastitis in Jordan showed a higher prevalence rate (35–45%) (Lafi et al. 1994; Hawari and Al-Dabbas 2008; Al-Tarazi et al. 2011), whereas in other parts of the world a lower rate was reported (0.5–14%) (De Vliegher et al. 2012). The reasons for this discrepancy may lie in the warmer weather in Jordan as well as the lack of implementation of hygiene control measures, fly control, optimal nutrition, inter-sucking among young stock in addition to the comfort measures, especially around calving. On the other hand, the lower rate reported in this work compared to other works that have been carried out in the same region might be attributed to fluctuating climatic conditions throughout different seasons of the year when the samples were collected, and may also be influenced by the geographical area of sample collection. Since much of the world is selecting for mastitis resistance, the genetic differences between the heifers in this study and previous studies may also explain some of the differences.

In this study most *Staphylococcus aureus*, *Streptococcus uberis* and *Corynebacterium bovis*-positive samples had SCCs of more than 1×10^6 cell/ml; their properties as major pathogens that robustly trigger activation of the immune system may be

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responsible for this. However, the coagulase-negative Staphylococci did not have a distinct pattern with respect to elevated SCC values. There was a strong correlation ($P \leq 0.05$, correlation coefficient 0.213) between the results of bacterial culture (culture-positive vs. culture-negative) and SCC class but not between SCC class and the type of isolated bacteria. These results were expected and indicate that increased SCCs in infected udders is a result of a non-specific immune response. In general, the increase in the SCC might depend on the host immune system at the time of the infection in addition to the pathogenicity of the isolated organism. This might explain why CNS isolates did not exhibit any pronounced tendency with respect to elevated SCC values, although they are considered less pathogenic to the mammary gland and theoretically produce persistent subclinical mastitis with a modest increase in the SCC.

Interestingly, in this study a majority of the culture-negative samples (53.6%) had elevated SCCs. An elevation in the normal SCC values in the absence of bacterial growth on blood agar may be attributed to many factors; presence of antibiotic traces in the milk, incorrect handling, prolonged time required for transport of samples resulting in a fall in the number of mastitis-causing bacteria to non-detectable levels, numbers of bacteria being below detectable levels (10–100 organisms/ml) at collection time. This may occur with *Staphylococcus aureus*, coliforms and *Mycoplasma* spp. Additionally, it is possible that bacteria may not be viable or that clinical signs may be due to bacterial products or, alternatively, that the organisms may have been killed by the cow's immune system. Finally, infection may be caused by organisms not cultivable in routine culture (anaerobes, or organisms requiring special nutrients).

Also, it is probable that SCC values might change with the stage of bacterial insult. Moreover, in several studies statistical analysis on complete SCC and bacterial culture data were performed and showed no association between SCC and bacterial isolation (Alekish et al. 2014; Al-Majali and Jawabreh 2003; Hariharan et al. 2004).

REFERENCES

- Alekish MO, Kenyon SJ (2006): A review of milking-machine-induced teat-end lesions and susceptibility to mastitis. *Bovine Practitioner* 40, 18–24.
- Alekish MO, Alshehabat MA, Abutarbush SM (2014): The prevalence and etiology of subclinical mastitis in Awassi sheep; emphasis on the relationship between the isolated organisms and the somatic cell count. *European Journal of Veterinary Medicine* 2014, 8. Available online at <http://scik.org> issn 2051-297x.
- Al-Majali AM, Jawabreh S (2003): Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. *Small Ruminant Research* 47, 243–248.
- Al-Tarazi YH, Chakiso AY, Lafi SQ (2011): Prevalence and distribution of bovine mastitis pathogens and their antimicrobial resistance in primiparous dairy heifers in Northern Jordan. *Jordan Journal of Agricultural Sciences* 7, 529–539.
- Andersen SI, Dohoo R, Riekerink O, Stryhn H (2010): Diagnosing intramammary infections: Evaluating expert opinions on the definition of intramammary infection using conjoint analysis. *Journal of Dairy Science* 93, 2966–2975.
- Archer SC, McCoy F, Wapenaar W, Green MJ (2013): Association between somatic cell count early in the first lactation and the longevity of Irish dairy cows. *Journal of Dairy Science* 96, 2939–2950.
- Barkema HW, Schukken YH, Lam TJGM, Beiboer ML, Benedictus G, Brand A (1999a): Management practices associated with the incidence rate of clinical mastitis. *Journal of Dairy Science* 82, 1643–1654.
- Barkema HW, Deluyker HA, Schukken YH, Lam TJGM (1999b): Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Preventive Veterinary Medicine* 38, 1–9.
- Bowers SS, Gandy K, Graves S, Eicher, Willard S (2006): Effects of prepartum milking on postpartum reproduction, udder health and production performance in first-calf dairy heifers. *Journal of Dairy Research* 73, 257–263.
- Compton C, McDougall S (2008): Heifer mastitis – A review of recent studies. *Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA*, 77–90.
- De Vliegher S, Laevens H, Opsomer G, De Muelenaere E, De Kruif A (2001): Somatic cell counts in dairy heifers during early lactation. *Vlaams Diergeneeskundig Tijdschrift* 70, 212–215.
- De Vliegher S, Barkema HW, Stryhn H, Opsomer G, De Kruif A (2005a): Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. *Journal of Dairy Science* 88, 938–947.
- De Vliegher S, Barkema HW, Opsomer G, De Kruif A, Duchateau L (2005b): Association between somatic cell count in early lactation and culling of dairy heifers using Cox frailty models. *Journal of Dairy Science* 88, 560–568.
- De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW (2012): Invited review: Mastitis in dairy heifers: Na-

- ture of the disease, potential impact, prevention, and control. *Journal of Dairy Science* 95, 1025–1040.
- DOS – Department of Statistics (2014): http://www.dos.gov.jo/dos_home_e/main/agriculture/census/tables/tab7_7.pdf Access date, March 1st, 2014. Amman, Jordan.
- Embert HC (ed.) (1986): Mastitis diagnosis. In: *Veterinary Clinical Pathology*. Saunders, Philadelphia. 395–453.
- Erskine RJ (2011): Mastitis in cattle. *Merck Veterinary Manual*. Merck & Co., New York. 1010–1015.
- Fox LK (2009): Prevalence, incidence and risk factors of heifer mastitis. *Veterinary Microbiology* 16, 82–88.
- Godden S, Bey R, Lorch K, Farnsworth R, Rapnicki P (2008): Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. *Journal of Dairy Science* 91, 151–159.
- Hariharan H, Donachie W, Macaldowie C, Keefe G (2004): Bacteriology and somatic cell counts in milk samples from ewes on a Scottish farm. *Canadian Journal of Veterinary Research* 68, 188–192.
- Hawari AD, Al-Dabbas F (2008): Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan. *American Journal of Animal and Veterinary Sciences* 3, 36–39.
- Hogan JS, Gonzalez RN, Harmon RJ, Nickerson SC, Oliver SP, Pankey JW, Smith KL (1999): *Laboratory Handbook on Bovine Mastitis*. Rev. ed. NMC Inc., Madison, WI.
- Lafi SQ (2006): Use of somatic cell counts and California Mastitis Test results from udder halves milk samples to detect subclinical intramammary infection in Awassi sheep. *Small Ruminant Research* 62, 83–86.
- Lafi SQ, Hailat NQ (1998a): Bovine and ovine mastitis in dulail valley of Jordan. *Veterinariski Arhiv* 68, 51–57.
- Lafi SQ, Hailat NQ (1998b): Incidence and antibiotic sensitivity of bacteria causing bovine and ovine clinical mastitis in Jordan. *Pakistan Veterinary Journal* 18, 88–94.
- Lafi SQ, Al-Rawashdeh O, Nawas T, Hailat NQ (1994): National cross-sectional study of mastitis in dairy cattle in Jordan. *Tropical Animal Health and Production* 26, 168–174.
- Nickerson SC (2009): Control of heifer mastitis: antimicrobial treatment-an overview. *Veterinary Microbiology* 16, 128–135.
- Nickerson SC, Owens WE, Boddie RL (1995): Mastitis in dairy heifers: Initial studies on prevalence and control. *Journal of Dairy Science* 78, 1607–1618.
- NMC – National Mastitis Council (1997): *A Look at Physiological and Regulatory SCC Standards in Milk*. NMC Newsletter Udder Topics.
- Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH, Jaenicke EC, Roberts RK (2003): Prepartum antibiotic treatment of heifers: Milk production, milk quality and economic benefit. *Journal of Dairy Science* 86, 1187–1193.
- Oliver SP, Gonzales RN, Hogan JS, Jayarao BM, Owens WE (2004): *Microbiological Procedures for the Diagnosis of Bovine Udder Infection*. 4th ed. National Mastitis Council, Inc. Madison, WI.
- Quinn P, Carter E, Morley B, Carter R (eds.) (1994): *California Mastitis Test (CMT)*. In: *Clinical Veterinary Microbiology*. 1st ed. Wolfe Publishing, London. 333–334.
- Rupp R, Beaudeau F, Boichard D (2000): Relationship between milk somatic cell counts in the first lactation and clinical mastitis occurrence in the second lactation of French Holstein Cows. *Preventive Veterinary Medicine* 46, 99–111.
- Santman-Berends IM, Olde Riekerink RG, Sampimon OC, van Schaik G, Lam TJ (2012): Incidence of subclinical mastitis in Dutch dairy heifers in the first 100 days in lactation and associated risk factors. *Journal of Dairy Science* 95, 2476–2484.
- Sears M, Gonzalez N, Wilson J, Han R (1993): Procedures for mastitis diagnosis and control. *Veterinary Clinics of North America, Food Animal Practice* 9, 445–468.
- Seegers H, Fourichon C, Beaudeau F (2003): Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary Research* 34, 475–491.
- Valde JP, Lawson LG, Lindberg A, Agger JE, Saloniemi H, Osteras O (2004): Cumulative risk of bovine mastitis treatments in Denmark, Finland, Norway and Sweden. *Acta Veterinaria Scandinavica* 45, 201–210.
- Waage S, Skei HR, Rise J, Rogdo T, Sviland S, Odegaard SA (2000): Outcome of clinical mastitis in dairy heifers assessed by re-examination of cases one month after treatment. *Journal of Dairy Science* 83, 70–76.
- Waage S, Odegaard SA, Lund A, Brattgjerd S, Rothe T (2001): Case-control study of risk factors for clinical mastitis in postpartum dairy heifers. *Journal of Dairy Science* 84, 392–399.

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