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Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds

O.C. Sampimon [a,*], H.W. Barkema [b], I.M.G.A. Berends [a], J. Sol [a], T.J.G.M. Lam [a,c]

[a] GD Animal Health Service, Deventer, The Netherlands
[b] Department of Production Animal health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada
[c] Dutch Udder Health Centre, Deventer, The Netherlands

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ABSTRACT

In this study, the prevalence of intramammary infection (IMI) with coagulase-negative staphylococci (CNS) in The Netherlands was estimated on 49 randomly selected herds with at least 40 lactating cows. In total, 4220 quarter milk samples were collected. The prevalence of CNS IMI in The Netherlands was estimated at 10.8% at quarter level and 34.4% at cow level, making it the most frequently isolated group of pathogens. Fourteen species of CNS were identified; the most frequently isolated species was *Staphylococcus chromogenes* (30.3%) followed by *Staphylococcus epidermidis* (12.9%) and *Staphylococcus capitis* (11.0%). Prevalence of CNS IMI was higher in heifers compared to older cows. Geometric mean quarter SCC of CNS-positive quarters was 109,000 cells/ml, which was approximately twice as high as culture-negative quarters. Quarters infected with *S. chromogenes*, *S. capitis* and *Staphylococcus xylosus* had a higher SCC ($P < 0.05$) than culture-negative quarters, while quarters that were culture-positive for *S. epidermidis* and *Staphylococcus hyicus* tended to have a higher SCC than culture-negative quarters. An increased prevalence of CNS IMI was associated with the herd-level variables source of drinking water not being tap water, housing of dry cows in one group instead of multiple groups, measurement of cow SCC every month, udder health monitoring by the veterinarian, pasturing during outdoor season, percentage of stalls contaminated with milk, and BMSCC > 250,000 cells/ml. Although a causal relation between these factors and prevalence of CNS is not proven and for some factors not even likely, knowledge of the associations found may be helpful when approaching CNS problems on dairy farms.

1. Introduction

Mastitis is one of the most important diseases in dairy cattle, resulting in significant losses (Halasa et al., 2007). It is a multifactorial disease which is hard to manage, and numerous management factors, such as housing facilities, milking procedures, feeding programmes, treatment regimes and others influence udder health on a dairy farm. More than 100 bacterial species can cause bovine mastitis (Smith and Hogan, 2001). At the herd-level it is important to know which pathogens are involved in clinical and subclinical mastitis, to be able to implement the correct management practices in case of an udder health problem. Also at a population level, it is important to know which pathogens are the most important ones, and what their antibiotic sensitivity pattern is. This can help advisors in choosing the right strategy in problem solving and in giving specific preventive advice.

* Corresponding author at: GD Animal Health Service, Arnsbergstraat 7, PO Box 9, 7400 AA Deventer, The Netherlands. Tel.: +31 570660367; fax: +31 570660345.
E-mail address: o.sampimon@gddeventer.com (O.C. Sampimon).

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Coagulase-negative staphylococci (CNS) are historically considered to be minor mastitis pathogens and are frequently not reported in mastitis studies (Bramley and Dodd, 1984). However, CNS are isolated from cases of subclinical and clinical mastitis, and also from teat canals, teat skin and teat ducts (Devriese and Keyser, 1979; Barkema et al., 1998; De Vliegher et al., 2003; Bradley et al., 2007; Sampimon et al., 2007). Additionally, CNS are the most frequently isolated pathogens in heifer mastitis (Trinidad et al., 1990; Matthews et al., 1991). Recently, several prevalence studies have been performed around the world. In almost all studies CNS were the most frequently isolated group of species from milk samples of cows with high SCC (Pitkälä et al., 2004; Bradley et al., 2007; Piepers et al., 2007). However, prevalence of CNS IMI in Dutch dairy herds has never been quantified.

Control programmes such as the "five-point program" work well for control of intramammary infections (IMI) with contagious major pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* (Neave et al., 1969; Sampimon et al., in press). However, these programs seem to be less effective to reduce prevalence of CNS IMI (Taponen and Pyörälä, 2007). As a result of the decrease of the prevalence of IMI with contagious major pathogens, CNS have become relatively more important. Although CNS have not been proven causal in all mastitis cases, they are considered as the leading cause of subclinical mastitis on most dairy farms implementing mastitis control practices (Harmon and Langlois, 1995). However, in other studies CNS had a protective effect on IMI with major pathogens (Lam et al., 1997b; De Vliegher et al., 2004). Factors such as parity, stage of lactation, breed and numerous management practices can influence the prevalence of a specific pathogen. These factors, however, have rarely been studied in relation to CNS.

The aim of this study was to determine the prevalence of IMI with CNS in Dutch dairy cows, and possible herd-level risk factors.

2. Materials and methods

2.1. Herds and cows

Data were obtained from a national subclinical mastitis prevalence study of 49 herds of at least 40 lactating cows. Herds were stratified by province (12 provinces), and the number of randomly selected herds per province was proportional to the total number of herds per province that had at least 40 lactating cows. To select a representative sample of the population it was calculated that approximately 50 herds were needed (Sampimon et al., in press). A total of 61 herds was selected and 49 agreed to participate in the study. These herds were sampled across the period of the study within regions. Based on the currently used cut-off levels in The Netherlands by the Dutch DHI, all quarters of high SCC (HSCC) cows (SCC $>$ 250,000 cells/ml) and heifers (SCC $>$ 150,000 cells/ml) were sampled. Additionally, a subset of approximately 25% of cows and heifers with a low SCC (LSCC), cows (SCC $\leq$ 250,000 cells/ml) and heifers (SCC $\leq$ 150,000 cells/ml), which were randomly selected were sampled once. The milk samples were collected within 1 week after arrival of the milk recording results on the farm. Cows that were dried off, culled or treated with antibiotics in the mean time were excluded from sampling. Samples were collected between April 2003 and September 2004. Selected cows were sampled during regular milking times. Pre-milking udder preparation with a cotton or paper towel was carried out by the farmer. All samples were collected according to the NMC protocol by trained personnel of the GD Animal Health Service (GD) in Deventer, The Netherlands. Teat ends were disinfected with a cotton swab soaked in methylated alcohol, and after the third stripping, milk was collected. The milk samples were transported on ice to the GD for bacteriology and determination of SCC. Bulk milk SCC was measured once, in a period 2 weeks before the sampling of the individual cows.

2.2. Laboratory analyses

The samples were split and SCC was determined using a Fossomatic cell counter (Foss Electric, Hillerød, Denmark). Bacteriological culturing was carried out according to the NMC protocol (Harmon et al., 1990). Major pathogens were considered to cause an IMI if $\geq$100 cfu/ml was isolated, while isolation of $\geq$500 cfu/ml of a minor pathogen was defined as an IMI. A colony of CNS was distinguished from *S. aureus* using Slidex Staphytect Plus (Oxoid, UK). Milk samples from which more than two bacterial species were cultured were considered to be contaminated. Directly after culturing, all milk samples were stored at $-20\,^{\circ}$C. From the 530 milk samples in which CNS was found, 160 milk samples were randomly selected. These samples were thawed at room temperature and then cultured again. Coagulase-negative staphylococci were speciated with the API Staph ID 32 (BioMérieux, Lyon, France) using the procedures recommended by the manufacturer. Probability of the identification result in a range of 10–100% was calculated using the Apilab software. Suggested tests for the delineation of species were executed.

2.3. Questionnaire

A questionnaire was conducted to obtain information on management factors of the farms such as housing facilities, milking procedures, feeding and treatment regimes. The questionnaire was validated before use at 10 dairy farms. During the farm visit to collect milk samples, the questionnaire was conducted by personnel of GD. The questions were aimed at the year preceding the sampling date. The items included in the questionnaire are summarized in eight categories of management practices in Table 1.

2.4. Statistical analysis

Cow level prevalence of CNS IMI and 95% confidence intervals were calculated for the HSCC and LSCC groups separately. To determine a national prevalence, the