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1 **Coagulase-negative staphylococci – emerging mastitis pathogens**

2

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Accepted Manuscript

1 Abstract

2
3 Coagulase-negative staphylococci (CNS) have become the most common bovine mastitis
4 isolate in many countries and could therefore be described as emerging mastitis pathogens. The
5 prevalence of CNS mastitis is higher in primiparous cows than in older cows. CNS are not as
6 pathogenic as the other principal mastitis pathogens and infection mostly remains subclinical.
7 However, CNS can cause persistent infections, which result in increased milk somatic cell count
8 (SCC) and decreased milk quality. CNS infection can damage udder tissue and lead to decreased
9 milk production. *Staphylococcus simulans* and *Staphylococcus chromogenes* are currently the
10 predominant CNS species in bovine mastitis. *S. chromogenes* is the major CNS species affecting
11 nulliparous and primiparous cows whereas *S. simulans* has been isolated more frequently from
12 older cows. Multiparous cows generally become infected with CNS during later lactation whereas
13 primiparous cows develop infection before or shortly after calving. CNS mastitis is not a
14 therapeutic problem as cure rates after antimicrobial treatment are usually high. Based on current
15 knowledge, it is difficult to determine whether CNS species behave as contagious or
16 environmental pathogens. Control measures against contagious mastitis pathogens, such as post-
17 milking teat disinfection, reduce CNS infections in the herd. Phenotypic methods for
18 identification of CNS are not sufficiently reliable, and molecular methods may soon replace
19 them. Knowledge of the CNS species involved in bovine mastitis is limited. The dairy industry
20 would benefit from more research on the epidemiology of CNS mastitis and more reliable
21 methods for species identification.

22

23 **Key words:** Mastitis; Bovine; Staphylococci; Coagulase-negative staphylococci

1 **1. Introduction**

2
3 To date, more than 50 *Staphylococcus* species and subspecies have been characterized.
4 The genus is divided into coagulase-positive staphylococci and coagulase-negative staphylococci
5 (CNS) based on their ability to coagulate plasma. More than ten different CNS species have been
6 isolated from mastitic bovine milk samples, and the species most commonly reported are
7 *Staphylococcus chromogenes* and *Staphylococcus simulans* (Trinidad et al., 1990b; Matthews et
8 al., 1992). *Staphylococcus hyicus* and *Staphylococcus epidermidis* have also frequently been
9 isolated (Myllys, 1995; Thorberg et al., 2006). In routine mastitis diagnostics, CNS are normally
10 not identified to species level but treated as a uniform group. CNS have traditionally been
11 considered to be minor mastitis pathogens, especially in comparison with major pathogens such
12 as *Staphylococcus aureus*, streptococci and coliforms. The main reason for this is that mastitis
13 caused by CNS is very mild, and usually remains subclinical (Taponen et al., 2006). The
14 significance of CNS, however, needs to be reconsidered as in many countries they have become
15 the most common mastitis-causing agents (Pitkälä et al., 2004; Tenhagen et al., 2006). Cows and
16 heifers can be infected with CNS before calving (Boddie et al., 1987; Trinidad et al., 1990b;
17 Green et al., 2005). In lactation, CNS infection is associated with an increased milk somatic cell
18 count (SCC), which can result in economic losses due to milk price penalties incurred for reduced
19 quality. Increased SCC has also been shown to be associated with decreased milk production
20 (Timms and Schultz, 1987; De Vliegher et al., 2005).

21
22 The aim of this paper is to review the literature on general aspects of intramammary infection
23 caused by CNS and to assess its significance in dairy herds.

1

2 **2. Prevalence of CNS mastitis**

3

4 Some decades ago, CNS were seldom reported as a cause of mastitis, or they were
5 classified as “secondary bacteria” (Verhoeff et al., 1981). In a study from the U.K. in the late
6 1970s, 1.7% of clinical mastitis cases were reported to be due to *S. epidermidis* (Pearson and
7 Mackie, 1979). The main focus of mastitis control in the 1970s, 1980s and 1990s was on the
8 contagious major pathogens *Streptococcus agalactiae* and *S. aureus* (Myllys et al., 1998). In
9 early studies, staphylococci that could be distinguished from *S. aureus* on the basis of colony
10 morphology and the coagulase test were generally classified as *S. epidermidis* or “other
11 micrococci” (Klastrup and Madsen, 1974). Gradually, CNS have become the predominant
12 pathogen isolated from subclinical bovine mastitis in many countries (Myllys et al., 1998). In a
13 study from Germany, 35% of quarters with subclinical mastitis harbored CNS (Tenhagen et al.,
14 2006). In Tennessee in the USA, the average proportion of CNS infections in high SCC herds
15 was 28% (Roberson et al., 2006), and herd prevalence ranged from 12 to 41%. In Dutch herds,
16 CNS were isolated from 6% of quarters with bacterial growth in high SCC cows (Poelarends et
17 al., 2001). In a study carried out in the US and Canada, 15% of new intramammary infections
18 post partum were due to CNS (Dingwell et al., 2004). In an earlier Canadian study, quarter
19 prevalence of CNS infections ranged from 5% to 6% during early lactation and increased from
20 14% to 17% towards the end of lactation (Davidson et al., 1992). In a survey from Estonia, 16%
21 of the quarters positive for bacterial growth harbored CNS (Haltia et al., 2006). The highest
22 prevalence of intramammary infections with CNS was reported in Finland, where CNS were
23 isolated from 50% of the quarters positive for bacterial growth in a nationwide survey (Pitkälä et

1 al., 2004). In a similar survey in Norway, the prevalence of CNS was 16% (Østerås et al., 2006).
2 It is difficult to compare results from different countries because the number of colony forming
3 units (CFU) per ml that is used as cut-off to categorize samples as CNS-positive varies between
4 studies. In the Finnish survey with the high prevalence, detection of 500 CFU/ml was used to
5 classify a sample as CNS positive, whereas the cut-off value in the Norwegian survey was 4000
6 CFU/ml. Use of a high CFU/ml cut-off for diagnosis of CNS infections may contribute to
7 underreporting of CNS mastitis.

8 The proportion of CNS among bacteria isolated from clinical mastitis cases remains very
9 low in many countries. In a recent study from Canada, CNS were isolated from 6% of quarters
10 with clinical mastitis (Olde Riekerink et al., 2007). In a Wisconsin study on milk samples from
11 clinical and subclinical mastitis obtained between 1994 and 2001 (Makovec and Ruegg, 2003),
12 the proportion of CNS isolates increased from 12.7% to 17.5%, but separate results were not
13 provided for clinical mastitis. In Sweden, CNS comprised only 6% of bacteria isolated from
14 clinical mastitis (Ekman and Østerås, 2004). In Switzerland, the respective figure was 17%
15 (Schällibaum, 2001) and in Israel 9% (Shpigel et al., 1998). Among 77,051 routine mastitis
16 samples submitted to laboratories in Finland during 2004-2006, CNS were the most frequently
17 isolated bacteria in samples from clinical (18%) and subclinical (24%) mastitis cases (Koivula et
18 al., 2007). In the practice area of the Faculty of Veterinary Medicine, University of Helsinki,
19 Finland, more than 20% of bacterial isolates from milk samples from clinical mastitis were CNS
20 (Nevala et al., 2004). In a study on clinical mastitis carried out in the same area about 30 years
21 ago, the proportion of CNS was only 6.5% (Pyörälä and Syväjärvi, 1987). Seasonal differences in
22 occurrence of CNS mastitis have been reported. In Finland, the prevalence of CNS and *S. aureus*
23 mastitis was highest during winter and spring, i.e. during the indoor season (Koivula et al., 2007).

1 In Norway, too, the highest prevalence of CNS mastitis was found during the late indoor season
2 (Østerås et al., 2006). The proportion of CNS is generally high in samples collected from animals
3 with subclinical mastitis but low in samples from animals with clinical mastitis. In countries
4 where the biggest udder health problems are caused by major environmental mastitis pathogens,
5 CNS infections may often be ignored.

6 The prevalence of CNS mastitis is higher in primiparous cows than in older cows
7 (Matthews et al., 1992; Poelarends et al., 2001; Tenhagen et al., 2006). CNS can colonize the
8 mammary gland of pregnant heifers (White et al., 1989; Myllys, 1995), and CNS were isolated
9 from the mammary gland and teat apices of heifers as young as 10 months old (Boddie et al.,
10 1987; De Vliegher et al., 2003). In intensive management systems, the prevalence of quarters of
11 precalving heifers infected with CNS can exceed 50% (Trinidad et al., 1990b; Oliveira et al.,
12 2006). In pasture-based grazing systems a lower prevalence (16%) has been reported (Parker et
13 al., 2007). Even under grazing conditions, CNS were the predominant isolates in precalving
14 heifers (77% of the bacteriologically positive quarters).

15 CNS are important pathogens in cattle of all ages, but the predominant CNS species
16 causing infection seems to differ between age groups. *S. chromogenes* was the major CNS
17 species in pre-calving heifers and primiparous cows (Trinidad et al., 1990b; Rajala-Schultz et al.,
18 2006; Taponen et al., 2006), whereas *S. simulans* was mostly isolated from cows in later
19 lactations (Taponen et al., 2006). Multiparous cows generally become infected with CNS during
20 later lactation whereas primiparous cows usually already have the infection at the beginning of
21 lactation (Gröhn et al., 2004; Taponen et al., 2007).

22

1 3. Clinical characteristics and effects on milk quality and yield

2

3 CNS usually cause subclinical or mild clinical mastitis, but they have also been reported

4 to produce severe local and systemic signs (Jarp, 1991). Reports on the clinical characteristics of

5 CNS mastitis are scant as CNS have been ignored in many studies on clinical mastitis. In a recent

6 Finnish study, half of the intramammary infections due to CNS were clinical, but in the majority

7 of the cases the signs were very mild (Taponen et al., 2006). No significant differences in the

8 severity of clinical signs caused by the two most common CNS species were found in that study,

9 which agrees with the findings of a previous study (Jarp, 1991). CNS infection is generally seen

10 as an increase in the SCC in milk of the infected quarter. Milk SCC usually remains below

11 500,000 cells/ml (Djabri et al., 2002). In a study in which dairy cows were followed-up

12 throughout the whole lactation, the geometric mean SCC was over 600,000 cells/ml in quarters

13 with persistent CNS infection, and about 60,000 cells/ml in healthy quarters (Taponen et al.,

14 2007). Even a transient CNS infection caused a temporary increase in milk SCC, which is

15 consistent with the report of Laevens et al. (Laevens et al., 1997). In a study analyzing the

16 relationship between clinical mastitis and SCC patterns, a higher risk for occurrence of CNS

17 mastitis in lactations with high average SCC was found (de Haas et al., 2004). The direct

18 economical impact of high SCC depends on the violation of limits for poor quality milk or

19 possible quality premiums paid for high quality milk. These differ considerably between

20 countries. The current legal limit in the European Union (EU) is 400,000 cells/ml, but in the US it

21 is as high as 750,000 cells/ml, so increases in bulk milk SCC have a different effect in these

22 regions. Many EU countries pay quality premium for milk with less than 250,000 cells/ml

23 (International Dairy Federation, 2006). In general, increases in milk SCC over 100,000 cells/ml

1 are associated with reduced milk production. Elevated milk SCC theoretically results in less milk
2 per animal going into the bulk tank (Hortet and Seegers, 1998; Østerås et al., 2006). In studies on
3 the effect of CNS intramammary infections on milk production, a slightly decreased milk
4 production has been reported (Timms and Schultz, 1987; Gröhn, 2004; De Vliegher et al., 2005).

5 In heifers, quarters infected with “non-aureus staphylococci” exhibited leucocyte
6 infiltration and a higher percentage of interalveolar stroma as compared with healthy controls,
7 which can have deleterious effects on future milk production (Trinidad et al., 1990a). In a Finnish
8 study, heifers with mastitis had a slightly higher genetic potential for milk production but their
9 recorded milk yield was slightly lower than that of their healthy herd mates (Myllys and Rautala,
10 1995). Gröhn et al. (2004) showed that multiparous cows with clinical CNS mastitis were
11 originally higher producers than their herd mates without CNS mastitis. Milk production losses
12 due to CNS mastitis could be underestimated if animals were compared with their herd mates
13 rather than with their own pre-infection production levels or genetic potential.

14

15 **4. Does CNS mastitis protect the quarter from other infections?**

16

17 Some authors suggest that quarters infected with the minor pathogens *Corynebacterium*
18 *bovis* and CNS would be more resistant to subsequent natural infections by major pathogens,
19 including streptococci and *S. aureus* (Rainard and Poutrel, 1988; Schukken et al., 1989;
20 Matthews et al., 1990; Lam et al., 1997a). In contrast, Parker et al. (Parker et al., 2007) showed
21 that precalving intramammary infection with CNS in heifers increased the risk for post-calving
22 infection with CNS, *S. aureus* or *Streptococcus uberis*. Similar findings were reported in another
23 study from New Zealand (Compton et al., 2007). In a field study in three Dutch herds (Zadoks et

1 al., 2001), infection with CNS was neither a risk factor nor a protective factor for mastitis caused
2 by *S. aureus* or *S. uberis*. In another field study, staphylococcal infections did not protect quarters
3 against mastitis caused by major environmental pathogens (Hogan et al., 1988). In challenge
4 studies using *S. epidermidis* or *S. chromogenes* (Linde et al., 1980; Matthews et al., 1990),
5 protective effects against subsequent *S. aureus* infection were found. Contradictory results have
6 also been published, including the study by Nickerson and Boddie (Nickerson and Boddie, 1994),
7 in which quarters infected with *Staphylococcus* spp. were found to be less susceptible to
8 experimental challenge with *S. aureus*, but more susceptible to challenge with *Streptococcus*
9 *agalactiae*. De Vliegher et al. (De Vliegher et al., 2004) demonstrated through *in vitro* studies
10 that *S. chromogenes* inhibited growth of *S. aureus* and streptococci. They suggested that the
11 effect was due to inhibitory substances produced by *S. chromogenes*. Another study reported
12 similarly that some strains of CNS inhibited growth of the major mastitis pathogen *Str.*
13 *agalactiae* (dos Santos Nascimento et al., 2005). Possible mechanisms for this effect could be the
14 increased SCC in the milk or antibacterial peptides (bacteriocins) produced by the bacteria (dos
15 Santos Nascimento et al., 2005). We think that the postulated inhibitory effect of CNS
16 intramammary infection against growth of so-called major pathogens remains theoretical and that
17 it is unlikely that this phenomenon would be of practical significance in mastitis control.

18

19 **5. Antimicrobial treatment of CNS mastitis**

20

21 National policies and strategies for treatment of mastitis vary. In some countries,
22 subclinical mastitis is treated with antimicrobials during lactation. In other countries, subclinical
23 and mild clinical mastitis cases, including most CNS mastitis cases, are left untreated or they are

1 treated using non-antibiotic means such as frequent milking-out. Not many treatment studies
2 have been published that specifically report results for quarters infected by CNS. Based on
3 available reports, mastitis caused by CNS seems to respond well to antimicrobial treatment.
4 Bacteriological cure ranges from 80 to 90% (Pyörälä and Pyörälä, 1998; McDougall, 1998;
5 Waage et al., 2000; Taponen et al., 2003a; Taponen et al., 2006). In all these studies, except in
6 the study carried out in New Zealand (McDougall, 1998), penicillin G was used in the treatment
7 and isolates were shown to be susceptible to penicillin *in vitro*. Elimination rates for mastitis
8 caused by penicillin-resistant CNS have been reported to be about 20% lower than those for
9 mastitis caused by penicillin-sensitive CNS (Pyörälä and Pyörälä, 1998). It is known from *S.*
10 *aureus* mastitis that infections caused by penicillin-resistant isolates may not be cured even if the
11 isolate is sensitive *in vitro* to the antimicrobial compound that is used for treatment (Sol et al.,
12 2000; Taponen et al., 2003b). Cows with higher parity have significantly lower tendency to cure
13 (Pyörälä and Pyörälä, 1998; Deluyker et al., 2005). Treatment duration varied from 2 to 4 days in
14 the cited studies. There is no consensus about the optimum duration of treatment of CNS mastitis.
15 According to a recent study, extending treatment length to 8 days did not improve cure rates of
16 subclinical CNS mastitis, as compared with treatment of 2 days (Deluyker et al., 2005). In that
17 study, the cure rate of CNS mastitis was 44% without treatment. Higher chances of cure were
18 observed in groups treated with pirlimycin but the difference between groups was not statistically
19 significant. Based on review of the literature and the authors' experience, it is our opinion that
20 CNS mastitis generally responds well to antimicrobial therapy and that the customary
21 antimicrobial treatment duration of 2-3 days can be used for CNS mastitis.

22 A single isolation of CNS from a quarter does not economically justify antimicrobial
23 treatment, in particular if only low numbers of bacteria are detected in the milk sample. CNS are

1 common bacteria on the teat skin and can sometimes contaminate the milk sample. Furthermore,
2 the spontaneous elimination rate of CNS infections without any treatment is relatively high. If
3 moderate or severe clinical signs are evident, treatment can be recommended. Intramammary
4 treatment with antimicrobials can also be recommended for quarters with persistent CNS
5 mastitis. Selection of antimicrobial drugs should be based on susceptibility testing. If penicillin G
6 is the treatment of first choice, beta-lactamase production can be determined by a rapid
7 nitrocephin test to assess penicillin sensitivity of isolates (Pyörälä, 2006). Nitrocephin tests were
8 recently shown to be sufficiently reliable to be recommended for routine clinical use to test beta-
9 lactamase production of mastitis staphylococci (Pitkälä et al., 2007). For persistent CNS
10 infections, antimicrobial treatment at drying-off remains a good tool, as cure rates of dry cow
11 therapy are generally very high for CNS infections (Newton et al., 2008).

12

13 **6. Prevention of CNS mastitis**

14

15 For CNS mastitis, as for all other types of mastitis, prevention is the key to combating the
16 problem. However, more knowledge and experience is needed to find the most effective
17 strategies for prevention of CNS mastitis. CNS have long been regarded as opportunistic skin
18 microbiota that occasionally can cause mastitis (Devriese and De Keyser, 1980) Control
19 measures against contagious mastitis pathogens such as post-milking teat disinfection reduce
20 CNS infections in the herd (Hogan et al., 1987). Discontinuation of teat dipping significantly
21 increased prevalence of infections with *Corynebacterium bovis* and CNS (Lam et al., 1997b).
22 Some CNS isolated from mastitis may be opportunists from the environment, but in the authors'
23 opinion it is very likely that at least the main species infecting the bovine mammary gland are

1 specifically adapted to the udder environment. Species of CNS may differ in this respect, but
2 information is lacking. In most herds, pregnant heifers are more likely to be infected with CNS
3 than cows. In solving CNS mastitis problems, focus should therefore be on the heifers, i.e. their
4 environment, feeding and management, before calving. The authors believe that welfare and
5 comfort of heifers may be significant factors for good udder health.

6 Prepartum intramammary antibiotic therapy for heifers reduced the number of CNS
7 infections during first lactation, but the effect on SCC was variable (Oliver et al., 2003;
8 Middleton et al., 2005). In a recent study of several herds from the U.S. and Canada (Borm et al.,
9 2006), no clear advantage from this practice was demonstrated. In addition, antimicrobial
10 treatment of heifers as a routine management procedure cannot necessarily be considered as
11 prudent use of antimicrobials. In a meta-analysis on the efficacy of dry cow treatment (Robert et
12 al., 2006), no significant benefit was established regarding the prevention of CNS infections with
13 blanket dry cow therapy.

14 Among other preventive measures against mastitis, non-antibiotic internal teat sealants
15 have come into use as an alternative or complement to antimicrobial dry cow therapy. Most
16 studies have focused on the prevention of intramammary infections caused by major pathogens,
17 and CNS have not been specifically addressed (Huxley et al., 2002; Sanford et al., 2006). Internal
18 teat sealants have also been tested in pre-partum heifers, in which they significantly reduced the
19 risk of intramammary infections and clinical mastitis post-calving (Parker et al., 2007). In that
20 study, a protective effect was demonstrated against CNS infections. Another approach to protect
21 teats against infections during the dry period would be the use of external teat seals administered
22 at drying-off, before calving or at both times (Godden et al., 2006). There is little peer-reviewed
23 evidence of the efficacy of external teat sealants, and short duration of adherence of the sealant to

1 the teat can be a problem. In a Norwegian study, external teat sealants did not provide protection
2 from clinical mastitis caused by CNS (Whist et al., 2006). Development of vaccines for bovine
3 mastitis has been difficult and even natural intramammary infection does not provide protection
4 against subsequent infections (Talbot and Lacasse, 2005). Vaccination against CNS mastitis has
5 not been investigated.

6

7 **7. Conclusions**

8

9 CNS have become the most common mastitis pathogens in many countries. CNS mastitis
10 mostly remains subclinical or shows only mild clinical signs. CNS can cause persistent
11 infections, resulting in increased milk SCC which affects milk quality, and may be related to
12 decreased milk production. The economical impact of the increase in bulk milk SCC depends on
13 the regulatory limits for milk SCC and quality premiums for milk with low SCC in individual
14 countries. CNS mastitis responds well to antimicrobial therapy. *S. simulans* and *S. chromogenes*
15 are probably the predominant CNS species in bovine mastitis. The knowledge on CNS species
16 involved in mastitis is still very limited and benefits would accrue from having more reliable
17 diagnostic methods for species identification. It is important to determine the predisposing factors
18 for CNS mastitis at herd and cow levels. Efficient strategies for prevention of CNS mastitis can
19 then be designed.

20

21 **Conflict of interest**

22

1 None of the authors (S. Pyörälä, S. Taponen) has a financial or personal relationship with
2 other people or organizations that could inappropriately influence or bias the paper entitled
3 “Coagulase-negative staphylococci – emerging mastitis pathogens”.

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