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Original article

# Coagulase-negative staphylococci (CNS) as an aetiological factor of mastitis in cows

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## Abstract

The aim of the present study was to determine the proportions of individual coagulase-negative *Staphylococcus* species in clinical and subclinical mastitis. The material consisted of 100 CNS isolates obtained from 223 milk samples collected from cows with clinical and subclinical mastitis. Coagulase-negative staphylococci constituted 44.8% of all isolated microorganisms. CNS were isolated from the mammary gland secretions of 86 cows from farms in the Lublin region (Poland). Clinical mastitis was found in 20 whereas subclinical mastitis in 66 study cows (23.3% and 76.7%, respectively). The symptoms of clinical mastitis were mild. The clinical forms of mastitis concerned mainly the first or second lactation. Subclinical mastitis was most commonly observed during the second lactation. Four CNS species (*S. xylosus*, *S. chromogenes*, *S. haemolyticus* and *S. sciuri*) were isolated from clinical and subclinical mastitis. *S. xylosus* was the commonest CNS species isolated from cows with clinical mastitis whereas *S. chromogenes* was the most prevalent one in subclinical mastitis cases. The three CNS species (*S. warneri*, *S. hominis* and *S. saprophyticus*) caused only subclinical mastitis.

**Key words:** cows, clinical and subclinical mastitis, CNS

## Introduction

For many decades, coagulase-negative staphylococci (CNS), widely spread in the natural environment and colonizing the skin and mucosa of animals and humans, have been considered non-pathogenic. At present, they are the predominant aetiological factor of bovine mastitis in many countries (Honkanen-Buzalski et al. 1994, Myllys et al. 1998, Chaffer et al. 1999, Makovec and Ruegg 2003, Pitkälä et al. 2004, Rajala-Schultz et al. 2004, Taponen et al. 2007, Pyorala et Taponen 2009, Malinowski and Kłossowska 2010). Recently, the incidence of CNS intramammary infections of the udder

in cows, sheep and goats has markedly increased (Deinhofer and Pernthaner 1995, Contreras et al. 2005, Winter et al. 2005).

In the majority of cases, CNS cause subclinical mastitis (Taponen et al. 2006, Persson Waller et al. 2011). However, they can also cause clinical mastitis, characterised by mild symptoms but associated with increased somatic cell count (SCC) (Taponen et al. 2007) and decreased milk production, which results in economic losses. It is difficult to control CNS mastitis because this group of pathogens is heterogeneous. Fifteen CNS species have been identified as aetiological factors of mastitis (Thorberg et al. 2009, Persson Waller et al. 2011). Moreover, coagulase-negative

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staphylococci are always present on the udder skin and in teat canals; under favourable conditions they permeate the galactogenic pathway to the quarter. The pathogenic mechanisms of CNS are expressed by two parameters: invasiveness (ability to permeate through protective barriers, to adhere to host cells and to form a biofilm) and toxicity (capacity to produce enzymes and toxins, including haemolysins and proteases) (Bartoszewicz-Potyrała and Przondo-Mordarska 2002, Bochniarz and Wawron 2012).

The key problems associated with the treatment and prevention of bovine mastitis caused by coagulase-negative staphylococci are their exceptional adaptive capacity, ability to form a biofilm and mechanisms of drug resistance (Łopaciuk and Dzierżanowska 2002, Bartoszewicz-Potyrała and Przondo-Mordarska 2002, Luthje and Schwarz 2006). In recent years, increased numbers of  $\beta$ -lactamase-producing CNS and *mecA*- gene positive CNS (MRCNS) resistant to all groups of  $\beta$ -lactam antibiotics have been observed (Suzuki et al. 1992, Van Duijkeren et al. 2004, Moon et al. 2007, Bochniarz and Wawron 2011, Persson Waller et al. 2011).

The aim of the study was to determine the proportions of individual coagulase-negative *Staphylococcus* species causing clinical and subclinical mastitis.

## Materials and Methods

The study material consisted of 100 CNS isolates obtained from 223 milk samples from cows affected by mastitis. Coagulase-negative staphylococci constituted 44.8% of all isolated microorganisms. CNS were isolated from the mammary gland secretions of 86 cows from farms located in the Lublin region (Poland). In 72 cows, they were isolated only from one udder quarter, in 14 – from two udder quarters. Cows with CNS were from 18 herds of various housing systems (4 free-stall or loose and 14 tie-stall housing systems) and of different sizes of herds (10-96 cows, 46 animals in a herd, on average). Clinical examination of cows and macroscopic evaluation of milk were carried out prior to collection of milk samples for bacteriological testing. Based on the anamnesis, the number of lactations of each cow was determined. The study cows did not receive any medicines during the ongoing lactation.

Milk was bacteriologically tested according to the generally accepted procedures. Milk samples brought to room temperature were thoroughly mixed and cultured on agar medium (BTL, Łódź, Poland) supplemented with sterile, defibrinated sheep blood (5% of the agar solution volume). After 24-hour incubation at 37°C under aerobic conditions, pathogens were in-

itially identified based on colony morphology and Gram-stained microscopic specimens.

**Identification of coagulase-negative staphylococcus species** was performed using a commercial API STAPH test (bioMérieux, France) based on determinations of 19 biochemical features of bacteria. The procedure followed the manufacturer's recommendations.

The types of mastitis were classified according to the standard principles. Based on clinical examinations, milk bacteriological testing and somatic cell counts in milk samples (using a Fossomatic device-Denmark), two types of mastitis were observed. SCC > 200 000/ml of milk and the presence of bacteria in bacteriological cultures without general symptoms were considered subclinical mastitis. Visible macroscopic milk changes and/or local changes within the mammary gland, occasional general symptoms and SCC > 200 000/ml of milk indicated clinical mastitis (Gentilini et al. 2002, De Vliegher et al. 2003, Moon et al. 2007).

## Statistical analysis

Significance of differences in percentages of CNS species with the phenotypic features tested, isolated from milk of cows with clinical and subclinical mastitis, was assessed using Statistica 6.0 software based on normal distribution for fraction comparisons. P-values of probability associated with the test fraction used were included (statistical significance was set at  $p \leq 0.01$ ,  $p \leq 0.001$ ).

## Results

In the study population of 86 cows, clinical symptoms of mastitis were observed in 20 (23.3%). The majority of symptoms were mild (slightly increased temperature – 2 cows, oedema and/or pain of the mammary gland – 8 cows, macroscopic changes in milk and mean SCC of 432 000/ml of milk – in all cows with clinical mastitis). The group of cows with clinical mastitis included 9 (43.6%) cows in first lactation, 8 (22.2%) cows in second, 2 (10.5%) cows in third and 1 (20.0%) cow in fourth lactation (Table 2). In cows with clinical mastitis, CNS infection developed in two quarters (2 cows) or in one quarter (18 cows). From 20 cows with clinical mastitis, 22 coagulase-negative staphylococci isolates were obtained (22.0% of all isolated CNS species) (Table 2). The following species were isolated: *S. xylosus*, *S. chromogenes*, *S. haemolyticus* and *S. sciuri*.

In the remaining 66 cows (76.7%), the presence of bacteria in milk and increased SCC were found

Table 1. Proportions of CNS species in clinical and subclinical mastitis.

Type of mastitis	CNS species														Total	
	<i>S. xylosus</i>		<i>S. chromogenes</i>		<i>S. haemolyticus</i>		<i>S. sciuri</i>		<i>S. warneri</i>		<i>S. hominis</i>		<i>S. saprophyticus</i>		n=100	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Clin.	8	28.6	4	15.4	7	28.0	3	21.4	0	0.0	0	0.0	0	0.0	22	22.0
Subclin.	20	71.4	22	84.6	18	72.0	11	78.6	4	100.0	2	100.0	1	100.0	78	78.0

n – number of isolation

Clin. – clinical mastitis

Subclin. – subclinical mastitis

Table 2. Relationship between type of mastitis and number of lactation.

No. of lactation	1		2		3		4		Total	
	(n=26)		(n=36)		(n=19)		(n=5)		(n=86)	
	n	%	n	%	n	%	n	%	n	%
Clin.	9	34.6	8	22.2	2	10.5	1	20.0	20	23.3
Subclin.	17	65.4	28	77.8	17	89.5	4	80.0	66	76.7

Clin. – clinical mastitis

Subclin. – subclinical mastitis

(mean SCC in milk in the affected quarters was 272 000/ml) yet no macroscopic milk changes or any general or local mammary gland lesions were observed. From the milk of these cows, 78 coagulase-negative staphylococci isolates were obtained, which constitutes 78.0% of all isolated CNS species (Table 1). Subclinical types of mastitis were caused by 7 CNS species: *S. xylosus*, *S. chromogenes*, *S. haemolyticus*, *S. sciuri*, *S. warneri*, *S. hominis* and *S. saprophyticus* (Table 1). Cows with subclinical mastitis had the following lactations: first – 17 (65.4%) cows, second – 28 (77.8%) cows, third – 17 (89.5%) cows and fourth – 4 (80.0%) cows (Table 2). In cows with subclinical mastitis the CNS infection affected two quarters (12 cows) or one quarter (54 cows).

## Discussion

Our findings and literature data reveal that the highest incidence of mastitis is caused by bacteria, including coagulase-negative staphylococci prevalent in many countries and considered non-pathogenic for many years (Oliver et al. 1997, Myllys et al. 1998, Makovec et al. 2003, Rajala et al. 2004, Taponen et al. 2007, Malinowski and Kłossowska 2010).

According to studies carried out in Finland in 1988-1995, the percentage of CNS species isolated from milk of cows with mastitis increased from 26.6%

to 53.5% (Myllys et al. 1998). Similar results were obtained during a study conducted in 2001, i.e. 49.6%, with coagulase-negative staphylococci outnumbering all other isolated pathogens (Pitkala et al. 2004).

Moreover, numerous reports indicated that CNS was the major aetiological factor of mastitis in various countries. High causes of mastitis in dry cows and immediately after calving during the first lactation were recorded (Boddie et al. 1987, Trinidad et al. 1990, Waage et al. 1999, Gentilini et al. 2002, De Vlieghe et al. 2003). Our results demonstrated that CNS mastitis could affect cows at various ages, yet the highest numbers of mastitis cases were observed during the first and second lactation. Likewise, clinical mastitis occurred during the first and second lactation (85% of clinical mastitis cases). Subclinical mastitis was most commonly found in second lactation cows (42.4%).

Our findings showed that *S. xylosus* constituted the highest percentage of CNS species isolated from the milk of cows with mastitis, which correlates with the results published by Malinowski et al. (2006) regarding the western part of Poland. In their study, *S. xylosus* was also the dominating species of CNS isolated from clinical and subclinical mastitis. Contrary to these findings, the study carried out in Finland by Jarp (1991) demonstrated that the most common coagulase-negative *Staphylococcus sp.* isolated from the milk of cows with clinical and subclinical mastitis was *S. simulans* (44.6%). All species of CNS obtained by

Jarp (1991) were similar in clinical and subclinical mastitis, except for *S. warneri*, *S. hominis* and *S. epidermidis*, which were responsible only for subclinical mastitis. Furthermore, the author observed that symptoms of acute mastitis occurred in 52.0% of CNS mastitis; however, the differences in their severity associated with various *Staphylococcus* species were not significant. Local changes in the mammary glands were found in mastitis caused by *S. haemolyticus*, *S. xylosus* and *S. simulans*. Elevated body temperature was observed only in cows infected with *S. haemolyticus*.

In the study carried out by Holmberg (1985), *S. simulans* was the dominant CNS species in Sweden. Another study conducted in this country by Birgersson et al. eight years later (1992) also demonstrated that *S. simulans* was the most common species of coagulase-negative staphylococci isolated in both clinical and subclinical mastitis in cows. The results regarding other countries were slightly different. In Japan, the major aetiological factor of clinical mastitis was *S. epidermidis* (Baba et al. 1980), whereas in Portugal this species was isolated most frequently in cows with subclinical mastitis (Nunes et al. 2007). Furthermore, Hodges et al. (1984), who identified species in milk of New Zealand cows with increased SCC but no other clinical symptoms, showed that the majority of isolates had biochemical features of *S. chromogenes*.

In Sweden, a study performed by Persson Waller et al. (2011) revealed that 7 out of 14 identified species (*S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. hyicus*, *S. warneri/pasteuri* and *S. xylosus*) were aetiological factors of both clinical and subclinical mastitis. The three predominant species in clinical mastitis cases included: *S. chromogenes*, *S. simulans* and *S. haemolyticus*; in subclinical mastitis cases: *S. epidermidis*, *S. chromogenes*, *S. simulans* and *S. haemolyticus* were identified. Moreover, the authors found that CNS were isolated in a substantially higher number of subclinical than clinical mastitis cases. In Finland, however, the causes of CNS subclinical and clinical mastitis were comparable (18% and 24%, respectively) (Koivula et al. 2007).

The species differences of CNS causing mastitis in cows may thus relate not only various countries and continents but also individual regions of a particular country. Furthermore, various proportions of CNS species were isolated in the same area at different periods. According to Rather et al. (1986), *S. simulans* was the most commonly isolated species in cases of CNS mastitis in cows in Illinois (USA). Several years after the publication of these results, Watts and Owens (1989) reported the highest proportion of *S. hyicus* in subclinical mastitis in cows in the United States. The study conducted at the Kentucky Univer-

sity by Matthews et al. in 1991 demonstrated that the most prevalent species of CNS obtained from milk was *S. chromogenes*. Likewise, Rajala-Schultz et al. (2004), who studied milk samples from cows affected by mastitis in Ohio in 2001-2002, identified 158 CNS species, with the highest percentage of *S. chromogenes*.

In the present study, four CNS species isolated from the milk of cows with clinical mastitis included *S. xylosus*, *S. chromogenes*, *S. haemolyticus* and *S. sciuri*. In clinical mastitis cases, *S. xylosus* was most commonly isolated while in subclinical mastitis cases – *S. chromogenes*. The additional three remaining species (*S. warneri*, *S. hominis*, *S. saprophyticus*) were responsible only for subclinical mastitis. The symptoms of acute mastitis (elevated body temperature, mammary gland lesions, macroscopic milk changes) were visible in 2 cows whose milk contained *S. haemolyticus* and *S. sciuri*. Despite transiently, slightly elevated body temperature, the course of diseases in these cows was mild. In the remaining cases diagnosed as clinical mastitis, the clinical symptoms were also not severe.

Our findings confirm the results reported by Pyorala and Syvajarvi (1987), i.e. the inflammatory reaction in the udder quarter affected by coagulase-negative staphylococci was milder than in mastitis caused by other pathogens. In general, the data published by other authors demonstrate that CNS caused only slightly elevated body temperature and slightly increased somatic cell counts in milk, compared to SCC in unaffected udders (Chaffer et al. 1999). SCC in the unaffected quarter is assumed to be lower than 200 000/ml of milk (Gentilini et al. 2002, De Vliegher et al. 2003, Moon et al. 2007). However, coagulase-negative staphylococci caused damage to the secretory tissue of the mammary gland (Trinidad et al. 1990), resulting in reduced milk production (Trinidad et al. 1990, Costa et al. 2000, Gentilini et al. 2002, Gröhn et al. 2004, De Vliegher et al. 2005), which is often neglected while estimating mastitis-related losses in the herd.

Our results reveal that coagulase-negative staphylococci can cause clinical mastitis; nonetheless, they primarily induce poorly noticeable subclinical conditions. In the group of 86 cows studied, subclinical mastitis was observed in 66 animals (76.7%). According to Czerw et al. (2007), cows with subclinical mastitis pose a threat to the entire herd and the percentage of subclinical infections in individual dairy cattle farms reaches 40-80% annually (Czerw et al. 2007). Due to the lack of external symptoms visible to the owner, the conditions remain unrecognised and left untreated, which leads to exacerbation of the disease or development of changes typical of chronic processes (Aarestrup and Jensen 1997, Chaffer et al.

1999, Taponen et al. 2006). Aarestrup and Jensen (1997) report that *S. chromogenes*-induced infections occurred shortly after delivery and subsided quickly; *S. simulans* infections lasted for a long time whereas *S. epidermidis*-attributable infections occurred occasionally during the same lactation. The above results demonstrate that in some cases CNS could be present in the mammary gland throughout the lactation period (Taponen et al. 2007).

Considering the fact that coagulase-negative staphylococci are a part of physiological bacterial flora inhabiting the animal skin, special caution should be paid to milk sampling and interpretation of culture results. The colonisation of teat skin and its opening by CNS has been abundantly described in the literature (Boddie 1987, White et al. 1989, Trinidad et al. 1990).

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