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Original article

Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland

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Abstract

The aim of this study was to evaluate the prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. In total, 387 Polish HF were involved in the study. The diagnosis of mastitis was performed on the basis of clinical examination of the udder, macroscopic evaluation of milk, determination of somatic cell count and bacteriological examination of milk. Subclinical mastitis was found in an average of 36.7% (range from 21.0% to 53.1%) of cows and of 15.7% (range from 9.6% to 25.2%) of quarters. Coagulase negative staphylococci (CNS; 31.6% of quarters), *Streptococcus (Str.) agalactiae* (15.6% of quarters), *Staphylococcus (Staph.) aureus* (12.1% of quarters) and fungi (12.2% of quarters) were most frequently isolated from subclinical mastitis. Etiological agents of subclinical mastitis differed strongly between herds. The results of this study showed that the incidence of subclinical mastitis at the end of lactation in dairy herds in North-East Poland is high. CNS were the most frequently isolated from subclinical mastitis cases, however mastitis caused by the contagious pathogens *Str. agalactiae* and *Staph. aureus* is still a problem. The fungal infections of the mammary gland also play an important role.

Key words: cows, drying off, subclinical mastitis, etiological agents

Introduction

Mastitis is characterized by an inflammation in one or more quarters of the udder and can be either clinical or subclinical (International Dairy Federation 1987). Clinical cases give rise to visible symptoms such as swelling, heat, pain in the affected quarters or clots and discoloration of the secretion. Cows with subclinical

mastitis do not show a visible udder inflammation and can be diagnosed by somatic cell count (SCC) and by detection of the presence of pathogens (Malinowski and Kłosowska 2002, Pyörälä 2003, Hamann 2005). Subclinical mastitis is 15 to 40 times more prevalent than the clinical form of mastitis (Seegers et al. 2003).

Mastitis is the most costly disease in dairy production, due to its high incidence and losses. Losses caused by clinical mastitis include reduced milk yield and quality, costs of veterinary care, discarded milk and shortening of productive life (Kossaibati and Esselemont 1997, Nielsen et al. 2010, Hogeveen et al. 2011). However, the majority of the economic losses caused by subclinical mastitis are due to reduced milk production (Seegers et al. 2003, Huijps et al. 2008). Milk and dairy products made from mastitic milk may cause foodborne diseases, infections or intoxications (Twardoń et al. 2005, Malinowski and Gajewski 2009).

Several causative agents have been implicated in mastitis in dairy cows including bacterial, mycoplasma and yeast pathogens. Streptococci, CNS and *Staph. aureus* are the most frequently isolated pathogens from subclinical cow mastitis. Infections caused by yeasts are also important (Malinowski et al. 1993, Giannechini et al. 2002, Barański et al. 2008, Wawron et al. 2010). However, in many countries, including Poland, changes in the relative and absolute importance of the main mastitis pathogens have been observed over time, probably because of marked changes in the dairy industry (Myllys et al. 1998, Pitkälä et al. 2004, Malinowski et al. 2006).

Efficacious treatment is an important component of any mastitis control program. The clinical cases in lactating cows should be treated promptly with an appropriate intramammary antibiotic. In severe cases systemic antibiotic may be used, either alone or in combination with an intramammary infusion (Malinowski 1997, Owens et al. 1997). The most effective time to treat subclinical mastitis is at drying off. Dry cow therapy with a long-acting intramammary preparation serves to eliminate subclinical infections present at the end of lactation and to prevent many new dry period infections (Osteras et al. 1994, Malinowski 1997, 2000). Treatment of all cows at drying off, independent of their infectious stage (blanket or total therapy) continues to be the standard procedure in most countries, except the Nordic European countries and the Netherlands. Most herds have been shown to benefit from blanket therapy at drying off with an antimicrobial infusion (Dingwell et al. 2003, Robert et al. 2006).

However, routine treatment of all cows has been questioned because of global concerns for developing antimicrobial drug resistance. Overuse of antibiotics in veterinary medicine is considered one of the main factors responsible for the emergence of antibiotic resistance in bacteria. The majority of antibiotics used in dairy herds are related to udder health, mainly as dry cow products (de Briyne et al. 2014). Recently, selective dry cow therapy has been recommended (van Werven 2014). In this strategy, only cows or quarters

assumed to be infected are treated. Studies have shown (Halasa et al. 2009) that cure rates were no different from blanket dry cow therapy when selective dry cow therapy was used, and selective dry cow therapy successfully eliminated intramammary infections to the same degree as did blanket dry cow therapy (Cameron et al. 2014). In the Netherlands following legislation that antimicrobials should no longer be used to prevent infections, selective dry cow treatment was implemented on January 1st 2014 (van Werven 2014).

An alternative to the use of dry cow therapy in cows with uninfected quarters is to apply a nonantibiotic teat sealer (either internal or external) (Berry and Hillerton 2002, Huxley et al. 2002, Lim et al. 2007). In cows with infected quarters at drying off these sealers can be used in combination with a long-acting antibiotic infusion to achieve elimination of the existing infections (Godden et al. 2003, Bradley et al. 2010). Therefore, knowledge of the health status of the mammary gland at the end of lactation has a significant impact on the choice of the best strategy for the treatment of existing subclinical infections and prevention of new intramammary infections during the dry period.

There are only a few studies about the incidence of subclinical mastitis before drying off in Poland (Malinowski et al. 1993, Barański et al. 2008). Thus, the aim of this study was to evaluate the prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland.

Materials and Methods

The study was carried out on 387 Polish HF cows from nine dairy herds in North-East Poland. Herd size ranged from 21 to 100 milking cows. All herds were housed in a loose housing barn and fed total mixed ration based on grass silage, maize silage and concentrate. The cows were milked twice daily in a milking parlor. The average milk yield ranged from 6000 to 8000 l per year. Blanket dry cow therapy was implemented in all herds.

The study was conducted according to good veterinary practice. The diagnosis of mastitis was performed on the basis of clinical examination of the udder, macroscopic evaluation of milk, determination of somatic cell count and bacteriological examination of milk. None of the cows suffered from clinical mastitis. Double milk samples were collected aseptically from 1538 quarters a week before drying off. Ten quarters were atrophic. The teats were cleaned and the first few streams were discarded. The teats were then dipped in disinfectant and the teat ends were wiped with

Table 1. Prevalence of subclinical mastitis in nine dairy herds.

Herd	Cows			Quarters		
	number of examined cows	subclinical mastitis		number of examined quarters	subclinical mastitis	
		n	%		n	%
A	32	17	53.1 ^{a,b}	127	32	25.2 ^a
B	57	15	26.3 ^c	227	23	10.1 ^{b,c}
C	45	23	51.1 ^{a,b}	179	30	16.8 ^{a,b}
D	41	19	46.3 ^{a,b}	163	38	23.3 ^a
E	36	13	36.1 ^{b,c}	143	29	20.3 ^{a,b}
F	25	11	44.0 ^{b,c}	100	19	19.0 ^{a,b}
G	21	10	47.6 ^{b,c}	82	18	22.0 ^{a,b}
H	30	9	30.0 ^{b,c}	120	14	11.7 ^{b,c}
I	100	25	21.0 ^c	397	38	9.6 ^c
Total	387	142	36.7	1538	241	15.7

^{a, b, c} – values within the same column with different superscript letters differ significantly at $p < 0.05$

Table 2. Microorganism (%) isolated from subclinical mastitis in nine dairy herds at the end of lactation.

Herd	Microorganisms %										
	Staph. aureus	CNS	Str. agalactiae	Str. dysgalactiae	Str. uberis	Other Str.	T. pyogenes	E. coli	C. bovis	Fungi	No growth
A	15.5	15.6	12.5	0	15.6	0	0	9.4	9.4	25.0	0
B	13.0	60.9	8.7	0	8.7	0	0	0	0	0	8.7
C	13.3	16.7	23.3	0	6.7	0	0	3.3	0	23.3	20.0
D	13.1	34.2	7.9	0	23.7	0	0	10.5	7.9	7.9	2.6
E	0	68.9	13.8	0	6.9	10.3	3.4	0	10.3	10.3	6.9
F	0	15.8	10.5	10.5	0	10.5	0	10.5	10.5	21.0	15.8
G	5.5	11.1	27.8	11.1	11.1	5.5	0	0	0	22.2	5.5
H	35.7	43.0	7.1	0	0	0	0	7.1	0	0	7.1
I	13.1	18.4	28.9	5.3	0	2.6	0	0	10.5	0	31.6
Total	12.1	31.6	15.6	2.9	8.1	3.2	0.4	4.5	5.4	12.2	10.9

Percentages do not total 100 as some quarters had mixed infection with two pathogens.

alcohol swabs and allowed to dry. 10 ml of the secretion was then collected into sterile tubes. The samples were cooled and immediately transported to the laboratory. SCC was measured with BactoCount IBm (Bentley, USA). Bacteriological examinations were performed according to commonly accepted rules (Malinowski and Kołosowska 2002). Briefly, 10 µl of milk were cultivated on Blood Agar Base (bioMérieux Poland), Mac Conkey Agar (BTL, Poland), Mueller-Hinton Agar (BTL, Poland) and Edwards Medium (Oxoid Ltd, England). Plates were incubated at 37°C and read 24 and 48 hours later. Bacteria were identified by their gross colony morphology and by Gram staining. Detailed identification of isolated bacteria

was performed using API tests (bioMérieux Poland). Sabouraud Dextrose Agar (bioMérieux, Poland) was used for isolation of fungi. Incubation took 5 days and 1ml of milk was used. Subclinical mastitis was diagnosed in a quarter, when clinical signs were not present and the SCC was higher than 400 000/ml with or without positive isolation of udder pathogens.

Statistical analyses were performed using GraphPad PRISM 6.0 (GraphPad Software Inc., San Diego, Ca, USA). Differences in incidence of mastitis among herds were statistically analyzed using Yates corrected Chi-square test. The level of significance was set at $p < 0.05$.

Results

Prevalence of subclinical mastitis at the end of lactation is presented in Table 1. The elevation of SCC above 400 000/ml was found in an average of 36.7% of cows and of 15.7% of quarters. The incidence of subclinical mastitis varied among herds from 21.0% to 53.1% cows, and from 9.6% to 25.2% quarters, respectively. The differences in incidence of subclinical mastitis in cows and quarters among herds were partially statistically significant ($p < 0.05$). Microorganisms isolated from subclinical mastitis are presented in Table 2. Bacteria or fungi were isolated from 89.1% of quarters with subclinical mastitis, 10.9% of samples from mastitis cases were culture negative. CNS (31.6% of quarters), *Str. agalactiae* (15.6% of quarters), *Staph. aureus* (12.1% of quarters) and fungi (12.2% of quarters) were most frequently isolated from subclinical mastitis. Etiological agents of subclinical mastitis differed between herds. In herd E subclinical mastitis were caused mainly by CNS, in herd A by fungi, whereas in herd I the predominant pathogen was *Str. agalactiae*.

Discussion

The presence of subclinical mastitis before drying off can have profound effects on cow health and productivity after calving. There is a high risk that subclinical cases will become clinical after calving (Green et al. 2002).

Studies on the prevalence of subclinical mastitis in Polish dairy cows before drying off are limited. Malinowski et al. (1993) found subclinical mastitis in 22.6% of quarters, Barański et al. (2008) reported 38.5% of cows and 12.6% of quarters infected before drying off. In the present study, 36.7% of cows and 15.7% of quarters were diagnosed with subclinical mastitis at the end of lactation. The prevalence of subclinical mastitis before drying off was higher in other studies from the UK (34.5% of quarters) and the USA (33 % of quarters) (Godden et al. 2003, Huxley et al. 2002). There was a large variation in the prevalence of subclinical mastitis at drying off between herds (range from 21.0% to 53.1% of cows and from 9.6% to 25.2% of quarters). This may be explained by the difference in milking management and environmental factors between the herds.

Over 137 different organisms have been identified as being causative agents of bovine mastitis, including bacteria, viruses, mycoplasma, yeasts and algae (Watts 1988). In the present study, most of the mastitis cases (31.6%) were caused by CNS. Similar results were reported from Poland by Malinowski et al.

(2006), Barański et al. (2008) and Bochniarz et al. (2013). CNS have become the predominant pathogen isolated from subclinical bovine mastitis in many countries, and could therefore be described as emerging mastitis pathogens (Myllys et al. 1998, Pyörälä and Taponen 2009). In the study from Germany, 35% of quarters with subclinical mastitis harbored CNS (Tenhagen et al. 2006). In Finland, CNS were isolated from 50% of the quarters positive for bacterial growth (Pitkälä et al. 2004).

Generally, the percentage of particular etiological agents of mastitis has changed during recent decades in many countries. A decrease in frequency of mastitis caused by *Str. agalactiae* as an effect of introduction of mastitis control programs has been observed (Myllys et al. 1998, Pitkälä et al. 2004, Hillerton and Berry 2005). In Poland, a decrease in *Str. agalactiae* infections was reported by Malinowski et al. (2006). However, in the present study *Str. agalactiae* was found in an average of 15.6% (range 7.9-28.9%) of quarters with subclinical mastitis. The high incidence of *Str. agalactiae* indicates the low efficacy of dry cow therapy in some herds.

Staph. aureus is permanently present in dairy herds. In our study, this pathogen was isolated from 12.1% of quarters with subclinical mastitis. Similar results were obtained by Malinowski et al. (2006), who found *Staph. aureus* in 8.6% cases of clinical and subclinical mastitis in the western part of Poland. This is in accordance with data from other countries (Giannechini et al. 2002, Pitkälä et al. 2004, Tenhagen et al. 2006).

An increasing problem is mastitis caused by fungi. In our study the presence of fungi was found in 12.2% of quarters with subclinical mastitis. Krukowski et al. (2000) isolated fungi from 9.6% of milk samples from cows with mastitis, Wawron et al. (2010) showed that fungi constituted 7.07% of all etiological agents of mastitis. In other studies the frequency of mastitis caused by fungi was lower (Malinowski et al. 2006). Fungal infections are associated mainly with prolonged intramammary administration of antibiotics, improper conditions in cow barns and poor milking hygiene (Malinowski 1997, Wawron et al. 2010).

The results of our study showed that the incidence of subclinical mastitis at the end of lactation in dairy herds in North-East Poland is high. CNS were the most frequently isolated from subclinical mastitis cases; however, mastitis caused by the contagious pathogens *Str. agalactiae* and *Staph. aureus* is still a problem. Fungal infections of the mammary gland also play an important role.

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