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Public health hazard due to mastitis in dairy cows*

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Milk from cows with sub-clinical *mastitis* accidentally mixed into bulk milk enters food chain and poses a threat to human health. Milk and other dairy products are reported to be frequently infected with *Staphylococcus aureus*. Also *Streptococcus agalactiae* has been described as one of the most common agents of invasive infections. The present study was aimed at discussing the economy and public health importance of *mastitis* in cattle as well as the health hazard of the causative organisms (*S. aureus* and *Str. agalactiae*).

Seven-hundred-and-four composite milk samples were collected (two or three times per cow per lactation) from 275 cows kept in two herds. The samples were analysed for the presence of *S. aureus, Str. agalactiae* and other *mastitis*-causing organisms. For pooled data of latent and subclinical *mastitis* the frequency of samples containing *S. aureus* and *Str. agalactiae* was 16.6 and 1.4%, while of those containing *Str. dysgalactiae, Escherichia coli* and other *mastitis*-causing organisms – 4.7, 2.9, and 14.7%, respectively. Quality of milk was found ranging within the European standard. However, high *mastitis* prevalence in both herds suggests that hygienic control measures should be applied during milk production.

KEY WORDS: cows / mastitis / milk quality / Staphylococcus aureus / Streptococcus agalactiae

Milk and milk products have the potential to transmit pathogenic organisms to humans. All the nutritional components that make milk and milk products an important

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part of the human diet also support the growth of pathogenic organisms. Early in this century, it was discovered that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever, and Q fever to humans. Fortunately, over the decades, the threat of these diseases and the incidence of outbreaks involving milk and milk products have been greatly reduced due to improved sanitary of the milk production practices and pasteurization technique. However, a variety of microorganisms still contribute to illnesses and disease outbreaks. Raw (unpasteurized) milk has been found to participate in spreading out of illnesses caused by *Listeria, Campylobacter, Yersinia, Salmonella, Staphylococci species,* and *E. coli*.

With severe clinical *mastitis*, abnormalities of milk are easily observed and milk is discarded by the producer. Such milk normally would not enter the food chain. But when milk of cows with sub-clinical *mastitis*, *i.e.* with no visible changes, is accidentally mixed into bulk milk, it enters food chain and can be dangerous to humans. Although pasteurization is likely to destroy all human pathogens, there is concern when raw milk is consumed or when pasteurization is incomplete or faulty.

Milk and other dairy products are frequently infected with with *S. aureus*. According to Gilmour and Harvey [1990] milk of infected animals is the main source of enterotoxigenic *S. aureus* of animal origin. For example certain *S. aureus* strains produce heat-resistant enterotoxins, which cause nausea, vomiting and abdominal cramps when ingested by humans and are responsible for staphylococcal food poisoning outbreaks [Kluytmans *et al.* 1997].

Toxins are produced due to improper cooling of milk, during cheese manufacture from raw milk and also due to post-processing contamination. These toxins can not be destroyed by heating or drying [National Mastitis Council 1996].

The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of S. aureus. Two different types of toxin with super-antigen activity can be produced: enterotoxins and toxic shock syndrome toxin (TSST-1). The staphylococcal enterotoxins (SEs) have been divided into five serological types (SEA, SEB, SEC, SED, and SEE) on the basis of their antigenic properties [Dinges et al. 2000]. The strains producing the staphylococcal enterotoxin type C (SEC) have been widely isolated from mastitis-afflicted cows [Matsunaga et al. 1993, Lee et al. 1998, Cardoso et al. 1999]. Recently, the occurrence of new types of SEs (SEG to SEJ) has been reported [Su and Wong 1995, Munson et al. 1998]. Kenny et al. [1993] reported a significant correlation between the SEC and TSST-1 in S. aureus from mastitic milk. However, the relationship between these new SEs and bovine *mastitis* has not been sufficiently clarified. According to Hayakawa et al. [1998] the production of exfoliative toxins by S. aureus isolated from mastitic cow's milk or from bulk milk seems to be rare. The importance of pathogenity of toxin formation of S. aureus for udder remains unclear. However, the super-antigenic toxins might overstimulate or reduce the host immune response.

The SEs belong to a family of the so-called pyrogenic toxins originating from *S. species* and *Str. species*. Pyrogenic toxins include SEs, TSST, exfoliants A and B and

Streptococcus pyrogenic toxins. These toxins have some common structure, functions and sequences. Although pyrogenic toxins are involved in distinct pathologies, they have some biological activities in common: they are pyrogenic, and they cause immunosuppression and non-specific T-cell proliferation. These activities are referred to super-antigen activity [White *et al.* 1989, Marrack and Kappler 1990]. Super-antigen activity results from direct interaction of SEs with T-cell antigen receptors and major histocompatibility complex (MHC) of antigen-presenting cells (APC). They bind to MHC class II molecules on antigen-presenting cells. Subsequently they activate a high percentage of T-cells after interacting with defined, variable sequences on the β chain of the T-cell receptors. This type of interaction has been shown to occur also in cattle [Schmaltz *et al.* 1995].

Str. agalactiae is an important bovine pathogen, especially as a cause of both clinical and sub-clinical mastitis in dairy cows [Keefe 1997]. Mastitis constitutes a source of economic loss for the dairy industry due to its effects on milk quality. It lowers the quality of cheese and other manufactured milk products [Politis and Ng -Kawai-Hang 1988] and decreases milk yield. It also reduces nutritive value of milk due to the changes in its composition, increases processing problems and off flavours [Oz et al. 1985]. Decreased is also the shelf life of fluid milk products, due to the growth of spoilage bacteria [Oz et al. 1985]. Moreover, the impact of mastitis involves the additional cost of therapeutic strategies and laboratory and veterinary services [Guérin-Faublée et al. 2002]. Str. agalactiae is considered a major cause of elevated SCC as related to standards in bulk tank milk. As SCC rise because of *mastitis*, milk quality decreases due to the drop lactose and casein. Milk yield of a cow with an infected quarter may drop by as much as 40 % while the cow does not show any apparent clinical signs of *mastitis*. A reduction in milk quality ultimately leads to loss of income of the dairy farmer as milk prices are related to milk composition and premiums are lost when SCCs and bacteria counts increase.

In humans, *Str. agalactiae* has been described as one of the most common factors of invasive infections in neonates, but it also causes invasive and non-invasive infections in adults [Schuchat 2001]. *Str. agalactiae* also causes significant morbidity and mortality in humans, both infants and adults, all over the world [Blumberg *et al.* 1992]. In neonates, *Str. agalactiae* is mostly acquired from the mother's vagina in early-onset disease, although community and breast milk transmissions have been reported [Bingen *et al.* 1992]. In adults, *Str. agalactiae* occurs preferentially in certain individuals, such as diabetics, pregnant and post-partum women, and immunocompromised patients, emphasizing the opportunistic nature of the infection [Lerner *et al.* 1977]. Furthermore, humans act as a significant reservoir of *Str. agalactiae*, since these bacteria may be carried in the vaginas of women without apparent clinical signs [Huet *et al.* 1993].

Another public health concern regarding *mastitis* are antibiotic residues in milk due to extensive use of antibiotics in the treatment and control of the disease. Antibiotic residues in foods can lead to severe reactions in people allergic to antibiotics and,

at low levels, can cause sensitization of normal individuals and development of antibiotic-resistant strains of bacteria. Compliance with recommended withholding time helps minimizing the risk of antibiotic residues to occur in milk and meat which is the producers' responsibility.

The objectives of this study were to show the economy and public health importance of *mastitis* as well as the health hazard of the causative organisms (*S. aureus* and *Str. agalactiae*) on the example of the quality of milk in two Polish dairy herds.

Material and methods

Two herds (K and P) of Polish Black-and-White (Polish Friesian) cows were selected to carry out the study within two years (2004 and 2005). In each herd the mean number of cows was 150. Cows were offered *ad libidum* the total mixed ration (TMR) based on corn and grass silage, with concentrates and vitamin-mineral supplements, and were milked twice a day. Pre- and post-dipping was applied for all milked cows in both herds.

Cows from herd K were kept in loose barn. Cubicles were bedded with either straw (during winter) or sand (during remaining seasons) Milking was performed in a herringbone low line-milking parlour with automatic take-off of clusters.

Cows from herd P were kept in tied-up stalls. The barn had high-line pipeline installation of Alfa-Laval units.

During the years 2004 and 2005 a total of 704 composite milk samples (352 from each herd) were collected from 275 lactating cows (138 from herd K and 137 from herd P) two or three times/lactation from a cow. The samples were then analysed for the presence of *Staphylococcus aureus*, *Streptococcus agalactiae* and other *mastitis*-causing species. Each milk sample was cultured on Columbia agar with 5% sheep blood (Bio-Merieux) to determine the somatic cell count (SCC) using Fossomatic (FOSS ELECTRIC, Denmark) counter.

The plates were incubated at 37°C for 24 h before inoculation with milk to check their purity; plates shoving any growth were eliminated. Composite milk samples were mixed by shaking and examined according to the methods published by National Mastitis Council [Hogan *et al.* 1999]. Inoculated plates were incubated at 37°C and bacterial growth was recorded after 24 and 48 h. Suspected colonies were identified according to International Commission on Microbiological Specifications for Food [1978]. The number of colonies of each bacterial species per ml milk was recorded. Bacterial species were identified according to National Mastitis Council as described by Hogan *et al.* [1999]. Gram staining and the preliminary assays with catalase for the gram-positive organisms and of potassium hydroxide test for the gram-negative ones were carried out in all cases.

Catalase test for the **gram-positive** organisms was performed by transferring one colony of the organism to be identified to the surface of glass slide, and adding a drop of 3% hydrogen peroxide (H₂O₂). A positive result was indicated by immediate formation

of gas bubbles. All *staphylococci* are catalase producers, while all *streptococci* do not produce the enzyme. The **gram-negative** organisms were identified using potassium hydroxide (KOH) test by transferring one colony of the organism to the surface of glass slide and adding one drop of 3% KOH. A positive result was indicated by release of viscous "stringy" material. The KOH test is based on the differences in the chemistry of the bacterial cell wall. The cell wall of gram-negative bacteria is easily disrupted when exposed to alkali weak solutions resulting in the release of viscous "stringy" DNA [Gregerson 1978]. Thus, all KOH-positive bacteria were classified as gram-negative organisms. More details about identification of bacteria species are given by Hameed [2006].

Quality of milk samples was evaluated by recording the total bacteria count (TBC) per ml of milk and then comparing the result to the European countries standard [International Dairy Federation 1996]. Only plates with 30-300 colonies were selected, colonies were counted and mean TBC in milk calculated.

Cases of *mastitis* were classified as clinical or sub-clinical according to the definitions given by International Dairy Federation [1987]. Infectious (clinical or sub-clinical) *mastitis* was assumed to occur when pathogens and inflammatory changes were detected in milk. *Mastitis* was classified as non-specific (clinical or sub-clinical) when there were inflammatory changes but no pathogens appeared in milk. A latent infection was assumed to occur when the secretion contained pathogens but showed a normal SCC [International Dairy Federation 1987]. The distinction between a normal and a high SCC in milk, as estimated by Fossomatic cell counter, was based on guidelines for diagnosis for composite milk samples issued by International Dairy Federation [1987].

The bacterial isolates were assorted to seven following groups: *S. aureus*, CNS (coagulase negative *staphylococci*), *Str. agalactiae*, *Str. dysgalactiae*, *E. coli*, other coli forms (*Klebsiella* and *Enterobacter*) and other bacterial species (environmental *streptococci*, *Bacillus cereus*, *Corynebacterium species*, *Pseudomonas species*). Then the bacteria were classified into two groups, contagious and environmental. The diagnosis of *mastitis* in the examined milk samples was done according to the criteria given below.

Clinical *mastitis* diagnosed by gross abnormalities in the milk such as flakes, clots, or a watery appearance, and also by inflammatory symptoms such as swelling and oedema of the mammary gland, fever and rapid heart rate.

Sub-clinical *mastitis* diagnosed when somatic cell count was \geq 400 000 cells/ml of milk. It was classified into contagious when there were \geq 500 cfu (colony forming units)/ml of *S. aureus* or *Str. agalactiae*. The environmental sub-clinical *mastitis* was diagnosed when there were \geq 2000 cfu/ml of CNS, *Str. dysgalactiae*, *E. coli*, other coli forms (*Klebsiella* and *Enterobacter*), and other bacterial species (environmental *streptococci*, *Bacillus cereus*, *Corynebacterium species*, *Pseudomonas species*).

Latent *mastitis* diagnosed when SCC was < 400 000 cells/ml milk and the presence of bacteria was confirmed. It was differentiated into contagious and environmental

according to the criterion used for classification of contagious and environmental subclinical *mastitis*.

Aseptic mastitis diagnosed when there was no bacterial growth at the end of incubation period and SCC was \geq 400 000 cells/ml.

Healthy cows diagnosed when there was no bacterial growth in the milk samples or the samples did not supply meaningful bacterial growth at the end of incubation period. Less than 500 cfu/ml for *S. aureus* and *Str. agalactiae* and <2000 cfu/ml for remaining bacterial species; SCC <400 000 cells/ml.

Results and discussion

The use of the estimates of bacteria count in milk samples as an index of sanitary condition of the mammary gland is commonly accepted. Table 1 shows that the TBC /ml in the examined milk samples ranged from 1.0×10 to 3.6×10^4 with a mean value of $2.6 \times 10^3 \pm 2.8 \times 10^2$ in herd K. In herd P the respective values for TBC/ml ranged from 1.0×10 to 1.0×10 to 1.0×10^5 with a mean value of $4.0 \times 10^3 \pm 4.2 \times 10^2$.

Table 1. Total bacteria count (TBC/ml) of the milk samples

Herd	TBC			
	no. of samples	minimum	maximum	mean/SE
Κ	352	1.0×10	3.6×10^{4}	$2.6 \times 10^3 / 2.8 \times 10^2$
Р	352	1.0×10	1.0×10^{5}	$4.0 \times 10^3 / 4.2 \times 10^2$

During the two years composite milk samples were examined bacteriologically (n=704). Different types of *mastitis* were identified – clinical, sub-clinical, latent and aseptic. Figure 1 shows the per cent of healthy udders and incidence of four *mastitis* types in cows (herds pooled). The share of samples yielded by cows with all four *mastitis* types pooled was estimated to be 60.81%. The remaining 39.19% of examined samples were classified as yielded by healthy cows. Of these samples 2.49% showed no bacterial growth (data not tabulated). As illustrated by Figure 1, the most frequent *mastitis* was that of a latent type, followed by sub-clinical type. The lowest frequency was recorded for clinical *mastitis*.

Figure 2 shows the incidence of latent *mastitis* caused by individual bacterial species. *S. aureus*, CNS and *E. coli* predominated in both herds. *Str. agalactiae* was the least frequent pathogen causing latent *mastitis*. In herd K the incidence of latent *mastitis* differed from that in herd P. Latent *S. aureus* and *Str. dysgalactiae* infections in herd K were more frequent than in herd P.

The incidence of sub-clinical *mastitis* caused by different bacterial species is shown in Figure 3. *S. aureus*, CNS and *Str. dysgalactiae* were the most frequently isolated bacteria. Both herds were afflicted equally by sub-clinical *mastitis* attributed to *S. aureus*. However, sub-clinical *mastitis* due to the rest of bacterial species was higher in herd P than in herd K (Fig. 3).



Fig. 1. Prevalence of different types of mastitis.



Fig. 2. Bacterial species causing latent mastitis.

Milk is a complex biological fluid containing a wide variety of constituents and possessing unique physical and chemical properties. In addition of being a nutritive medium, it also presents a favourable environment for the multiplication of various contaminating microorganisms. Milk obtained from a healthy animal and a disease-



Fig. 3. Bacterial species causing sub-clinical mastitis.

free udder contains small number of bacteria. *Mastitis* resulting from major pathogens causes considerable compositional changes in milk, including increase in SCC.

SCC indicator is widely used to predict the suitability of milk for human consumption [Heeschen 1996, Smith and Hogan 1999]. There is no evidence that any particular cell count *per se* has any significant effect on human health. However, the higher the cells count the greater the risk of raw milk contamination with pathogens and antibiotic residues [Heeschen 1996, Smith and Hogan 1999, Savelle *et al.* 2000]. Furthermore, increased SCC is also associated with reduced suitability of the raw milk for manufacturing and processing [Barbano 1999, Ma *et al.* 2000].

Bacteriological examination showed that most of the milk samples examined complied standards recommended by European Union [International Dairy Federation 1996]. According to that standard method bacteria count on plate must not exceed 1 \times 10⁵ cfu/ml of milk. The low total counts of bacteria may reflect a good sanitation practices applied during milking. However, 6.1% and 8.2% (data not tabulated) of the examined milk samples in herd K and P, respectively, exceeded the upper border line of standards recommended by the United States Milk Quality Program which amounts to <2.5 \times 10⁴/ml for aerobic plate count [Barbano 1992].

For latent and sub-clinical *mastitis* the pooled frequencies of samples containing \geq 500 cfu/ml of *S. aureus* and *Str. agalactiae* were 16.6% and 1.4%, respectively (joint percentage from Fig. 2 and 3). In turn, frequencies of samples containing \geq 2000 cfu/ml of *Str. dysgalactiae*, *E. coli*, other *coli* forms and other bacterial species were 4.7, 2.9, 1.2 and 14.7% respectively (joint percentage from Fig. 2 and 3). *S. aureus* is one of the most important etiological factors of bovine *mastitis*, a disease widely recognized

as being of great importance for public health and economic significance [Lopes *et al.* 1990]. The mechanism by which *S. aureus* evades immune elimination and causes disease is not clear. Colonization by *S. aureus* and other *staphylococci* typically occurs in the external area of the teat canal prior to the illness [Cullen and Hebert 1967]. After colonization, *mastitis* leads to severe damage to the mammary gland epithelial cells [Almeida *et al.* 1996], consequently increasing the inflammatory response within the gland. This phenomenon is probably a result of various bacterial exonerations, which exhibit super antigen (SAg) activity and are potentially responsible for the severity of the disease. Evidence suggests that SAgs of *S. aureus* have a suppressive effect on the host immune responce by activation of lymphocytes with immunosuppressive activity [Park *et al.* 1993].

The high counts of *coli* form in some of milk samples are attributed to *mastitis*. Such milk may often be used directly as a drink, and constitute a public health concern. It is epidemiologically significant that not only animals, but also humans carry *E. coli* in their gastrointestinal tract. The occurrence of *coli* forms in milk may, therefore, be considered as a real indicator of the faecal pollution with the possibly existing associated pathogens. The public health hazard of *E. coli* has been emphasized by many authors, because these bacteria have been implicated in human cases of gastroenteritis, epidemic diarrhoea in infants, sporadic diarrhoea in children as well as in cases of food poisoning [Marier *et al.* 1973, Mossel 1982].

Mastitis pathogens in milk pose a lower threat to public health if milk is pasteurized. On the other hand, the improper use of antibiotics to eliminate *mastitis* pathogens become a public health concern. The careless therapy with antibiotics against *mastitis* can lead to their residues in milk. Very little information is available concerning the effect of *mastitis* treatment in cows on human health and welfare. Therefore, a need exists to assure that *mastitis* control and treatment procedures do not have adverse impact on public safety.

Effective programmes for *mastitis* control that promote dairy food safety are based on identifying the pathogens present, developing effective tools to control *mastitis* pathogens and observing practices that reduce the risk of antibiotic contamination of bulk milk. Controlling *mastitis* is important because the condition has significant implications such as financial losses of dairy farmers, adverse effects on cow welfare and potential effects on public health.

Sub-clinical *mastitis* enhances the growth and multiplication of pathogens resulting in impaired utility or even deterioration of their milk. Possible losses in both herds could be asociated with culling either due to lower milk production, or *mastitis* as a result of uncured intra-mammary infections when the bacteria causing the disease fail to respond to the commonly used antibiotics (resistance development). Culling leads to two kinds of losses, namely a reduced slaughter value of a cow and an increased replacement cost and lost production time following prematured removal from the herd before the animal reaches its optimum production age [Radostits *et al.* 2000].

It is notoriously difficult to estimate the losses associated with clinical mastitis, which arise from the costs of treatment, culling, and decreased milk production. It is even more difficult to quantify the losses associated with sub-clinical *mastitis*, which arise as a result of treatment, decreased milk yield and quality, and an increase in the risk of culling. Significant losses in milk production from individual cows and herds have been shown to be associated with elevated SCC (higher cell counts mean greater loss). The loss concerning an individual cow rises from 6 to 30% as cell counts increase from 100 000 to 1 600 000 cells/ml while the loss of the entire herd increases from 6 to 29% for cell counts from 500 000 to 1 500 000 cells/ml [Philpot and Nickerson 1991]. De Graaf and Dwinger [1996] reported raw milk production losses per cow with sub-clinical mastitis to be about 1.56 kg daily. Average milk mean production loss per affected quarter due to sub-clinical mastitis was estimated to reach 17.6% [De Graaf and Dwinger 1996]. In addition to the costs outlined above, both clinical and sub-clinical mastitis have been shown to adversely affect the animals' subsequent fertility [Schrick et al. 2001]. Cows with multiple lactation experience had greater milk production losses as compared to lactating heifers. Blowey [1986] assumed no decrease in milk production due to sub-clinical mastitis. However, a 10% decrease in milk vield in the following lactation was shown after acute *mastitis*.

The present study showed that the quality of the examined milk samples as evaluated according to TBC fits European standard $(1.0 \times 10^5 \text{ cfu/ml milk})$. However, high SCC and *mastitis* prevalence were found in both herds. Thus, hygienic control measures should be considered during milk production.

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Zagrożenie zdrowia człowieka wynikające z zapaleń wymion krów dojnych

Streszczenie

Mleko krów z podklinicznym zapaleniem wymienia może zagrażać zdrowiu człowieka. Stwierdzono, że mleko i jego produkty mogą być źródłem pokarmowych zatruć ludzi gronkowcem złocistym. Infekcje bakteryjne u dzieci i dorosłych może wywoływać także paciorkowiec bezmleczności występujący w mleku chorych krów. Celem pracy było wskazanie znaczenia *mastitis* jako czynnika zagrażającego zdrowiu ludzi, ze szczególnym uwzględnieniem bakterii najczęściej wywołujących tę chorobę, a więc gronkowca złocistego i paciorkowca bezmleczności. W latach 2004 i 2005 przeanalizowano 704 próbki mleka pobrane od 275 krów dwu- lub trzykrotnie w czasie jednej laktacji. Krowy utrzymywano w dwóch stadach. Mleko badano pod kątem wystąpienia podklinicznych stanów zapalnych wymienia wywołanych paciorkowcami, gronkowcami i innymi bakteriami. Podkliniczne i utajone *mastitis* wywołane gronkowcem złocistym występowało łącznie u 16,6%, podczas gdy wywołane paciorkowcem bezmleczności – u 1,4% badanych krów. Podkliniczny i utajony stan zapalny wywołany przez inne bakterie występował u 14,7% badanych zwierząt. Podsumowując można stwierdzić, że aczkolwiek jakość badanych próbek mleka mieściła się w standardach europejskich, to jednak stopień rozpowszechnienia *mastitis* w badanych populacjach okazał się znaczny, co świadczy o konieczności prowadzenia kontroli warunków higienicznych pozyskiwania mleka w stadach krów mlecznych.