



Mastitis pathogens in dairy cattle – a review

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ABSTRACT

Udder inflammations are one of the most common diseases of high-yielding dairy cows that cause high economic losses and have negative influence on milk technological value. Nowadays, pathogens involved in the inflammation process present lower susceptibility to antibiotics and around 90% of mastitis cases is caused by environmental bacteria. This situation forces researchers for seeking new solutions in mastitis prevention and treatment. The aim of this paper was to describe bacteria, fungi and algae are most common reasons of mastitis in dairy cows.

Keywords: dairy cows mastitis udder bacteria fungi, *Staph. aureus*, *E. coli*, *Klebsiella*, *A. pyogenes*

1. INTRODUCTION – MASTITIS, WHY DOES IT MATTER?

Mastitis, also known as udder inflammation, is a common problem in dairy herds causing increase in costs of milk production (Bramley et. al 1996, Halasa et al. 2007) and also have negative impact on milk composition and its technological value (Biggs, 2009).

Milk from cows with healthy udder should contain <200.000 somatic cells count (SCC) in 1 ml of milk. SCC is an indicator of the udder health problems. Most somatic cells are white blood cells (e. g. macrophages and neutrophils) that influx mammary gland tissue from blood during inflammation (Akers and Nickerson, 2011). Conditions decreasing the resistance and susceptibility of cow udders to inflammations can be e.g. prolonged intra-udder antibiotics administration, increased incidence of udder mycosis results from mineral-vitamin

deficiencies, antioxidant deficiencies, imbalanced diet, poor environmental conditions and even weather changes (Wawron et al., 2010).

Genetic correlation between clinical mastitis and milk yield are unfavourable (Heringstad et al., 2000; Carlén et al. 2004). Estimated genetic correlations between clinical mastitis and SCC were ranged from 0.3 to 0.8, with an average around 0.6 (Heringstad et al., 2000; Koeck et al. 2012).

This paper contains present information about pathogens – bacteria, fungi and algae – responsible for most mastitis cases in dairy cows.

2. 1. Costs and consequences of mastitis

Both stages, clinical and subclinical mastitis also influence veterinary costs and have negative impact on milk technological value. The first signal suggesting mastitis occurrence in herd can be increased somatic cell count (SCC). Clinical mastitis is connected with high losses in cows yields (Jones et al. 1984; Houben et al. 1993; Hortet and Seegers 1998; Gröhn et al. 2004). Calculations of the mastitis economic losses and the benefits of mastitis management vary distinctly according to different researches (Halasa et al. 2007). Calculations based on farm-specific and pathogen-specific analysis can balance economic losses and benefits in mastitis management. However, Halasa et al. (2007) also pointed out that some calculations from different studies included varied factors.

In most of cases cows which presented subclinical form of mastitis decreased milk production by 10-15% from cow. Results provided by St. Rose et al. (2003) showed that milk production did not improve after cows were recovered from subclinical mastitis, that may underestimate losses in production. Lammers et al. (2001) revealed that, mastitis pathogens have different target cell specificity and use different mechanisms to adhere to cells of the bovine mammary gland. Too fast milking can increase somatic cell count and udder injuries which can effect on higher mastitis frequency (Rupp and Boichard 1999; Berry et al. 2005; Dodenhoff et al. 2000; Sewalem et al. 2010).

Previous studies revealed that clinical mastitis frequency in different countries was 12-20% (Zwald et al. 2004; Mrode et al. 2012; Koeck et al. 2014). Udder diseases increases level of herd replacement. Percentage of dairy cows culled because of udder diseases in Poland is 5.38-16.13% (Pokorska et al. 2012; Sawa et al. 2012; Czaplicka et al. 2013; Neja et al. 2015; Kalińska and Slószarz, 2016). However, these problems are definitely more seldom if lactations last longer than 305 days (Sawa et al. 2012).

2. 2. Bacterial mastitis

About 90% of pathogens responsible for udder inflammations are environmental pathogens (Lassa et al., 2013) which presence is common in cow-barn environment. Increase in animal amount, humidity and pollutions in cow environment increases also bacteria and other pathogens pression on animals.

First of all, it should be emphasized that, except *A. pyogenes* that at present is the etiological agent of clinical mastitis only, all the microorganisms can be the reason of both, clinical and subclinical form of mastitis (Malinowski et al., 2006a).

Staph. aureus, *E. coli* and *Klebsiella* sp. causes the greatest losses of milk in primiparous cows that suffered from mastitis. The most significant losses in older cows are

caused by *Streptococcus* sp., *Staph. aureus*, *A. pyogenes*, *E. coli* and *Klebsiella* sp. (Gröhn et al., 2004).

Milk samples with SCC <200.000 were in around 60% culture negative (Malinowski et al., 2006a). According to Wernicki et al. (2014) common mastitis pathogens were *Staphylococcus aureus* and *Streptococcus agalactiae*, less frequent *Corynebacterium bovis* or *Mycoplasma bovis* infections. Similar results received e. g. Lassa et al. (2013) – streptococci and staphylococci bacteria were most frequently isolated (38,5% and 17,9%, respectively) and Malinowski et al. (2006a) – most frequently isolated pathogens were *Streptococcus* sp., Coagulase-negative staphylococci, *Staphylococcus aureus*, Gram-negative bacilli and *Corynebacterium* sp. Studies conducted by Lassa et al. (2013) suggested that coliforms, fungi, *Arcanobacterium pyogenes*, algae and *Corynebacterium* spp. were responsible for 16,4%, 3,3%, 31,%, 0,9% and 0,8% of mastitis cases, respectively. Different results presented Krukowski et al. (2001), their study revealed that *Staphylococcus aureus* was isolated only from 10.4% samples, *Streptococcus agalactiae* was isolated in 4.9% cases, and *Streptococcus* spp. in 6.4%, but coagulase-negative staphylococci were in 36.6% samples, and *Escherichia coli* was isolated from 3.5% samples.

Analysis of mastitis cases divided according to SCC results presented by Malinowski et al. (2006a) suggest that samples with SCC from 200.000 to 2.000.000 usually were infected by Coagulase-negative staphylococci, *Staphylococcus aureus* and *Streptococcus* sp. In samples with SCC >2.000.000 expected pathogens were mainly CAMP-negative and CAMP-positive streptococci and Gram negative bacilli. *Prototheca* sp. (64.5%), yeast-like fungi (60.2%) and *Streptococcus* sp. (55.1%) were isolated from samples which SCC was $\geq 5.000.000$. The highest milk SCC ($\geq 10.000.000$) was associated with intramammary infections caused by *Arcanobacterium pyogenes* (95.5%), *Streptococcus agalactiae* (57.6%) and Gram-negative organisms (46.5%). Similar results were reported in Wilson et al. (1997) studies and SCC was the highest for infections caused by Gram-negative bacteria, *Streptococcus agalactiae*, *Streptococcus* sp., *Arcanobacterium pyogenes*, and *Prototheca* sp. Several authors pointed out that the heaviest mastitis forms were connected with infections by *Arcanobacterium pyogenes*, *Streptococcus agalactiae*, coliforms, CAMP-negative *Streptococcus*, yeast-like fungi and *Prototheca* sp. (Wilson et al. 1997; Lammers et al. 2001; Leitner et al., 2000; Djabri et al., 2002; Kłossowska et al., 2005; Malinowski et al., 2006a).

2. 3. Mycotic bovine mastitis

Previously antibiotics were widely used what could influence on increased incidence of mycotic bovine mastitis. Authors suggested that treatment with antibiotic in case of bacterial mastitis can results in higher mycotic udder inflammations rate (Elad et al., 1995a; Elad et al., 1995b; Gonzales, 1996; Wawron et al., 2010). Elimination of antagonistic bacterial flora during antibiotic therapy stimulate fungi to multiplication and also decreased amounts of vitamin A (antioxidant) in the glandular tissue and epithelium have positive impact on fungi growth (Wawron et al., 2010).

In general, fungi are rare cause of mastitis but sometimes can occur in epizootic proportions (Gonzales, 1996), usually in farms with poor environmental and hygienic conditions. Poor quality of materials used as bedding (e.g. straw) with high humidity can be source of fungi causing mastitis in cattle (Lassa and Smulski, 2013). Fungi are also reason of udder inflammation when udder is washed with water but was not dried (Blowey and Edmondson, 2010).

According to the present results, the percentage of yeasts inducing mastitis increased in comparison to e.g. studies carried out by Krzyżanowski and Sielicka (1996) (Wawron et al., 2010). Around 1-13% of mastitis cases was caused by bovine mycotic mastitis (Malinowski and Krzyżanowski, 1982; Costa et al. 1993; Krzyżanowski and Sielicka, 1996; Lagneau et al. 1996; Moretti et al., 1998; Krukowski et al., 2006; Lassa and Smulski 2013). In Poland it's about 2-9% of all mastitis cases (Krukowski et al. 2001; Malinowski et al., 2003; Wawron et al., 2010). But yeasts were more frequently isolated in the end of winter and in spring (Krukowski et al. 2006; Wawron et al. 2010). Bovine mycotic mastitis was also more frequent in small herds (up to 50 cows) (Smulski and Malinowski 2011).

Previous studies reported several species of yeast that can cause bovine mastitis (Richard et al. 1980; Watts 1988; Costa et al. 1993; Jensen and Aalbaek B. 1994; Elad et al. 1995a; Lagneau et al. 1996). In Krukowski et. al. (2001) research 9,6% of samples were positive for fungi and yeasts species were of the genera *Candida*, *Rhodotorula* and *Trichosporon*. Malinowski and Krzyżanowski (1982) received similar results as well as Krukowski et al. (2006), but in their research isolated yeasts were classified into 4 genera (*Candida*, *Trichosporon*, *Saccharomyces* and *Rhodotorula*). However, Spanamberf et al. (2008) isolated 68 species of yeast in Brasil and the most frequent genera were *Candida* (37.9%), *Pichia* (19.1%), *Cryptococcus* (10.3%) and *Rhodotorula* (10.3%). In the infected mammary glands in tropical countries were also found species like *Aspergillus* sp., *Penicillium* sp., *Epicoccum* sp., *Phoma* sp. and *Alternaria* sp. (Costa et al. 1993).

Previous researches showed that *Candida* sp. is responsible for most cases of mastitis (Watts, 1988; Aalbaek et al., 1994; Elad et al. 1995b; Krzyżanowski and Sielicka, 1996; Lagneau et al. 1996; Keller at al., 2000; Malinowski and Kłossowska, 2002; De Casia dos Santos and Marin, 2005; Kivaria and Noordhnizen, 2007; Wawron et al., 2010). Also Krukowski et al. (2006) confirmed that results and the most frequently isolated yeasts during a confinement-housing season were *Candida* sp., *C. kefir*, *C. humicola*, *C. rugosa* and *C. inconspicua*. *C. krusei*, *C. kefir* and *C. lusitaniae* were the most common in Wawron et al. (2010) analysis and only 3.33% were fungi from other genera (*Trichosporon*, *Rhodotorula*, *Cryptococcus*).

2. 4. Protothecal mastitis

Udder inflammations can also be connected with organisms from *Prototheca* genus (e.g. algae). Studies carried out by Lassa et al. (2013) revealed that algae were responsible for only 0,9% of mastitis cases. Even lower results were obtained by Krukowski et al. (2006). Their analysis showed that algal mastitis occurrence was 0.35%. Poor environmental conditions, inappropriate milking hygiene and prolonged antibiotic therapy can be the reason of increased protothecal mastitis occurrence (Janosi et al., 2001; Roesler et al., 2003; Marques et al., 2006; Lopes et al., 2008; Marques et al., 2010) that can reach even over 30% (Milanov et al., 2006).

An interesting relationship between *Prototheca* sp. species and SCC in milk samples were revealed by Malinowski et al. (2006a). The occurrence of these microorganism was the highest in samples with SCC >2.000.000 (22.5-39%). Similar results were also confirmed by other authors (Wilson et al., 1997; Lammers et al., 2001; Leitner et al., 2001; Djabri et al., 2002; Kłossowska et al., 2005). However, Wawron et al. (2010) did not find any case of algae-related mastitis, that were reported e.g. by: Aalbaek et al. 1994; Gonzales 1996; Malinowski et al., 2002; Krukowski et al., 2006; Malinowski et al., 2006b.

Presence of the algal species *Prototheca zopfii* was demonstrated in analyzed samples from cows with mastitis in different papers (Aalbaek et al., 1994, Krukowski et al., 2006; Milanov et al., 2006; Möller et al., 2007; Jagielski et al. 2011; Zaini et al., 2012; Wawron et al., 2013). In further researches *P. zopfii* genotype 2 was investigated as the aetiological agent of bovine *Prototheca* mastitis (Möller et al., 2007). Recently, Jagielski et al. (2011) presented third *P. zopfii* genotype but their findings clearly show the predominance of the *P. zopfii* genotype 2 in aetiology of the disease in comparison to new genotype (*P. blaschkeae*).

Available references pointed out on high resistance of *P. zopfii* to antibiotics which affects the effectiveness of mastitis treatment (Melville et al., 1999; Milanov et al., 2006; Lopes et al., 2008; Wawron et al., 2013).

2. 5. Mastitis – future prognosis in prevention and treatment

Mastitis is a multifactorial disease and its prevention requires proper management on both stages (clinical and subclinical), but should concentrate on mastitis prevention. It is clear that inappropriate and excessive antibiotics use in treatment of dairy cows contributed to the increased antibiotic resistance of mastitis pathogens (Oliver and Murinda, 2012). Nowadays, authors pointed out that potential treatments were limited to only few options (Aarestrup, 1999; Levy, 2001). Hendriksen et al. (2008) presented comparison of resistance to several antimicrobial agents for selected bacteria: *Staphylococcus aureus*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli* (eight strains of each species) in different European countries. Their results suggested that frequency occurrence of resistance to several antimicrobial agents varied among countries which can reflect differences in the antimicrobial use between countries and veterinarian procedures. Therefore, the best solution should be treatment of animals based on the observed resistance patterns and local knowledge (Hendriksen et al., 2008).

This situation forces researchers in their surveys to two-ways directions:

1. looking for new, innovative solutions of mastitis treatment
2. enhance in methods of prevention.

Reduction of antibiotics seems to be a necessary step. Therefore, scientists must find alternative substances that can replace antibiotics. The most promising are metallic nanoparticles because of their good antibacterial properties, which is current interest in the researches because of growing development of resistant strains (Gong et al., 2007).

Nanotechnology is a rapidly growing field used in science and technology due to possibility of manufacturing new materials at the nanoscale level (Albrecht et al., 2006). Nanoscale materials have high surface area to volume ratio and the unique chemical and physical properties that make them promising antimicrobial agents (Morones et al., 2005; 2007; Kim et al., 2007).

Nanoparticles have low toxicity for mammary gland tissue. Their highest reactivity is observed when nanoparticles are placed in new environment and were not covered by other substances or structures (bacteria, proteins, lipids) (Saptarshi et al., 2013).

Metal nanoparticles e.g. of silver and copper could be alternative for traditional antibacterial agents like antibiotics (Castellano et al., 2007). One of their most important advantages is lack of risk of resistance against nanoparticles (Rai et al., 2012). Nanoparticles can be effective solution in mastitis prevention and treatment in dairy cows because of antibiotics reduction necessity or diagnosing bacteria susceptibility to antibiotic in each case

(Oliver and Murinda, 2012; Hendriksen et al., 2008). Silver nanoparticles are bactericidal and fungicidal agents that damage cell membranes by the formation of micro-environment rich in silver ions, protein denaturation, formation of reactive oxygen species, disorders in DNA replication and proteins and enzymes expression in the respiratory chain (Lee et al., 2014; Li et al., 2010).

Nanoparticles may also have toxic effect on bacteria because of formation reactive oxygen species (in Fenton reaction), lipids and proteins peroxidation and DNA degradation (Li et al., 2012). Nanoparticles activity is connected with bacteria cell membrane. Gram-positive bacteria (e.g. *Staphylococcus aureus*) have thick peptidoglycan layer that makes them less susceptible on toxicity of silver nanoparticles in comparison to Gram-negative bacteria (Radzig et al., 2012). Copper nanoparticles are more toxic for Gram-positive bacteria what results in damaging their cell membranes (Azam et al., 2012). It is connected with high amount of amine and carboxyl groups that are part of cell membranes in Gram-positive bacteria (Beveridge and Murray, 1980). Different properties of silver and copper nanoparticle should be the most effective solution in mastitis management due to their synergistic effect on various pathogens.

3. CONCLUSIONS

Mastitis is one of most common diseases in dairy herds causing high economic losses for milk producers. Increasing microbial resistance against antibiotics forces scientist to looking for new solutions. Researchers should concentrate on estimating the most effective alternatives. Nanoparticles could be the most promising answer for modern dairy cows problems due to their properties and low toxicity for mammary gland tissue. However, authors should also support milk producers in improving mastitis prevention methods.

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