MASTITIS IN DAIRY HERDS: AN SAFETY AND QUALITY HAZARD FOR RAW MILK AND MILK PRODUCTS IN REPUBLIC OF MACEDONIA

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Abstract

Mastitis, milk quality and dairy food safety are all very much interrelated. The present study was aimed at discussing the epidemiology of mastitis in dairy herds and their public health importance as well as the health hazard of the udder pathogens. Mastitis, an inflammation of the mammary gland caused by bacterial infection, trauma, or injury to the udder, remains the most common and most expensive disease affecting dairy cattle throughout the world. Mastitis risk factors or disease determinants can be classified into three groups: host, pathogen and environmental determinants. Many studies conducted in different countries under varying conditions reported that annually 20 to 40% of dairy cows have expressed clinical mastitis during lactation. Data from dairy farms in Republic of Macedonia reported that annual prevalence of clinical mastitis was 45.86%. Major mastitis pathogens are classified as being either environmental or contagious. Treatment of the cows suffering from mastitis, ejection of chronic illness and reduction of the number of new infections is the main goal of the mastitis control program. Mastitis is the most common cause of the use of antimicrobials in dairy cows. The biggest problem in the treatment of mastitis in dairy cows is the resistance of microorganisms to the specific group of antibiotics. 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. Developing uniform national regulations including microbial standards for raw milk, labeling of raw milk, improving sanitation during milking, and enhancing and targeting educational efforts are potential approaches to this issue.

Keywords: dairy cow, mastitis, milk safety, milk quality, public health.

Introduction

Mastitis, milk quality and dairy food safety are all very much interrelated. Mastitis, an inflammation of the mammary gland caused by bacterial infection, trauma, or injury to the udder, remains the most common and most expensive disease affecting dairy cattle throughout the world. Mastitis is caused by several different bacteria that can invade the udder, multiply there and produce harmful substances that result in inflammation.

The National Mastitis Council estimates that mastitis costs dairy producers in the United States over \$2 billion dollars annually. Per lactation, costs ranged from \$138 to \$1,169 (Oliver and Calvindo, 1995; Heikkila, et al., 2012). Consequently, mastitis continues to be one of, if not, the most significant limiting factor to profitable dairy production. Actually, losses due to decreased milk production and milk quality had the biggest economic influence in dairy industry (Schukken et al. 1997).

The relationship of mastitis with milk yield and milk composition has received considerable research attention because of tremendous economic implications. Mastitis reduces milk yield and alters milk composition. There is two types of mastitis: clinical and subclinical. Clinical mastitis is characterized by abnormal milk and/or visible abnormalities of the udder such as hot and swollen udders. However, subclinical infections, the most common form of mastitis, are not readily apparent because there are no visible signs of the disease. Important components hereof are the daily decision regarding mastitis control program with exception of good hygiene practice (Trajcev and Nakov, 2009).

The measurement used most commonly to detect subclinical mastitis is the somatic cell count (SCC) of milk. One characteristic feature of mammary gland inflammation is an elevation in the number of somatic cells in milk. According to national legislation in Republic of Macedonia (FVA, 2012) milk from uninfected mammary glands contains \leq 100.000 somatic cells per milliliter. A milk SCC from 200.000 - 400.000/ml is a clear indication that milk has reduced manufacturing properties, which means is not for consummation. This, an increase in the SCC of milk is a reasonably good indicator of inflammation in the udder. Objectives of this paper are to briefly review the influence of mastitis on milk yield and composition, to describe how dairy producers can control mastitis by using proven methods of mastitis prevention and control, and to discuss the relationship of mastitis with dairy food safety.

Epidemiology of mastitis in dairy herds

Mastitis is one of the major diseases in dairy herds, causing economic losses to the entire milk production chain. Mastitis is a multi-factorial disease, closely related to the production system and environment in which the cows are kept. Mastitis risk factors or disease determinants can be classified into three groups: host, pathogen and environmental determinants.

National data from countries which are the biggest milk producers, informed that annually 20 to 40% of dairy cows have expressed clinical mastitis during lactation (Bartlett et al. 2001). According to Sviland and Waage (2002) the incidence of clinical mastitis in Norway for the period from 1992 to 1995 was 49 cases per 100 cow/year, and lactation incidence risk was 32-34%. According the research of Workineh et al. (2002), prevalence of clinical mastitis in Ethiopia, the most populated cattle country in Africa, varied from 1.2 to 23.5%.

Mastitis commonly occurs in cows with high milk production and has a long lasting effect on milk yield. The disease has a big influence on productivity and utilization of genetic potential of dairy cows. The effect of mastitis on milk yield can differ, depending on the parity and stage of lactation at which the disease occurs (Grohn et al. 2006). The biggest milk yield losses were observed when clinical mastitis was occurred in early lactation (Hagnestam et al. 2007). The cows that were suffered from clinical mastitis never ever were reached current milk yield during the rest of the lactation.

There were numerous studies which reported effects of clinical mastitis on milk production. Some reviews about the relationship between clinical mastitis and milk yield was reported variability between 3% to 8.1% for decrease in the milk yield per lactation and estimates of milk yield loss ranged from 160 to 850 kg/cow per lactation (Rajala et al. 1999). Revealing by Hortet and Seegers (1998), the estimated lactational milk loss was found to vary between 0 and 9.5% across parities.

According the research performed in the dairy farms in Republic of Macedonia, the lactation incidence risk (LIR) in entire population of dairy cows was 45.86% (Trajchev et al., 2013). This research reported that that LIR in entire population tended to increased with increasing the parity, or consecutive lactation, beginning from cows in first lactation (36.04) up to cows in third lactation (54.83%), and then decreasing. but without linearity. Another research carried out at the dairy farms in Republic of Macedonia revealed that the total annual prevalence of clinical mastitis for entire observed population of dairy cows, calculated per 100 lactations was 19.97% (Nakov at el., 2014) while lactation incidence risk at the same research was 21.49%. Estimated risk of mastitis incidence in the population of dairy cows observed was 85.02 cases per 100 cow-years at risk (Nakov at el., 2014). This research showing that the prevalence of clinical mastitis and LIR are increased with increasing the parity, or consecutive lactation, beginning from cows in the first lactation (12.55% and 13.36%) up to cows in the fifth and higher lactation (34.92% and 34.96%).

According the data from another research performed in Macedonian dairy herds, the quarter level prevalence of clinical mastitis was 15.06% per lactation, out of which 3.32% were front left, 3.10% front right, 4.28% rear left and 4.28% were rear right quarters (Nakov et al., 2014). The prevalence of udder quarters affected with clinical mastitis tended to increased with increasing the parity, from cows in first to the third parity, and then begins to decline slightly. The rear quarters frequently manifested form of clinical mastitis (49.39%) in relation to the front one (33.04%), and in 17.55% of the cases there were affected either, front and rear quarters.

Prevalence of milkborne pathogens in raw milk

Whilst over 100 different micro-organisms have been identified as causative agents of mastitis, only a few species of staphylococci, streptococci and Gram-negative

organisms are of economic or epidemiological significance. The importance of the various mastitis pathogens has also markedly changed throughout the past 50 years as a result of different control and husbandry methods used. Major mastitis pathogens are classified as being either environmental or contagious.

The routine use of antibiotics and improved understanding of the complex aetiology of mastitis have meant that the targeting of control, and even eradication, of some mastitis pathogens has became more efficient. Increased emphasis on somatic cell count reduction and targeting certain contagious micro-organisms (i.e. Streptococcus agalactiae may have changed the relative importance of the principal mastitis pathogens in the national herd. Low SCC herds may be more susceptible to environmental mastitis caused by *Escherichia coli*, which are becoming more important, whilst Str. agalactiae is rapidly disappearing (Jones, 1998). There is also increasing evidence that bacteria that until recently have been considered non-pathogenic or opportunistic udder pathogens are becoming more common as primary mastitis pathogens. These bacteria include Corynebacterium bovis and coagulase negative staphylococci. The contagious pathogens usually have a mechanism to adhere to the epithelial cells of the udder or to become intracellular, in order to protect themselves from the intramammary defense mechanisms. Staphylococcus aureus, Streptococcus agalactiae and Streptococcus dysgalactiae belong to this group of pathogens. Actinomyces pyogenes is an opportunistic, contagious mastitis pathogen, usually spread by flies. Mastitis caused by these microbes is often chronic and causes elevated SCC levels. It is possible to eradicate contagious mastitis pathogens from a herd by aggressive antimicrobial therapy and/or culling and biosecurity (Pyorala, 2003). Antibiotic dry cow therapy has been seen as a major factor in diminishing the significance of these pathogens in British dairy herds (Jones, 1998).

One study from England (Barkema et al., 1999), revealed that the various aerobic bacteria that were isolated from the milk included coliforms (27.7%), *S. aureus* (35.3%), other *Staphylococcus spp.* (20.8%), *Micrococcus spp.* (5.8%), *Streptococcus spp.* (9.8%) and others (0.6%). Coliforms that were isolated included 33 *Pseudomonas aureginosa* (68.8%),10 *Klebsiella edwardisii* (20.8%), *3 Escherichia coli* (6.3%) and 2 unidentified *Enterobacteriaceae* isolates (4.2%). Other less common microorganisms isolated were *Nocardia asteroides* and *Aspergillus fumigatus*. Anaerobic bacteria were not isolated from any of the milk samples.

The two years research preformed in Republic of Macedonia for determination of udder pathogens prevalence and their antimicrobial profile in dairy cows during the period of early lactation find out that 162 udder quarters that have abnormal milk secretion, of them: 56.17% were microbiologically negative and 43.83% microbiologically positive: *Streptococcus agalactiae* was isolated in milk samples from 19.14% quarters, *Enterococcus spp.* in 8,02%, *Candida non-albicans* in 6,79%, *Staphylococcus aureus* in 6,17%, *Escherichia coli* in 1,85%, *Aspergilus*

niger in 1,23% and *Pseudomonas aeruginosa* was isolated in milk samples from 0,62% of quarters. (Trajchev., et al., 2014).

Biosecurity practices for udder health and dairy food safety

Control of inflammation of the mammary gland and the reduction in their appearance in the herd is based on the number of somatic cells in milk and the incidence of clinical mastitis (IDF, 1997). Thus, in order to minimize the appearance of mammary inflammation, the main goal should be the number of somatic cells below 200,000 / ml of milk, and the incidence of clinical mastitis below 20% (Pyorala, 2003; Schukken et al., 2003). Klastrup et al. (1987) found that 25% of factors affecting the occurrence of mastitis account for ambient conditions, 20% of genetic factors and 50% of farm management. The goal of the strategy for controlling mastitis is to reduce new infections and eliminate the existing ones. However, particular attention should be paid to the prevention. A good effect in the fight against contagious mastitis caused by Staphylococcus aureus and Streptococcus agalactiae is achieved by implementing certain measures that relate primarily to the hygiene of the mammary gland before and after milking, the correct drying of cows, and the timely and appropriate treatment of cows with clinical mastitis (Barnouin et al., 2005). Equally important is the proper maintenance of milking equipment (Manninen, 1995). In cases of persistent chronic mastitis, it is necessary to remove such cows from further utilization (Barnouin et al., 2005). However, the practice of these measures, according to the literature data, despite the good effect on Staphylococcus aureus and Streptococcus agalactiae infections, posed another problem. Namely, there was an increase clinical forms of mastitis caused by other pathogens, such as coagulase negative staphylococci, Streptococcus uberis, Streptococcus dysgalactiae, Mycoplasma bovis, Serratia Spp.

The intensive systems for breeding of dairy cows in Republic of Macedonia, in accordance with the National legislation for Animal Welfare (FVA, 2014), practice a free posture system. Such breeding technology requires the existence of a central mill site on farms. It is this segment of the complex objects of farms for milking cows that is one of the key control point for reduction of incidence of mammary gland inflammation. According the research by Nakov., et al. (2014) caried out into the three dairy farms in Republic of Macedonia was found that the most important risk factors that should be monitored are: udder conformation, udder teat conformation and measurement of the distance from teat end to floor. Trajchev and Nakov (2009) suggested mastitis control that outlines these procedures: Systematic examination of herd for detection clinical and subclinical forms of mastitis; Laboratory tests for identification of mastitis causative microorganisms and selection of most appropriate antimicrobial substance for cow therapy; Application of suitable therapy and procedures for mastitis cow treatment; and Preventive measures on farms, which is consist of standard methods and procedures, with main goals to prevent or reduce the influence of new intramamary infections in the herds.

Antimicrobial residues in raw milk

Treatment of mastitic cows, ejection of chronic illness and reduction of the number of new infections is the main goal of the mastitis control program. Mastitis is the most common cause of the use of antimicrobials in dairy cows. The biggest problem in the treatment of mastitic cows is the resistance of microorganisms to the specific group of antibiotics (Rajala-Schultz et.al. 2004.). Mastitis, milk quality and dairy food safety are indeed all interrelated. An increase in the incidence of mastitis in a herd will generally result in increased use of antibiotics, which in turn increases the potential for antibiotic residues in milk and the potential for increased bacterial resistance to antibiotics. This clearly illustrates the importance of effective herd mastitis prevention and control programs. A safe, wholesome, abundant and nutritious milk supply should be the goal of every dairy producer in the world. Effective mastitis control strategies including prudent use of antibiotics will help dairy producers achieve these important goals.

Streptococcus agalactiae was a major cause of mastitis in the pre-antibiotic era. It remains a significant cause of chronic mastitis in many herds, even though it can be readily eliminated. Procedures for the diagnosis and treatment of intramammary infections due to the bacterium are well established. Since it can survive for long periods only within the mammary gland and is susceptible to penicillin therapy, eradication within a closed herd is possible. Herds can be maintained free of infection with S. agalactiae under field conditions. The therapeutic and preventative effectiveness of antimicrobial drugs for bovine mastitis is dependent upon the etiological agent, proper use of the drug under consideration, dairy husbandry, sanitation procedures, and the phase of the disease. The excessive and inappropriate use of antimicrobial agents for the treatment of mastitic cows, such as antibiotics and sulphonamide preparations, can produce resistant strains of pathogenic microorganisms to a particular group of antibiotics, which can complicate the treatment. In addition, to failure to comply the procedure with the milk of dairy cows, which have mastitis and they are in process of medical tretman, may cause seriously problem in the human health.

The research performed in Republic of Macedonia by Trajchev et al., (2017) showed that the isolates of *Streptococcus agalactiae* were mostly susceptible to antimicrobials tested, with some exception. The isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus spp.* were resistant to the most of the antimicrobials (*Penicillin, Ampicillin, Cefuroxim, Cefixim, and Amoxicillin.*).

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 producer's responsibility.

National legislation for milk quality control

The National legislation for milk quality control are very important in order to increase the quality and safety of raw milk production. According to national legislation in Republic of Macedonia for quality of raw milk (FVA, 2012), the milk is classified in terms of the total number of bacteria and the total number of somatic cells. According to the total number of microorganisms, raw milk is classified into three classes: extra class (≤ 100.000 /ml), first class (100.001/ml – 700.000/ml) and second class (700.001/ml – 1.500.000/ml). According to the total number of somatic cells, raw milk also is classified into three classes: extra class (≤ 400.000 /ml), first class (400.001/ml – 500.000/ml) and second class (500.001/ml – 600.000/ml). According this classes, depends the value of raw milk and the quality. The goal of legislation for milk quality is to stimulate farmers to produce hygienically proper milk.

The application of the HACCP - food safety system in cattle farms is still debatable, especially in terms of its implementation. As an alternative, improving the safety of primary cow's milk production is aimed at finding and applying new and more effective codes in good agricultural practice (Morgan, 2004). These codes contain qualitative guidelines for the whole process of milk processing in the farm, in order to produce milk from healthy animals in generally accepted conditions. However, practice has shown that farmers are unable to comply with all instructions, but only for some of them, which they can fulfil (Vissers, 2007). Angelovski et al. (2009) point out the insufficient hygiene in the storage and utilization of dairy cows, the inadequate treatment of milk after milking, as a result of insufficient education of the farmers for hygiene in primary livestock production.

The basic criteria for assessing the safety of raw cow's milk are lying down in the "legislation on the special requirements for safety and hygiene and the manner and procedure for performing official controls of milk and dairy products" (FVA, 2012),. The only difference in these documents is the transitional period of 4 years, which is included in our regulation. This period provides for a gradual reduction in the number of microorganisms and the number of somatic cells in raw milk. Thus, the maximum permitted number of microorganisms in raw cow's milk during 2010 should be up to 400,000 cfu/ml. According to the research conducted by Srbinovska (2007) carried out in the Bitola, Skopje - Kumanovo and Ohrid - Struga regions, the raw cow milk was of poor hygienic quality, since even 76.3% of the milk samples were registered more than 3.000.000 cells of microorganisms / ml bull tank milk. Otherwise, the number of microorganisms was ranged from 15,000/ml to 70,000,000/ml of bull tank milk (Srbinovska, 2007). These data point the fact that the hygienic quality of raw milk and its valorization have more significance for the dairies, but also have an economic interest.

Future assurance to consumers

The most frequent disease in the world production of milk is mastitis. Therefore, it needs more scientific upgrade and application work in our country. The main objective of our mastitis controlling program is therapy of dairy cows with mastitis, culling of chronically mastitis cows, as well as reducing of total number of new infections of mastitis. One of the most important objective of the mastitis control program in dairy herds is the control of raw milk quality and safety.

Milk with clinical mastitis is easy to recognize, but when milk of cows with subclinical mastitis without 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. 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).

All farmers in the Republic of Macedonia should aspire to the legislation for milk quality control to produce more quality milk. The milk producer or, where appropriate, the operator who collects the milk should ensure compliance with the specific safety requirements for the production of raw milk: animal health requirements for the production of raw milk for human consumption, requirements for premises and equipment of hygiene of the holdings for production of raw milk, hygiene requirements during milking, collection and transport and requirements for raw milk.

One of the more clearly defined parts of the farming operation to which the HACCP concept can be applied is in the production of milk. According to the Codex Alimentarius, the validation of the HACCP system is defined as a procedure for gathering evidence that control measures, if they are implemented properly, they are able to control the dangers of certain results.

The most important thing is the regular revision of the documentation and the entire HACCP plan, so the documentation should always follow all the changes that occur in different processing (Khandhke and Meyes, 1998). Only in this way this system can have the long-term ensure of the functioning of food safety - the HACCP plan.

A major health problem in the dairy industry in Republic of Macedonia is mastitis, either clinical or subclinical. HACCP concept should be used in our country as well, and more common. This concept can increase the milk yield and reducing the infections of mammary gland in dairy cows.

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