The importance of bacteriological investigation of milk from cows suffering from mastitis

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Abstract

The aim of this work was to demonstrate the importance of bacteriological investigation of milk from cows suffering from mastitis before the commencement of antibiotic treatment. Individual milk samples (10% of total milked cows; n = 36) from a dairy farm in Eastern Slovakia were tested for the presence of contagious and environmental mastitis pathogens. The following bacteria were identified in ten cows (27.77%): *Streptococcus agalactiae, Staphylococcus lentus, Staphylococcus xylosus, Staphylococcus sciuri* and *Staphylococcus equorum.* Bacteria such as *Staphylococcus epidermidis, Pseudomonas aeruginosa, Bacillus* spp. and members of the Enterobacteriaceae family were sporadically isolated and identified in other individual milk samples (n = 26). No *Staphylococcus aureus* or *Streptococcus agalactiae* were isolated in the milk from these cows. All isolates of *Streptococcus agalactiae* were resistant to amoxicillin-clavulanate, azithromycin, penicillin and oxacillin and partially sensitive to cephalothin and cefazolin antibiotics.

Bacteriological investigation, cow mastitis, milk

Introduction

Inflammation of the mammary glands continues to represent significant economic losses for primary milk producers even at the present time, not merely as the result of reduced milk quality and the associated low prices of milk, but also as a result of the costs arising from the treatment of cows suffering from mastitis and the services of veterinarians. The figures on the occurrence of mastitis on dairy farms in the individual regions vary, and range from 5 to 50% of all dairy cows, in which one or more quarters of the udder may be affected. Although the use of antimicrobial therapy improves the health and comfort of animals, the economic losses associated with the additional financial costs and the spoilage of milk are large (Erskine et al. 2003; Kayitsinga et al. 2017).

In accordance with Regulation (EC) No. 853/2004 of the Parliament and of the Council, as amended, raw milk for processing for human consumption must, among other things, come from dairy cows that show no evident signs of impairment to their overall state of health or evident signs of inflammation or injury to the mammary glands or the skin of the mammary glands. The milk must be fresh, from one or more milkings, and obtained by the complete emptying of the udders of the cows. In addition, milk that contains foreign substances or their residues, substances with pharmacological and hormonal effects, antibiotics, cleaning and disinfecting agents, or substances that may cause technological and organoleptic imperfections to milk, etc. must also be excluded from deliveries for dairy treatment and processing because such substances may pose a threat to human health.

It follows from the above that operators of food companies that produce raw milk must ensure the fulfilment of the conditions stipulated by the legislation of the European Union and assure that the lowest possible (or preferably no) occurrence of mastitis is recorded in their herd in dairy cows producing milk for further processing for human consumption.

Mastitis is defined as inflammatory change to the mammary glands that is characterised by physical, chemical and microbiological changes to milk, as well as pathological

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changes to the tissue of the mammary gland resulting in changes to the composition of milk and changes to the basic and characteristic properties of milk (Dudriková 2000).

The microorganisms contributing to the onset of mastitis are divided into (1) contagious organisms that colonise the mammary gland and are transmitted by milking equipment and milkers, e.g. *Streptococcus agalactiae, Streptococcus dysgalactiae, Staphylococcus aureus* and *Mycoplasma* spp., with their primary reservoir being infected mammary glands, and (2) environmental pathogens (e.g. *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter aerogenes, Serratia* spp., *Proteus* spp., *Pseudomonas* spp. and other Gram-negative bacteria, coagulase-negative staphylococci, environmental streptococci, microscopic filamentous fungi and yeasts, and other pyogenic bacteria such as *Trueperella pyogenes* and *Corynebacterium bovis* or members of the genus *Prototheca*), with their primary reservoir being the environment.

The most important thing is to recognise which group of agents of mastitis occurs in the herd most frequently or causes clinical or subclinical mastitis. Not just the clinical diagnosis of mastitis, but also its treatment with antibiotics and the exclusion of raw milk from supplies for further processing for human consumption during treatment and the stipulated withdrawal period for the given type of medicament, follow from this. Injudicious use of broad-spectrum antibiotics without a bacteriological diagnosis of the agent/agents of mastitis may result in the development of highly resistant strains of microorganisms that subsequently result in the high ineffectiveness of treatment and an increase in the costs of keeping dairy cows. The bacteriological investigation of milk represents a practical solution to the identification of agents of illness and a plan for the effective treatment of mastitis with antibiotics.

In view of growing problems in the primary production of milk associated with the use of antibiotic treatment of mastitis and the possible spread of strains of agents of mastitis highly resistant to the antibiotics used in practice, the aim of our pilot study was to determine the following in a herd of dairy cows in deep-litter housing: (1) the occurrence of agents of mastitis in individual samples of milk in 10% of the dairy cows in the total herd, and (2) the sensitivity of the identified isolates of microorganisms to selected types of antibiotic used most frequently in the treatment of mastitis in veterinary practice.

Materials and methods

We collected individual samples of raw milk (10% of the total number of milked cows; n = 36) at the beginning of morning milking, after discarding the first squirts of milk and disinfecting the ends of the teats with 70% alcohol, in sterile test tubes from each quarter *ana partes,* while observing the principles for the bacteriological investigation of samples of milk. The dairy cows are reared in deep litter on a commercial farm in the Eastern Slovak Region. This producer of raw milk has recently been experiencing problems with a large number of somatic cells contained in the milk (more than 400 000 ml^{-1} of milk) and with the reduced effectiveness of antibiotic treatment of cows affected by mastitis. We transported the samples of milk taken in a cool box and subjected them to immediate bacteriological investigation at the University of Veterinary Medicine and Pharmacy in Košice in accordance with the principles of Oliver et al. (2004). The following cultivation media for the isolation of bacteria were used: Blood agar base No. 2 (Oxoid) with the addition of 5% of defibrinated sheep blood, which we used for the detection of the haemolytic activity of the investigated bacteria. We also used the selective growth media Endo agar (Oxoid) for detection of enteric bacteria and Baird-Parker agar (Oxoid) for detection of coagulase positive staphylococci. The surface of these selective media was inoculated with 0.1 ml of each undiluted pre^{a} growth sample of milk. Then the plates were incubated at 37 °C for 24 – 72 hours under aerobic conditions. We identified the bacteria on the primary cultivation medium on the basis of the morphology of the grown colonies, the type of haemolysis, and catalase and oxidase tests. After microscopical examination the Gram-positive and Gram-negative bacteria were differentiated. In suspected streptococcal species also CAMP test on blood agar was performed and *Streptococcus agalactiae* was proven. Further tested isolates presumptively identified as staphylococci were tested for coagulase activity (Coagulase Slide Test, Merck) and staphylococcal species were subsequently identified according to biochemical indicators (STAPHYtest 24, Erba Lachema, CZ). On the other hand, suspected colonies of streptococci were assessed on the basis of growth in 6.5% NaCl, aesculin hydrolysis and a CAMP test and identification was done on the basis of biochemical reactions using the API 20 System (bioMérieux Inc., Hazelwood, MO). Gram-negative bacteria were detected in the same way on the basis of their biochemical reaction and species were identified by using the API 20E System (bioMérieux Inc., Hazelwood, MO).

We tested bacteria identified as *Streptococcus agalactiae* by a disk diffusion method for sensitivity to the following types of antibiotics: cefotaxime, cephalothin, cefazolin, amoxicillin-clavulanate, azithromycin, penicillin and oxacillin. Resistance of strains were being given by a zone of inhibition with size less than 18 mm (EUCAST 2018).

Results and Discussion

Individual samples of raw cow's milk (n = 36) were taken at a commercial farm from dairy cows reared on deep litter. *Streptococcus agalactiae* was isolated and identified in five of these cows. Strains of the genus *Staphylococcus* (*S. lentus, S. xylosus, S. sciuri* and *S. equorum*) were confirmed by the STAPHYtest 24 in another five cows. The bacterial species like *Staphylococcus epidermidis, Staphylococcus sciuri, Pseudomonas aeruginosa* and also members of *Bacillus* spp. and members of the family *Enterobacteriaceae* were sporadically isolated and identified in other individual samples of raw milk (n = 26). Neither *S. aureus* nor *Streptococcus agalactiae* were isolated in any of these samples of milk.

All the isolates of *Streptococcus agalactiae* were resistant to amoxicillin-clavulanate, azithromycin, penicillin and oxacillin and partially sensitive to cefotaxime, cephalothin and cefazolin (the size of the zones of inhibition around these antibiotic discs ranged from 18 to 20 mm).

The determination of resistance, or partial sensitivity, to the tested antibiotics in the identified strains appears a big problem in the given herd, as these antibiotics have been used for treating dairy cows for a relatively long time. It is, therefore, necessary to draw up a recovery plan in the herd of dairy cows, to perform bacteriological diagnostics of all the cows, including cows that do not show clinical symptoms of mastitis or a large number of somatic cells in the milk (Tančin et al. 2007), for the presence of pathogenic microorganisms causing mastitis, and to determine the sensitivity of isolates to a wider range of the antibiotics that can be used in practice to treat mastitis. In addition, it is also necessary to exclude from the herd all cows with a chronic and lasting problem with mastitis and focus on the thorough sanitation (cleaning and disinfecting) of all areas in which the cows are kept. The observation of milking hygiene, udder cleaning and the personal hygiene of milkers is also essential, as the isolates we found and confirmed indicate that there is, in all probability, a high prevalence of both contagious and environmental agents of mastitis in the given herd.

Reduction to the use of antibiotics for the treatment of mastitis resulting from the performance of the bacteriological investigation of milk before the commencement of antibiotic treatment instead of empirical therapy (the traditional method of treatment with the immediate use of broad-spectrum antibiotics) is stated by authors including Hess et al. (2003), Lago et al. (2011) and Wagner and Erskine (2013).

Conclusions

On the basis of our results from the isolation and identification of bacteria from individual samples of raw cow's milk that contribute to the onset of contagious and environmental mastitis (*Streptococcus agalactiae, S. lentus, S. xylosus, S. sciuri* and *S. equorum*), and in view of the antibiotic resistance or partial sensitivity to the tested antibiotics (cefotaxime, cephalothin, cefazolin, amoxicillin-clavulanate, azithromycin, penicillin and oxacillin, according to EUCAST) determined in isolates of *Streptococcus agalactiae* in the samples of raw cow's milk we tested, we can confirm the necessity of using the bacteriological investigation of milk for the presence of agents in the herd of dairy cows, including determination of sensitivity to antibiotics by a private veterinarians, before treatment that will not have the desired effect is commenced. Such ineffective treatment ultimately results not merely in stress caused to animals as a result of illness (pain, trauma to the mammary glands, the application of antibiotics by a vet), but also in increased costs in assuring a raw material (milk) of a high quality intended for further processing for human consumption.

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