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CONTROLLING OF *MYCOPLASMA BOVIS* AT A FARM IN UKRAINE AS A PART OF ERADICATION PROGRAM OF MASTITIS

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Summary. The article analyses the approaches to the diagnosis of mycoplasmal mastitis in cows. Despite decades of research in the field of etiology and pathogenesis of cattle mycoplasmosis, the diagnosis of this infection remains a topical issue in veterinary medicine. In time detection of infected cows, compliance with preventive measures in the milking parlors and in the newborncalves facilities, and the setting the order of milking are the main methods for preventing outbreaks of mycoplasmosis in farms. The necessity of proper sampling using and the order of the procedure for milking cows for the diagnosis of *Mycoplasma bovis* and other species of mycoplasmas are substantiated. Basing on the analysis, the following algorithm for determining the infectious status for *Mycoplasma* spp. and sampling for diagnosis, as well as the procedure for milking cows, was offered.

The data obtained as a result of the study indicate the necessity to plan a set of preventive and therapeutic measures in the farms to prevent the infection of healthy animals with a contagious mycoplasmal infection.

Keywords: mastitis, *Mycoplasma bovis*, contagious pathogen, cows, Ukraine, control

Introduction. Mastitis is a well-known and economically significant disease of dairy cattle. Mycoplasmosis is a common disease in cattle-breeding industry, that bring to farms many economic problems such as culling milk, spending money for treat unhealthy cows, culling animals, decreasing of milk products' cost and other. Contagious organisms are well adapted to survive and grow in the mammary gland and frequently cause infections lasting weeks, months or years (Maunsell et al., 2011; Barkema et al., 2009).

Mycoplasma species with the exception of some mycoplasmal infections that may originate in the other parts of the body and spread systemically, these three organisms gain entrance into the mammary gland through the teat canal. Thus, it is necessary to consider, that mycoplasmas can also cause inflammations of a mammary gland secretory tissue and aggravate process of treatment (Fox, Kirk and Britten, 2005). Mycoplasmosis is a disease of cattle, which is widespread in farms around the world. The most pathogenic and often diagnosed pathogen is *Mycoplasma bovis* (Pothmann et al., 2015; Lysnyansky et al., 2016; Aebi et al., 2015; Spergser et al., 2013) although there is evidence of the absence of this pathogen in the Czech Republic (Surýnek, Vrtková and Knoll, 2016).

Mycoplasmas belong to the simplest free-living prokaryotes. Their sizes are very close to viruses, but they have their own DNA replication, transcription and protein synthesis systems, are able to reproduce in conditions of an artificial nutrient medium. The size of the genome within the Mollicutes class can vary greatly, from 580 to 2,200 thousand base pairs (Stukolkina, 2005).

Mycoplasmosis has extensive clinical manifestations; it causes not only udder lesions, but also problems of the reproductive and musculoskeletal system, inflammation of the eyes and ears of animals. The carrier of the disease can be any mastitis-sick animal, which initially had a respiratory or reproductive form of mycoplasmosis because microorganisms enter the mammary gland through the circulatory system. The contagiousness and persistence of bacteria in the herd usually depend on many factors: the number of infected animals and the general immunity status, the effect of stress factors (Stipkovits et al., 2000; Houlihan et al., 2007).

The lack of a wall of Mollicutes cells makes them more sensitive to the environment, so they have a reduced ability to survive outside the animal's body (Jones and Simecka, 2003).

During the infectious process, they are mainly localized in immunocompetent cells (macrophages). *M. bovis*, entering the epithelial cells of the mammary gland, causes a cellular immune response of the body, which is manifested by an increase in the expression of mRNA of tumor necrosis factor-alpha, interleukins (IL) IL-1β, IL-6, IL-8, lactoferrin, Toll-like receptor-2, RANTES chemokine, and serum amyloid (Zbinden et al., 2015).

The chronic nature of this infection indicates that all components of the immune system can participate in response to *Mycoplasma* spp.; however, T cells are the main component of the immune response against mycoplasma infection. Progression of mastitis depends on the balance between the components of the cellular immune response, which can contribute both to an

increase in the resistance of the host organism, and cause immune-mediated pathogenesis (Rodríguez et al., 2015).

Determining the presence of *Mycoplasma* spp. in milk is an important part of the mastitis control program (Fox, 2013). Sampling, in this case, should occur in accordance with the rules of asepsis and depend on the methodology of further analysis. In the field of applied research, an urgent need is to develop cost-effective, sensitive and specific diagnostic tests that can provide accurate identification of infected animals. Data on the prevalence of pathogens are necessary in order to determine the consequences of mycoplasma infections and develop recommendations for the eradication of contagious mastitis (Rossetti, Frey and Pilo, 2010; Justice-Allen et al., 2011).

The aim of the study was to improve the diagnostics algorithm to control the epizootic situation in the farms. For this purpose, the following tasks were set: to develop a scheme for controlling herd infection using an approach for detecting the *Mycoplasma* spp. and *M. bovis* genomes in the milk of cattle by polymerase chain reaction and to approve this approach at farms in Ukraine.

Materials and methods. Research carried out in LLC 'Center of Veterinary Diagnostics' (Kyiv, Ukraine). Milk samples were taken from the different region of Ukraine and taken into sterile 30 ml phials and kept at –20 °C. Containers with selected for research samples were marked with the date of sampling, the age of the animal, the name of the farm and the area.

To identify the genetic material of bacteria of the genus *Mycoplasma* spp., primers were selected in the milk samples using the Vector NTI Advanced 11 software package ('Invitrogen', USA). Primers were tested on test strain *Mycoplasma* spp. (State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine) selected by analyzing their level of homology to the selected DNA template of the pathogen:

F: 5'-ACTCCTACGGGAGGCAGCAGTA-3'
R: 5'-TGCACCATCTGTCACTCTGTTAACCTC-3'

The test system 'LSIVetMAX Screening Pack Real-Time PCR Kit, ruminant respiratory diseases' (France) was used for *M. bovis* detection. Amplification was conducted on a 7500 Fast Real-Time PCR-System device ('Applied Biosystems', USA).

Interpretation of the results was carried out by analyzing the curves obtained by the thermocycler, based on the presence or absence of the intersection of the fluorescence curve with the threshold line set at a certain level. A sample was considered positive if the Ct value on the FAM/Green channel was less than 45. The sample was considered negative if there was no fluorescence curve on the FAM/Green channel, and the detected fluorescence curve VIC/channel and the Ct value were less than 45 (internal endogenous reaction control).

Results and discussion. Samples of milk were selected from cows according to the clinical signs (milk was apparently with clots and containing 'flakes') and after carrying out a special test for the presence of somatic cells ('PortaChek', USA) and lactate dehydrogenases ('PortaChek', USA). It was also known in advance that the studied farms were unfavorable for mycoplasmosis (up to 30% of animals had vulvovaginitis).

The analysis of 144 milk samples obtained from 20 dairy farms for the presence of the *Mycoplasma* spp. genome revealed the presence of a pathogen DNA in 61 samples.

Positive samples were combined together into prefabricated samples according to the name of the farm, but no more than 5 samples per sample. Analysis of these samples for the presence of DNA of *M. bovis* revealed the DNA of the pathogen in 10 samples obtained from 15 farms.

Further positive samples were analyzed for the quantitative determination of the DNA content of the pathogen using real-time PCR. The results of such analysis are shown in the Tables 1 and 2.

Table 1 — DNA detection of *Mycoplasma* spp. in different region of Ukraine

Region	Number of farms examined	Number of samples examined	Number of *Mycoplasma* spp. positive samples
Poltava	5	17	4
Kharkiv	3	16	16
Sumy	1	8	8
Khmel-nytskyi	2	11	7
Kyiv	4	47	9
Vinnytsia	1	2	2
Mykolaiv	1	14	0
Cherkasy	3	19	5
Total	20	144	61

Thus, the results of a study of pathological milk for the presence of *Mycoplasma* spp. and *M .bovis* DNA showed that most cows with the identified genetic material of the pathogen in milk had also problems with infectious diseases of the reproductive and respiratory tract. The obtained data allow planning a complex of preventive and therapeutic measures in farms to prevent infection of healthy animals.

Table 2 — Results of quantitative determination of *M. bovis* genetic material in the researched cow's milk using real-time PCR (Ct value)

Region	Number of farms examined	Number of samples examined	Quantitative determination of *M. bovis*
Poltava	1	2	*Ct = 36.98
Kharkiv	3	5	Ct = 24.97
Sumy	1	2	0
Khmel-nytskyi	2	3	0
Kyiv	4	5	Ct = 37.97 Ct = 24.97 Ct = 28.97 Ct = 34.17 Ct = 22.33
Vinnytsia	1	2	0
Cherkasy	3	3	Ct = 37.97 Ct = 25.85 Ct = 32.20
Total	15	20	10 (positive)

Note: * — the value of 'Ct' is a relative indicator of the amount of the desired genetic material in the sample and is expressed in cycles from 1 to 35: the higher the cycle, the less the primary amount of material in the sample and vice versa. Ct = 16–24 — '+++', Ct = 25–31 — '++', Ct = 32–37 — '+', Ct ≥ 38–40 — '+/–'.

Basing on the analysis, the following algorithm for determining the infectious status for *Mycoplasma* spp. and sampling for diagnosis, as well as the procedure for milking cows was proposed (Fig. 1).

To control the infected status of the herd for contamination by *Mycoplasma* spp. it is necessary to collect milk samples regularly for PCR analysis from a common milk bulk tank, and it is needed to take samples from each cow with an increase in somatic cell counts and lactate dehydrogenase in a mammary gland secretion (Group № 3).

In addition, samples should be taken from all cows transferred to the milking herd immediately after calving or during lactation (Group № 2).

In order to prevent transmission of mycoplasmas through the milking machine, the newly acquired cows and calves must be quarantined for testing on *Mycoplasma* spp. before entering the herd. Cows with clinical signs of mycoplasmal infection (arthritis, conjunctivitis, vulvovaginitis) should be checked for the presence of *Mycoplasma* spp. and after confirmation of infection should be distributed to the appropriate milking group (Group № 1).

Cows that are positive for *Mycoplasma* spp. should be milked after healthy cows and after cows with mastitis of another etiology. Negative herds that regularly acquire cattle should diagnose the milk from a tank on the mycoplasma, twice a month.

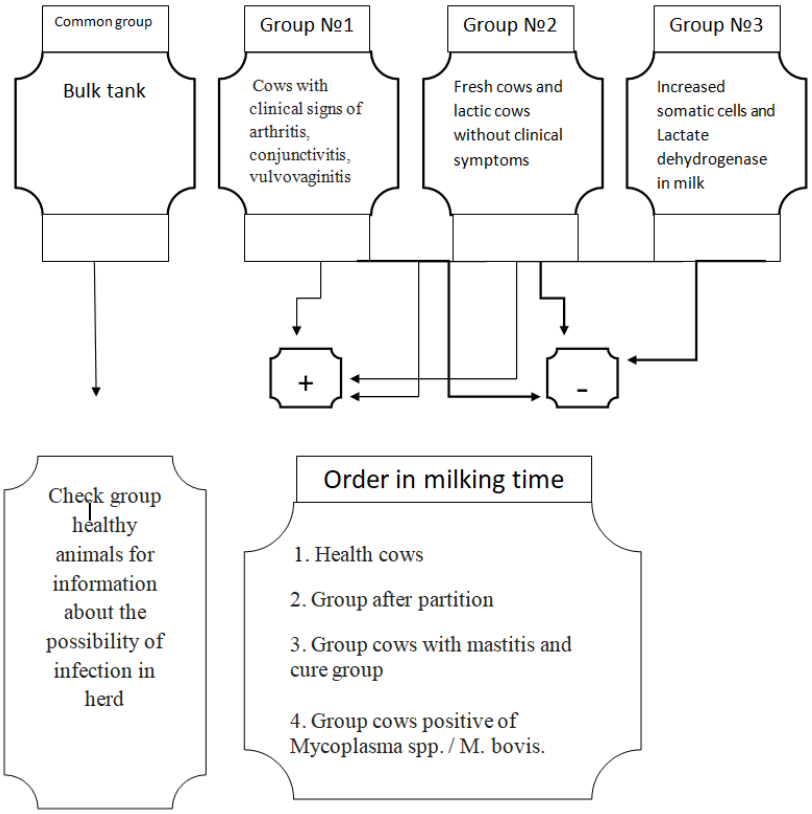

Figure 1. Algorithm for determining the status of the herd infection and sampling for the diagnosis, and the procedure milking procedure.

Conclusions. As a result of the conducted studies the present of *Mycoplasma* spp. was detected in 63 samples of all milk samples. *Mycoplasma bovis* pathogens were detected in 10 samples of milk samples. Basing on the findings, an algorithm for sampling for carrying out molecular diagnostics of mastitis of *Mycoplasma* etiology, as well as a sequence of milking cows in unfavorable farms, is proposed to prevent the spread of the disease. The results can be applied to mastitis controlling plan at farm.

The detection of infected cows, the observance of preventive measures in the milking parlor and in the rooms with new cows and the order of milking are the main methods of preventing outbreaks of mycoplasmosis.

Each farm can be exposed to breast diseases, and the number of infections is increasing if the pathogenic microorganisms associated with mastitis are not detected in time.

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