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Received on 28.07.2020

Approved for publication on 14.10.2020

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ORIGINAL ARTICLES | BOVINE DISEASES ОРИГИНАЛЬНЫЕ СТАТЬИ | БОЛЕЗНИ КРС

DOI: 10.29326/2304-196X-2020-4-35-255-260

UDC 619:618.19-002:636.2:615.371:637.12.05

Quality profile of milk from high producing dairy cows vaccinated against mastitis

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SUMMARY

One of the raw milk quality criteria is the count of somatic cells, produced by the cow's immune system to fight infectious diseases of the mammary gland. The paper presents the analysis of somatic cell count and total bacteria count of milk from cows, vaccinated against mastitis using Startvac vaccine. Tests were performed as a comparison between a dairy unit and a farm under different management conditions and using different milking techniques. Six months after the start of the vaccine application the somatic cell count at the dairy unit decreased by 60 thousand/ml, at the farm by 182 thousand/ml. The agent profile was represented by the following bacteria: *Enterococcus faecium*, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Bacillus*, *Lactobacillus* were also isolated from udder secretion. After a year of immunization somatic cell count both at the unit and on the farm decreased by 245 and 216 thousand/ml respectively; it is noteworthy that 43.75% of microflora isolated from mammary gland secretion was represented by *Streptococcus* spp. After two years of the vaccine use the somatic cell count was equal to 371 and 725 thousand/ml at the unit and on the farm respectively. Tests of mammary gland secretions revealed *Streptococcus* spp. in 27.27% of cases, *Staphylococcus aureus* and *Enterococcus faecium* were isolated in 18.18% of tested samples. It was established that after three years of the vaccine use the major cause of mastitis in cows was *Streptococcus* spp. (55.00%). During four years of tests, a downward trend in somatic cell count of bulk milk from high producing dairy cows as well as in the number of agents responsible for inflammation in a mammary gland was detected. Somatic cell count of milk from vaccinated animals decreased by 286 and 432 thousand/ml at the unit and on the farm respectively. During the test period *Staphylococcus aureus* isolation rate declined by 19.41%.

Key words: mastitis, Startvac mastitis vaccine, somatic cells, mastitis agents, milk quality.

Acknowledgements: The studies were performed with the financial support of the Ministry of Education and Science of the Russian Federation within the framework of the Program of Fundamental Research at the State Scientific Academies for 2013–2020 using Molecular, Biological and Nanobiotechnological Techniques for the Development of Next Generation Biologicals, Technologies and Methods of Their Use to Control Highly Dangerous Infectious, Parasitic and Non-Contagious Animal Diseases.

For citation: Isakova M. N., Sivkova U. V., Ryapsova M. V., Shkuratova I. A., Lysov A. V. Quality profile of milk from high producing dairy cows vaccinated against mastitis. *Veterinary Science Today*. 2020; 4 (35): 255–260. DOI: 10.29326/2304-196X-2020-4-35-255-260.

Conflict of interest: The authors declare no conflict of interest.

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УДК 619:618.19-002:636.2:615.371:637.12.05

Показатели качества молока высокопродуктивных коров на фоне применения противомаститной вакцины

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РЕЗЮМЕ

Одним из качественных показателей сырого молока является содержание в нем соматических клеток, играющих защитную роль против инфекционных заболеваний молочной железы коров. В статье приведен анализ уровня соматических клеток и бактериальной обсемененности молока на фоне применения противомаститной вакцины Startvac. Исследования проводились в сравнении: на базе комплекса и фермы, различающихся условиями содержания и технологией доения. Через 6 месяцев с начала применения вакцины уровень соматических клеток в комплексе снизился на 60 тыс./мл, на ферме – на 182 тыс./мл. Структура возбудителей была представлена такими бактериями, как *Enterococcus faecium*, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*. В секрете вымени также были выделены *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Bacillus*, *Lactobacillus*. После года иммунизации животных количество соматических клеток в комплексе и на ферме снизилось на 245 и 216 тыс./мл соответственно, при этом выделенная из секрета молочной железы микрофлора в 43,75% случаев была представлена *Streptococcus* spp. Спустя два года с начала применения вакцины показатель соматических клеток в сборном молоке в комплексе и на ферме составил 371 и 725 тыс./мл соответственно. Исследование секрета молочной железы показало наличие в 27,27% случаев *Streptococcus* spp.; *Staphylococcus aureus* и *Enterococcus faecium* выделены в 18,18% исследуемых проб. Установлено, что спустя три года иммунизации основной причиной мастита у коров было наличие *Streptococcus* spp. (55,00%). За четырехлетний период исследований выявлена тенденция к снижению показателя соматических клеток в сборном молоке высокопродуктивных коров, а также спектра возбудителей, вызывающих воспаление в молочной железе. Количество соматических клеток в сборном молоке на фоне иммунизации животных снизилось в условиях фермы и комплекса на 286 и 432 тыс./мл соответственно. За период исследования наблюдается снижение высеваемости *Staphylococcus aureus* на 19,41%.

Ключевые слова: мастит, противомаститная вакцина Startvac, соматические клетки, возбудители мастита, качество молока.

Благодарность: Работа выполнена при финансовой поддержке Минобрнауки России в рамках Программы фундаментальных научных исследований государственных академий наук на 2013–2020 гг. по направлению «Молекулярно-биологические и нанобиотехнологические методы создания биопрепаратов нового поколения, технологии и способы их применения с целью борьбы с особо опасными инфекционными, паразитарными и незаразными болезнями животных».

Для цитирования: Исакова М. Н., Сивкова У. В., Ряпосова М. В., Шкуратова И. А., Лысов А. В. Показатели качества молока высокопродуктивных коров на фоне применения противомаститной вакцины. *Ветеринария сегодня*. 2020; 4 (35): 255–260. DOI: 10.29326/2304-196X-2020-4-35-255-260.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

One of the most essential prerequisites for the increase in milk yields and dairy farming performance is the improvement of existing breeds and their genetic potential [1]. Herewith, milk quality in dairy production is prioritized.

A quality parameter of raw milk is the number of somatic cells, which are in fact cells of different tissues and organs, playing a protective role against udder infectious diseases. They include white blood cells (WBCs), red blood cells (RBCs), columnar, squamous and cuboidal epithelial cells [2–5]. Numerous genetic and environmental factors influence the number and types of WBCs, which compose the majority of somatic cells in milk from healthy animals. Quality of milk from high producing dairy cows is adversely affected by infection-induced mammary gland inflammation, which is manifested by increased levels of somatic cells. The overwhelming majority of mammary gland somatic cells is represented by neutrophils [6, 7]. A low somatic cell count is a reliable indicator of high quality milk, free from pathogenic organisms.

Antimicrobial therapy, notwithstanding the presence of sensitive pathogens, is often unsuccessful; moreover, the drug components are secreted with milk for a long time. Search for different approaches that allow producing milk with a low somatic cell count and maintaining this count at the optimal level is a vital task in modern ve-

terinary practice. One of the measures to prevent mastitis, i.e. to maintain low somatic cell counts, is the vaccination of cows [2, 8].

The study objective was to evaluate the effect of mastitis vaccine Startvac (Laboratorios Hipra, Spain) on somatic cell count and total bacterial count of milk from high-producing dairy cows.

MATERIALS AND METHODS

The study was conducted in 2016–2020 in the Laboratory for Reproductive Pathology and Young Animal Diseases and Department of Veterinary and Laboratory Diagnosis under Testing Laboratory of the FSBSI UrFASRC UrB of RAS.

Experimental studies were performed at the breeding farm, located in the Sysertsky Raion of the Sverdlovsk Oblast. 1,400 Holstein-Friesian cows with annual milk production rate of 9,299 kg are kept on the farm. The average period of the cow's use for milk production is 3.8 lactations. The tests were conducted as a comparison between the dairy unit (free stall housing and milking in parallel system milking parlors) and on the farm (tie stall housing and milking using pipeline milking machines).

Mastitis vaccine Startvac for cows (Laboratorios Hipra, Spain) was registered in the Russian Federation in 2010. Externally the vaccine is a homogenous white-yellow emulsion. It is filled in glass bottles 1, 5 and 25 doses per each;

the bottles are sealed using rubber stoppers and aluminum caps. The vaccine is produced from inactivated cells of *Escherichia coli* (J5) and *Staphylococcus aureus* (CP8), containing slime associated antigenic complex (SAAC), with the following excipients added: liquid paraffin 9.5 mg/ml and benzyl alcohol 10.5 mg/ml. One inoculation dose (2 ml) contains at least 50 effective immunogenic doses of *Escherichia coli*, strain J5 and at least 50 effective immunogenic doses of *Staphylococcus aureus* (CP8) strain SP 140. The vaccine is administered intramuscularly in the amount of 2 ml, its temperature shall be +15 to +25 °C. The solution shall be shaken before use.

The first immunization using Startvac vaccine of all cows on the breeding farm was conducted in December 2016. Three weeks later, all animals were revaccinated and then boosted every three months. By the time when the paper was ready, 14 vaccinations had been performed with the most recent one to occur in April 2020.

Bulk raw milk was tested every month for somatic cell count using DCC cell counter (GMU Tumba DeLaval International AB, Sweden).

During the test period milk samples were collected for further molecular, genetic and microbiological tests and evaluation of vaccination effect on the presence of agents, responsible for inflammation in the mammary gland. The samples collected were tested by real-time polymerase chain reaction using Rotor-Gene 3000 (Corbett Research, Australia) and the following test-kits “Vetscreen. STREPTOPOL-V”, “Vetscreen. STAPHYPOL”, “Vetscreen. COLYPOL”, “Vetscreen. STREPTOPOL” (OOO “IDS”, Moscow). For the purposes of bacteriological and microbiological testing milk samples were seeded onto liquid and solid nutrient media, in particular beef extract broth, beef extract agar, Endo agar, Sabouraud agar, mannitol salt agar, enterococci agar, Hiss serum sugars. The recovered isolates were identified using Bergey's Manual of Systematic Bacteriology and manual of pathogenic and opportunistic fungi. During the study of mastitis vaccine effectiveness, 125 milk samples from high producing cows were tested.

RESULTS AND DISCUSSION

The previous studies, conducted on the breeding farm, showed numerous mastitis cases in cows during the year. The morbidity in 2015 was 12.2% out of the total number of tested cows; in 2016, the number of udder inflammations grew up to 22.1%, which is 1.8 times higher as compared with the previous year. In 2015, clinical mastitis was detected in 6.8% and subclinical mastitis was found in 5.4% of animals. In 2016 there was a change in this ratio: more animals suffered from subclinical mastitis (17.4%) and clinical mastitis was identified in 4.8% of animals [9]. Before vaccination somatic cell count in bulk milk from high producing cows, kept at the dairy unit, was 695 thousand/ml, and on the farm this value was equal to 916 thousand/ml. The most commonly detected microorganisms were *Staphylococcus aureus* (29.42%) and *Streptococcus* spp. (23.53%). *Enterococcus* bacteria were recovered from 11.76% of samples. 17.65% of tested samples contained *Aspergillus* mold. Gram-negative bacteria of *Klebsiella* spp. (5.88%), *Pseudomonas* (5.88%), *Enterobacter* (5.88%) were also isolated. Moreover, the microorganisms were detected as monocultures (27.3%), and as mixed cultures of bacteria (55.6%), fungi and yeasts (17.1%). These tests demonstrated that mastitis in animals, and as a result

increased somatic cell counts in milk, were caused by a rather wide range of agents.

Due to a high incidence of subclinical mastitis in lactating cows, responsible for increased somatic cell counts in milk, and a large number of animals suffering from udder inflammations, caused by *Staphylococcus aureus*, it was decided to vaccinate animals with Startvac mastitis vaccine.

When the vaccination started, the somatic cell count in milk samples from cows, kept at the dairy unit and milked using special milking equipment, was 559 thousand/ml. The somatic cell count in bulk milk from cows, kept on the farm and milked using a stationary pipeline equipment, was 822 thousand/ml.

After vaccination, the somatic cell count started decreasing gradually, and then grew insignificantly, but the general declining trend continued. In six months after vaccination had started the somatic cell count at the dairy unit and on the farm decreased by 60 and 182 thousand/ml correspondingly. At that moment, the mastitis in cows was caused predominantly by *Enterococcus faecium* (20.00%), *Staphylococcus aureus* (17.33%), *Streptococcus* spp. (17.33%), *Pseudomonas aeruginosa* (14.67%), *Streptococcus agalactiae* (8.02%), *Staphylococcus saprophyticus* (5.33%), *Staphylococcus epidermidis* (5.33%), *Enterococcus faecalis* (5.33%), *Escherichia coli* (1.33%), *Bacillus* (1.33%), *Lactobacillus* (1.33%) were also isolated from milk. In 2.67% of tested milk samples, no pathogenic and opportunistic microorganism growth was observed.

After one year of mastitis vaccine application, tests of bulk milk showed stable decrease in somatic cell counts at the dairy unit with free stall housing and milking in parallel system milking parlors, SCC was equal to 450 thousand/ml, which is 245 thousand/ml less as compared to the value, obtained before vaccination. On the farm with a tie stall housing and milking using pipeline milking machines no steady tendency in decline of somatic cell counts was observed. After one year of Startvac vaccine use the somatic cell count decreased by 261 thousand/ml, but in the following month a rise in this value by 227 thousand/ml was observed (Fig. 1). The microflora isolated from milk in 43.75% cases was represented by *Streptococcus* spp., and by *Streptococcus agalactiae* in 31.25% of tested samples. The proportion of *Staphylococcus aureus* and *Escherichia coli* positive samples, responsible for mastitis, was the same (12.50% each).

After two years of vaccination against mastitis somatic cell counts in bulk milk at the dairy unit and on the farm were 371 and 725 thousand/ml correspondingly (Fig. 2). Thus, a gradual decrease of this indicator was observed at the dairy unit, whereas on the farm this decrease was intermittent. The major part of the mastitis agents was represented by *Streptococcus* spp. (27.27%). The numbers of *Staphylococcus aureus* (18.18%) and *Enterococcus faecium* (18.18%) were equal in the tested samples. The percentage of *Escherichia coli* among the pathogens isolated from milk was 9.10%. The number of samples, in which no microorganism growth was detected, was 27.27%.

After three year-immunization of high-producing cows, the somatic cell count in bulk milk was at the level of 630 thousand/ml on the farm and 263 thousand/ml at the dairy unit, which is 286 and 432 thousand/ml lower than this value before the vaccination program was applied. Results of molecular genetic and microbiological studies showed that most cases of mastitis in cows were caused by *Streptococcus* spp. (55.00%). *Staphylococcus aureus* was

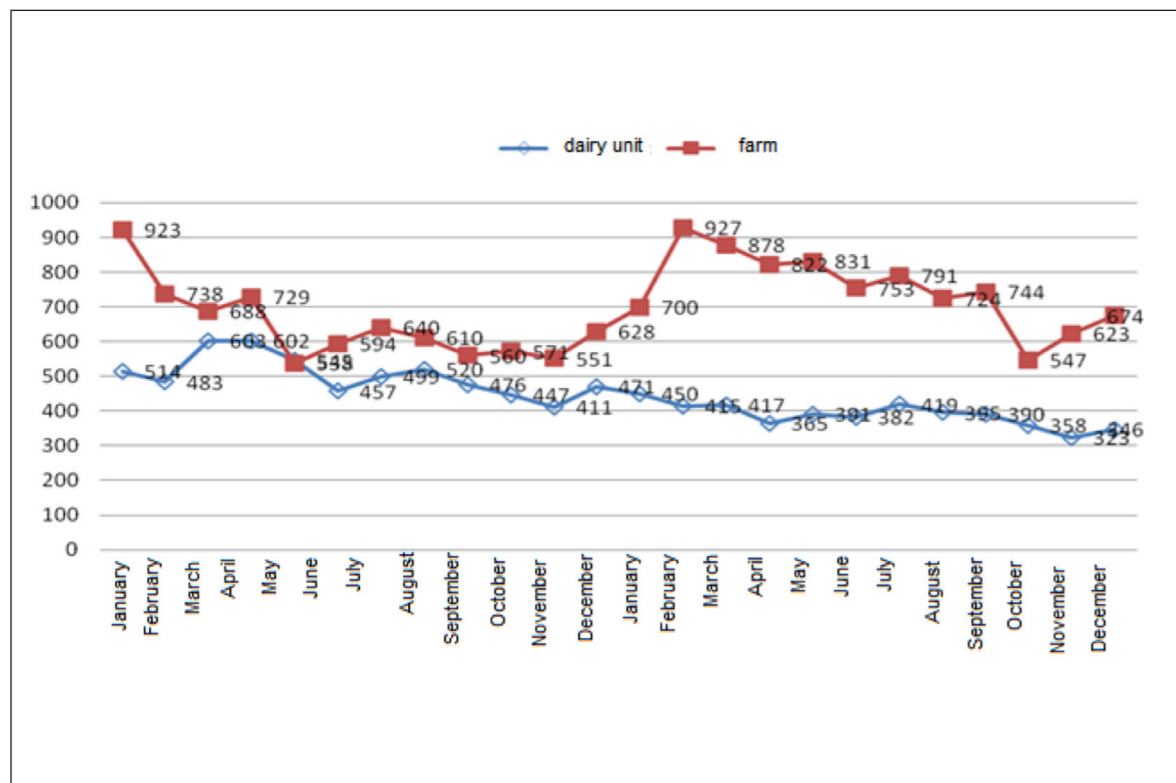


Fig. 1. Dynamics in somatic cell counts in 2017–2018

Рис. 1. Динамика уровня соматических клеток в период с 2017 по 2018 г.

isolated in 10.00% of the tested samples, *Escherichia coli* and *Streptococcus agalactiae* were detected at the same levels (5.00%). In 25.00% of the studied samples, there was no growth of pathogenic and opportunistic microorganisms.

The results of molecular genetic and microbiological tests performed during vaccination of highly producing cows with Startvac mastitis vaccine showed a significant reduction in the range of pathogens, causing inflammation in the mammary gland. There was a 19.41% decrease in the isolation rate of *Staphylococcus aureus*, which is explained by an increase in antibodies levels in vaccinated animals. White blood cells, contained in milk, perform one of the main protective functions against mastitis agents. For the normal functioning of the immunity protective properties, antibodies must induce the production of white blood cells and be targeted against certain bacterial agents. Earlier studies on the immunobiological status of cows established that inflammatory reactions occurring in the mammary gland are accompanied by changes in the overall resistance of the animal body, including immunological blood parameters. When studying the body's response to the vaccine administration, evaluated by overall resistance rates, an increase in the number of T-lymphocytes by 12.1% and B-lymphocytes by 7.0% was noted. The opsonophagocytic assay showed an increase in the phagocytic activity of neutrophils by 14.3%. Animals showed a persistent decrease in the level of circulating immune complexes in the blood to (106.8 ± 3.4) c. u., which is explained by suppression of inflammatory response in the mammary gland due to the activation of humoral immunity factors [10].

CONCLUSION

Application of the mastitis vaccine Startvac and use of the major mastitis therapy regime used by the breeding establishment for high producing cows result in a positive downward trend in somatic cells counts in bulk milk. Somatic cell counts in milk of vaccinated cows from the dairy unit with free stall housing and milking in parallel system milking parlors decreased by 432 thousand/ml, while in milk of high-producing cows from the farm with a tie stall housing and milking using pipeline milking machines, the level of somatic cells decreased by only 286 thousand/ml. The difference in somatic cells counts in bulk milk of high-producing cows kept in the dairy unit and farm conditions can be explained by differences in the husbandry practices and milking technologies, which, in turn, can affect the formation and number of pathogenic and opportunistic microorganisms responsible for mastitis. This assumption will serve as the target for further research in this direction. Vaccination of animals facilitated the reduction of milk bacterial contamination caused by *Staphylococcus aureus* by 19.41%. Thus, the obtained results showed promising use of the vaccine in future and its introduction into the mastitis control and milk quality improvement program.

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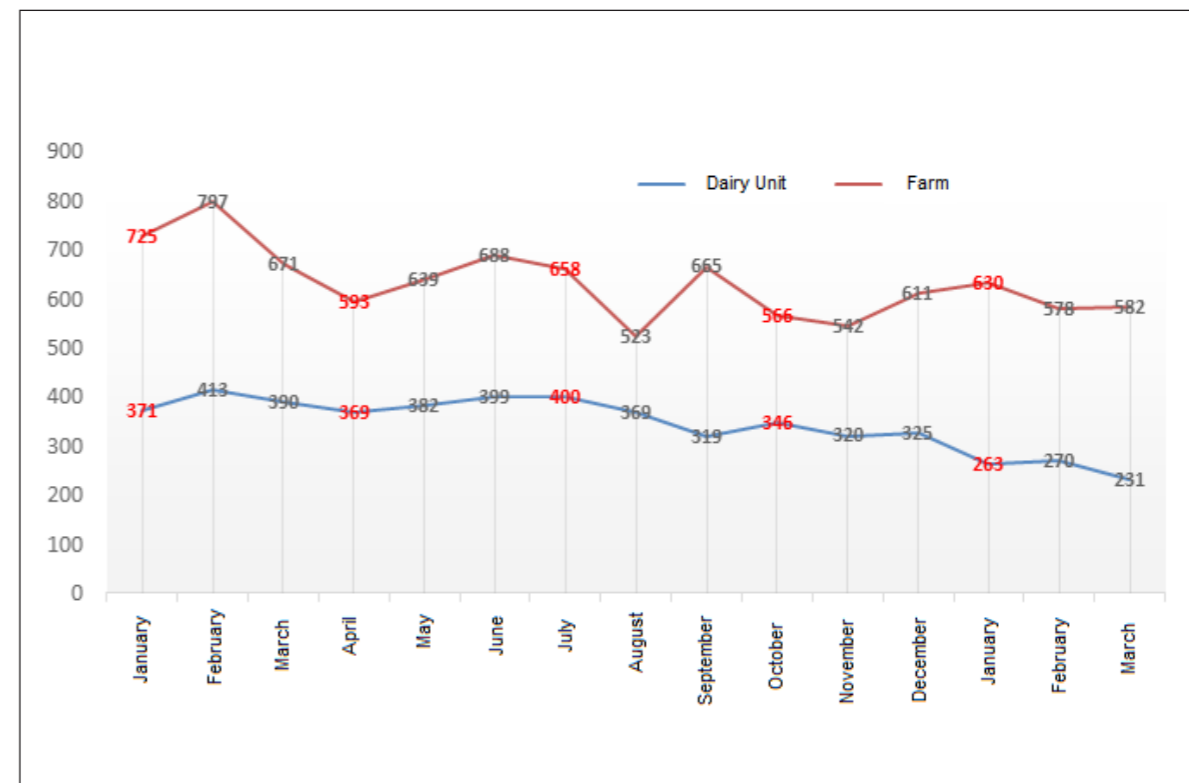


Fig. 2. Dynamics in somatic cells counts in 2019–2020

Рис. 2. Динамика уровня соматических клеток в период с 2019 по 2020 г.

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Received on 27.07.2020

Approved for publication on 04.09.2020

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DOI: 10.29326/2304-196X-2020-4-35-261-265
UDC 619:616.98:579.873.21:636.2:616-078

Improvement of bovine tuberculosis diagnosis

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SUMMARY

Detection of animals with non-specific reactions to tuberculin is one of the major problems in bovine tuberculosis (TB) diagnosis. There is a need to find and improve methods for detection of the sensitization causes. This paper presents the results of comparative studies of different ways to stabilize red blood cells in order to obtain diagnosticums for indirect hemagglutination (IHA) test. The article describes the stages of red blood cells stabilization and sensitization and demonstrates the diagnostic significance of Fili stabilization method using formaldehyde as a fixative. The highest antibody titers (1:3000 and 1:4000) were received in hyper-immune sera of rabbits immunized with *Mycobacterium bovis* using a homologous diagnosticum. Practical importance of the sensitins homologous to the infection is shown during testing of 1,911 serum samples collected from animals of different categories (diseased; healthy and reacting to tuberculin; healthy and not reacting to tuberculin) with IHA test using diagnosticums produced from *Mycobacterium bovis* and *Mycobacterium fortuitum*. Based on the positive results of the IHA test, TB was diagnosed in 87.5% of animals originating from an infected farm during post-mortem examination. The results of the IHA test agreed with those of the intradermal tuberculin test in 37.7% of cases. Diagnostic antibody titers were found in 206 TB infected animals with no reaction to the intradermal test. However, the post-mortem examination revealed TB changes in internal organs. The obtained data suggest a possibility to use the IHA test to detect TB infected animals with non-specific reactions to tuberculin.

Key words: tuberculosis, indirect hemagglutination test, differentiation, cattle, standardization, antibodies, red blood cells, sensitization.

For citation: Baratov M. O. Improvement of bovine tuberculosis diagnosis. *Veterinary Science Today*. 2020; 4 (35): 261–265. DOI: 10.29326/2304-196X-2020-4-35-261-265.

Conflict of interest: The author declares no conflict of interest.

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УДК 619:616.98:579.873.21:636.2:616-078

К совершенствованию диагностики туберкулеза крупного рогатого скота

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РЕЗЮМЕ

Выявление животных с неспецифическими реакциями на туберкулин – одна из наиболее актуальных проблем в диагностике туберкулеза. Очевидна необходимость поиска и совершенствования методов для выявления причин сенсibilизации. В работе представлены результаты сравнительного изучения различных способов стабилизации эритроцитов с целью получения диагностикума для проведения реакции непрямого гемагглютинации. Отражены этапы стабилизации и сенсibilизации эритроцитов. Показана диагностическая значимость метода стабилизации Фили с использованием формальдегида в качестве фиксатора. Наиболее высокие титры антител (1:3000 и 1:4000) получены в гипериммунных сыворотках крови кроликов, иммунизированных *Mycobacterium bovis*, с гомологичным диагностикумом. Практическая значимость гомологичных заражению сенситинов показана при исследовании 1911 проб сывороток крови животных из хозяйств различных категорий (больные; здоровые, реагирующие на туберкулин; здоровые, не реагирующие на туберкулин) в реакции непрямого гемагглютинации с диагностикумами, изготовленными из *Mycobacterium bovis* и *Mycobacterium fortuitum*. В благополучном по заболеванию хозяйстве, на основании полученных в реакции непрямого гемагглютинации позитивных результатов, при проведении патологоанатомического вскрытия диагноз на туберкулез установили у 87,5% животных. Отмечено совпадение результатов реакции непрямого гемагглютинации с показаниями внутрикожной туберкулиновой пробы в 37,7% случаев. У 206 больных туберкулезом животных обнаружены диагностические титры антител при отсутствии реакции на внутрикожную пробу. Однако при проведении патологоанатомического исследования были выявлены изменения туберкулезного характера внутренних органов. Полученные данные указывают на возможность использования реакции непрямого гемагглютинации при выявлении больных туберкулезом животных с неспецифическими реакциями на туберкулин.

Ключевые слова: туберкулез, реакция непрямого гемагглютинации, дифференциация, крупный рогатый скот, стандартизация, антитела, эритроциты, сенсibilизация.