DOI: 10.29326/2304-196X-2022-11-4-296-302

Comparative analysis of species composition and quantitative analysis of udder microflora in cows with subclinical and clinical mastitis

N. N. Avduevskaya[1], A. V. Kapustin[2], A. V. Gorbatov[3], E. V. Ivanov[4]

[1] Vologda Branch of the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV" (Vologda Branch of the FSC VIEV), Vologda, Russia

[2, 3, 4] Federal State Budget Scientific Institution "Federal Scientific Centre VIEV" (FSC VIEV), Moscow, Russia

[1] *https://orcid.org/0000-0002-6392-5823, e-mail: Natali.Avduevskaya@mail.ru*

[2] *https://orcid.org/0000-0003-0136-2487, e-mail: kapustin_andrei@mail.ru*

[3] *e-mail: incidentor@yandex.ru*

[4] *https://orcid.org/0000-0001-6602-5313, e-mail: doctor2112@yandex.ru*

SUMMARY

Due to the great relevance of the problem of mastitis in cows, the diversity of udder microflora in the affected animals, as well as to develop therapeutic and preventive measures on farms, studies were conducted to identify similarities and differences in the species composition of microorganisms in animals with subclinical and clinical breast inflammation, their proportion, to establish the correlation between the secreted microflora and the type of mastitis, as well as to study and compare the enzymatic properties of *Staphylococcus aureus* isolated from animals with clinical and subclinical mastitis. It was found that on all the studied farms the number of cows and heifers with subclinical mastitis exceeded the number of animals with clinical udder inflammation. As a result of microbiological studies of 182 mammary gland secretion samples collected from cows with subclinical and clinical mastitis from 13 agricultural establishments of the Vologda, Yaroslavl and Kostroma regions, 70 cultures of pathogenic and opportunistic microflora were isolated. It was demonstrated that, in case of subclinical mastitis, the following cultures were most often isolated from milk: *Staphylococcus aureus* (17.9% of cases), pathogenic *Streptococcus* (9.8% of cases), of which the proportion of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* was 6.5 and 3.3%, respectively. Opportunistic *Staphylococcus* (6.5%) and *Enterobacteria* (6.5%) were isolated in equal proportions. In case of cows with clinical mastitis, *Staphylococcus aureus* was isolated in 16.9% of cases, pathogenic *Streptococcus* – in 10.2% of cases, of which the proportion of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* was 6.8 and 3.4%, respectively. Opportunistic *Staphylococcus* and *Enterobacteria* were found in equal amounts – 3.4% of cases each. No growth of *Mycoplasma* on special nutrient media was registered in both cases. It was established that similar pathogenic and opportunistic microorganisms are isolated from animals with subclinical and clinical mastitis. The main causative agent is *Staphylococcus aureus*, the incidence of which in case of latent mastitis is slightly higher (by 1.0%). It is followed by *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, which are detected more often in case of clinical udder inflammation – by 0.2% on average. The frequency of isolation of opportunistic *Staphylococcus* is 1.9 times higher in case of subclinical mastitis. It is worth noting that with clinical udder inflammation, enterobacteria were detected only at one of the thirteen studied agricultural establishments.

Keywords: subclinical and clinical mastitis, cows, pathogenicity factors, *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae*

Acknowledgements: The study was carried out within the framework of the State Assignment of the Ministry of Education and Science of the Russian Federation on the subject No. FGUG-2022-0009.

For citation: Avduevskaya N. N., Kapustin A. V., Gorbatov A. V., Ivanov E. V. Comparative analysis of species composition and quantitative analysis of udder microflora in cows with subclinical and clinical mastitis. *Veterinary Science Today*. 2022; 11 (4): 296–302. DOI: 10.29326/2304-196X-2022-11-4-296-302.

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

For correspondence: Natalia N. Avduevskaya, Researcher, Vologda Branch of the FSC VIEV, 160009, Russia, Vologda, ul. Chekhova, 10, *e-mail: Natali.Avduevskaya@mail.ru.*

УДК 619:616.98:618.19-002:636.22/.28

Сравнительный анализ видового состава и количественное соотношение микрофлоры при субклиническом и клиническом мастите коров

Н. Н. Авдуевская[1], А. В. Капустин[2], А. В. Горбатов[3], Е. В. Иванов[4]

[1] Вологодский филиал ФГБНУ «Федеральный научный центр – Всероссийский научно-исследовательский институт экспериментальной ветеринарии имени К. И. Скрябина и Я. Р. Коваленко Российской академии наук» (Вологодский филиал ФГБНУ ФНЦ ВИЭВ РАН), г. Вологда, Россия

[2, 3, 4] ФГБНУ «Федеральный научный центр – Всероссийский научно-исследовательский институт экспериментальной ветеринарии имени К. И. Скрябина и Я. Р. Коваленко Российской академии наук» (ФГБНУ ФНЦ ВИЭВ РАН), г. Москва, Россия

[1] *https://orcid.org/0000-0002-6392-5823, e-mail: Natali.Avduevskaya@mail.ru*

[2] *https://orcid.org/0000-0003-0136-2487, e-mail: kapustin_andrei@mail.ru*

[3] *e-mail: incidentor@yandex.ru*

[4] *https://orcid.org/0000-0001-6602-5313, e-mail: doctor2112@yandex.ru*

РЕЗЮМЕ

В связи с актуальностью проблемы маститов коров, разнообразием выделяемой при этом заболевании микрофлоры, а также для планирования лечебно-профилактических мероприятий в хозяйствах были проведены исследования по выявлению сходства и различия видового состава микроорганизмов при субклинической и клинической форме воспаления молочной железы, их количественного соотношения, установлению зависимости выделяемой микрофлоры от формы мастита, а также изучению и сравнению ферментативных свойств золотистого стафилококка, выделенного при клиническом и субклиническом мастите. Установлено, что во всех обследованных хозяйствах количество коров и нетелей, больных субклиническими маститами, превышало число животных с клиническими формами воспаления молочной железы. В результате проведения микробиологических исследований 182 проб секрета молочной железы, полученных от больных субклиническими и клиническими формами мастита коров из 13 сельскохозяйственных предприятий Вологодской, Ярославской и Костромской областей, изолировано 70 культур патогенной и условно-патогенной микрофлоры. Показано, что при субклиническом мастите из молока чаще всего выделяли культуры золотистого стафилококка (17,9% случаев), патогенные стрептококки (9,8% случаев), из них доля *Streptococcus agalactiae* и *Streptococcus dysgalactiae* составила 6,5 и 3,3% соответственно. В равных соотношениях изолированы культуры условно-патогенных стафилококков (6,5%) и энтеробактерий (6,5%). От коров с клиническим маститом культуры золотистого стафилококка были выделены в 16,9% случаев, патогенных стрептококков – в 10,2% случаев, из них доля *Streptococcus agalactiae* и *Streptococcus dysgalactiae* составила 6,8 и 3,4%. Условно-патогенные стафилококки и энтеробактерии обнаружены в равных количествах – по 3,4% случаев. Рост микоплазм на специальных питательных средах при указанных формах мастита не отмечен. Установлено, что при субклинических и клинических формах мастита выделяются идентичные патогенные и условно-патогенные микроорганизмы. Основным возбудителем является *Staphylococcus aureus*, индикация которого при скрытой форме мастита незначительно выше (на 1,0%). За ним следуют *Streptococcus agalactiae* и *Streptococcus dysgalactiae*, обнаружение которых превалирует при клинической форме воспаления молочной железы в среднем на 0,2%. Частота выделения условно-патогенных стафилококков в 1,9 раза выше при субклиническом мастите. Стоит отметить, что при клиническом воспалении молочной железы энтеробактерии обнаруживали только в одном из тринадцати обследованных сельскохозяйственных предприятий.

Ключевые слова: субклинический и клинический маститы, коровы, факторы патогенности, *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae*

Благодарности: Исследования выполнены в рамках Государственного задания Минобрнауки России по теме № FGUG-2022-0009.

Для цитирования: Авдуевская Н. Н., Капустин А. В., Горбатов А. В., Иванов Е. В. Сравнительный анализ видового состава и количественное соотношение микрофлоры при субклиническом и клиническом мастите коров. *Ветеринария сегодня.* 2022; 11 (4): 296–302. DOI: 10.29326/2304-196X-2022-11-4-296-302.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Авдуевская Наталья Николаевна, научный сотрудник Вологодского филиала ФГБНУ ФНЦ ВИЭВ РАН, 160009, Россия, г. Вологда, ул. Чехова, д. 10, *e-mail: Natali.Avduevskaya@mail.ru.*

INTRODUCTION

Mastitis has been and remains one of the most serious problems in dairy farming. The disease is widespread throughout Russia in cows of different breeds. On average, from 17 to 20% of cows suffer from clinical and latent forms of mastitis in all countries around the world, and in some regions the number reaches 50% and above.

The economic damage caused by mastitis is significant, due to the loss of animal productivity, deterioration of the biological and technological properties of milk, which, due to the content of pathogenic microbes, also becomes dangerous for humans and young farm animals. It is estimated that a cow which has had mastitis reduces milk yield by 150–200 kg during the current lactation. Taking into account the massive number of livestock, losses due to mastitis in the dairy industry amount to 10–12% of the products produced [1].

According to RASKhN academician E. S. Voronin [2], mastitis is in the first place among other non-contagious diseases of cows in terms of economic damage caused to the world livestock industry.

Regardless of the root cause of inflammation of the mammary gland in cows, later, the organ gets infected with various pathogenic and opportunistic microflora in almost 100% of cases [3]. According to V. I. Mutovin [4], microflora is detected in 80% of clinical and subclinical mastitis cases. In most cases, microbes are either direct causative agents of mastitis and can independently cause mammary gland pathology, or complicate its course contributing to other etiological factors. The microbial factor may be the main cause of outbreaks of mastitis in cows at agricultural enterprises.

The most important and frequent causative agents of mastitis are staphylococci and streptococci [5–7].

Semina L. K. et al. has determined that the largest share in the structure of microflora isolated on the Vologda Oblast farms belongs to the coccal microflora – 79.6% of the total number of isolated cultures, with *Staphylococcus aureus* amounting to 23.2%, coagulase-negative staphylococci – 30.2%, streptococci – 26.2%. The proportion of enterobacteria was small – 9.2% [7]. Demidova L. D. in her study has established that among the coccoid microflora *S. aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* are isolated most often [8]. The data provided by G. N. Kuzmin also speak about the most frequent isolation of the following coccal microflora from mastitis milk: *S. aureus* – in 30.5–29.3% of cases, *S. agalactiae* – in 22.0–17.7% of cases, *S. dysgalactiae* – in 16.6–15.9% of cases [9]. Some researchers have found mycoplasma in cows' milk [10].

The course of disease is diverse, it mainly occurs in two forms: clinical and subclinical. Clinical mastitis is manifested by clear signs of inflammation of the mammary gland, such as pain, swelling, heat, as well as changes in the color and consistency of milk. Such inflammation of the mammary gland is quite rare and, according to V. I. Mutovin [4], accounts for about 20% of the total number of udder diseases.

Subclinical mastitis is latent or has poorly expressed signs. The most characteristic symptoms of latent mastitis are: a gradual decrease in the milk yield from the affected quarter of the udder, milk takes on a watery appearance, which is the reason to suspect the disease [11]. On dairy farms, the latent inflammation of the mammary gland in cows is much more common and creates the greatest economic problem. According to some data, subclinical mastitis occurs 4–5 times more often than the clinical one [12]. Mastitis occurring in a subclinical form is the most dangerous and is the main cause of agalactia and hypogalactia of individual udder lobes, which can lead to the reduction in milk yields. When one udder lobe is affected by subclinical mastitis, on average, each sick cow produces up to 10–15% less milk per lactation [13].

It is proved that pathogenic and opportunistic microorganisms are found in the milk obtained from cows with both clinical and subclinical mastitis. According to some reports, in clinical mastitis, *S. aureus*, *S. agalactiae*, *S. dysgalactiae*, *Streptococcus uberis* [12] is isolated in 90–95% of cases; according to B. L. Belkin et al. [14], *Staphylococcus aureus* is more often detected (40.5–48.95%), and in case of subclinical mastitis – coagulase-negative and coagulase-positive staphylococcus (*S. aureus*) in the same proportion, as well as agalactic streptococcus.

Researcher M. Roguinsky [15] found that in 30% of cases subclinical mastitis is caused by staphylococci, in 62% – by streptococci, in 1% – by enterobacteria.

Murska S. D. during the study determined that in case of clinical mastitis, the main pathogens were *Escherichia coli* – 41.1% and *Staphylococcus epidermidis* – 33.4%, in case of subclinical mastitis, the dominant microflora was staphylococcus – it accounted for 90% of all the infectious agents of bovine mastitis [16].

To plan therapeutic and preventive measures at agricultural enterprises, it is necessary to take into account the species composition of pathogens isolated in the above-mentioned forms of mastitis. For example, treatment of mastitis with beta-lactam antibiotics (penicillins, cephalosporins, carbapenems) when the disease is caused by *S. aureus* will be useless, since this pathogen is multidrug-resistant, on the opposite, they are effective in case *S. agalactiae*, *S. dysgalactiae* are detected. It is well known that complex drugs for intracisternal administration are currently used to treat mastitis, and the action of these drugs is determined by the period of administration (lactation and start-up) rather than the type of the disease.

The novelty of this work consists in the study of the species composition of the microflora in cows with subclinical and clinical mastitis.

Due to the urgency of the problem of cow mastitis, diversity of the secreted microflora, as well as for planning therapeutic and preventive measures on farms, the purpose of this work was to identify similarities and differences in the species composition of microorganisms in case of subclinical and clinical forms of udder inflammation, determine their quantitative ratio, establish the correlation between the secreted microflora and the form of mastitis. Based on the fact that there is little information in scientific sources about the comparison of the pathogenicity factors of *Staphylococcus aureus* in both forms of udder inflammation, the objective was also to study and compare the enzymatic properties of this pathogen in case of clinical and subclinical mastitis.

MATERIALS AND METHODS

Milk from cows with subclinical and clinical mastitis from thirteen livestock farms in the Vologda, Yaroslavl and Kostroma regions was subjected to microbiological studies.

Milk sampling and study were carried out in accordance with the "Guidelines for the bacteriological studies of milk and udder secretion of cows" [17], identification of isolated microorganisms – according to GOST 30347-2016 "Milk and dairy products. Methods for determination of *Staphylococcus aureus*" [18].

Catalase activity of *Staphylococcus aureus* was determined using 3% hydrogen peroxide, coagulase activity of *S. aureus* was determined using rabbit citrate dry plasma (ECOlab Company, Russia). Mannitol fermentation test was carried out using mannitol-salt agar (medium No. 10 GRM). Hemolytic activity was tested by inoculating *Staphylococcus aureus* on blood agar, DNase activity was studied using an elective medium and 1 N hydrochloric acid solution. Hyaluronidase activity was determined using a standardized preparation of hyaluronic acid and a 1% rivanol solution.

Streptococci were identified using a reagent kit for detecting group A, B, C, G, D, F streptococci (Aquapast Company, Russia).

Mathematical processing of the obtained results was performed using methodological manual "Biometric processing of laboratory, clinical and epidemical data" [19].

RESULTS AND DISCUSSION

According to the data obtained from five agricultural enterprises, it was found that subclinical mastitis is much more common than the clinical one (Table 1).

It was found that on all the studied farms the number of cows and heifers with subclinical mastitis exceeded the number of animals with clinical forms of udder inflammation. The highest excess was noted on the third and fifth farm – by 5.9 and 2.5 times, respectively.

Terentyeva N. Yu. and Ermolaev B. A. when studying the structure of inflammatory processes and conducting study on the farms in the Ulyanovsk region, also noted that subclinical mastitis (70.83–83.75%) prevailed over the clinical one (16.25–29.17%) [20].

Khalipaev M. G. and Sakidibirov O. P. in their studies also found that cows suffered from clinical mastitis less often (26 animals, or 8%) than from subclinical (66 animals, or 20%) [21].

As a result of microbiological studies of 182 mammary gland secretion samples collected from cows with subclinical and clinical mastitis from 13 agricultural establishments in the Vologda, Yaroslavl and Kostroma regions, 70 cultures of pathogenic and opportunistic microflora were isolated.

A comparative analysis of the species composition and the quantitative analysis of the isolated microflora in the above-mentioned forms of udder inflammation was carried out (Table 2, Fig.).

It was found that in subclinical mastitis, cultures of *Staphylococcus aureus* are most often isolated from udder secretions – in 17.9% of cases, pathogenic streptococci – in 9.8% of cases, of which *S. agalactiae* – in 6.5% and *S. dysgalactiae* – in 3.3%. Cultures of opportunistic staphylococci (in 6.5% of cases) and enterobacteria (in 6.5% of cases) were isolated in equal proportions.

Table 1
Comparison of the number of cows with subclinical and clinical mastitis on different farms

Farm number	Total number of cows	Cows with mastitis, anim/%			Subclinical to clinical mastitis ratio
		in total	with subclinical form	with clinical form	
1	1,232	346/28.1	228/18.5	118/9.6	1:1.9
2	452	134/29.6	75/16.6	59/13.0	1:1.3
3	800	282/35.2	241/30.1	41/5.1	1:5.9
4	1,000	319/31.9	205/20.5	114/11.4	1:1.8
5	598	226/37.8	162/27.1	64/10.7	1:2.5

Cultures of *Staphylococcus aureus* were isolated from cows with clinical mastitis in 16.9% of cases, pathogenic streptococci – in 10.2% of cases, of which the proportion of *S. agalactiae* was 6.8%, *S. dysgalactiae* – 3.4%. Opportunistic staphylococci and enterobacteria were found in equal amounts – 3.4% of cases, respectively.

No growth of mycoplasma on special nutrient media was registered in both cases.

Studies have shown that similar pathogenic and opportunistic microorganisms are isolated from animals

Table 2
Species diversity of microorganisms in case of subclinical and clinical mastitis

Farm number	Types of microorganisms, %									
	subclinical mastitis					clinical mastitis				
	total samples	*S. aureus*	*Staphylococcus* spp.	*Streptococcus* spp.	*Enterobacteria*	total samples	*S. aureus*	*Staphylococcus* spp.	*Streptococcus* spp.	*Enterobacteria*
1	9	1/11.1	1/11.1	0	0	13	1/7.7	0	1**/7.7	0
2	13	3/23.1	1/7.7	0	2/15.4	5	2/40.0	0	0	2/40.0
3	8	0	0	3*/37.5	1/12.5	2	0	0	2*/100.0	0
4	29	9/31.0	2/6.9	0	1/3.4	11	2/18.2	1/9.1	0	0
5	12	1/8.3	0	0	3/25.0	3	1/33.3	0	0	0
6	10	2/20.0	3/30.0	1*/1** 2/20.0	0	6	3/50.0	0	1*/16.7	0
7	11	3/27.3	0	4*/36.4	0	1	0	0	1*/100.0	0
8	6	0	1/16.7	1**/16.7	0	3	0	0	1**/33.3	0
9	8	1/12.5	0	1**/12.5	0	4	0	0	0	0
10	5	0	0	0	0	6	0	0	0	0
11	7	2/28.6	0	0	0	2	1/50.0	0	0	0
12	1	0	0	0	0	2	0	1/50.0	0	0
13	4	0	0	1**/25.0	1/25.0	1	0	0	0	0
In total	123	22/17.9	8/6.5	12/9.8 8*/6.5 4**/3.3	8/6.5	59	10/16.9	2/3.4	6/10.2 4*/6.8 2**/3.4	2/3.4

** S. agalactiae, ** S. dysgalactiae.*

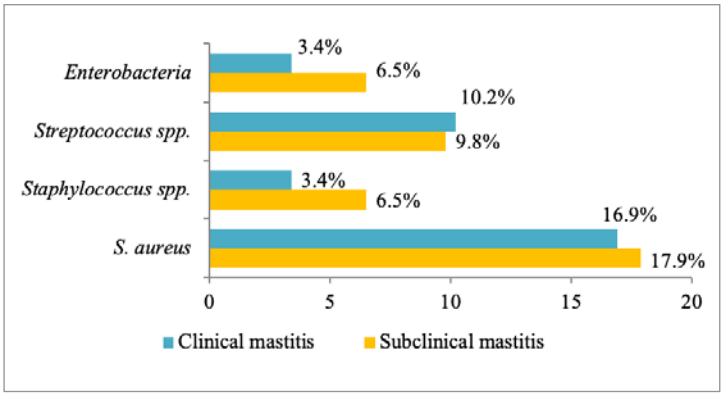

Fig. Quantitative analysis of udder microflora in cows with subclinical and clinical mastitis

with subclinical and clinical mastitis. The main causative agent is still *S. aureus*, whose identification in case of latent mastitis is slightly higher – by 1.0%. It is followed by *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, which are detected more often in case of the clinical form of udder inflammation – by 0.2% in both cases. The frequency of isolation of opportunistic staphylococci is 1.9 times higher in case of subclinical mastitis. It is worth noting that in cases of clinical inflammation of the mammary gland, enterobacteria were detected only at one of the thirteen studied agricultural establishments.

Bala S. S. describes similar results on the identity of the microflora, as well as the frequency of detection of *Staphylococcus aureus* in cases of subclinical and clinical mastitis [22]. Thus, in the clinical form of mastitis, according to his studies, staphylococci (*S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. auricularis*) were isolated in 75.1% of cases, enterobacteria (*E. coli*) and streptococci in 8.2 and 16.7% of cases, respectively. As for the subclinical form of the disease, the author did not find significant changes in the frequency of bacteria detection – staphylococci (*S. aureus*, *S. epidermidis*, *S. hominis*, *S. warneri*) were registered in 76.7% of cases, streptococci – in 23.3% of the examined animals. S. S. Bala established that the leading species was *S. aureus*, which was most often isolated in monoculture in both forms of mastitis (in 52.8 and 50.0% of cases, respectively).

Due to the fact that *Staphylococcus aureus* remains the main causative agent of both clinical and subclinical mastitis in cows, comprehensive studies have been conducted to identify some of its pathogenicity factors. For this purpose, 10 isolates of *S. aureus* were obtained from three farms from cows with mastitis, both with pronounced clinical signs and in a latent form.

The data presented in Table 3 show that all the studied *S. aureus* isolates had a number of pathogenicity factors specific for the representatives of their species: catalase, coagulase activity, enzymatic activity against mannitol, hemolytic, DNase activity, which indicates their virulence. Hyaluronidase activity was not identified in all the isolates, but only in two cultures of *Staphylococcus aureus* isolated on different farms from the milk obtained from cows with subclinical mastitis, and in one culture isolated from the udder secretion of animals with clinical mastitis.

High hemolytic activity (zone of complete lysis, alpha-hemolysis) was noted in isolates No. 2 and 5 obtained from cows with latent mastitis, and in pathogens No. 7, 9, 10 isolated from the samples collected from cows with pronounced symptoms of mastitis. Other *Staphylococcus aureus* cultures had beta-hemolytic activity (incomplete lysis zone), and both alpha- and beta-hemolytic *S. aureus* were detected on the same farm.

CONCLUSION

Thus, on dairy farms in the Vologda, Yaroslavl, Kostroma regions, the most widespread form of mastitis in cows is subclinical. A comparative analysis of the species composition of microorganisms isolated from the udder secretion of cows with subclinical and clinical mastitis showed that the detected microflora is identical, therefore, the form of the disease does not depend on the microorganism species.

Table 3
The severity of some pathogenicity factors in *S. aureus* isolates

Isolate number	Catalase	Coagulase	Mannitol fermentation	Hemolysis	DNase	Hyaluronidase
Subclinical mastitis						
1	+	+	+	β	+	–
2	+	+	+	α	+	+
3	+	+	+	β	+	–
4	+	+	+	β	+	–
5	+	+	+	α	+	+
Clinical mastitis						
6	+	+	+	β	+	–
7	+	+	+	α	+	–
8	+	+	+	β	+	–
9	+	+	+	α	+	–
10	+	+	+	α	+	+

The quantitative ratio of pathogenic staphylococci and streptococci (*S. aureus*, *S. agalactiae*, *S. dysgalactiae*) varies depending on the form of mastitis, but slightly – the detection of *S. aureus* was from 17.9% in subclinical mastitis to 16.9% in clinical mastitis, *S. agalactiae* – from 6.5 to 6.8%, *S. dysgalactiae* – from 3.3 to 3.4% of cases, respectively.

The number of isolated cultures of opportunistic staphylococci was 1.9 times higher in the subclinical form of mastitis, and enterobacteria were detected in clinical mastitis only on one of the thirteen studied farms.

Cultures of *Staphylococcus aureus* with a number of pathogenicity factors, including high hemolytic activity (alpha-hemolysis), were detected in cows with both latent and clear signs of udder inflammation. On the same farm, some cultures of *S. aureus* caused alpha- and beta-hemolysis, which may indicate the circulation of various strains of the pathogen with different virulence on that farm.

REFERENCES

1. Polyantsev N. I., Podbereznyi V. V. Veterinary obstetrics and biotechnics of animal reproduction: a textbook. Rostov-on-Don: Feniks; 2001. 480 p. (in Russ.)

2. Kuz'min G. N. Infectious mastitis of cows. Voronezh: Istoki; 2004. 146 p. (in Russ.)

3. Kudelina N. A., Tuzov I. N. Profilaktika infektsionnogo mastita u doinykh korov na promyshlennoi ferme uchkhoz "Krasnodarskoe" = Prevention of infectious mastitis in dairy cows on the instructional farm "Krasnodarskoye". *Nauchnoe obespechenie agropromyshlennogo kompleksa: materialy X Vserossiiskoi konferentsii molodykh uchenykh, posvyashchennoi 120-letiyu I. S. Kosenko = Scientific support of the farming sector: materials of the X All-Russian Conference of young scientists dedicated to the 120th anniversary of I. S. Kosenko*. Krasnodar: KubSAU; 2020; 223–224. eLIBRARY ID: 29131073. (in Russ.)

4. Mutovin V. I. Cow mastitis control. Moscow: Sel'khozizdat; 1963. 160 p. (in Russ.)

5. Terentyeva N. Y., Ermolaev V. A. Spread of mastitis in cows in farms of Ulyanovsk region. *Vestnik of Ulyanovsk State Agricultural Academy*. 2015; 2 (30): 141–147. DOI: 10.18286/1816-4501-2015-2-141-147. (in Russ.)

6. Ivanyuk V. P., Bobkova G. N. Milk microflora and complex therapy of cows with mastits. *Sostoyanie, problemy i perspektivy razvitiya sovremennoi nauki: materialy natsional'noi nauchno-prakticheskoi konferentsii = State, problems and prospects of development of modern science: materials of the national scientific and practical conference*. Bryansk: Bryansky SAU; 2021; 61–68. (in Russ.)

7. Semina L. K., Avduevskaya N. N., Skulyabina Z. A., Voroshilova T. G., Baldycheva G. A. Indication of coccal microflora of the udder secretion of cows with mastitis. *Russian Journal "Problems of Veterinary Sanitation, Hygiene and Ecology"*. 2018; 3 (27): 56–60. DOI: 10.25725/vet.san.hyg.ecol.201803010. (in Russ.)

8. Demidova L. D. Obsemenennost' moloka patogennymi streptokokkami na fermakh promyshlennogo tipa = Contamination of milk with pathogenic streptococci on industrial farms. *In: Hygiene on animal farms, veterinary microbiology and production of high sanitary quality animal products.* Moscow: VNIIVS; 1980; 98–100. (in Russ.)

9. Kuzmin G. N. Epizooticheskie osobennosti mastita kokkovoi etiologii u korov = Epidemic features of mastitis of coccal etiology in cows. *Nauchnye aspekty profilaktiki i terapii boleznei sel'skokhozyaistvennykh zhivotnykh: materialy nauchnoi konferentsii, posvyashchennoi 70-letiyu fakul'teta veterinarnoi meditsiny Voronezhskogo gosudarstvennogo agrarnogo universiteta = Scientific aspects of prevention and therapy of diseases of farm animals: materials of a scientific conference dedicated to the 70th anniversary of the Faculty of Veterinary Medicine of the Voronezh State Agrarian University.* Voronezh: Voronezhskii SAU;1996; Part 1: 185–186. eLIBRARY ID: 30602442. (in Russ.)

10. Laptev G. Yu., Novikova N. I., Ilyina L. A., Yildirim E. A., Soldatova V. V., Nikonov I. N. Molecular-genetic investigation of microorganisms corresponding mastide in cattle. *Actual issues inagricultural biology*. 2017; 4 (6): 30–34. eLIBRARY ID: 30779620. (in Russ.)

11. Ilyina A. I., Pospelov A. I. Udder diseases. Leningrad; Moscow: Sel'khozgiz; 1961. 151 p. (in Russ.)

12. Isakova M. N., Riaposova M. V., Bezborodova N. A., Britsina O. A. Microbiological background with inflammation of breast of high-productive cows. *Russian Journal "Problems of Veterinary Sanitation, Hygiene and Ecology"*. 2017; 2 (22): 63–67. eLIBRARY ID: 29716327. (in Russ.)

13. Tanbaeva G. A. Diagnosis of subclinical forms of mastitis in cows. *Aktual'nye problem zhivotnovodstva v usloviyakh importozameshcheniya: materialy mezhdunarodnoi nauchno-prakticheskoi konferentsii, posvyashchennoi pamyati doktora biologicheskikh nauk, professora, Zasluzhennogo deyatelya nauki RF A. P. Bulatova = Actual problems of animal husbandry in the context of import substitution: materials of the international scientific and practical conference dedicated to the memory of Doctor of Biological Sciences, Professor, Honored Scientist of the Russian Federation A. P. Bulatov*. Kurgan: Kurgan SAA; 2018; 399–404. (in Russ.)

14. Belkin B. L., Cherepahina L. A., Sotnikova V. M., Popkova T. V., Skrebneva E. N. Cow mastitis: etiology, pathogenesis, diagnosis, treatment and prevention. Orel: Orel SAU; 2009. 216 p. (in Russ.)

15. Roguinsky M. Etiologie et traitement des mammites. *Cahiers Med. Vet*. 1977; 46: 8–13.

16. Мурська С. Д. Дослідження секрету молочної залози у корів господарств Івано-Франківської області. *Науковий вісник ЛНУВМБТ імені С. З. Гжицького*. 2014; 16 (2-2): 244–250. (in Ukrainian)

17. Methodological guidelines for the bacteriological study of milk and udder secretion of cows: approved by the Main State Veterinary Department of the Ministry of Agriculture of the USSR on December 30, 1983. No. 115-69. Available at: https://base.garant.ru/72125912/89300effb84a59912210b23abe10a68f. (in Russ.)

18. GOST 30347–2016 Milk and milk products. Methods for determination of *Staphylococcus aureus*. Moscow: Standartinform; 2016. 14 p. (in Russ.)

19. Marinin E. A. Biometric processing of laboratory, clinical and epidemic data: a methodological guide. Novocherkassk: NCZSRVI; 1980. 38 p. (in Russ.)

20. Terentyeva N. Y., Ermolayev V. A. Spread of mastitis in cows in farms of Ulyanovsk region. *Vestnik of Ulyanovsk State Agricultural Academy*. 2015; 2 (30): 141–147. DOI: 10.18286/1816-4501-2015-2-141-147. (in Russ.)

21. Khalipaev M. G., Sakidibirov O. P. Diagnostics and treatment of subclinical mastitis at cows. *Problems of development of the agro-industrial complex of the region*. 2019; 3 (39): 202–206. eLIBRARY ID: 41223342. (in Russ.)

22. Bala S. S. Biological properties of microflora secreted from milk produced by cows with clinical and subclinical forms of mastitis. *Izvestia Orenburg State Agrarian University*. 2010; 4 (28): 287–289. eLIBRARY ID: 15509299. (in Russ.)

Received 23.05.2022
Revised 17.06.2022
Accepted 22.07.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Natalia N. Avduevskaya, Researcher, Vologda Branch of the FSC VIEV, Vologda, Russia.

Andrey V. Kapustin, Doctor of Science (Biology), Associate Professor, Deputy Director for Scientific Work, FSC VIEV, Moscow, Russia.

Alexander V. Gorbatov, Candidate of Sciences (Veterinary Medicine), Leading Researcher, Head of Laboratory of Microbiology, FSC VIEV, Moscow, Russia.

Evgeny V. Ivanov, Candidate of Science (Biology), Senior Researcher, Laboratory of Microbiology, FSC VIEV, Moscow, Russia.

Авдуевская Наталья Николаевна, научный сотрудник Вологодского филиала ФГБНУ ФНЦ ВИЭВ РАН, г. Вологда, Россия.

Капустин Андрей Владимирович, доктор биологических наук, доцент, заместитель директора по научной работе ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.

Горбатов Александр Вениаминович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории микробиологии ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.

Иванов Евгений Валерьевич, кандидат биологических наук, старший научный сотрудник лаборатории микробиологии ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.