DOI: 10.12731/2658-6649-2025-17-6-2-1553 **EDN: WJMYSA**
UDC 591

Original article

ZOOPATHOGENIC ROLE OF *MORAXELLACEAE* AND *CORYNEBACTERIACEAE* BACTERIA IN MASTITIS IN RUMINANTS

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Abstract

Background. The article examines the incidence and pathogenic significance of Moraxella and Corynebacteria in mastitis of ruminants using the example of large and small cattle in various farms in the regions of the Russian Federation. As a result of the conducted studies, the sporadic presence of Moraxella microorganisms in the microbiota of mastitis milk among opportunistic pathogens in cows was established. No Moraxella was found in goats. Several cases were identified at once in which Acinetobacter spp., belonging to the Moraxellaceae family, was isolated in associations. The incidence of pathogenic corynebacteria as the leading representative of the clinical microbiota was noted at the level of 60% in the goats of LLC « Pikaev » in the Republic of Morodovia and 26.7% in cows from various farms in the Moscow region, the Republic of Morodovia and Bashkortostan.

Purpose. The purpose of this work is to study the incidence and pathogenic significance of bacteria Moraxella and Corynebacterium in mastitis in cattle and small cattle.

Materials and methods. The research was carried out at livestock enterprises in the Moscow region, the Republic of Mordovia, and the Republic of Bashkortostan. In the period from March to August 2024, clinical trials were conducted in the field and in production conditions for mastitis of large and small cattle with sampling of pathological material. The clinical (pathological) material was milk, which was collected in sterile containers. Screening bacteriological studies for indication of microorganisms (20 studies) and identification of bacteria were conducted. The samples were seeded onto Petri dishes with 10% blood and chocolate agar content. Petri dishes were incubated at 37 °C for 48 hours. Putative colonies of Moraxella, identified by their smooth, rounded and grayish-white appearance with a hemolysis zone, were subjected to Gram staining and biochemical tests,

then positive colonies were further confirmed using MALDI–TOF, other bacterial pathogens were also identified using biochemical tests and mass spectrometry. For this purpose, after sampling, a well-isolated colony was removed using a sterile loop, then re-suspended in 300 µl of distilled water, then the suspension was centrifuged for 10 minutes at 10,000 rpm, after which 2 µl of a reactogenic solution was added to the precipitate, transferred to a MALDI plate and loaded into an ionization chamber.

Results. The following types of microorganisms have been detected and identified in cattle with a clinical picture of serous, catarrhal mastitis: Streptococcus uberis, Staphylococcus capitis, Trueperella pyogenes, Bacillus pumilus, Brevibacterium celere, Corynebacterium xerosis, Bacillus licheniformis, Acinetobacter junii, Escherichia coli, Paraclostridium bifermentans, Psychrobacter immobilis, Enterococcus faecium, Serratia liquefaciens, Citrobacter freundii, Pseudomonas spp., Corynebacterium spp., Enterococcus faecalis, Psychrobacter phenylpyruvicus, Raoultella ornithinolytica, Moraxella catarrhalis (1 isolate). The following types of microorganisms were found in goats with a clinical picture of serous catarrhal mastitis: Aerococcus viridians, Corynebacterium stationis, Staphylococcus haemolyticus, Staphylococcus chromogenes, Corynebacterium xerosis, Corynebacterium stationis, and Staphylococcus epidermidis. The results are presented in table 1. No growth was detected from samples of biological material from mastitis milk of sheep and goats, during screening bacteriological studies on blood agar. To determine the pathogenic properties of the isolated cultures, a coagulase test for staphylococci and a bioassay on white mongrel mice were used. The inoculum diluted to the desired concentration (10^8 CFU/ml), OD = 600 nm = 1, obtained from a pure culture of Moraxella bovis and Moraxella bovoculi, was administered subcutaneously to mice at a dose of 100 µl.

Conclusion. The nosological role of moraxella microorganisms in mastitis in cows and goats has not been confirmed at this stage of research. Cases of indication of Moraxella catarrhalis and Acinetobacter spp. have been noted among the clinical microbiota, which does not exclude the nosological role of microorganisms of the Moraxellaceae family in these pathologies, as early studies occasionally noted.

Keywords: Moraxella; Corynebacteria; mastitis; ruminants; cattle

For citation. Pimenov, N. V., Ivannikova, R. F., & Pozyabin, S. V. (2025). Zoopathogenic role of *Moraxellaceae* and *Corynebacteriaceae* bacteria in mastitis in ruminants. *Siberian Journal of Life Sciences and Agriculture*, *17*(6-2), 328-340. https://doi.org/10.12731/2658-6649-2025-17-6-2-1553

Научная статья

ЗООПАТОГЕННАЯ РОЛЬ БАКТЕРИЙ СЕМЕЙСТВА *MORAXELLACEAE* И *CORYNEBACTERIACEAE* ПРИ МАСТИТЕ У ЖВАЧНЫХ ЖИВОТНЫХ

Н.В. Пименов, Р.Ф. Иванникова, С.В. Позябин

Аннотация

Обоснование. В статье рассматривается частота встречаемости и патогенная значимость Moraxella и Corynebacteria при маститах жвачных животных на примере крупного и мелкого рогатого скота в различных хозяйствах регионов Российской Федерации. В результате проведенных исследований установлено спорадическое присутствие микроорганизмов Moraxella в микробиоте маститного молока среди условно-патогенных микроорганизмов у коров. У коз моракселла не обнаружена. Было выявлено сразу несколько случаев, когда в ассоциациях выделяли Acinetobacter spp., относящийся к семейству Moraxellaceae. Частота встречаемости патогенных коринебактерий как ведущего представителя клинической микробиоты отмечена на уровне 60% у коз ООО «Пикаев» в Республике Мородовия и 26,7% у коров из различных хозяйств Московской области, Республики Мородовия и Башкортостана.

Цель. Цель работы – изучить частоту встречаемости и патогенную значимость бактерий Moraxella и Corynebacterium при маститах крупного и мелкого рогатого скота.

Материалы и методы. Исследования проводились на животноводческих предприятиях Московской области, Республики Мордовия и Республики Башкортостан. В период с марта по август 2024 года проведены клинические испытания в полевых и производственных условиях при мастите крупного и мелкого рогатого скота с отбором проб патологического материала. Клиническим (патологическим) материалом служило молоко, которое собирали в стерильные емкости. Проводились скрининговые бактериологические исследования на индикацию микроорганизмов (20 исследований) и идентификацию бактерий. Образцы высевали на чашки Петри с 10% кровяным и шоколадным агаром. Чашки Петри инкубировали при 37°С в течение 48 часов. Предполагаемые колонии Moraxella, идентифицированные по гладкому, округлому и серовато-белому виду с зоной гемолиза, подвергались окрашиванию по Граму и биохимическим тестам, затем положительные колонии подтверждались

методом MALDI-TOF, другие бактериальные патогены также идентифицировались с помощью биохимических тестов и масс-спектрометрии. Для этого после отбора проб хорошо изолированную колонию удаляли стерильной петлей, затем повторно суспендировали в 300 мкл дистиллированной воды, суспензию центрифугировали в течение 10 минут при 10000 об/мин, после чего к осадку добавляли 2 мкл реактогенного раствора, переносили на MALDI-пластину и загружали в ионизационную камеру.

Результаты. У крупного рогатого скота с клинической картиной серозного, катарального мастита были обнаружены и идентифицированы следующие виды микроорганизмов: Streptococcus uberis, Staphylococcus capitis, Trueperella pyogenes, Bacillus pumilus, Brevibacterium celere, Corynebacterium xerosis, Bacillus licheniformis, Acinetobacter junii, Escherichia coli, Paraclostridium bifermentans, Psychrobacter immobilis, Enterococcus faecium, Serratia liquefaciens, Citrobacter freundii, Pseudomonas spp.., Corynebacterium spp., Enterococcus faecalis, Psychrobacter phenylpyruvicus, Raoultella ornithinolytica, Moraxella catarrhalis (1 изолят). У коз с клинической картиной серозно-катарального мастита были обнаружены следующие виды микроорганизмов: Aerococcus viridians, Corynebacterium stationis, Staphylococcus haemolyticus, Staphylococcus chromogenes, Corynebacterium xerosis, Corynebacterium stationis и Staphylococcus epidermidis. Результаты представлены в таблице 1. При скрининговых бактериологических исследованиях на кровяном агаре в пробах биологического материала из маститного молока овец и коз роста не обнаружено. Для определения патогенных свойств выделенных культур использовали коагулазный тест на стафилококки и биопробу на белых беспородных мышах. Инокулят, разведенный до нужной концентрации (108 КОЕ/мл), OD = 600 нм = 1, полученный из чистой культуры Moraxella bovis и Moraxella bovoculi, вводили мышам подкожно в дозе 100 мкл.

Заключение. Нозологическая роль микроорганизмов моракселлы при мастите у коров и коз на данном этапе исследований не подтверждена. Среди клинической микробиоты отмечены случаи индикации Moraxella catarrhalis и Acinetobacter spp., что не исключает нозологической роли микроорганизмов семейства Moraxellaceae при данных патологиях, как это периодически отмечалось в ранних исследованиях.

Ключевые слова: Moraxella; Corynebacteria; мастит; жвачные животные; крупный рогатый скот

Для цитирования. Пименов, Н. В., Иванникова, Р. Ф., & Позябин, С. В. (2025). Зоопатогенная роль бактерий семейства *Moraxellaceae* и *Corynebacteriaceae* при мастите у жвачных животных. *Siberian Journal of Life Sciences and Agriculture*, *17*(6-2), 328-340. https://doi.org/10.12731/2658-6649-2025-17-6-2-1553

Introduction

Dairy cattle breeding plays a leading role in productive animal husbandry and is a high-tech branch of agriculture. Mastitis is one of the most common and studied diseases, which leads to a decrease in milk productivity and significant economic losses associated with a shortage of milk and a decrease in its quality. Among productive dairy cattle in Russia, mastitis affects from 15 to 60% of the livestock, and milk losses reach up to 30% of the annual milk yield [1-5]. Even after successful treatment of mastitis, hypogalactia or agalactia most often develops, and treatment costs and losses from animal culling account for significant economic losses. At the same time, significant investments aimed at eliminating mastitis do not lead to the development of reliable preventive measures, and losses from the disease continue to be global. In addition, numerous studies indicate that milk from sick animals can be a source of infection of young animals, and if ingested by humans.

Mastitis occurs in animals at any age, while it is less common in the first three lactation periods. The proportion of mastitis increases with age. This disease occurs in all seasons of the year [6; 7].

Pathogenic and opportunistic microorganisms play a leading role in the occurrence of mastitis, and mechanical damage associated with impaired milking technology, cracks and udder wounds also provoke the development of the disease [8-10]. Taking into account the role of the microbial factor in the development of breast pathology, researchers constantly point out the sanitary and epidemiological significance of mastitis, especially in lactating cows. Currently, it is difficult to distinguish a boundary in determining the pathogenic properties of some microorganisms. They have a high prevalence, the ability to multiply extremely quickly, and most types of microorganisms become unpretentious in the use of food and energy sources [11-14]. Many previously considered conditionally pathogenic microorganisms have acquired new properties and ecological niches and today play an important role in the development of diseases, including mastitis in productive animals [15].

In this regard, the study of the issues of timely bacteriological diagnosis of mastitis with the determination of the etiological structure remains relevant today.

Purpose. The purpose of this work is to study the incidence and pathogenic significance of bacteria Moraxella and Corynebacterium in mastitis in cattle and small cattle.

Materials and methods

The research was carried out at livestock enterprises in the Moscow region, the Republic of Mordovia, and the Republic of Bashkortostan. In the period

from March to August 2024, clinical trials were conducted in the field and in production conditions for mastitis of large and small cattle with sampling of pathological material. The clinical (pathological) material was milk, which was collected in sterile containers. Screening bacteriological studies for indication of microorganisms (20 studies) and identification of bacteria were conducted. The samples were seeded onto Petri dishes with 10% blood and chocolate agar content. Petri dishes were incubated at 37 °C for 48 hours. Putative colonies of Moraxella, identified by their smooth, rounded and grayish-white appearance with a hemolysis zone, were subjected to Gram staining and biochemical tests, then positive colonies were further confirmed using MALDI–TOF, other bacterial pathogens were also identified using biochemical tests and mass spectrometry. For this purpose, after sampling, a well-isolated colony was removed using a sterile loop, then re-suspended in 300 µl of distilled water, then the suspension was centrifuged for 10 minutes at 10,000 rpm, after which 2 µl of a reactogenic solution was added to the precipitate, transferred to a MALDI plate and loaded into an ionization chamber.

Results

The following types of microorganisms have been detected and identified in cattle with a clinical picture of serous, catarrhal mastitis: Streptococcus uberis, Staphylococcus capitis, Trueperella pyogenes, Bacillus pumilus, Brevibacterium celere, Corynebacterium xerosis, Bacillus licheniformis, Acinetobacter junii, Escherichia coli, Paraclostridium bifermentans, Psychrobacter immobilis, Enterococcus faecium, Serratia liquefaciens, Citrobacter freundii, Pseudomonas spp., Corynebacterium spp., Enterococcus faecalis, Psychrobacter phenylpyruvicus, Raoultella ornithinolytica, Moraxella catarrhalis (1 isolate). The following types of microorganisms were found in goats with a clinical picture of serous catarrhal mastitis: Aerococcus viridians, Corynebacterium stationis, Staphylococcus haemolyticus, Staphylococcus chromogenes, Corynebacterium xerosis, Corynebacterium stationis, and Staphylococcus epidermidis. The results are presented in table 1. No growth was detected from samples of biological material from mastitis milk of sheep and goats, during screening bacteriological studies on blood agar. To determine the pathogenic properties of the isolated cultures, a coagulase test for staphylococci and a bioassay on white mongrel mice were used. The inoculum diluted to the desired concentration (10^8 CFU/ml), OD = 600 nm = 1, obtained from a pure culture of Moraxella bovis and Moraxella bovoculi, was administered subcutaneously to mice at a dose of 100 µl.

Table 1.

Results of microbiological examination of biomaterial samples for mastitis in cows and goats.

№	Kind of animal	Age	Isolated mocrjjrganisms	Pathogenic microorganisms
1	Goat	2 years	Aerococcus viridians Corynebacterium stationis Staphylococcus haemolyticus	Corynebacterium stationis
2	Goat	1.5 years	Staphylococcus chromogenes Staphylococcus haemolyticus Corynebacterium xerosis	Corynebacterium xerosis
3	Goat	2.3 years	Staphylococcus chromogenes	-
4	Goat	2.4 years	Staphylococcus chromogenes Aerococcus viridians Staphylococcus haemolyticus Corynebacterium xerosis Corynebacterium stationis	Corynebacterium xerosis Corynebacterium stationis
5	Goat	2.1 years	Staphylococcus epidermidis	Staphylococcus epidermidis
6	Cow	2 years	Pseudomonas sp. Enterococcus faecalis Serratia liquefaciens Citrobacter freundii	Serratia liquefaciens
7	Cow	2.7 years	Serratia liquefaciens Psychrobacter phenylpyruvicus Raoultella ornithinolytica	-
8	Cow	3 years	Enterococcus faecalis Citrobacter freundii Trueperella pyogenes	-
9	Cow	2.1 years	Bacillus licheniformis Moraxella catarrhalis	Bacillus licheniformis Moraxella catarrhalis
10	Cow	3 years	Streptococcus uberis Staphylococcus capitis Trueperella pyogenes Bacillus pumilus	Bacillus pumilus
11	Cow	2.6 years	Bacillus licheniformis Acinetobacter junii Escherichia coli Corynebacterium xerosis	Corynebacterium xerosis
12	Cow	2,2 years	Paraclostridium bifermentans Psychrobacter immobilis Enterococcus faecium Escherichia coli	Paraclostridium bifermentans Psychrobacter immobilis
13	Cow	2 years	Serratia liquefaciens Citrobacter freundii	-
14	Cow	3.4 years	Escherichia coli Pseudomonas sp. Corynebacterium spp.	Corynebacterium sp.
15	Cow	3 years	Bacillus licheniformis Escherichia coli	Bacillus licheniformis
16	Cow	3 years	Streptococcus uberis Staphylococcus capitis Trueperella pyogenes Bacillus pumilus	Bacillus pumilus
17	Cow	2.5 years	Brevibacterium celere Corynebacterium xerosis	Corynebacterium xerosis
18	Cow	2.2 years	Bacillus licheniformis Escherichia coli	Bacillus licheniformis Escherichia coli
19	Cow	2.3 years	Staphylococcus spp.	Staphylococcus spp.
20	Cow	3 years	Brevibacterium celere Corynebacterium xerosis	Corynebacterium xerosis

Discussion

A study of the nosological role of the moraxella group of bacteria in mastitis in ruminant productive animals revealed, during screening of pathological material (n=20), the sporadic presence of moraxella microorganisms in the clinical microbiota of mastitis milk among other representatives, opportunistic pathogens: S. uberis, Staph. capitis, haemolyticus, chromogenes, epidermidis, T. pyogenes, Corynebacterium xerosis, stationis, E. coli. Thus, in 2 cases, Moraxella catarrhalis was identified (1 isolate was obtained). Aerococcus viridans was also detected in goats with a clinical picture of serous catarrhal mastitis, but no moraxella was detected. Several cases were identified at once in which Acinetobacter spp., belonging to the Moraxellaceae family, was isolated in associations, colonies of which formed hemolysis on blood agar. However, Acinetobacter spp. and representatives of the genus Moraxella – M. catarrhalis have been identified together with Staphylococcus and Pseudomonas aeruginosa. The detection of this combination in various samples may indicate a probable zoopathogenic role of Acinetobacter spp. and Moraxella spp., however, further research is required for more definitive conclusions. In addition, Psychrobacter bacteria, known as opportunists, which are important in the pathogenesis of a number of inflammatory diseases of humans and small domestic animals, were isolated from samples of clinical material in the associations. The role of psychrobacteria in zoonosology has not yet been disclosed. The data obtained with the isolation of pathogenic Psychrobacter immobilis in association may indicate its developing pathogenetic significance.

In contrast to Moraxellaceae, bacteria of the Corynebacteriaceae family played a more significant role in the primary screening of mastitis secretions in ruminants. The incidence of pathogenic corynebacteria as the leading representative of the clinical microbiota was noted at the level of 60% in the goats of LLC «Pikaev» in the Republic of Morodovia and 26.7% in cows from various farms in the Moscow region, the Republic of Morodovia and Bashkortostan. This indicates the leading role of corynebacteria in the nosology of mastitis in cattle and small cattle, with Corynebacterium xerosis showing the dominant etiotropic value. Of course, the problem of mastitis is not limited to the range of microorganisms involved in etiopathogenesis, broader screening and monitoring studies by various authors identify Streptococcus uberis, Trueperella pyogenes, Staphylococcus epidermidis and a number of other pathogens – staphylococcus, streptococcus, E. coli. However, the results obtained develop an idea of the participants of the microbiome in serous mastitis of productive livestock and require biological control and medicinal application, at least against corynebacteria.

Conclusion

The nosological role of moraxella microorganisms in mastitis in cows and goats has not been confirmed at this stage of research. Cases of indication of Moraxella catarrhalis and Acinetobacter spp. have been noted among the clinical microbiota, which does not exclude the nosological role of microorganisms of the Moraxellaceae family in these pathologies, as early studies occasionally noted.

Conflict of interest information. The authors declare that they have no conflict of interest.

Sponsorship information. The materials were prepared within the framework of the 2023 Russian Science Foundation "Conducting Basic Scientific Research and exploratory scientific research by small individual scientific groups" (Agreement No. 24-26-00168 of 12/29/2023).

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ВКЛАД АВТОРОВ

Все авторы сделали эквивалентный вклад в подготовку статьи для публикации.

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Поступила 07.07.2025 Received 07.07.2025
После рецензирования 29.08.2025 Revised 29.08.2025
Принята 10.09.2025 Accepted 10.09.2025