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Role of bovine respiratory syncytial virus in etiology of respiratory diseases on milk farms

S. V. Koteneva[1], A. V. Nefedchenko[2], T. I. Glotova[3], A. G. Glotov[4]
Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SFSCA RAS),
Institute of Experimental Veterinary Medicine of Siberia and the Far East, Krasnoobsk, Novosibirsk Region, Russia
[1] *ORCID 0000-0003-2649-7505, e-mail: koteneva-sv@mail.ru*
[2] *ORCID 0000-0002-4181-4268, e-mail: homeovet@yandex.ru*
[3] *ORCID 0000-0003-3538-8749, e-mail: t-glotova@mail.ru*
[4] *ORCID 0000-0002-2006-0196, e-mail: glotov_vet@mail.ru*

SUMMARY
Bovine respiratory syncytial virus (BRSV) is one of the etiological agents of respiratory diseases. The agent spreads widely in all the countries with intensive livestock farming and can cause pathologic changes in respiratory system either alone or in combination with other viruses and bacteria. It is a matter of crucial importance to study spread of the agent on large milk farms, to detect it in the internal organs of infected animals, and to quantify virus accumulation in them. The purpose of the research was to study peculiarities of RS infection spread, frequency of the virus detection in biomaterial samples (both alone and in associations with infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea/mucosal disease viruses (BVDV) and with *Pasteurellaceae* bacteria) on large milk farms affected by respiratory animal diseases; and to determine virus concentration in the respiratory organs. BRSV alone was reported in 9.2% of the tested biomaterial samples, as associated with IBR and BVDV it was reported in 1.4% and 5.2% of samples, correspondingly. The number of samples containing simultaneously BRSV and *Pasteurellaceae* bacteria was 10.8%. The virus was reported in a maximum of 26.6% of the tested samples. With the help of real-time PCR the virus genome was detected in lungs (13.1%), in exudate from trachea, bronchi and nasal sinuses (6.0%), in nasal discharge (4.0%) and in bronchi (1.7%). The virus was seldom detected in trachea and bronchial mucosa (1.1%) and in pulmonary lymph nodes (0.8%). Quantification of BRSV RNA demonstrated that maximum virus accumulation was observed in lungs and nasal charges and it confirms data on its tropism to pulmonary interstitium.

Keywords: Cattle, respiratory diseases, respiratory syncytial virus (RSV), polymerase chain reaction, synergism.

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For correspondence: Tatyana I. Glotova, Doctor of Science (Biology), Professor, Chief Researcher, Laboratory of Biotechnologies, Diagnostic Center, Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SFSCA RAS), Institute of Experimental Veterinary Medicine of Siberia and the Far East, 630501, Russia, Novosibirsk Region, Novosibirsk Raion, Krasnoobsk, *e-mail: t-glotova@mail.ru.*

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Роль респираторно-синцитиального вируса крупного рогатого скота в этиологии респираторных болезней на молочных комплексах

С. В. Котенева[1], А. В. Нефедченко[2], Т. И. Глотова[3], А. Г. Глотов[4]
ФГБУН Сибирский федеральный научный центр агробиотехнологий Российской академии наук (СФНЦА РАН),
Институт экспериментальной ветеринарии Сибири и Дальнего Востока, пос. Краснообск, Новосибирская обл., Россия
[1] *ORCID 0000-0003-2649-7505, e-mail: koteneva-sv@mail.ru*
[2] *ORCID 0000-0002-4181-4268, e-mail: homeovet@yandex.ru*
[3] *ORCID 0000-0003-3538-8749, e-mail: t-glotova@mail.ru*
[4] *ORCID 0000-0002-2006-0196, e-mail: glotov_vet@mail.ru*

РЕЗЮМЕ

Одним из этиологических агентов респираторных болезней является респираторно-синцитиальный вирус крупного рогатого скота (РСВ КРС). Возбудитель широко распространен во всех странах мира с интенсивным типом ведения животноводства и может вызывать патологию респираторного тракта самостоятельно или взаимодействуя с другими вирусами и бактериями. Актуальным является изучение распространения возбудителя на крупных молочных комплексах, выявление его во внутренних органах инфицированных животных, в том числе с количественной оценкой накопления в них. Целью работы было изучение особенностей распространения респираторно-синцитиальной инфекции, частоты выявления вируса в пробах биологического материала как в моноварианте, так и в ассоциациях с вирусами инфекционного ринотрахеита (ИРТ КРС) и вирусной диареи – болезни слизистых оболочек (ВД-БС КРС) крупного рогатого скота, бактериями семейства *Pasteurellaceae* на крупных молочных комплексах, неблагополучных по респираторным болезням животных, с определением концентрации вируса в органах респираторного тракта. В моноварианте РСВ КРС присутствовал в 9,2% исследованных проб биоматериала, в ассоциациях с вирусами ИРТ и ВД-БС КРС – в 1,4 и 5,2% проб соответственно. Количество проб, содержащих одновременно РСВ КРС и бактерии семейства *Pasteurellaceae*, составило 10,8%. Максимально вирус присутствовал в 26,6% проб от числа исследованных. Методом полимеразной цепной реакции в реальном времени геном вируса выявляли в легких (13,1%), в экссудате трахеи, бронхов и носовых синусов (6,0%), носовых выделениях (4,0%), бронхах (1,7%). Реже вирус присутствовал в пробах слизистой оболочки трахеи и бронхов (1,1%) и легочных лимфатических узлах (0,8%). Количественная оценка РНК РСВ КРС показала, что максимальное накопление вируса происходило в легких и носовых выделениях, что подтверждает данные об его тропизме к интерстицию легочной ткани.

Ключевые слова: Крупный рогатый скот, респираторные болезни, респираторно-синцитиальный вирус, полимеразная цепная реакция, синергизм.

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Для корреспонденции: Глотова Татьяна Ивановна, доктор биологических наук, профессор, главный научный сотрудник лаборатории биотехнологии – диагностический центр СФНЦА РАН, Институт экспериментальной ветеринарии Сибири и Дальнего Востока, 630501, Россия, Новосибирская обл., Новосибирский район, пос. Краснообск, *e-mail: t-glotova@mail.ru.*

INTRODUCTION

Respiratory diseases of calves take one of the leading places among bovine pathologies in the Russian Federation. They cause death or a decrease in the growth rate of animals, result in additional expenditure on treatment, diagnostic and preventive measures [1, 2]. Bovine respiratory syncytial virus (BRSV) plays an important role in the structure of infectious respiratory diseases and this infection is widespread in all the countries with intensive animal husbandry [3, 4].

The causative agent of bovine respiratory syncytial virus infection (BRSV infection) is an enveloped RNA virus belonging to the *Pneumoviridae* family, the *Orthopneumovirus* genus, which mainly replicates in the cells of the respiratory epithelium.

Calves under 6 months of age are most susceptible to infection; however, adult animals can also get sick. The incubation period of BRSV infection is 2–5 days. At the beginning of the disease, animals may demonstrate the following symptoms: depression, fever, cough, rhinitis, rhinopharyngitis. In severe cases, a secondary infection can occur resulting in bronchitis, bronchiolitis, and pneumonia. The main complications of the infection may include emphysema, respiratory failure and acute fibrinous pneumonia. Post-mortem lesions are observed only in lungs.

Laboratory diagnosis of BRSV infection includes detection of the virus antigen in the pathological material from infected animals using immunofluorescence assay (IFA); or of the virus ribonucleic acid (RNA) using polymerase chain reaction (PCR), isolation of the virus in cell culture and determination of seroconversion to the virus in convalescent animals [5]. Due to great instability and weak ability of the pathogen to replicate in cell cultures, virological studies are not effective enough and require a lot of time and effort [6].

At present, it is crucial to study the BRSV infection epizootic situation in large dairy establishments (where imported cattle are held), as well as to study the tropism of the virus to respiratory tract with a quantitative assessment of its accumulation in them. The disease can occur independently or in association with other viral infections. Initial infection with BRSV in epithelial cells reduces the level of protection of the respiratory tract of animals and facilitates colonization and secondary infection of the lower respiratory tract by bacteria. The synergistic interaction between the virus and *Pasteurellaceae* bacteria is described [7, 8].

The aim of the research was to study BRSV infection spread, the frequency of the pathogen detection in samples of biological material alone and in associations with the viruses of infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea/mucosal disease (BVDV), as well as with *Pasteurellaceae* bacteria in large dairy establishments affected by animal respiratory diseases; as well as to determine BRSV concentration in the respiratory tract.

MATERIALS AND METHODS

The studies were carried out in 2010–2020 in 7 large dairy establishments in Siberia, where specific prevention of BRSV infection was not carried out or only inactivated vaccines were used. The following samples of biological material were tested: nasal discharge, tracheal and bronchial exudates, pieces of bronchi and lungs, taken from dead animals or animal subject to emergency slaughter due to signs of respiratory failure. The samples were delivered frozen to the laboratory within 24 hours. Totally, 1,040 samples were tested; they were first ground in separate porcelain mortars with sterile sand, homogenized; then 10% suspensions were prepared on saline solution, centrifuged at 3,000 rpm for 15 minutes, then 100 µl of clarified supernatant was used to isolate RNA.

The virus RNA was isolated using the "RIBOsorb" kit (the Federal Budget Institution of Science of the Central Scientific Research Institute of Epidemiology of the Rospotrebnadzor) in accordance with the manufacturer's recommendations. Reverse transcription to obtain cDNA was performed using the "REVERTA-L" kit of the same manufacturer.

PCR with electrophoretic detection of results was used to detect genomes of three viruses in biomaterial samples, and real – time PCR was used to quantify BRSV RNA [6, 9]. The concentration of viral RNA in samples of biological material from sick animals was quantified relative to the mRNA level of the bovine GAPDH gene and expressed in $\log_{10}$ copies of viral RNA per 10^5 copies of GAPDH ($\log_{10}$ BRSV/GAPDH) [10].

Serological methods were used to study 6,000 sera samples. Antibodies to IBR and BVD viruses were detected using microneutralization assay in the continuous MDBK cell line according to the World Animal Health Organization (OIE, 2019) standard using "TK-A" strain and the cytopathogenic NADL strain as an antigen, and to BRSV – in the indirect hemagglutination test. For the purposes of serological screening, sera samples were taken from animals once, and to determine seroconversion they were taken twice with a 30-day interval. Persistent infection with BVDV was diagnosed when the viral RNA was detected in paired sera samples taken with a 30-day interval.

Pasteurellaceae bacteria were isolated in artificial culture media according to the guidelines for laboratory diagnosis of pasteurellosis in animals and birds, and their genotyping was performed using PCR [11].

RESULTS AND DISCUSSION

In order to determine how frequently BRSV is detected alone and in associations with IBR and BVD viruses and *Pasteurellaceae* bacteria (using PCR), we tested 1,040 biomaterial samples taken from dead and emergently slaughtered calves under 6 months of age with the signs of respiratory damage. In all animals post-mortem examination revealed acute fibrinous or catarrhal bronchopneumonia, and sometimes interstitial and pulmonary emphysema and signs of lung parenchyma destruction. The results of the study are presented in the Table.

The table shows that BRSV alone was present in 9.2% of the studied biomaterial samples, and in the associations with IBR and BVD viruses it was detected in 1.4 and 5.2% of the samples, respectively. The maximum number of samples (10.8%) contained both BRSV and *Pasteurellaceae* bacteria. In total, BRSV was detected in 26.6% of the tested samples [12, 13].

Respiratory syncytial virus alone was more often detected in lungs and bronchi, and in association with the IBR – in trachea and nasal mucosa, less often – in bovine lungs. Together with BVDV and *Pasteurellaceae* bacteria, BRSV was detected in lungs.

Table
Frequency of BRSV detection: alone and in association with IBR, BVDV and *Pasteurellaceae* bacteria using PCR with electrophoretic detection [12, 13]

Таблица
Частота выявления РСВ КРС в моноварианте и в ассоциациях с вирусами ИРТ, ВД-БС КРС и бактериями семейства *Pasteurellaceae* при помощи ПЦР с электрофоретической детекцией [12, 13]

n = 1,040

Pathogen, association of pathogens	Number of positive samples	Percent of positive samples from the number of samples tested
BRSV alone	96	9.2
BRSV + IBR	15	1.4
BRSV + BVDV	54	5.2
BRSV + *Pasteurellaceae* Including bacteria: *Pasteurella multocida* *Mannheimia haemolytica*	112 42 70	10.8 4.0 6.7
Total	277	26.6

Many authors assign a special role in the synergism of infectious agents to BVDV, which, due to its immunosuppressive effect, can increase the susceptibility of animals to infection with other viral and bacterial pathogens [7, 8, 14, 15].

According to our data, the level of infection of animals with BVDV is 90% and above in large dairy establishments, where about 3% of calves are persistently infected. The percentage of animals (of all sex and age groups) seropositive to BRSV in such herds is 67.5% on average, and the virus genome is detected more often than in herds without persistently infected animals [9].

Thus, large dairy establishments demonstrate a mixed presence of BVDV and BRSV. A correlation was revealed between the level of BVDV in animals, i.e. between the presence of animals persistently infected with this pathogen in the examined establishments, and the frequency of clinical signs of respiratory diseases in calves that occur due to BRSV [12].

Often, after a predisposing viral infection, secondary bacterial bronchopneumonia develops, since damage to the epithelium of the respiratory tract leads to a violation of mucociliary clearance and facilitates the movement of bacteria to the lower respiratory tract. In addition, respiratory viruses suppress the phagocytic activity of alveolar macrophages and disrupt intracellular bactericidal processes. Also, respiratory viruses can promote bacterial adhesion, enhance the expression of surface proteins of host cells, which can then be joined by bacteria [7, 16].

Basically, secondary bacterial bronchopneumonia develops due to the presence of *Pasteurellaceae* bacteria, namely *Mannheimia haemolytica* and *Pasteurella multocida* [8, 9, 15].

An important aspect of the BRSV infection pathogenesis is that it suppresses non-specific mechanisms of the respiratory immune defense, and it initiates and enhances bacterial colonization of the lungs after its primary replication. The virus can independently cause bronchitis, pneumonia and emphysema, but its main feature is immunosuppression and an ability to provide preconditions for bacterial pneumonia, in particular, pulmonary pasteurellosis [5, 8].

The results of the conducted research show that BRSV infection is common in large dairy establishments in Siberia and can occur both alone and in various associations [17]. The synergistic interaction of microorganisms of different classes plays a significant role in the occurrence of mass bronchopneumonia in cattle under natural conditions. When developing effective measures of specific prophylaxis for this bovine disease, especially when importing animals from different sources, it is important to understand and decipher the mechanisms that contribute to the development of mixed forms of infections.

Previously, we studied BRSV distribution in the respiratory tract of calves with respiratory syncytial infection using PCR with electrophoretic detection of amplification products, but the concentration of the virus RNA could not be determined due to the limitations of the method [3].

To study the virus distribution in the upper and lower respiratory tract, positive samples of biomaterial were additionally tested in real-time PCR and it was found that the BRSV genome was more often present in lungs (13.1% of the number of tested samples). In addition, the virus was detected in the exudate from trachea, bronchi and nasal sinuses, which accounted for 6.0% of the samples. The percentage of virus detection in nasal discharge was 4.04%, and in the bronchi – 1.7%. The virus was less often detected on the tracheal and bronchial mucosa (1.1%) and in pulmonary lymph nodes (0.8%). The data obtained demonstrate a wide distribution of the virus in the organs of the upper and lower respiratory tract.

Virus quantification in different parts of the respiratory tract of infected animals was of great interest. The maximum concentrations of the virus genome were detected in lungs ($1.3 \pm 0.5 - 4.8 \pm 0.47$ $\log_{10}$ copies of BRSV RNA/GAPDH), nasal discharge ($1.5 \pm 0.75 - 2.1 \pm 0.25$ $\log_{10}$ copies of BRSV RNA/GAPDH) and exudates from trachea, bronchi, and nasal sinuses ($0.3 \pm 0.21 - 2.8 \pm 0.15$ $\log_{10}$ copies of BRSV RNA/GAPDH). Different virus concentrations detected during the research in the biomaterial samples may indicate that animals were sampled at different stages of the infectious process.

The above facts show how important respiratory syncytial virus is among bovine respiratory diseases and demonstrate its role in the pathogenesis of mixed respiratory diseases.

CONCLUSION

The results of the conducted research improve understanding of respiratory syncytial infection in animals in large dairy establishments and of its role in the pathogenesis of mono- and mixed infections of the bovine respiratory tract. BRSV was detected in 26.6% of biomaterial samples from sick and dead calves under 6 months of age; samples were taken during mass outbreaks of respiratory diseases, in particular during acute fibrinous bronchopneumonia. The virus alone was detected in 9.2% of cases, and in associations with IBR and BVDV in 1.4 and 5.2% of biomaterial samples, respectively. The number of samples containing BRSV and *Pasteurellaceae* bacteria was 10.8%, which confirms the synergistic interaction between infectious agents of different nosological groups.

In some establishments, the frequency of BRSV infection directly depended on the level of animal infection with BVDV, as well as on the presence of animals persistently infected with this virus in herds [12].

Quantitative analysis of BRSV RNA in the tested biomaterial samples showed its maximum accumulation in lungs and nasal discharge, and it confirms the data on the virus tropism to the pulmonary interstitium, and this contributes to the occurrence of acute fibrinous bronchopneumonia. Quantification of viruses and bacteria with the help of real-time PCR can be a useful tool to study pathogenesis of mixed viral-bacterial infections in the wild.

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Svetlana V. Koteneva, Candidate of Science (Veterinary Medicine), Senior Researcher, Laboratory of Biotechnologies, Diagnostic Center, Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SFSCA RAS), Institute of Experimental Veterinary Medicine of Siberia and the Far East, Krasnoobsk, Novosibirsk Region, Russia.

Alexey V. Nefedchenko, Doctor of Science (Veterinary Medicine), Associate Professor, Leading Researcher, Laboratory of Biotechnologies, Diagnostic Center, Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SFSCA RAS), Institute of Experimental Veterinary Medicine of Siberia and the Far East, Krasnoobsk, Novosibirsk Region, Russia.

Котенева Светлана Владимировна, кандидат ветеринарных наук, старший научный сотрудник лаборатории биотехнологии – диагностический центр ФГБУН Сибирский федеральный научный центр агробиотехнологий РАН (СФНЦА РАН), Институт экспериментальной ветеринарии Сибири и Дальнего Востока, пос. Краснообск, Новосибирская обл., Россия.

Нефедченко Алексей Васильевич, доктор ветеринарных наук, доцент, ведущий научный сотрудник лаборатории биотехнологии – диагностический центр ФГБУН Сибирский федеральный научный центр агробиотехнологий РАН (СФНЦА РАН), Институт экспериментальной ветеринарии Сибири и Дальнего Востока, пос. Краснообск, Новосибирская обл., Россия.

Tatyana I. Glotova, Doctor of Science (Biology), Professor, Chief Researcher, Laboratory of Biotechnologies, Diagnostic Center, Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SFSCA RAS), Institute of Experimental Veterinary Medicine of Siberia and the Far East, Krasnoobsk, Novosibirsk Region, Russia.

Alexander G. Glotov, Doctor of Science (Veterinary Medicine), Professor, Chief Researcher – Head of Laboratory of Biotechnologies, Diagnostic Center, Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SFSCA RAS), Institute of Experimental Veterinary Medicine of Siberia and the Far East, Krasnoobsk, Novosibirsk Region, Russia.

Глотова Татьяна Ивановна, доктор биологических наук, профессор, главный научный сотрудник лаборатории биотехнологии – диагностический центр ФГБУН Сибирский федеральный научный центр агробиотехнологий РАН (СФНЦА РАН), Институт экспериментальной ветеринарии Сибири и Дальнего Востока, пос. Краснообск, Новосибирская обл., Россия.

Глотов Александр Гаврилович, доктор ветеринарных наук, профессор, главный научный сотрудник – заведующий лабораторией биотехнологии – диагностический центр ФГБУН Сибирский федеральный научный центр агробиотехнологий РАН (СФНЦА РАН), Институт экспериментальной ветеринарии Сибири и Дальнего Востока, пос. Краснообск, Новосибирская обл., Россия.