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Role of acute respiratory diseases in pathogenesis of distal limb infections in cattle

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SUMMARY
According to current concepts, ruminal and metabolic acidosis occur due to feeding cattle mainly with preserved acidic feeds such as silage and haylage. However, errors in feeding are not the only etiological factor leading to acidosis. In some cases, metabolic acidosis in cattle can develop along with respiratory infection caused by viral and bacterial agents. The main pathological processes resulting from acute respiratory diseases of cattle are bronchitises, tracheites and pneumonias. When the respiratory tract is affected in cattle, hypoxia occurs, causing intoxication and, thus, leading to ruminal acidosis. As a result, vasoactive substances (bacterial endotoxins, histamine, lactate) enter the bloodstream, the vascular endothelium is damaged due to the simultaneous expansion of arterioles and compression of venules, blood fluid is perfused from the vessels into the surrounding tissues, the blood flow in the microcirculatory bed is disrupted. An important role in the disturbance of blood circulation in small blood vessels is played by circulating immune complexes representing the «antigen-antibody» complex. Low molecular weight circulating immune complexes settle in various organs and tissues of the body, lead to inflammation and damage the normal tissue structure. Most frequently, immune complexes affect the endothelium of blood vessels, renal glomeruli and joints. Distal limb vessels are primarily affected in cattle, leading to disturbance of skin trophism of the limbs and hooves, development of laminitis, while the hoof horn is weakly keratinized and cannot resist aggressive mechanical and chemical environmental factors. Damaged hooves are the gateway of infection for the agents of necrobacteriosis (*Fusobacterium necrophorum*), staphylococcosis (*Staphylococcus* spp.), streptococcosis (*Streptococcus* spp.) and other pathogens. In addition, favorable conditions evolve for the development of mixed infection due to reduction in the overall organism resistance, which is observed for both respiratory and distal limb infections.

Keywords: acute respiratory diseases of cattle, distal limb infections of cattle, necrobacteriosis, staphylococcosis, streptococcosis, pasteurellosis, bovine infectious rhinotracheitis, bovine viral diarrhea, bovine parainfluenza-3, bovine respiratory syncytial infection

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Роль острых респираторных заболеваний в патогенезе инфекций дистального отдела конечностей крупного рогатого скота

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РЕЗЮМЕ

По современным представлениям ацидоз рубца и метаболический ацидоз являются следствием кормления крупного рогатого скота преимущественно консервированными кислыми кормами, такими как силос и сенаж. Вместе с тем погрешности в кормлении не единственный этиологический фактор, приводящий к ацидозу. В ряде случаев у крупного рогатого скота метаболический ацидоз может развиваться на фоне респираторной патологии, вызываемой вирусными и бактериальными агентами. Основными патологическими процессами, вызываемыми острыми респираторными заболеваниями крупного рогатого скота, являются бронхиты, трахеиты и пневмонии. При поражении респираторного тракта в организме животных возникает гипоксия, что ведет к развитию эндогенной интоксикации, приводящей к ацидозу рубца, в результате чего в кровь поступают сосудисто-активные вещества (эндотоксины бактерий, гистамин, лактат), за счет одновременного расширения артериол и сжатия венул повреждается эндотелий сосудов, наблюдается перфузия из сосудов в окружающие ткани жидкости крови, нарушается кровоток в микроциркуляторном русле. Немаловажную роль в нарушении циркуляции крови в мелких кровеносных сосудах играют циркулирующие иммунные комплексы, представляющие собой комплекс антиген – антитело. Низкомолекулярные циркулирующие иммунные комплексы, оседая в разнообразных органах и тканях организма, приводят к воспалению и повреждают нормальную структуру тканей. Наиболее часто иммунные комплексы поражают эндотелий кровеносных сосудов, почечные клубочки и суставы. У крупного рогатого скота в первую очередь поражаются сосуды дистального отдела конечностей, что ведет к нарушению трофики кожи конечностей и копытец, развивается ламинит, при этом копытный рог слабо кератинизирован и не может противостоять агрессивным механическим и химическим факторам внешней среды. Поврежденные копытца являются воротами инфекции для возбудителей некробактериоза (*Fusobacterium necrophorum*), стафилококкоза (*Staphylococcus* spp.), стрептококкоза (*Streptococcus* spp.) и других патогенов. Кроме того, благоприятные условия для развития микст-инфекции создаются за счет снижения общей резистентности организма, что отмечается как при респираторной патологии, так и при патологии дистального отдела конечностей.

Ключевые слова: острые респираторные заболевания крупного рогатого скота, инфекции дистального отдела конечностей крупного рогатого скота, некробактериоз, стафилококкоз, стрептококкоз, пастереллез, инфекционный ринотрахеит КРС, вирусная диарея КРС, парагрипп-3 КРС, респираторно-синцитиальная инфекция КРС

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INTRODUCTION

Bovine extremity diseases under the conditions of intensive animal farming are quite common and sometimes become widespread. Their main causes are gross violations of animal feeding and keeping, as well as infectious respiratory diseases.

According to our observations, 25.4% of cases of respiratory pathology in cattle are associated with infections of the distal extremities, primarily with necrobacteriosis, streptococcosis and staphylococcosis, which cause economic losses due to reduced performance and culling up to 30% of highly productive animals [1, 2].

One of the factors influencing the development of distal extremity infections in cattle is acute respiratory infections, such as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine parainfluenza-3 (BPI-3), bovine respiratory syncytial virus (BRSV) infection, pasteurellosis, chlamydiosis and salmonellosis. Farms, which reported necrobacteriosis outbreaks were infected with bovine respiratory infections.

The aim of the research was the theoretical and practical substantiation of the pathogenic role of acute respiratory disease in infections of bovine distal extremities.

MATERIALS AND METHODS

The research was conducted at the Department of Infectious and Non-Infectious Pathology of the Ural State Agrarian University and farms of the Ural Region in the period from 2014 to 2021. Epizootological, pathomorphological, immunological and bacteriological research methods were used in the work.

The epidemic situation related to acute respiratory diseases was assessed by the statistical method; the annual reports of the Information and Analytical Center of the Rosselkhoznadzor Veterinary Surveillance Department (FGBI "Federal Center for Animal Health"), the BI Udmurt Veterinary Diagnostic Center, the FSBI "Chelyabinsk Interoblast Veterinary Laboratory", the GBI "Sverdlovsk Oblast Veterinary Laboratory" were studied.

The objects of the study were commercially raised cattle (tethered), blood, serum, hoof scrapes as well as pathological material collected from a 14-day-old calf killed due to pneumonia signs (pieces of lung and bronchial lymph nodes).

The pathological material from the killed calf was fixed in a 10% neutral buffered formalin, embedded in paraffin and histological preparations were prepared according to the generally accepted method. The sections were stained with hematoxylin and eosin, as well as using Van Gieson method, then examined by light microscopy according to the generally accepted method.

The blood of cows and calves was used for immunological studies. The amount of circulating immune complexes (CICs) in the serum was calculated by immunoprecipitation in 4% polyethylene glycol (PEG-6000), followed by photometry using SF-2000 spectrophotometer (OKB Spectrum LLC, Russia).

Bacteriological studies were carried out according to generally accepted methods.

The digital data of epizootological and laboratory studies are processed using mathematical statistics methods adopted in biology and medicine. The reliability of the results was determined by statistical processing using the Student's paired *t*-test. The results were considered reliable at $p \leq 0.05$.

The obtained statistical and experimental data were processed using the Microsoft Excel software, which is included in the Microsoft Office package.

RESULTS AND DISCUSSION

Acute respiratory diseases (ARDs) in the bovine infectious pathology in the Ural region rank second after infectious gastrointestinal diseases [1]. The infectious respiratory diseases are mostly spread in animals on the farms of the Udmurt Republic, where the following diseases take a lead in the nosological picture of infectious respiratory pathologies by the number of infected sites: pasteurellosis – 41.5%, BPI-3 – 16.7%, chlamydiosis – 14.1%, BVD – 11.8%, IBR – 10.6%, BRSV infection – 5.3% [2, 3].

In the livestock farms of the Chelyabinsk Oblast, the following diseases were registered: pasteurellosis – 46.3%, IBR – 21.9%, BVD – 12.3%, chlamydiosis – 7.3%, BPI-3 – 7.3%, BRSV infection – 4.9%. In the Sverdlovsk Oblast, pasteurellosis – 63.7%, BPI-3 – 14.9%, BVD – 8.5%, IBR – 7.4%, chlamydiosis – 5.5% were detected on cattle farms (Table 1).

When assessing the BRSV infection prevalence on farms of the Ural Economic region, it was found that among all bovine infectious respiratory pathologies, the frequency of this disease detection in the Udmurt Republic is 5.3%, in the Chelyabinsk Oblast is 4.9%. In the Sverdlovsk Oblast, according to the data of the GBI "Sverdlovsk Regional Veterinary Laboratory", BRSV infection was not reported [4, 5].

Serological, molecular biological, microbiological and immunological tests suggest a great involvement of pathogens of different taxonomic groups in respiratory pathology. The most frequently detected associations of BRSV infection are with IBR, pasteurellosis and other infectious agents (Tables 2, 3).

In large dairy farms, the detection of BRSV antibodies in cattle depended on the level of the infection with IBRV and pasteurellosis agent, as well as the presence in herds of individuals latently infected with bovine respiratory syncytial virus. Herewith, the level of infection of animals with the IBRV ranged from 10.6 to 21.9%, with pasteurellosis – from 41.5 to 46.3% [5].

No differences in the geographical spread of the disease among cattle of different ages were established. The greatest number of positive serological reactions to IBR, BVD, BRSV viruses and wound infections of cattle distal extremities were detected in large dairy farms with a herd high density and milk yield. The infection rate of cattle in medium and small livestock farms was lower, but the higher the milk yield was, the higher was the risk of the above mentioned respiratory diseases occurring. The spread of diseases was facilitated by the crowding of animals and the presence of latent virus carriers in the herd [4].

The results of various studies have proved that the range of infectious agents that cause infectious respiratory diseases and wound infections of bovine distal extremities is quite wide. Infections of bacterial etiology are often secondary, but can be concomitant or independent. This depends on the animal density in facilities, the presence or absence of specific vaccination against viral and bacterial infections, as well as on-farm factors. In this regard, when planning anti-epidemic measures, it is essential to conduct a full range of laboratory diagnostic tests (virological, bacteriological) in order to determine the nosological structure of a concrete outbreak of respiratory infections and the etiological role of each infectious pathogen.

Table 1
Rate of acute respiratory infections in cattle at livestock establishments

Disease	Udmurt Republic, %	Chelyabinsk Oblast, %	Sverdlovsk Oblast, %
Pasteurellosis	41.5	46.3	63.7
Chlamydiosis	14.1	7.3	5.5
BPI-3	16.7	7.3	14.9
BVD	11.8	12.3	8.5
IBR	10.6	21.9	7.4
BRSV infection	5.3	4.9	–

The difference is reliable $p \leq 0.05$

Table 2
Viral and bacterial associations of BRSV in livestock establishments of the Republic of Udmurtia

Viral-bacterial associations BRSV in the Udmurt Republic	As % of the total ARD affected farms, %
BRSV infection + pasteurellosis + chlamydiosis	1.8
BRSV infection + IBR + pasteurellosis	1.6
BRSV infection + pasteurellosis	1.6
BRSV infection + IBR + BVD + pasteurellosis	1.5
BRSV infection + IBR + pasteurellosis + chlamydiosis	1.4
BRSV infection + IBR + BVD + pasteurellosis + chlamydiosis	0.8
BRSV infection + IBR + BVD + chlamydiosis	0.8
BRSV infection + IBR + BVD	1.5
BRSV infection + BVD + pasteurellosis	0.5
BRSV infection + IBR + BPI-3 + pasteurellosis	0.4

The difference is reliable $p \leq 0.05$.

Histological examination of the pathological material (lung and bronchial lymph nodes), collected in a livestock farm of the Chelyabinsk Oblast from a 14-day-old calf killed due to signs of respiratory disease, revealed signs of pneumonia characteristic of pasteurellosis (Fig. 1, 2), syncytia were found in the lung tissues, which is consistent with BRSV infection (Fig. 3, 4) [3–5].

Based on the results of the histological examination, it was concluded that the nature of the processes detected in the tested preparations corresponds to an associated viral-bacterial infection (BRSV infection + pasteurellosis) [3–5]. This diagnosis was confirmed by laboratory research methods (materials are not covered by this paper).

In addition to respiratory infections, an infectious pathology of the distal extremities was registered in the animals on the farm where the studies were conducted. The bacteriological test of hoof scrapings revealed the causative agent of necrobacteriosis – *Fusobacterium necrophorum*.

Thus, in this farm, a mixed infection was registered – acute infectious respiratory diseases in association with wound infections of the distal extremities (BRSV infection + pasteurellosis + necrobacteriosis).

At the next stage of the work, the concentration of CICs was calculated in the blood of pregnant cows two months before calving and of calves aged 1 month (Table 4). As it can be seen, the concentration of CICs in cows and calves was higher than the physiological norm, which is a sign of their insufficient elimination out of the body. This increases the risk of deposition of these complexes in normal tissues. An important pathogenetic factor in the development of wound infections of the bovine distal extremities in some cases are acute respiratory infections.

According to modern concepts, rumen acidosis and, as a consequence, metabolic acidosis are the result of feeding cattle mainly with preserved acidic feeds, such as silage and haylage [6–10]. Cellulolytic bacteria (*Ruminococcus*, etc.), as well as fungi of the *Neocallimastigaceae* family are sensitive to the pH of the environment: the acidic environment inhibits their growth, and therefore the cellulase activity of the rumen contents decreases. The bovine gastrointestinal tract stops digesting fiber, the digestibility of the diet decreases sharply. The acidic environment also suppresses the growth of *Streptococcus bovis* bacteria, which are representatives of the rumen microbiome, participate in the breakdown of pectin, protein and starch and thereby contribute to the acidification of the environment. *Streptococcus bovis* is replaced by lactic acid bacteria of the *Lactobacillus* genus, which are more resistant to acidic environments. Such conditions are favorable for the reproduction of *Fusobacterium necrophorum* bacteria in the rumen, which can enter the bloodstream, as well as of other microorganisms that can produce toxins.

Table 3
Viral and bacterial associations of BRSV in livestock establishments of the Chelyabinsk Oblast

Viral-bacterial associations BRSV in the Chelyabinsk Oblast	As % of the total ARD affected farms, %
BRSV infection + IBR + pasteurellosis	4.9
BRSV infection + IBR + BVD + BPI-3 + pasteurellosis + chlamydiosis	2.4

The difference is reliable $p \leq 0.05$.

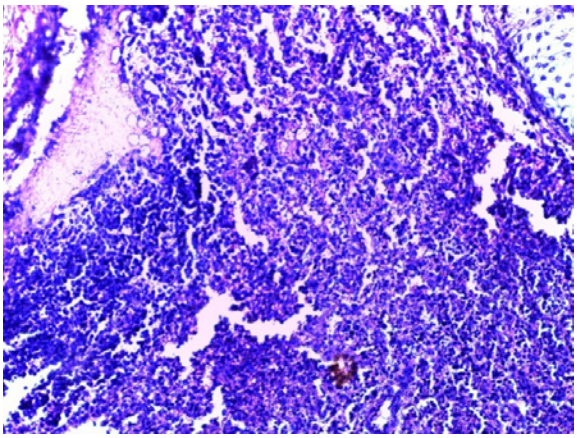

Fig. 1. Foci of purulent pneumonia (hematoxylin – eosin staining, magnification 200×)

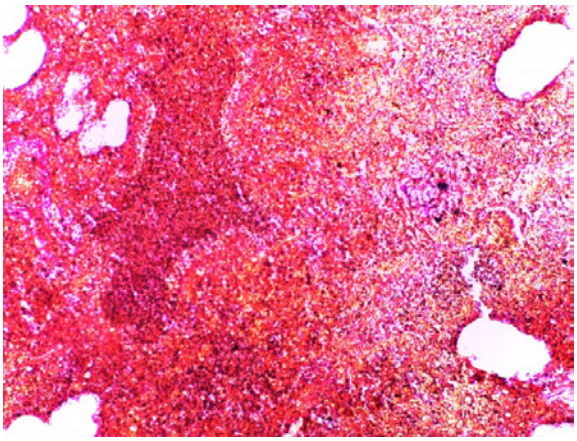

Fig. 2. Focal hemorrhagic pneumonia (staining according to Van Gieson, magnification 200×)

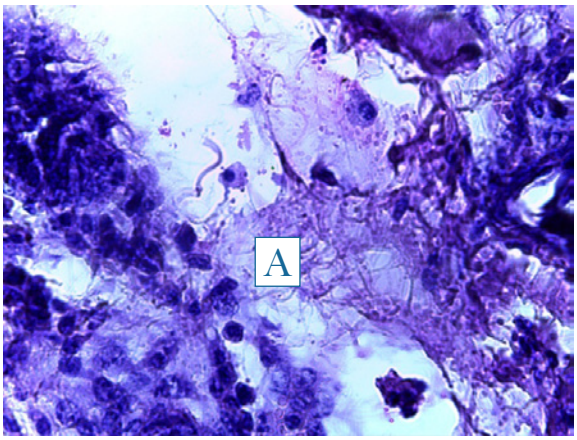

Fig. 3. Macrophages in the lung and syncytia (A), characteristic of respiratory syncytial infection (hematoxylin – eosin staining, magnification 630×)

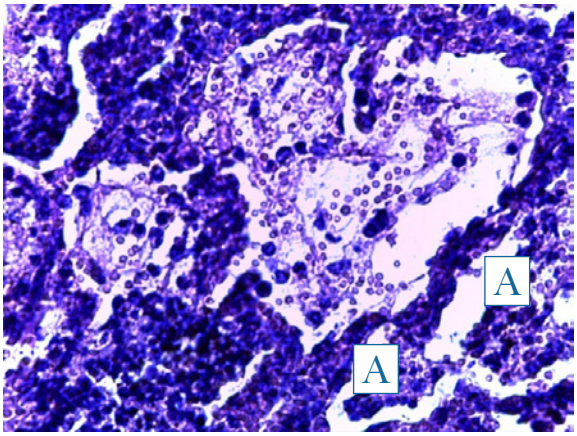

Fig. 4. Macrophages, erythrocytes and syncytia (A) in the lumen of the alveoli, typical of respiratory syncytial infection (hematoxylin – eosin staining, magnification 400×)

However, errors in feeding are not the only etiological factor leading to acidosis. For example, several authors have found that in parallel to respiratory pathology caused by viral and bacterial agents, acidosis develops in cattle [8, 9, 11, 12].

The main pathological processes in the respiratory tract caused by bovine acute respiratory infections are bronchites, tracheites and pneumonias. When the respiratory tract is affected, hypoxia occurs in the body of animals, which leads to the development of endogenous intoxication, resulting in rumen acidosis [11, 12], followed by introduction of vasoactive substances (bacterial endotoxins, histamine, lactate) into blood [7–10]. Due to the simultaneous expansion of arterioles and compression of venules, the vascular endothelium is damaged, perfusion of blood fluid from the vessels into the surrounding tissues occurs, blood flow in the microcirculatory bed is disrupted [7].

An important role in poor blood circulation in small blood vessels is played by CICs, which are antigen – antibody complexes. The CICs formation is one of the normal responses of the body's immune system to the introduction of a pathogen. At the same time, CICs increased concentration, which occurs during a high antigenic load or malfunction of their elimination mechanisms out of the body, leads to pathological changes in the tissues and organs of animals, which is caused by high biological activity of immune complexes. Most of the CICs are quickly removed from the bloodstream of the body due to the reticulohistiocytic system, which combines various heterogeneous groups of the body cells, in particular Kupfer

Table 4
Concentration of circulating immune complexes in the blood of cows and calves

Indicator	Cows ($n = 10$)	Calves ($n = 10$)
CICs, c.u.	212.400 ± 0.645	234.460 ± 5.905

cells capable of active phagocytosis. CICs have both immunostimulating and immunosuppressive properties. The greatest pathogenic effect is exerted by complexes capable of activating the complement system and reacting with blood cells that have receptors for binding immunoglobulins or complement. The main CICs damaging effect is complement and neutrophil-dependent mechanism. Complement associated CICs exhibit chemotactic properties, resulting in the accumulation of neutrophils in the affected areas and to the release of hydrolytic enzymes from them that destroy the body tissues. At the same time, CICs can cause pathology regardless of the presence of neutrophils and complement [13]. Low-molecular-weight CICs, settling in various organs and tissues of the body, lead to inflammation and damage the normal tissue structures. The blood vessel endothelium, renal glomeruli and joints are most often damaged by immune complexes [14].

In cattle, the vessels of the distal extremities are primarily affected, which leads to trophism disorder of hoof and limb skin. That's why laminitis develops, while the hoof horn tissue is weakly keratinized and cannot resist aggressive mechanical and chemical environmental factors [7, 10]. Damaged hooves are the gateway of infection for the agents of necrobacteriosis (*Fusobacterium necrophorum*), staphylococcosis (*Staphylococcus* spp.), streptococcosis (*Streptococcus* spp.) and other pathogens [7, 10, 12, 15]. In addition, favorable conditions for the development of mixed infection are created by reduced organism resistance, which is observed both during respiratory pathology and during pathologies of the distal extremities [15].

CONCLUSION

Studies have shown that one of the pathogenic factors contributing to the development of infections of bovine distal extremities is respiratory diseases, leading to reduced natural resistance of the body and subsequent metabolic acidosis.

Comprehensive prophylaxis of infections of bovine distal extremities should include mandatory laboratory diagnostics for acute respiratory diseases.

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