Development of a method for collecting bronchoalveolar lavage from calves for microbiological diagnosis of bronchopneumonia

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Abstract. A serious problem for the health of cattle, especially in highly productive animals, is infectious bronchopneumonia, which occurs when exposed to numerous adverse environmental factors and opportunistic microorganisms. Infectious bronchopneumonia is an important problem in the livestock industry and remains a major cause of significant economic losses in dairy herds and feedlots due to high morbidity and mortality rates, in addition to negatively affecting growth, reproductive performance and life expectancy. Therefore, the development of a new method for intravital minimally invasive diagnosis of calves with acute catarrhal bronchopneumonia using bronchoalveolar lavage sampling for subsequent microbiological studies is relevant. The invention relates to veterinary medicine and can be used in intravital diagnostics of bronchopneumonia in calves by isolating microorganisms that initiate the purulent-inflammatory process from samples of bronchoalveolar contents taken in the area of the tracheal bifurcation. A method for intravital diagnosis of bronchopneumonia in calves includes transnasal introduction to a sick animal of a sterile silicone medical tube with a diameter of 4 mm (the internal diameter of the hole is 3 mm), a wall thickness of 1.0 mm and a length of 150 cm, until slight resistance and a repeated cough reflex appear, upon reaching in the area of the tracheal bifurcation, the nasogastric tube is moved back 1 cm, a disposable syringe with a volume of 50 ml is attached to the free end, and with its help, 30-40 ml of sterile isotonic saline solution (0.9% NaCl solution, 37° C) is injected into the trachea, and then immediately aspirate up to 10 ml of bronchoalveolar contents.

1 Introduction

Recently, due to the intensification of dairy farming, there has been an increase in the concentration of cattle on livestock farms [1-3]. This, in turn, creates unfavorable conditions that contribute to a decrease in their resistance to various adverse environmental influences,

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including the negative influence of associations of opportunistic microflora that circulate in farming biogeocenosis [4, 5].

Factor infections in farmed cattle can be caused by a variety of pathogens, including bacteria, viruses and protozoa. Some of the most common factor infections in cows include obstetric and gynecological diseases in cows, as well as pneumoenteritis in calves [6–10]. To prevent factor infections in cattle, it is necessary to follow preventive measures, including regular vaccination, maintaining hygiene in stalls and around animals, monitoring feeding and drinking, as well as regular examination and treatment of sick animals [11-15].

Currently, in livestock farms, respiratory diseases are widespread among highly productive animals, which are most often diagnosed in young animals. These diseases lead to significant economic losses for the industry and consist of the death of animals, loss of production from sick or recovered animals, slowdown in their growth and development, and costs of treatment and prevention [4, 16—20]. Bronchopneumonia in calves is registered in almost all zones of our country and in terms of share among all pathologies on farms it ranks second after gastrointestinal diseases, reaching 20-30% [21-23]. The etiological factors of nonspecific bronchopneumonia in calves are a complex of reasons: crowded housing, decreased resistance and immunological reactivity of the body of newborn animals, exposure to unfavorable environmental factors, stress, unbalanced feeding, as well as conditionally pathogenic microbiota of the anterior respiratory tract, which under these unfavorable conditions can acquire pathogenic properties [24-26].

In this regard, improving the intravital diagnosis of bronchopneumonia in calves by determining microbiocenosis from bronchoalveolar contents selected from the lower respiratory tract of sick animals is a matter of paramount importance, which requires a timely and competent solution.

2 Materials and methods

The study was supported by the Russian Science Foundation Grant No. 24-26-00091, https://rscf.ru/project/24-26-00091/. The material for the study was calves aged 1-3 months, patients with acute catarrhal bronchopneumonia (n=37), which were located in the livestock farms of Babaevo LLC, Sobinsky district, Vladimir region and Delta-F LLC, Sergiev Posad urban district, Moscow region, with a total livestock of 3680 heads, including 1690 cows. This experiment was approved by the Academic Council of the Department of Veterinary Medicine, as well as the Institute Bioethical Commission of the Agricultural-Technological Institute of the Peoples' Friendship University of Russia. P. Lumumba, on the subject of humane treatment of animals.

Animals that were treated within 14 days prior to sampling were excluded from the study. To exclude causative agents of chlamydia and mycoplasmosis in the morning, blood was taken from sick animals from the jugular vein into sterile tubes for serological studies using the automatic “ALISEI” system for enzyme immunoassay on the basis of the Scientific and Educational Resource Center (REC) “Pharmacy” of RUDN University.

Bacteriological studies were carried out using generally accepted methods.

The technical result of the claimed invention is the development of a new safe method for intravital diagnosis of bronchopneumonia in calves by isolating microorganisms that initiate the purulent-inflammatory process from samples of bronchoalveolar contents taken in the area of the tracheal bifurcation. Bronchoalveolar lavage was collected from sick calves using silicone sterile catheters into sterile tubes. Before sampling the contents of the bronchi, the arms and both nostrils of the calves were treated with 70° ethyl alcohol. Sampling was carried out by the same veterinarian without sedation of sick animals using disposable silicone sterile catheters, 4 mm in diameter and 150 cm in length.

The purpose of the study is to conduct intravital minimally invasive diagnostics of calves with acute catarrhal bronchopneumonia by sampling bronchoalveolar lavage in the area of the tracheal bifurcation and a detailed study of microbiocenosis from the selected contents.

3 Results and discussion

Making a final diagnosis in the fight against any infectious disease is impossible without identifying the entire spectrum of its pathogens. Therefore, to make an accurate diagnosis, it is necessary to carefully conduct microbiological studies. When conducting intravital microbiological studies of pathological material in diseases of the respiratory tract, sampling of the contents of the oral and nasal cavities is still widely used. With this method, it is impossible to reliably determine the initiating pathogens that caused the development of the purulent-inflammatory process in the lower respiratory tract.

A well-known method for selecting biomarkers of the nasal microbiome to predict the onset of respiratory diseases in cattle and their treatment was chosen as a prototype [27]. In this method, to identify the causes of development and predict the occurrence of respiratory diseases in cattle, a swab is taken from the nasal opening of a cow to measure the level of at least one biomarker from the given antigens using PCR analysis: Fusobacterium mortiferum, Prevotella stercorea, Bacteroides vulgatus, Prevotella oris, Clostridium saudiense, Lactobacillus plantarum, Bacteroides uniformis, [Clostridium] clostridioforme, Lactobacillus mucosae, Gemmiger formicilis, Prevotella copri, Terrisporobacter petrolearius, Blautia obeum, [Clostridium] scindens, Lactobacillus caviae, Ruminococcus lactaris, Catenibacterium mitsuokai, Kineothrix alysoides, Streptococcus pasteurianus, Clostridium butyricum, Lactobacillus gasseri, Holdemanella biformis, Faecalibacterium prausnitzii, Ruminococcus faecis, Fusicatenibacter saccharivorans, [Eubacterium] eligens, Butyricicoccus pullicaecorum, Blautia wexlerae, Ruminiclostridium cellobioparum, Massihprevotella massiliensis, Prevotellamassilia timonensis.

The result is then analyzed to determine whether the animal should be treated. A cow is considered to be infected and should be treated if one or more of the following differences are detected in the sample content relative to the content of a healthy cow: decreased Fusobacterium mortiferum levels, decreased Prevotella stercorea counts, decreased Bacteroides vulgatus counts, decreased Prevotella oris counts , decrease or increase in Clostridium saudiense (where a decrease indicates that the cow is likely to develop respiratory disease, and an increase indicates that the cow is already developing the disease), an increase in Lactobacillus plantarum, a decrease in Bacteroides uniformis, a decrease in [Clostridium] clostridioforme , decrease or increase in Lactobacillus mucosae (where a decrease indicates that the cow is already sick and an increase indicates that the cow is likely to develop a respiratory disease), decrease in Gemmiger formicilis, decrease in Prevotella copri, decrease in Terrisporobacter petrolearius, increase in Blautia obeum , decrease in [Clostridium] scindens, increase in Lactobacillus caviae, increase in Ruminococcus lactaris, decrease or increase in Catenibacterium mitsuokai (where a decrease indicates that the cow is sick and an increase indicates that the cow is likely to develop respiratory pathology), an increase in Kineothrix alysoides, increase in Streptococcus pasteurianus, increase in Clostridium butyricum, decrease in Lactobacillus gasseri, decrease in Holdemanella biformis, decrease in Faecalibacterium prausnitzii, decrease in Ruminococcus faecis, decrease in Fusicatenibacter saccharivorans, decrease in [Eubacterium] eligens, decrease in Butyricicoccus pullicaecorum, decrease in Blautia wexlerae, increase in Ruminiclost ridium cellobioparum, decrease Massiliprevotella massiliensis or reduction of Prevotellamassilia timonensis.

However, according to domestic researchers and our data, the development of bronchopneumonia in calves on livestock farms of the Russian Federation is caused by a

completely different spectrum of microorganisms. In addition, it must be taken into account that the PCR diagnostic method, based on determining the indicated biomarkers of the entire livestock, is quite expensive and economically ineffective. The disadvantage of this method is also that when performing PCR analysis of biomarkers selected from nasal cavity smears, it is possible to determine only the microbial landscape of the nasal cavity, and not the initiating pathogens that caused the development of a purulent-inflammatory process in the lower respiratory tract.

The technical result of the claimed invention is the development of a new safe method for intravital diagnosis of bronchopneumonia in calves by isolating microorganisms that initiate the purulent-inflammatory process from samples of bronchoalveolar contents taken in the area of the tracheal bifurcation.

Upon achieving the technical result, associations of bronchopneumonia pathogens were isolated from sick calves from samples taken in the lower respiratory tract, their species and serological identification were carried out, and their pathogenic properties were studied. The method of intravital diagnosis of bronchopneumonia in calves can be used when conducting a complex of diagnostic studies for pathologies of the respiratory tract.

The material for the study was calves aged 1-3 months, patients with acute catarrhal bronchopneumonia (n=37). Animals that were treated within 14 days prior to sampling were excluded from the study.

The invention is carried out in the following way. Pathological bronchoalveolar contents are collected from sick calves using sterile silicone medical tubes with a diameter of 4 mm (the internal diameter of the opening is 3 mm), a wall thickness of 1.0 mm and a length of 150 cm, into sterile tubes.

Before sampling the bronchoalveolar contents, the researcher’s hands and both nostrils of sick calves are treated with 70° ethyl alcohol. Sampling is carried out by the same veterinary specialist without sedation of sick animals.

The animal is fixed in a standing position in a restraining machine. The assistant, holding the calf by the nasal septum and lower jaw, stretches the animal's head and neck as cranially as possible so that the silicone tube can easily enter the trachea during the inhalation phase of the respiratory cycle. In this case, the silicone tube is carefully advanced through the larynx and trachea until the bifurcation is reached.

If a silicone probe accidentally enters the esophagus while trying to advance to the trachea, it is immediately removed and a new sterilized instrument is used to avoid contamination of the sample.

The nasogastric tube is advanced transnasally until little resistance is encountered. An indicator of reaching the tracheal bifurcation area is a repeated cough reflex. Upon reaching the tracheal bifurcation area, the nasogastric tube is moved back 1 cm, a disposable syringe with a volume of 50 ml is attached to the free end and with its help 30-40 ml of sterile isotonic saline solution (0.9% NaCl solution, 37 °C), and then immediately aspirate up to 10 ml of bronchoalveolar contents. A sample of bronchoalveolar contents selected in the described manner is delivered to the laboratory within three hours for bacteriological studies.

The possibility of carrying out intravital diagnosis of calves with bronchopneumonia using sampling in the area of the tracheal bifurcation and a detailed study of microbiocenosis from the selected contents is confirmed by the presented examples, but is not limited to them.

When conducting bacteriological studies of 37 samples of bronchoalveolar contents, selected using the presented method, 115 microorganisms of 13 species, classified into nine genera, were isolated from calves with signs of acute catarrhal bronchopneumonia. Most often, in cases of bronchopneumonia in calves, Staphylococcus aureus and Mannheimia haemolytica were isolated from pathological material, 18 (15.6%) strains each, Escherichia coli – 15 (13.1%) strains, Pasteurella multocida and Klebsiella pneumoniae 11 (9.6%) strains each, as well as Streptococcus pyogenes and Klebsiella ozaenae, 7 (6.1%) strains each, of

their total number. Streptococcus uberis, Trueperella pyogenes and Pseudomonas aeruginosa were isolated less frequently, with 6 (5.2%) cultures each, respectively; Streptococcus faecalis – 4 (3.5%); Staphylococcus intermedius and Proteus mirabilis 3 (2.6%) strains each. The majority of isolates - 71 (61.7%) were classified as gram-negative when stained using the Gram method, and 44 (38.3%) bacteria isolated from the bronchotracheal mucus of calves were classified as gram-positive microflora.

Isolates of Escherichia coli cultures isolated from samples of bronchoalveolar contents are represented by five serotypes. O8 – 5 (33.3%), O26 – 4 (26.7%) and O111 – 3 (20.0%) serotypes of the total number of strains were most often recorded. Escherichia coli serotypes O127 and O119 were identified much less frequently – 2 (13.3%) and 1 (6.7%), respectively. It should also be noted that eleven isolated Escherichia coli cultures exhibited hemolysin-producing activity.

Of the 115 strains of microorganisms that initiate bronchopneumonia in calves, the majority had pathogenic properties - 75 (65.2%) cultures. At the same time, representatives of the genera Mannheimia sp. p. – 18 (24.0%), Staphylococcus sp. p. – 15 (20.0%), Pasteurella sp. p and Escherichia sp. p. 11 (14.7%), respectively, of the total number of pathogenic microorganisms. Representatives of the genera Klebsiella sp. p. – 9 (12.0%) strains, Streptococcus sp. p. – 7 (9.3%) crops and Pseudomonas sp. p. – 4 (5.3%) isolates.

Most often, the development of acute catarrhal bronchopneumonia in calves is caused by associations of opportunistic microorganisms, which include from 2 to 5 pathogens. At the same time, associations that included three joints – 21 (56.8%) and two joints – 8 (21.6%) were isolated more often from bronchoalveolar samples. Four-component and five-component associations of microorganisms were identified much less frequently - 4 (10.8%) cases each, respectively. It was established that in all five-component associations the main pathogen was always the Mannheimia haemolytica strain: Mannheimia haemolytica + Pasteurella multocida + Pseudomonas aeruginosa + Staphylococcus aureus + Streptococcus uberis; Mannheimia haemolytica + Pasteurella multocida + Klebsiella pneumoniae + Staphylococcus aureus + Streptococcus pyogenes; Mannheimia haemolytica + Pseudomonas aeruginosa + Escherichia coli O26 + Klebsiella ozaenae + Staphylococcus aureus and Mannheimia haemolytica + Proteus mirabilis + Escherichia coli O26 + Staphylococcus aureus + Streptococcus pyogenes. In addition, Mannheimia haemolytica was a co-member of the majority - 75.0% of four-component associations. It should also be noted that only two associations were represented by gram-positive microflora, namely the two-component Staphylococcus aureus + Streptococcus uberis and the three-component Staphylococcus aureus + Streptococcus faecalis + Streptococcus pyogenes.

The method of intravital diagnosis of bronchopneumonia in calves, including sampling of washings from natural cavities for microbiological research, differs from existing methods in that a sterile silicone medical tube with a diameter of 4 mm (the internal diameter of the hole is 3 mm), a wall thickness of 1, is administered transnasally to the sick animal. .0 mm and a length of 150 cm, until slight resistance and a repeated cough reflex appear, upon reaching the tracheal bifurcation area, the nasogastric tube is moved back 1 cm, a disposable syringe with a volume of 50 ml is attached to the free end and with its help 30-40 are injected into the trachea ml of sterile isotonic saline solution (0.9% NaCl solution, 37°C), and then immediately aspirate up to 10 ml of bronchoalveolar contents.

4 Conclusion

Respiratory diseases are widespread among highly productive animals, which are most often diagnosed in young animals. These diseases lead to significant economic losses, which consist of the death of animals, loss of production from patients or those who have recovered from the disease, slowdown in their growth and development, stress, and costs of treatment

and prevention. In this regard, improving the intravital diagnosis of bronchopneumonia in calves by determining microbiocenoses from bronchoalveolar contents selected from the lower respiratory tract of sick animals is a matter of paramount importance, which requires a timely and competent solution. The invention relates to veterinary medicine and can be used in intravital diagnostics of bronchopneumonia in calves by isolating microorganisms that initiate the purulent-inflammatory process from samples of bronchoalveolar contents taken in the area of the tracheal bifurcation. Therefore, the development of a new method for intravital minimally invasive diagnosis of calves with acute catarrhal bronchopneumonia using bronchoalveolar lavage sampling for subsequent microbiological studies is relevant. A method for intravital diagnosis of bronchopneumonia in calves includes transnasal introduction to a sick animal of a sterile silicone medical tube with a diameter of 4 mm (the internal diameter of the hole is 3 mm), a wall thickness of 1.0 mm and a length of 150 cm, until slight resistance and a repeated cough reflex appear, upon reaching in the area of the tracheal bifurcation, the nasogastric tube is moved back 1 cm, a disposable syringe with a volume of 50 ml is attached to the free end, and with its help, 30-40 ml of sterile isotonic saline solution (0.9% NaCl solution, 37° C) is injected into the trachea. , and then immediately aspirate up to 10 ml of bronchoalveolar contents. This method is simple in technical execution and makes it possible to isolate the microbial landscape directly from the focus of the purulent-inflammatory process. This is a more effective and cost-effective method of sampling pathological material for bronchopneumonia in calves, when compared with existing ones.

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