



Rheological properties of nasal mucus of calves during periods of development of bronchopneumonia

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Abstract.

Determination of viscosity and adhesion of nasal discharge is considered to be promising diagnostic tests to evaluate the mucociliary system in calves in case of respiratory diseases. The aim of the study was to find out the rheological properties of sputum in the development of bronchopneumonia. In clinically healthy animals, the viscosity of the nasal exudate amounted to 1.228 ± 0.0238 mm²/s, and the adhesion – to 1.018 ± 0.0375 g/cm². At the stage of manifestation of bronchopneumonia, the rheological properties of nasal secretion change with an increase in its viscosity and adhesion.

Key words: bronchopneumonia, mucociliary system, nasal discharge.

INTRODUCTION.

Mucociliary clearance is an important primary innate mechanism that protects the lungs from harmful substances contained in inhaled air [1]. In young animals, this protection may be impaired by respiratory infections, inhalation of toxic substances and allergens [2,3] or as a consequence of perinatal pathology [4]. With an increase in the functional load on the respiratory organs or the development of their pathology, the parameters of lung ventilation change [5], ciliated epithelial structure and the rate of mucociliary clearance. There is no doubt that the relevance of respiratory diseases in calves will increase in the coming years, therefore, it is necessary to search for new approaches to solving this problem. The aim of the study was to find out the rheological properties of sputum of development of bronchopneumonia in calves.

MATERIALS AND METHODS.

The research was conducted in the production laboratory of Veterinary Research Institute at the industrial complex for fattening young cattle. The object of the study were 440 clinically healthy calves of Holstein breed at the age of 4-5 months, which were placed in group stalls for 12-15 heads, where they got complete feeding. The laboratory air temperature was 17-20°C, relative humidity-62-66% and air velocity-0.10-0.18 m/s. During the first two weeks of the experiment 330 animals remained clinically healthy, but 110 calves developed symptoms of respiratory pathology. In 98 sick animals, bronchopneumonia was diagnosed, while in 5 animals it was in severe form, in 85-there was an average form and in 8 – a mild degree of the severity of the disease. Two groups were formed: I-clinically healthy (control, n=35), II - animals with moderate bronchopneumonia (n=85).

All sick animals at the stage of the manifestation of bronchopneumonia were appointed a course of treatment, which included intravenous injection of solutions of calcium chloride and glucose (1 and 3 days), intramuscular injections of multivitamin preparation "Tetravitam" (1 and 7 day) and antibiotics, selected on the basis of the evaluation of the sensitivity of pathogens (gentamicin sulfate, 1-7 days), by in addition, on 2 and 4.

At the stage of progression of the disease (prodromal period), a biomaterial was collected daily in the period from the appearance

of the first symptoms of the diseases of the respiratory organs until the formation of a specific complex of signs of pneumonia. Macroscopic, microscopic, and rheological properties of nasal secretion were evaluated. The secretion was selected with the help of swabs used. They were injected into the nasal cavity to a depth of 3-5 cm. The studies were carried out within 6 hours after the selection of the biomaterial, but before that it was contained for 1 hour in a thermal chamber at a temperature of 20°C. To determine the kinematic viscosity (Shabunin *et al.*, 2017).

The statistical analyses were performed using the SPSS version 22 (IBM Corp, Version 22.0, Armonk, NY, USA, 2013) statistical package.

RESULTS AND DISCUSSION.

During the experiment the indices of the calves from group I corresponded to the parameters of healthy animals: body temperature (BT) – $38.8 \pm 0.25^\circ\text{C}$ (range from 38.4 to 39.3°C), respiration rate (RR) – $18.5 \pm 1.03/\text{min}$ (from 16.0 to 20.0/min), heart rate (HR) - $78.0 \pm 3.17/\text{min}$ (from 72 to 84/min). In the auscultation of the chest in the projection of the lungs vesicular breathing was audited, which was the loudest in the first three intercostal spaces. Rheological parameters of nasal exudate were within the reference range: viscosity from 1.180 to 1.275 m²/s*10⁻⁶, adhesion from 0.98 to 1.060 g / cm². The exception were 5 calves, which from 95 to 102 days of the experiment have shown signs of pathology of the respiratory system: shortness of breath being ($21.9 \pm 0.40/\text{min}$), the allocation of serous nasal secretions and increased vesicular breathing.

In the calves from group II (n=85) on the first day of the experiment the parameters corresponded to the parameters of healthy animals: BT – $38.7 \pm 0.20^\circ\text{C}$ (range from 38.4 to 39.2°C), RR – $18.5 \pm 0.97/\text{min}$ (from 16.0 to 19.0/min) and HR – $78.0 \pm 2.50/\text{min}$ (from 74 to 82/min) were studied. During auscultation of the chest, vesicular breathing was heard, which was the loudest in the first three intercostal spaces. The rheological parameters of the nasal exudate were within the reference range [6]. The first symptoms of respiratory lesion appeared on the 5-7 day of the experiment in the form of moderate respiratory acceleration ($22.0 \pm 1.25 / \text{min}$) and pulse ($90.0 \pm 2.50/\text{min}$), as well as the increased vesicular respiration and liquid transparent nasal secretion, the viscosity of which decreased by 5%, and adhesion – by 4% (Table).

Table: Rheological parameters of nasal secretion in healthy and sick calves (Mean±).

Indicators	Healthy (group I)	Prodromal period of the disease (group II)		
		1 day	2-3 days	4-5 days
Viscosity, $\text{m}^2/\text{c} \cdot 10^{-6}$	1.210±0.006	1.150±0.013	1.293±0.010	1.315±0.008*
Adhesion, r/cm^2	1.020±0.007	0.98±0.011	1.080±0.014	1.234±0.005*

P≤0.05, ** - P≤0.01, *** - P≤0.001, compared to clinically healthy animals (group I).

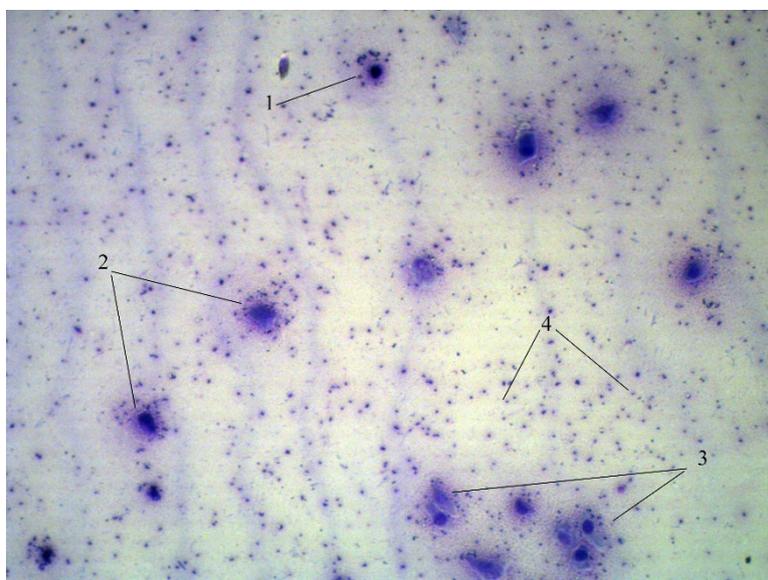


Figure - Bronchopneumonia, the height of the disease. Tracheobronchial exudate, Romanovsky-Giemsa staining (x 160): 1-lymphocyte, 2-polymorphonuclear leukocytes, 3-epithelial cells, 4-gram-positive and gram-negative bacteria

On 2-3 days of the disease (6-8 days of the experiment) there was an increase in RR ($24.8 \pm 1.50/\text{min}$) and HR ($102.0 \pm 2.80/\text{min}$). During auscultation of the chest, rigid vesicular breathing was heard, but in the upper and middle part of the projection of the lungs there were areas of vibration to the sound – the initial stage of formation of the rales. Mucosa of sputum, viscosity, which was higher than in healthy calves by 6.9%, and adhesion by 5.9%, was released from the nasal openings. Microscopy of sputum smears showed an increase in the number of microflora and neutrophils. These symptoms gave rise to a preliminary diagnosis – bronchitis. On the 4-5 day of the disease there was a mixed shortness of breath, productive (wet) cough, which in 22 (25.9%) calves was muffled and short, but in ones 63 (74.1%) – loud paroxysmal. During auscultation of the thorax, wet rales of different sizes were heard. Mucopurulent sputum with a volume fraction of pus from 20 to 45% was isolated. Its viscosity was higher than in healthy animals by 8.7% and adhesion by 21% .

Microscopy of sputum smears showed the presence of a large number of microflorae, lymphocytes and neutrophils. Basophils, alveolar macrophages and epithelial cells were always present, but in a smaller number (Figure).

Bacteriological studies of sputum showed the presence of strains of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, sensitive to gentamicin. Based on the KB lab test (Kirby-Bauer antibiotic testing), the larger zone of inhibition around the *S. aureus* (diameter of the zone of 21.5), *E. coli* (23) and *K. pneumoniae* (26) is gentamicin which indicates that these bacteria are more sensitive to the gentamicin. As a result, the final diagnosis was made - "Bronchopneumonia unspecified" (ICD-10: J18.0) of moderate severity of the course.

The results confirm the previously established reference interval of clinically healthy calves: nasal secretion viscosity-1.180-1.275 ($\text{m}^2/\text{s} \cdot 10^{-6}$ (cSt, mm^2/s), adhesion – 0.98-1.060 g/cm^2 (96,1-104,0 PA).

The development of the inflammatory process in the respiratory tract is accompanied by pathophysiological changes in the secretory elements of the mucous membrane and the mechanisms of sputum formation [7]. At the beginning of the prodromal period, serous secretion with the reduced viscosity and adhesion is found, which is caused by the activation of serous submucosal glands [8] and is a manifestation of the protective mechanism aimed at the removal of a pathological agent. In the future, there are structural changes in the mucous membrane with an increase in the number of goblet cells. In the nasal secret, the proportion of mucosal sputum increases, in which the content of neutral Mucins is increased, but acidic – reduced, which causes an increase in the volume of the gel with its predominance over the sol [9]. The result is the increased viscosity and to a lesser extent, the adhesion of the secret of the respiratory tract, there is sticking of the cilia of ciliated epithelium, formation of mucous conglomerates that impedes the movement of air [10]. Structural changes in the epithelium and bronchial secretion, resulting in local obstructive phenomena and pathological respiratory noise form a set of specific symptoms that allow to diagnose bronchopneumonia. Like inflammation, the formation of pus is a protective reaction of the body to the invasion of bacterial pathogens. With the increasing number of pus, sputum transforms from mucous to Muco-purulent, with higher adhesion. Therefore, at the final stage of the prodromal period, the adhesive properties of the nasal secretion increase to a greater extent than the viscosity, which is also facilitated by the activation of proteolytic enzymes of leukocytes and microbes [11].

CONCLUSION.

In the prodromal period of bronchopneumonia, the rheological properties of the nasal secretion change with an increase in its viscosity and adhesion, which reduces the effectiveness of mucus clearance and creates favorable conditions for the manifestation of

the disease. After the treatment even though the symptoms of the underlying disease (bronchopneumonia) disappeared, residual pathological changes in the respiratory system remain, the severity of which determines the effectiveness of the therapy and the risk of re-disease. The data presented in this paper are part of the results of the studies conducted in a fundamentally new direction of veterinary medicine - the study of the residual pathological phenomena after the disease, their clinical significance, methods of predicting and correcting the outcome.

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