Application of electro reflexotherapy in bronchopneumonia of calves against background of necrobacteriosis management

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Abstract. One of the main reasons for the high incidence of young cattle of bronchopneumonia is the low level of the immunological response due to the imperfect immune system of the young organism, as well as the ill-being of livestock farming with one or a number of diseases caused by pathogens related to opportunistic and/or stationary microflora. To obtain a positive result of the treatment of bronchopneumonia, it is necessary to provide the first line of nonspecific protection of the calves’ body, as well as reducing the effects of the infectious and inflammatory stressor on the sick organism. The object of the study was young cattle with florid clinical signs of catarrhal bronchopneumonia. Three groups were formed from the selected calves: control and two experienced. In the control, a generally accepted treatment regimen for bronchopneumonia was used, and in the experiment, electroreflexotherapy was additionally used. Combination treatment of catarrhal bronchopneumonia of calves with the use of electroreflexotherapy contributes to: activation of the inflammatory process, stimulation of specific and non-specific immunobiological surveillance, providing direct and indirect bacteriostatic and bactericidal effects and has some antiadrenergic effect.

1. Introduction
Bronchopneumonia, in conditions of industrial farming, are a very common pathology, the contributing factors of which are disorders in the nature and mode of feeding, housing conditions. Animals with imperfect immunity are mostly susceptible to this disease – young animals, as well as weakened animals. An important role in the development of the inflammatory process of the respiratory system is played by associations of purulent-necrotic microorganisms belonging to the opportunistic pathogenic microflora: Clostridium perfringens type A, Staphylococcus aureus, Corynebacterium pyogenes, Proteus, Pseudomonas, Streptococcus, including Fusobacterium necrophorum [1-3].

Fusobacterium necrophorum, according to statistics, constantly lives in 50-100% of cows and is a symbiont of the gastrointestinal tract and can cause a disease such as necrobacteriosis. It is worth noting that this disease is stationary in nature and is concomitant for livestock farms. The pathogen is released into the environment from the body of sick animals with feces, urine, purulent contents of necrotic lesions of the skin and hooves, and uterine discharges [4-6]. A particular danger is that during the life of the bacteria toxins causing diphtheria inflammation in tissues and organs are produced, therewith the main virulence factor of Fusobacterium necrophorum is a high molecular weight protein - leukotoxin, which has a toxic effect on white blood cells, that significantly reduces the immune system of adult and young animals [7-8]. In addition, it should be emphasized that the above
mentioned associations of microorganisms lead to the development of persistent infections and chronic inflammation, implying a long-term use of antibacterial agents, which cannot but affect the macroorganism. Often, the prolonged use of antibiotics and/or overestimated doses comes out in toxic reactions, namely, neurotoxic, hepatotoxic, nephrotoxic, etc. [9].

In connection with the foregoing, we conclude that for the effective treatment of bronchopneumonia in calves, it is necessary to normalize the key parameters of innate immunity, providing the first line of nonspecific defense of the body.

To increase the natural resistance and correct the immunological alterations, various methods are used, one of which is acupuncture, which is often used in complex treatment and prophylactic measures, and other types of effects on biologically active points [10].

2. Methods and materials

The aim of this work was to study the effect of electroreflexotherapy in bronchopneumonia of calves on the background of necrobacillosis on the farm.

Research was conducted within the same farm of the Amur region. The selected animals belonged to the same sex and age group and were accordingly kept under similar conditions. So, three equivalent groups of calves of Holstein breed were formed, aged 30-45 days with a florid clinical picture of catarrhal bronchopneumonia: control and experimental (experience 1 and experience 2), 12 animals each.

Assessment of clinical status, measurement of skin energy potential in the area of biologically active points (BAP), analysis of hematological, biochemical and immunochemical blood parameters of calves of the control and experimental groups was performed at the beginning and end of the study.

To study the morphological composition of the blood of calves, stabilized samples were used by adding an anticoagulant (heparin) to the tube, and biochemical and immunochemical parameters were studied in blood serum. Blood hematological and immunochemical parameters were determined using generally accepted methods in veterinary practice (counting the number of erythrocytes and leukocytes was carried out using a Goryaev camera; the level of hemoglobin was determined on a biochemical photometer Stat Fax 1904 + using the ‘Vital’ reagent (manufactured by ‘Vital Development’, Russia, 2013); differential counting of leukocytes was carried out visually in dry fixed stained blood smears (staining of blood smears by Pappenheim's method); a turbidimetric method was used to determine immunoglobulins in blood serum), and biochemical studies were performed using SPINREACT diagnostic kits (SANT ESTEVE DE BAS (GIRONA), Spain, 2013) on a Stat Fax 1904+ biochemical photometer (Awareness Technology Inc., USA, 2012). The data obtained were analyzed, mathematically processed and compared with generally accepted normative indicators [2, 11-12].

The animals of the control group received drug treatment according to the developed scheme on the farm, and experimental calves additionally received electroreflexotherapy using the dynamic electrostimulation apparatus (Limited Liability Company ‘Regional center for adaptive reception therapy’, Ekaterinburg, Russia, 2011) with a hybrid frequency (10 and 77 Hz) for five minutes once a day (in the morning) for seven days (table 1).

For the diagnosis and treatment of bronchopneumonia, BAPs projected onto the pectoral muscles, arteries, veins and nerves were used, namely, BAPs of the skin of the lateral walls of the chest [13], as well of the sternal and costal zones, localized symmetrically towards the sagittal line, on either side in the intercostal spaces (except for the BAP located on the ventral sagittal line in front of the manubrium of the sternum) [14].

The digital material available from the research was statistically processed using the computer program Statistica 6.0 (USA) and presented as arithmetic mean and its error (M±m). The confidence level was determined in relation to the background (by the first day of research) using the parametric Student’s t-test. The result was considered reliable at p<0.05.
Table 1. Research scheme.

|                         | Control          | Experience 1 (E1)                  | Experience 2 (E2)                  |
|-------------------------|------------------|------------------------------------|------------------------------------|
| **Diagnostic measures** | – Assessment of   | – Laboratory blood tests (hematological, biochemical, immunochemical) |                                    |
| before and after the    | clinical status  |                                    |                                    |
| experiment              |                  |                                    |                                    |
| **Drug treatment**      | – 0.9% solution  | – 40% solution for injection ‘Glucose’ |                                    |
| (according to the       | for injection  ‘NaCl’ | – 20% injection for calcium        |                                    |
| instructions for the    |                  | borgluconate injection             |                                    |
| use of drugs)           |                  | – Solution for injection ‘Ciprovet 5%’ |                                    |
| **Non drug treatment**  | Not applied      | Through BAPs of the skin of the    | Through BAPs of the skin of the    |
| (electroreflexotherapy) |                  | lateral walls of the chest         | sternum and costal areas of the    |
|                         |                  | [13]                               | chest [14]                         |

3. Results and discussion

The results of background studies of calves in the control and experimental groups showed a high tension of the body as a result of the inflammatory process, which was indicated by increased values of the skin electric potential in the BAP of the lateral walls of the chest, area of the sternum and costal areas (measuring level limit: experience 1 – from 83.8±1.51 to 87.9±1.11 conventional units; experience 2 – from 84.6±1.53 to 88.8±1.12 conventional units), as well as a high content of leukocytes (from 15.5±0.71 to 15.8±0.87 10³/l) and alpha – globulins ranging from 9.8±0.82 to 10.5±1.57 g/l, which corresponded to the upper limit of the norm. At the same time, an insufficient level of humoral immunity was recorded in all the animals studied, which was confirmed by the content of segmented neutrophils at the lower limit of normal (from 27.0±1.35 to 28.0±1.40%) and a reduced content of IgA immunoglobulins (from 1.6±0.35 to 2.3±0.13 mg/ml) and IgG (from 1.7±0.03 to 2.5±0.24 mg/ml).

In addition, it should be noted that the inflammatory process proceeded with the phenomena of hypoxia and anemia (a high content of red blood cells – from 9.9±0.37 to 10.0±0.37 10¹²/l on the background of a low hemoglobin index – from 100.7±1.50 to 103.7±1.00%), which is typical for respiratory diseases. At the same time, a high level of tension in the body could also be associated with the presence of a stationary infection, namely, the causative agent of necrobacteriosis, as evidenced by high IgM values (from 5.1±0.15 to 6.1±0.22 mg/ml), the growth of which often noted in response to the presence of an infectious factor as a result of disease and / or immunization of animals [15]. So, as a result of a bacteriological study conducted on the farm, it was found that bacteria sensitive to ciprofloxacin and tylosin: *Staphylococcus epidermidis, Proteus mirabilis*, including *Fusobacterium necrophorum* was egested in 34% of the livestock [13]

Considering the fact that any inflammatory process is a strong stressor for the body, which leads to overexcitation of the sympathetic nervous system and activation of RAS (renin-angiotensin system), an increase in the secretion of catecholamines, which block the glycogen synthetase enzyme, which contribute to an increase in the level of glucose in blood serum (from 4.4±0.18 to 5.1±0.28 mmol/l), due to an increase in the mobilization of glucose from glycogen and a decrease in its deposition [16]. Along with this, the activity of the lactate dehydrogenase (LDH) glycolytic enzyme is reduced, apparently due to a shift in the reaction of conversion of lactate to pyruvate towards the formation of lactate during the Krebs cycle [17], which may be caused by insufficient free oxygen, which is typical for diseases accompanied by impaired perfusion and microcirculation of the respiratory organs, by luminal occlusion and the presence of tissue edema. Another confounding factor in the accumulation of lactate may be a low degree of its utilization in the liver when its functions are impaired, as evidenced by high rates of bilirubin (from 7.0±1.43 to 8.2±1.23 μmol/l) and transferases (aspartate aminotransferase (AST) – from 110.0±2.52 to 113.0±2.10 and alanine aminotransferases (ALT) –
from 41.7±1.45 to 42.7±0.85 U/L) on a background of reduced levels of total protein (from 43.7±2.03 to 49.0±0.70 g/l) and albumin fractions (from 11.4±0.78 to 13.3±0.29 g/l) (figure 1-3).

**Figure 1.** Blood indicators of calves, control (C), n = 12.

**Figure 2.** Blood parameters of calves, experiment 1 (E1), n = 12.

**Figure 3.** Blood indicators of calves, experiment 2 (E2), n = 12.
Thus, the pre-experimental studies showed the presence of a causative agent of necrobacteriosis in the farm in 34% of cases, while catarrhal bronchopneumonia was recorded in calves on a background of low immunity, which was confirmed by the presence of characteristic clinical signs, the results of hematological, biochemical and immunochemical blood tests.

After the treatment measures with the use of electroreflexotherapy, an increase in the electric potential of the skin in the BAP of the lateral walls of the chest, of the sternum and costal areas (E1) from 91.8±1.35 to 95.6±0.56 srv. units; E2 – from 92.7±1.40 to 96.6±0.60 srv. units) and the level of alpha - globulins up to 16.9±1.36 g/l (E1, p<0.001) and 16.9±1.27 g/l (E2, p<0.001), were recorded in experimental calves, which indicated an exacerbation of the inflammatory process. At the same time, an increase in body temperature was noted in animals, which could promote an activation of gas exchange in the lungs and potentiation of specific and non-specific factors of the immunobiological surveillance system, providing direct and indirect bacteriostatic and bactericidal effects [1]. Thus, the transition of the inflammatory process to the acute phase ensured the secretion of lipopolysaccharide, thereby increasing the production of neutrophils and stimulating innate humoral immunity [18], as indicated by a 29 (E2, p<0.001) – 32% (E1, p<0.001) increase in segmented neutrophils. The growth of IgA immunoglobulins by 1.5 times (E1, p<0.01; E2 p<0.05) and SIgA by 29 (E2, p<0.05) - 35% (E1, p<0.001) indicated the stimulation of local phagocytic defense, which is expressed in the obstacle to adhesion of microorganisms on the epithelial cells of the mucous membranes of the respiratory organs, as well as the intestines and urinary tract [15, 19]. A significant decrease (E1 – by 80%, p<0.001; E2 - by 64%, p<0.001) of IgG immunoglobulin indicated that the infectious component was still present at the time of the study [20].

A slight increase in LDH activity (by 5% in E1, p<0.05; by 7% in E2, p<0.01) on the background of a decrease in glucose level (by 35-43%, p<0.001) indirectly indicated a decrease in the hypertonicity of the sympathetic part of the autonomic nervous system, which could develop under the influence of a stressor, as well as normalization of gluconeogenesis, restoration of the level of metabolism and liver function, as evidenced by a decrease in bilirubin level by 43-46% (E1, p<0.001), AST – by 15-17% (E2-E1, p<0.001) and ALT – by 13-14% (E2-E1, p<0.001).

The results of studies conducted in the control group after applying the treatment regimen generally accepted on the farm were statistically unreliable and were not significant. A comparative analysis of the dynamics of the clinical condition and blood picture in experimental calves with bronchopneumonia did not show significant differences when using electroreflexotherapy using biologically active skin points in different areas of the chest, which is explained by an increase in the diameter of the BAP and the formation of extensive trigger zones.

As a result of studies and analysis of the results obtained, it was found that the complex treatment of catarrhal bronchopneumonia of calves with the use of electroreflexotherapy in the area of biologically active points of the skin of the lateral walls of the chest, of the sternum and costal areas using the DENS apparatus helps to activate a indolent inflammatory process, stimulate local humoral immunity and has an anti-adrenergic effect.

4. Conclusions
The complex treatment of catarrhal bronchopneumonia of calves using electroreflexotherapy in the field of biologically active points of the skin of the lateral walls of the chest, sternum and costal areas using a dynamic electrical stimulation apparatus with hybrid frequencies (10 and 77 Hz) contributes to: activation of the inflammatory process, which was confirmed by a 1.7 times increase in alpha globulins and an increase in body temperature; stimulation of specific and non-specific immunobiological surveillance, providing direct and indirect bacteriostatic and bactericidal effects, which is reflected in an increase in the percentage of segmented neutrophils by 29-32%, IgA by 1.5 times and SIgA by 28-35%. An important point is that electroreflexotherapy has an anti-adrenergic effect, thereby, positively impacts on the body of a sick calf with catarrhal bronchopneumonia in a necrobacteriosis ill-beinng farm, reducing negative consequences caused by the inflammatory and infectious components by restoring microcirculation and perfusion of the respiratory system and
metabolic processes in the liver, which was manifested in the normalization of such blood parameters as bilirubin level (decreased by 43-46%) and transferases (AST decreased by 15-17% and ALT - by 13-14%), LDH (increased by 5-7%) and glucose (decreased by 35-43%).

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