Etiology and diagnosis of the gastrointestinal and respiratory diseases of calves in the farms of the Vologda region

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Abstract. The article reflects the statistical, bacteriological, virological and molecular genetic research results. In 2019, the proportion of gastrointestinal pathologies in the total incidence of young animals was 37.6 ± 0.7%. On average, over the past five years, 46.6 ± 0.7% of born calves have been ill. Gastrointestinal diseases are recorded mainly in young animals under the age of 3 months. Of the number of cases, 43.3% die from birth to 10 days, 31.7% from 11 to 30 days, and 15.8% of calves from one to three months. The proportion of infectious diseases is 0.36% of the total incidence of animals. 152 studies were carried out, including: 39 fecal specimen by ELISA, 30 molecular genetic (PCR), bacteriological - 83 samples of biomaterial from dead and sick animals. 107 microorganism cultures have been identified. According to the ELISA, the most frequently detected antigens were causative agents of coronavirus - 33.3%, rotavirus - 30.8%, the causative agent of viral diarrhea - in 15.4% of cases. In 96.8% of cases, the causative agents of viral infections were identified in the associations: coronavirus and rotavirus (43.3%), coronavirus, rotavirus and viral diarrhea (36.7%), coronavirus and viral diarrhea (20.0%). In 22.2% of the samples, PCR was used to detect the herpes virus type 5 gene in rinses from calves’s nasal cavity. As a result of bacteriological studies, a change in the spectrum of cultures of microorganisms isolated from pathological material was noted. The percentage of unidentified crops decreased by 2.6 times. Given the results, we propose to include in the scheme of diagnostic studies for mixed infections additional studies: feces for viral infections by ELISA; crops of primary pathological material and feces should be carried out additionally on accumulation media; PCR analysis of swabs from the nasal cavity, blood; during bacteriological examination, consider the allocation of microorganisms: Pseudomonas, Streptococcus, Staphylococcus.

1. Introduction
Among the diseases of farm animals that are ubiquitous and cause enormous economic damage to livestock farms, the main ones are gastrointestinal and respiratory diseases of young animals, accompanied by high mortality [1,2,3,5]. Official statistics, most diseases of the digestive system in young farm animals refers to non-infectious etiology. But numerous studies have found that in the vast majority of cases, these diseases are infectious in nature and proceed as mixed infections [12,13,15].

The wide spread of mixed infections caused by various associations of microorganisms makes special demands on diagnostic studies - the diagnostic scheme should be based on the results of a comprehensive study [14]. Since several genera of the Enterobacteriaceae family and other types of microorganisms, as well as the rota and corona viruses, simultaneously take part in the development of diseases, the results of laboratory studies are crucial in determining the nosological profile of diseases [4,9,11].
A wide range of pathogens, rapid coverage of livestock disease, lack of effectiveness of preventive and therapeutic measures [6,7] indicate the need to develop an optimal diagnostic scheme for mixed infections of young cattle using express research methods with a high degree of sensitivity and specificity.

2. Materials and methods
Epizootological, bacteriological, virological, molecular genetic and statistical research methods were used in the work. 152 studies were carried out, including: 39 fecal specimen using the ELISA method (Rodicor-IFA-TEST VIEV) for the detection of viral antigens, 30 molecular genetic (PCR) K-Sorb (production Synthol, Russia), bacteriological method - 83 samples of biomaterial from dead and sick animals. 107 cultures of microorganisms were identified in accordance with the “Guidelines for the bacteriological diagnosis of mixed intestinal infection of young animals caused by pathogenic enterobacteria” (approved by the Department of Veterinary Medicine of the Ministry of Agriculture and Food of the Russian Federation, 1999).

3. Research objective
Improving the diagnostic scheme for mixed diseases of young cattle.

4. Research results
Gastrointestinal and respiratory diseases of young animals are the most acute problem of modern animal husbandry in the Vologda Oblast [8, 10]. The spread of mixed infections makes special demands on laboratory diagnostic methods, which are often crucial in establishing the main etiological factors of infectious diseases.

Analysis of statistical data showed that the incidence and mortality of young animals in the farms of the Vologda Oblast remain high. So, in 2019, the proportion of gastrointestinal pathologies in the total incidence of young animals was 37.6 ± 0.7%. This indicator is quite stable, the coefficient of variation is insignificant (Cv = 4.2%). On average, over the past five years, 46.6 ± 0.7% of born calves have been ill.

Gastrointestinal diseases are recorded mainly in young animals under the age of 3 months. Of the number of cases, 43.3% die from birth to 10 days, 31.7% from 11 to 30 days, and 15.8% of calves from one to three months. The method of correlation analysis revealed a direct strong relationship between the incidence and mortality of young animals (h = 0.74, P > 0.99), which indicates a high pathogenicity of etiologically significant microorganisms in the disease.

In the Vologda Oblast, the following infectious diseases of young animals are currently officially registered: Escherichiosis (coli bacteriosis) and malignant edema, but their specific gravity, according to official data, is not elvik - 0.36% of the total incidence of animals. In our opinion, the majority of diseases of young animals were classified as non-infectious diseases by official veterinary statistics.

| Name               | 2014 | 2015 | 2016 | 2017 | 2018 |
|--------------------|------|------|------|------|------|
| **Colibacillosis** |      |      |      |      |      |
| Dysfunctional Items| 51   | 55   | 51   | 41   | 45   |
| Sick               | 124  | 106  | 112  | 72   | 80   |
| Пало               | 73   | 75   | 64   | 61   | 57   |
| Vaccinated         | 53,219 | 51,861 | 57,884 | 62,667 | 58,994 |
| **Malignant edema**|      |      |      |      |      |
| Dysfunctional items| 36   | 40   | 34   | 36   | 55   |
| Sick               | 46   | 52   | 43   | 49   | 60   |
| Died               | 46   | 52   | 43   | 43   | 60   |
| Vaccinated         | 11,505 | 10,477 | 16,867 | 37,649 | 47,251 |
From the data presented in table 1, it is clear that during the analyzed period, 494 young animals suffered from colibacillosis, 330 animals fell, or 66.8% of the number of sick calves. Similar indicators for malignant edema amounted to 250 goals and 244 heads, or 97.6%, respectively, which indicates a high mortality rate among calves.

Figure 1 shows the focal rates for colibacteriosis and malignant edema of calves in the farms of the Vologda Oblast for 2014-2018.

![Figure 1. Coefficient of foci for colibacteriosis and malignant edema of young cattle](image)

In 2018, the focal rate for colibacteriosis decreased by 1.4 times compared with 2014, which indicates the effectiveness of preventive measures. For malignant edema in 2018, the focal rate also slightly decreased compared to 2017. The data obtained indicate that the causative agent of malignant edema of animals, which was previously considered a saprophytic inhabitant of the intestine, was not harmless for farm animals, capable of causing food intoxication - enterotoxemia under favorable conditions.

The viral agents that cause gastrointestinal and respiratory tract diseases in calves include pathogens: rota-, corona-, pestivirus, herpesvirus and others. It should be noted that studies on these diseases that are epizootically significant for young animals are not conducted in the region or are carried out extremely rarely.

The results of our studies of biomaterial for the presence of viral antigens are shown in table 2.

**Table 2. Results of virological examination of the material by ELISA and PCR methods.**

| Indicators           | Detection of viral antigens in feces, (ELISA) | PCR         |
|----------------------|---------------------------------------------|-------------|
|                      | Coronavirus                   | Rotavirus   | Pestivirus (VD-BS) | Viral associations |
| Samples investigated | 39                            | 39          | 39                   | 39              |
| Positive samples, %  | 33.3                          | 30.8        | 15.4                 | 96.8            |

According to the results of ELISA, antigens of the causative agents of coronavirus were most often detected - in 33.3% of the studied samples, rotavirus - in 30.8%. The causative agent of viral diarrhea, a disease of the mucous membrane of cattle, was found in calves in 15.4% of cases. In 96.8% of cases, the causative agents of viral infections were identified in the associations: coronavirus and rotavirus (43.3%), coronavirus, rotavirus and viral diarrhea (36.7%), coronavirus and viral diarrhea (20.0%).
Studies indicate a relationship between diseases of calves with diarrhea and the detection of antigens of rota-, corona- and pestivirus in them. In one of the farms examined by PCR, a genome of type 5 herpes virus was found in swabs from calves' nasal cavities in 22.2% of samples.

Of the many laboratory diagnostic methods, bacteriological, based on the indication of pathogens from the animal organism and the environment, is of the greatest importance.

The results of bacteriological studies of pathological material taken from sick and dead calves in 2016 and 2019 are presented in figure 2.

![Figure 2. Spectrum of microorganisms isolated from the fallen young cattle.](image)

From the data presented in the figure, it can be seen that in 2016 20.6% of the selected cultures were E. coli microorganisms, then by 2019 their share decreased to 0.9%. And also the percentage of unidentified crops decreased from 26.9% to 10.2%. Isolation of cultures of the genus Salmonella was almost at the same level of 1.3-1.8%.

At the same time, an increase in the number of conditionally pathogenic cultures such as: Citrobakter - from 1.5% to 10.0%, Proteus - from 3.0% to 14.0%, Pseudomonas - from 0.8% to 7.5%, Streptococcus from 8.4% to 13.8%, the presence of which in the pathological material during diagnosis is not taken into account by practical doctors, therefore, it requires determination of their role in the etiology of gastrointestinal diseases of young animals.

Under these conditions cultures of the genus Pasteurella increased from 3.3% to 5.3%, as well as the genus Clostridium from 13.1% to 20.8%. However, this does not affect either the structure or the growth of the share of infectious diseases in the structure of morbidity.

Cases of the release of Klebsiella, Yersinia, and others also began to be recorded. We isolated Y. enterocolitica, pathogenicity, which was proved by a positive reaction of agglutination (RA) with serum of virulent yersinia (SVI) and in a biological test on white mice. Currently, these species are recognized as capable of causing pathology of the gastrointestinal and respiratory tract in young animals both
independently and complicate the course of nutritional diseases, such as dyspepsia, colostrum toxicosis, rickets, and also form associations with pathogenic species. The use of accumulation media for the initial seeding of pathological material ensured the isolation of these microorganisms.

Thus, as our studies have shown, recently there has been a change in the spectrum of cultures of microorganisms isolated from pathological material. The percentage of unidentified crops decreased by 2.6 times. From sick and dead animals, infectious agents are released more often than is reflected in the statistical reporting. As a rule, mixed bacterial flora and viral-bacterial associations are distinguished.

Considering the results of our studies, we propose to include in the scheme of diagnostic studies for mixed infections:

- study of feces for viral infections by enzyme immunoassay. This method during the day will allow us to establish the presence of pathogens of viral diarrhea, rota-, corona-virus enteritis in sick animals;
- sow of primary pathological material and feces in addition to accumulation media (peptone-potassium and selenite) to increase the excretion of Salmonella and Yersinia;
- PCR study of swabs from the nasal cavity, blood.
- during bacteriological examination, in order to obtain more reliable diagnostic results, the allocation of microorganisms should be taken into account: Pseudomonas, Streptococcus, Staphylococcus, since they can cause a mixed infection.

5. Conclusion
Gastrointestinal and respiratory diseases of young animals in all examined households proceeded mainly in the form of mixed infections. Their complex etiological structure necessitates an integrated approach to the diagnosis and rehabilitation of dysfunctional farms. The data obtained must be taken into account when carrying out preventive measures in livestock farms in the region to prevent the occurrence of bacterial and viral diseases in calves.

The additions that we propose for inclusion in the standard scheme for conducting research on mixed infections of young cattle have revealed a wider range of microorganisms, reduced the percentage of unidentified crops by 2.6 times - from 26.0% to 10.2%, more fully evaluate their role in the occurrence of gastrointestinal diseases of calves.

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