Molecular-genetic analysis of bovine herpesvirus-5 (BoHV-5) in the milk of cows in farms of Vologda region

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Abstract. The results of molecular-genetic analyses of milk sample are presented in this article. The experimental studies were carried out in the dairy cattle of livestock farms of the Vologda region suspected to the respiratory infections. Samples of milk were obtained from the cows with the following clinical signs: cough and mucosal discharges from the nasal cavity. The laboratory studies were conducted in the virology laboratory of Federal scientific center-all-Russian research Institute of experimental veterinary medicine. Polymerase chain reaction and Reverse transcription polymerase chain reaction were used for the detection of the genome of the viruses. The samples were tested for the presence of following viral infections: bovine herpesvirus, bovine viral diarrhea virus – mucosal disease, parainfluenza-3 virus and coronavirus. A comparative analysis of the nucleotide sequences of the studied virus and the reference strains of the GenBank database showed the similarity of the detected virus in cow milk of the Vologda region with the Bovine herpesvirus-5 (BoHV-5). During the differential diagnostics, the coronavirus, bovine viral diarrhea virus and parainfluenza-3 virus were excluded. Thus, as a result of the studies, Bovine herpesvirus-5 (BoHV-5) was detected for the first time in the milk of cows of livestock farms of the Vologda region.

1. Introduction
Today, one of the relevant problems of livestock is respiratory diseases of cattle of viral etiology as infectious bovine rhinotracheitis, parainfluenza-3, bovine viral diarrhea, bovine coronavirus which causes significant economic damages [1]. Respiratory diseases in young cattle predominate over all other diseases in terms of prevalence, mortality, and forced slaughter [9]-[10]. In some farms, the death of es in combination with forced slaughter reaches 40-55% [8]. Despite the constant updating and expansion of information on viral respiratory diseases of animals, these infections continue to cause a serious problem to livestock worldwide. Respiratory diseases of calves are a group of diseases emerging by a wide range of infectious, genetic and sanitary factors. The causative agents of these diseases can affect the respiratory tract and lymphoid system independently or in associations between themselves [3]-[6]. The laboratory methods as PCR (polymerase chain reaction) which can detect the pathogen in the samples play a special role in the differentiation diagnosis.
In recent years, herpesviruses have become increasingly important in infectious pathology of cattle. Among viral infections, herpesvirus takes one of the leading places due to the ubiquitous distribution among humans and animals, the variety of clinical manifestations, the chronic course, as well as various transmission routes. Herpesviruses are united in the extended family of Herpesviridae [5]-[10-13]. According to the literature [2]-[9], there are more than 100 type of herpesviruses. All representatives of the family Herpesviridae are divided into three subfamilies by their biological properties: Alphaherpesvirinae, Betaherpesvirinae, Gammaherpesvirinae, according to the International Committee on taxonomy of viruses (2015). There exist an unassigned genus, not included to any of three subfamilies. A typical representative of the Alphaherpesvirinae subfamily is bovine herpesvirus type 5 (currently classified as BoHV-5). BoHV-5 is a linear double-stranded DNA virus which encodes 70 proteins (138 000 base pairs). The BoHV-5 genome has 82% similarity with the BoHV-1 (Infectious bovine rhinotracheitis) proteins. Bovine herpesvirus- 5 is widespread in Brazil and Argentina and is considered as the main cause of neurological disorders in these regions [2]-[3]-[6]-[7]. When infected with BoHV-5, primary replication of the virus occurs in the epithelial cells of the nasal mucosa, then the virus spreads through the axons to the brain, undergoes secondary replication in the central nervous system cells, and meningoencephalitis develops. Based on the restriction analysis all BoHV-5 strains are divided into three types: "a", "b", "non-a-non-b". The strains of type “a” are detected more often [10]. The same strain can cause a fatal infection in a newborn calves and an asymptomatic course of the disease with prolonged virus carrier in 5-6-month-old animals.

During acute course of the disease calves die quickly as they do not have time to form an immune response [8]-[10]. The increasing complexity of the epizootic situation of respiratory viral diseases has led to a decrease in progress in the development of specific prevention of the above mentioned infections. Live recombinant vaccines, which are used to prevent bovine infectious rhinotracheitis, the causative agent of which is bovine herpesvirus type 1 (BoHV-1), are not effective against Bovine herpesvirus type 5 (BoHV-5), although they are used when importing breeding stock by suppliers [12].

The uniqueness of herpesviruses is that they can remain latent in the host organism, integrate into the host cell genome and persist for a long time. When immunodeficiency occurs or resistance decreases, the latent virus is reactivated and excreted into the external environment [5]-[9].

Currently, the specific weight of dairy farms is increasing in Russia. High milk productivity of cows is often accompanied by metabolic disorders and resistance reduction of animals which leads to the activation of various infectious agents [1-4]-[9], mainly mass respiratory diseases [1]-[3]-[4], which are one of the main causes of economic damage.

Vologda region is a large region in which dairy farming is rapidly developing. For several years, in some livestock farms of the region, the outbreaks of mass respiratory infection were recorded. According to the literature [1]-[8]-[9], the provision of newborn young animals with high-value nutrients and protective factors in the postnatal period of development belongs to the secret of the mammary glands. Therefore, we carried out the study of the secret of the udder of cows for the presence of viral infections.

The purpose of the research was to identify the causative agent of respiratory infections in the milk of cows of the livestock farms of the Vologda region.

2. Materials and methods
The laboratory studies were conducted in the virology laboratory of FSC VIEV. The samples of milk were collected from cows from farms of Vologda region with the following clinical signs: coughing, mucous discharge from the nasal cavity. Milk samples for the study were prepared as follows: test tubes with samples (10 ml each) were centrifuged at 2500 x g for 15 minutes. Sediment containing the largest number of cells was used in the study. The samples were tested for the presence of following viral infections: bovine herpesvirus, bovine viral diarrhea virus – mucosal disease, parainfluenza-3 virus and coronavirus. PCR and RT-PCR were used to detect the viral genome in milk. DNA was extracted from biological material by a “DNA Extran” reagent kit (Syntol ZAO, Russia), according to the manufacturer’s recommendations. For RNA isolation “A set of reagents for the isolation of total RNA from whole blood, cell cultures and tissue” samples were used. For the bovine herpesvirus identification
we have used primers to the fragment of glycoprotein B gene. The information about primers and amplification modes are presented in the table 1 and 2.

Table 1. Primers used in the study.

| No | Causative agent         | Direct Primers                           | Reverse Primers                          |
|----|-------------------------|-----------------------------------------|------------------------------------------|
| 1  | Bovine herpesvirus-5    | 5'-ACGACGGGACGATGTG-TAC-3'             | 5'-CTCTCGTCTCGCAG-CAT-3'                |
| 2  | Bovine viral diarrhea virus | 5'-GATTTCAAGGGGACTTT-TTT-3'           | 5'-TACTACTTAGTAGGAGA-TGT-3'             |
| 3  | Parainfluenza-3 virus   | 5'-GATCAGGAACTCTTTAAG-GGC-3'           | 5'-TTTCCCCGACCCCCTTC-TAT-3'             |
| 4  | Coronavirus             | 5'-GCAGGTTTAATCCTTCTACTTT-GGA-3'       | 5'-CACCAAGAATTATGTCTGTGGTT-TGA-3'       |
| 5  | Reverse transcription   | Random 6                                |                                          |

Table 2. Amplification modes.

| No | Causative agent        | Primers annealing   | PCR modes         | Elongation        |
|----|------------------------|---------------------|-------------------|-------------------|
|    |                        | temperature | time in sec. | temperature | time in sec. | number of cycles | temperature | time in sec. |
| 1  | Bovine herpesvirus-5   | –          | –             | 94         | 60          | 30              | 72          | 300           |
|    |                        | –          | –             | 55         | 90          |                  | 72          |               |
|    |                        | –          | –             | 72         | 120         |                  | 72          |               |
| 2  | Bovine viral diarrhea virus | 94        | 120            | 94         | 60          | 50              | 72          | 420           |
|    |                        | 55         | 60             | 72         | 60          |                  | 72          |               |
| 3  | Parainfluenza-3 virus  | 94         | 900            | 95         | 60          | 30              | 72          | 600           |
|    |                        | 50         | 60             | 72         | 60          |                  | 72          |               |
| 4  | Coronavirus            | –          | –             | 94         | 10          | 35              | 68          | 420           |
|    |                        | –          | –             | 55         | 30          |                  | 68          |               |
|    |                        | 68         | 45             |            |             |                  |             |               |
| 5  | Reverse transcription  | –          | –             | 25         | 600         | 1               | –           | –             |
|    |                        | –          | –             | 42         | 2400        |                  |             |               |
|    |                        | –          | –             | 92         | 300         |                  |             |               |

For amplification, a reaction mixture was prepared to comprise 15 μl of H2O; 4 μl MgCl2; 4 μl dNTP; 2.5 μl of PCR buffer; 0.5 μl of each primer; 0.5 units of Taq polymerase; 3 μl of the DNA template. To the reaction mixture, 5% DMSO was added and Hot Start PCR method was used. A thermostat of DNA-technology (Russia) was used for preheating the reaction mixture. Amplification was carried out in a solid-state amplifier of the DNA-technology (Russia). Electrophoresis was carried out in a 1.5% agarose gel by adding ethidium bromide in Tris-acetate buffer solution. The results were taken into account on a transilluminator.

Sequencing was performed at ZAO Syntol using the ABI Prism Genetic Analyzer 3100 genetic analyzer (Applied Biosystems, USA). Local alignment of the studied nucleotide sequences was
performed by BLAST (GenBank, INSDC). Multiple alignment and clustering pattern was determined by the neighbor-joining method with the “Bootstrap” values based on 1000 replicates.

3. Results
In the livestock enterprises of the region where the studies were conducted respiratory diseases of both adult and young cattle are annually recorded. Experimental studies were carried out in livestock farms of Vologda region of dairy direction suspected to respiratory infections. Acute form of the disease with high temperature (41.5 ° C), rapid breathing, severe cough, serous nasal discharges were observed in the beginning of disease manifestation. Subsequently, the nasal discharges became serous-purulent and breathing became difficult. Along with respiratory syndrome conjunctivitis was observed in many animals (cows and calves), accompanied by lacrimation and purulent exudate discharges. Simultaneously with the above mentioned clinical signs, vulvovaginitis, endometritis and mass abortions were also observed in cows. In all examined farms the herd reproduction occurs due to the young animals. Therefore, we assumed that an established circle of pathogens circulates in these farms.

In the study of milk samples obtained from cows belonging to the farms of the Vologda region for the first time, the fragments of the bovine herpesvirus-5 (BoHV-5) gene were detected. As a result of electrophoresis of amplification products, a 400 bp DNA fragment as bright stripes of amplicons was identified.

Using the computer program “BLAST”, a comparative analysis of the nucleotide sequences of the studied virus and the reference strains of the INSDC database was performed. They showed the similarity of the virus found in the milk of cows in farms in the Vologda region with the herpes virus of cattle type 5. In the etiopathogenesis of cattle diseases of a number of bovine viruses, the participation of pathogens such as coronavirus, viral diarrhea and parainfluenza-3 was excluded. The obtained amplicons were studied by phylogenetic analysis.

![Figure 1](image_url)

The dendrogram in figure 1 shows the phylogenetic relationship between the Vologda mol 2017 isolate and reference strains of bovine herpes virus (BoHV).

4. Conclusion
Outbreaks of mass respiratory infections of both adult and young cattle in livestock farms of the region have been observed for several years.

This was the reason for studying the secret of cow udders for the presence of viral infections.
Thus BoHV-5 was detected for the first time in the milk of cows from livestock farms of the Vologda region as a result of molecular genetic analysis.

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