Identification of Bovine Herpesvirus type 5 in nasal swabs of naturally infected calves by Polymerase Chain Reaction

I N Simanova¹, S V Alexeyenkova², A M Anoyatbekova² and K P Yurov²

¹Vologda branch of The Federal research center-all-Russian research Institute of Experimental veterinary medicine named after K. I. Scriabin and Ya. R. Kovalenko of the Russian Academy of Sciences, Vologda, 160000, Russia
²Federal scientific center-all-Russian research Institute of experimental veterinary medicine named after K. I. Scriabin and Ya. R. Kovalenko of the Russian Academy of Sciences, Moscow, 109428, Russia

E-mail: Irina_simanova@mail.ru

Abstract. In this article, the results of molecular-genetic analyses of nasal swabs are presented. The experimental studies were carried out in the dairy farms of Vologda region suspected to the respiratory infections. The samples were obtained from the asymptomatic calves and from calves with cough and nasal discharges. The laboratory studies were conducted in the laboratory of virology of the FSC VIEV. The genome of the virus was detected by a polymerase chain reaction. The comparative analyses of identified nucleotide sequences of the virus with the reference strains of the International Nucleotide sequence database has shown the similarity with Bovine herpesvirus-5. Accordingly, as a result of our studies, the Bovine herpesvirus type 5 was identified in the nasal swabs of calves in farms of Vologda regions.

1. Introduction

The respiratory diseases of cattle are widespread in almost all countries of the world and cause significant economic damage due to the death of young animals, a decrease in milk production of a cow and a loss of animal body weight. The leading role in the etiology of the disease belongs to viruses and herpesviruses are among them [1,2].

Bovine Herpesvirus type 5 (BoHV-5) is a DNA virus of the Herpesviridae family, the Alphaherpesvirinae subfamily and the Varicellovirus genus. The BoHV-5 genome consists of a linear double-stranded DNA that encodes about 70 proteins. Together with bovine herpesvirus type 1 (BoHV-1), it causes significant economic loss to livestock. Based on DNA restriction analysis, serological studies, in particular the cross-neutralization reaction, it was determined that the identified viral strains and isolates differ in genomic and antigenic structure. In this regard, already in 1992, the International Committee on Taxonomy of Viruses was classified BoHV-5 as a separate virus.

BoHV-5 is known as the causative agent of fatal meningoencephalitis of cattle and is widespread in the USA, Australia, Hungary, Argentina, Italy, Canada and etc. All BoHV-5 strains are subdivided into three types: "a", "b", "non-a- non-b ". The strains of type "a" are most identified [3,4].

The mechanism of transmission of herpesvirus type 5 is poorly understood. It is known that BoHV-5 primarily infects epithelial cells; the virus replicates in the nasal mucosa and vagina. There is a report on the detection of viral DNA in the semen of subclinically infected bulls [5], which indicates the
transmission of the virus through artificial insemination. In our previous research, we have confirmed the presence of virus DNA of BoHV-5 in the milk of naturally infected cows [6]. During the acute course of the disease, the virus is secreted through the nasal discharge in large quantities. The immune response during infection with BoHV-5 is not known well. It is believed that the immune mechanisms in the host organism upon infection with BoHV-5 and BoHV-1 are similar [7]. Therefore, due to the high incidence of respiratory infections in young animals in livestock farms of the Vologda region, it is necessary to identify their pathogen.

This study aimed to conduct the molecular genetic analysis for identification of bovine herpesvirus-5 in calves in the farms of Vologda region.

2. Materials and methods

2.1. Samples collection, preparation and transportation

Nasal swabs from the mucosa of sick and healthy calves were collected from several farms of the Vologda region. General symptoms of respiratory diseases were observed in animals. A swab from the nasal mucosa was taken with a dry sterile probe, which was introduced with light and rotating movements along the nasal mucosa. After collecting the material, the working part of the probe was broken off and placed in a special test tube with a transport medium for storing and transporting swabs from the respiratory organs. The tubes were stored at +8 °C and transported in a special thermal container with refrigerants. The samples were examined by PCR at the Virology Laboratory of the Federal Scientific Center VIEV.

2.2. Polymerase Chain Reaction

2.2.1. Extraction of total DNA. For the extraction of viral DNA, a commercial kit of ZAO Syntol was used. The extraction was carried out following kit’s instruction. The modes of amplification, primers to glycoprotein B were selected in accordance with the data of the literature (table 1). The “Hot-start” method was used with the addition of 5% DMSO in order to obtain a higher product yield.

| No | Causative agent | Primers | Reverse |
|----|----------------|---------|---------|
| 1  | Bovine herpesvirus type 5 | 5'-ACGACGGACGATGTGTAC-3' | 5'-CTCTCGTGTCCTCGACGAT-3' |

The amplification products were analyzed by electrophoresis in 1.5% agarose gel with the addition of ethidium bromide in Tris-acetate buffer solution.

2.2.2. Sequencing and phylogenetic analysis. Sequencing was carried out in ZAO Syntol on a genetic analyzer ABI Prism Genetic Analyzer 3100 (Applied Biosystems, USA). The phylogenetic tree was
constructed by Mega 7.0 software. An alignment of nucleotide sequences was performed by ClustalW program.

2.3. Isolation of the virus in cell culture

The virus was isolated in a calf kidney (CK) cell culture. For this purpose, a 10% suspension of nasal discharge was prepared in phosphate-buffered saline with the addition of antibiotics (amikacin 1 mg/ml). After adding antibiotics, the suspension was kept for 2 hours at room temperature and then centrifuged at 2500 g for 10 minutes. The supernatant in a volume of 1 ml was used for the infection of cells monolayer. For the adsorption of the virus, the infected cells were kept for 1 hour at 37 °C. After adsorption, the suspension was removed, the monolayer was rinsed 2 times and a cultural medium (Eagle's MEM medium with Hank’s balanced salt solution and Lactalbumin medium) was added. Infected cell cultures were incubated at 37 °C in a thermostat for 3-5 days and were daily viewed by a microscope.

3. Results and discussion

Respiratory diseases in cattle are widespread all around the world including Russia. The damage mainly consists of the lack of offspring, the cost of medical and preventive measures. When studying the etiology of these diseases, the herpesvirus infection is often described, in particular the causative agent of infectious rhinotracheitis (BoHV-1) [8, 9]. According to N.N. Kryukov and co-authors (1968), this pathogen was introduced into the territory of Russia during the import of cattle. It is explained by the ability of herpesviruses to persist in the organism of susceptible animals. In this regard, there is a high probability of spreading not only BoHV-1 but also BoHV-5 in a free of the disease regions. Consequently, the purpose of this study was to identify BoHV-5 in dairy farms of the Vologda region affected to respiratory infections. The research was conducted in the period of 2018-2019 years.

The material was obtained from calves aged 10-16 days with visible clinical signs of respiratory infection: rhinitis, cough, dyspnea, conjunctivitis. Increase in body temperature up to 41.50C was observed in animals. At the beginning of the disease, serous discharge from the nasal cavity was observed, which later became purulent. The samples were investigated by molecular genetic methods, in particular, the polymerase chain reaction. During the reaction, a highly conserved gene-glycoprotein B, which is an important component of bovine herpesvirus, was amplified. During the study, 214 samples of nasal swabs were collected from young cattle in six of the nine studied farms in the region. The results of the study are presented in table 3.

| Animal species | Biological material | studied sample/ years | Identified positive samples / % |
|----------------|---------------------|-----------------------|---------------------------------|
| Calves         | Nasal swabs         | 2018 114              | 12/10.5 %                       |
|                |                     | 2019 100              | 15/15.0 %                       |
| Total for two years: |                   | 214                  | 27/25.5 %                       |

While electrophoresis of amplification products the DNA fragments 400 bp in length was identified. Positive samples were submitted for sequencing. During phylogenetic analysis, it was determined that the nucleotide sequences of the virus circulating in the farms of the Vologda region are similar to herpesvirus type 5. The phylogenetic tree is presented in figure 1.
Figure 1. Phylogenetic tree reflecting the degree of phylogenetic relationship between the Vologda isolates and reference strains of bovine alphaherpesvirus, based on the analysis of the nucleotide sequences of a gene fragment encoding glycoprotein B (gB).

The nucleotide sequences of strains and isolates from GenBank database, and our identified isolates of BoHV-1 and BoHV-5 are used in the article.

The phylogenetic tree was inferred by the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The distances were computed using the Maximum Composite Likelihood method. The analysis involved 30 nucleotide sequences and was conducted in MEGA7.

During phylogenetic analysis, it was determined that the detected nucleotide sequences are in 98.33% similar to Brazilian strains P190 / 96, ISO 97 / 45, SV507 / 99, EVI-190 and strain N565. They have also a 98.05% similarity with the UL 27 strain.
In the study of nasal discharge of calves in cell culture, the cytopathic effect of the virus was observed on 3-5 days after infection. Given the sensitivity and specificity of the PCR reaction and its ability to quickly detect the DNA of the virus, this method has advantages over virological methods mainly virus isolation.

The differential diagnosis from other viral infections as coronavirus, bovine viral diarrhea virus and parainfluenza-3 was performed by the RT-PCR test. Differential diagnosis is very important, since the clinical signs of respiratory diseases are generally similar [10].

4. Conclusion
Thus, the results of conducted studies confirmed the presence of bovine herpesvirus type 5 in calves. The data obtained indicate that bovine herpesvirus type 5 is widely spread in the farms of Vologda region as it was found in six out of nine studied farms and is one of the most relevant problems in the conditions of livestock enterprises.

References
[1] Yurov K P and Shulyak A F 1999 Veterinaria 4 34-8
[2] Yurov K P, Alexeyenkova S V and Pchelnikov A V 2013 Veterinaria 8 23-9
[3] Armstrong J A., Pereira H G and Andrewes C H 1961 Virology 14(2) 276-85
[4] Glotov A G, Glotova T I, Semenova O V and etc. 2016 Materials of IV International Congress Sochi 9
[5] Yurov G K, Alexeyenkova S V and Yurov K P 2012 Russian Veterinay journal. Farm animals 3 14-6
[6] Simanova I N, Alekseyenkova S V and Yurov K P 2020 IOP Conf. Series: Earth and Environmental Science 548
[7] Cascio K E, Belknap E B, Schultheiss P C et al. 1999 J. Vet. Diagn. Invest. 11 134-9
[8] Dewals B, Dewals L, Gillet T, Gerdes et al. 2005 Vet. Microbiol. 110 209-20
[9] Keuser V, Schynts F, Detry B et al. 2004 Journal of Clinical Microbiology 42(3) 1228-35
[10] Anoyatbekova A M, Alexeenkova S V and Yurov K P 2020 IOP Conf. Series: Earth and Environmental Science 548 022066 IOP Publishing doi:10.1088/1755-1315/548/2/022066