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Molecular Genetic Assessment of Nodular Dermatitis Virus in Cattle Herds

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Abstract

Background: The study highlights the significance of data availability in molecular genetic research, focusing on cattle nodular dermatitis virus. Limited data on Russian genetic variants hinders comprehensive virus characterization. Molecular genetic methods are crucial for accurate diagnosis, considering cross-reactions with poxviruses. Increased data availability is essential to improve diagnostics and biosafety in the region.

Method: The researchers conducted a molecular genetic analysis of the gene sequences of the nodular dermatitis virus to assess the variability of the virus. The authors used the GenBank database to compare sequences and used the MEGA X program for phylogenetic analysis and sequence alignment.

Results: The sequences of the nodular dermatitis virus closest to the Russian genetic variants have been determined. The volumes of available epizootiological and molecular genetic data were compared, and the circulation of the vaccine strain was confirmed.

Conclusion: The article emphasizes the need to increase the availability of data on the genetic sequences of Russian samples to fully characterize the genetic diversity of the nodular dermatitis virus. Despite the limited data, the authors observed a tendency to increase genetic diversity. They recommend isolating and storing the virus in the cell culture to enhance genetic information.

Introduction

Analytical work is an integral part of any science, including veterinary studies, as it helps summarize practically obtained data and deduce patterns. The degree of availability of this information (epizootic, clinical, and molecular genetic data) can vary greatly, due to many factors, such as the development of the topic, the availability of open databases of different localization levels, and updating and prompt replenishment of the data entered into these databases [1-3]. To improve measures for the prevention and control of infectious diseases, as well as to improve the biosafety situation in the Russian Federation and closely related countries of the Asian region, specialists in these regions need to have access to the maximum possible amount of data from their colleagues. Analytical works on any relevant topic, in particular, infectious diseases, confirm this.

As an example, let us consider (infectious) nodular dermatitis (ND) which belongs to the category of particularly dangerous vector-borne animal diseases, causing serious damage to the economy. The virus, depending on the situation and the presence of vectors, can cause both local outbreaks and mass epizootics in cattle, causing serious damage [4-7]. The degree of economic damage is estimated based on the costs of antiepizootic measures and assessment of the general tension of the epizootic situation, the death of productive and breeding animals, and a sharp decrease in the quality of skins, meat, and milk of sick animals. In the RF, as of the end of December 2022, 14 settlements are unsafe regarding ND, the vast majority of which are in border regions [8].

For the effective diagnosis of infectious ND, there is not enough clinical and epizootiological data, and the key role belongs to laboratory diagnostic methods.

In the modern diagnosis of ND, it is necessary to consider such a factor as cross-reactions with other poxviruses. The most reliable methods in such a situation are molecular genetic methods, in particular, the polymerase chain reaction. Nevertheless, without considering the variability of circulating strains, it will be impossible to correctly select the appropriate specific primers for test systems [9-13].

Thus, the purpose of our work was to indicate the importance of the availability of data for molecular genetic analysis in veterinary studies on the example of the ND virus in cattle.

Methods

To assess the epizootic situation for the ND virus, data for all federal districts of the RF were evaluated. We synthesized and analyzed existing research findings related to the nodular dermatitis virus and its genetic diversity. To facilitate genetic analysis, the virus was cultured in a controlled laboratory environment. This involved the propagation of the virus in suitable cell cultures to obtain a sufficient quantity for genetic analysis.

Viral DNA extraction was performed on the cultured virus samples using established molecular biology techniques. The extracted viral DNA served as the genetic material for subsequent analysis.

Selection Protocol for the Genes

Several genes were selected for analysis to assess the genetic variability of the ND virus. These genes were chosen based on their relevance to virus characterization and epidemiological studies:

- RNA Polymerase
- GPCR Gene (G Protein-Coupled Receptor Gene)
- LSDV155 Gene
- LSDV127 Gene
- LSDV117 Gene
- LSDV095 Gene
- OPC103 Gene

Gene Amplification and Sequencing Protocols

Specific protocols were employed to amplify the selected genes from the viral DNA. This involved the use of polymerase chain reaction (PCR) techniques with gene-specific primers designed for each target gene.

The amplified gene segments were then subjected to DNA sequencing, which generated the nucleotide sequences of these genes. High-throughput sequencing technologies were utilized to ensure accuracy and reliability.

Forward Primer: 5'-AAACCTGTCTTCTTGCTACCTTGC-3'
Reverse Primer: 5'-AGCACAGAAACATCCAGCTC-3'
GPCR Gene (G Protein-Coupled Receptor Gene):
Forward Primer: 5'-GTTAGGGATCTGGACCTGTTTTAC-3'
Reverse Primer: 5'-GTACGTGTTATGTCGGGTATC-3'

These primers were designed to target specific regions of interest in the ND virus genome, enabling us to amplify and sequence the relevant genes accurately.

Sequence Alignment and Phylogenetic Analysis

The obtained gene sequences were aligned to identify similarities and differences among them. Sequence alignment was conducted using the Clustal W algorithm within the MEGA X software. Phylogenetic analysis was performed to assess the evolutionary relationships among the ND virus strains. The construction of phylogenetic trees was carried out using the MEGA X software, which determined the most appropriate methods and models based on the data. Free Microsoft Excel 2007 software was used for statistical data processing.

Results

Upon obtaining the gene sequences, we conducted a comprehensive analysis to assess genetic diversity within the ND virus population. Notably, we observed a trend towards increased genetic diversity when comparing the sequences of select genes. This observation suggests potential evolutionary changes within the ND virus in Russia.

In total, of all the sequences of the ND virus obtained from samples from the RF, sequences of seven genes out of 156 are presented in the GenBank database. The entire list is presented in Table 1.

Name of the gene	Number of sequences in the GenBank database
ND virus	
Total sequences	66
Complete genome	1
RNA polymerase	15
GPCR gene	15
LSDV155 gene	7
LSDV127 gene	8
LSDV117 gene	8
LSDV095 gene	9
OPC103 gene	3

Table 1: Summary number of gene sequences of ND virus from the GenBank database.

Due to the small amount of open data, it is extremely difficult to determine the variability of the virus within the RF using phylogenetic analysis. Only a comparative analysis with sequences from other countries is possible.

These data were confirmed when using the BLAST software, which shows samples as close as possible to the selected sequence [10]. This suggests potential regional similarities among these virus strains. In contrast, sequences from other countries formed a separate cluster, indicating genetic divergence. The cluster formed by sequences from Russia is highlighted with bold lines and the opposite one with broken lines. It is joined by a sample from Turkey (marked with a circle in the cluster of Russian samples) and a sample from Kazakhstan (marked with a square in the cluster of Russian samples). With a direct letter-by-letter comparison of several genes whose sequences are presented in the database, the following results were obtained. For the subunit of the RNA polymerase of the ND virus, high conservativeness was noted, which is generally characteristic of poxvirus enzymes. Single substitutions were noted in several strains from the Volga region and Siberia (Figure 1). These substitutions are not synonymous and lead to amino acid substitutions in the protein, in particular, substitutions K24E and S87P.

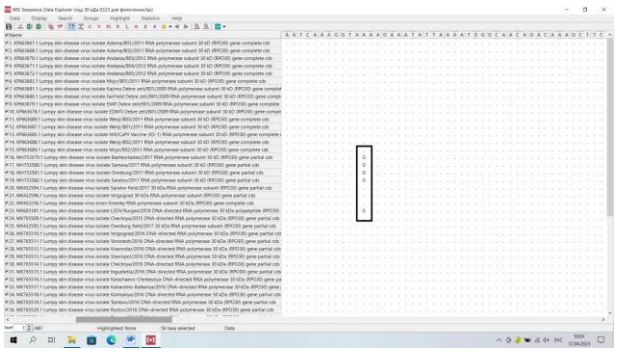

Figure 1: Nucleotide substitutions in the region of the gene encoding the 30 kDa RNA polymerase subunit.

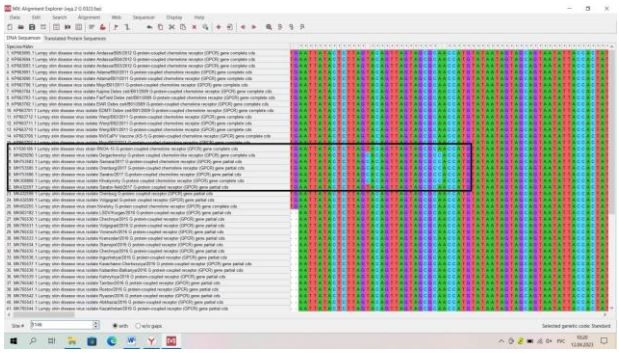

Figure 2: Nucleotide substitutions in the GPCR gene.

For the GPCR gene, the letter-by-letter comparison showed the presence of substitutions in the same isolates as for the RNA polymerase subunit gene (Fig. 2). The GPCR gene also exhibited substitutions in the same isolates as the RNA Polymerase gene, indicating a potential genetic relationship between these two regions of the ND virus genome. These observations suggest that while the ND virus maintains a relatively stable genetic profile, localized variations and substitutions may occur, contributing to its genetic diversity.

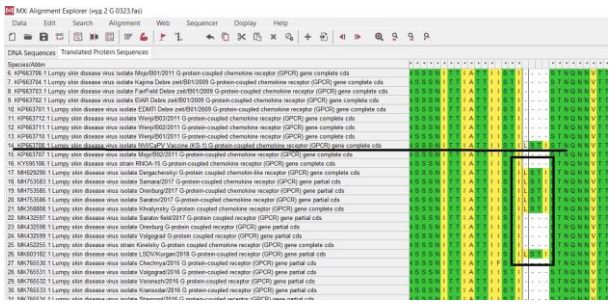

Figure 3: The correspondence of the amino acid insertion of the analyzed sequences to the insertion of the vaccine strain.

Upon further comparison, it was determined that these samples corresponded to a widely used vaccine strain, which corresponds to the data of publications on its possible uncontrolled spread (Fig. 3) [14-16]. The

presence of the vaccine strain raises concerns about its potential uncontrolled spread and its impact on epidemiological safety. This finding underscores the importance of stringent monitoring and control measures for vaccine distribution and use to prevent unintended consequences in ND virus epidemiology.

Discussion

When analyzing the data related to the molecular genetics of the ND virus, we can say that the open international database contains too few sequences obtained from Russian virus samples to be able to characterize the genetic diversity of this virus in Russia well enough. Nevertheless, despite this fact, when comparing the sequences of some genes letter-by-letter with the first strains and with the vaccine strain, one can notice a tendency to increase genetic diversity. Comparing the sequences of select genes, we observed a noteworthy tendency toward increased genetic diversity. This observation suggests that the ND virus in Russia may be undergoing evolutionary changes, leading to genetic divergence. These findings are consistent with existing literature that underscores the dynamic nature of poxviruses, which includes the ND virus [11]. The genetic variability observed in our study may be attributed to factors such as host immune pressure, geographical distribution, and co-evolution with potential vectors.

To expand our understanding of the ND virus's genetic makeup and evolution, it is advisable to isolate and cultivate the virus in cell culture. This approach has been successfully employed in previous studies on poxviruses, allowing for the accumulation of larger quantities of virus material for in-depth genetic analysis [17]. Future research should build upon this strategy to investigate the genetic dynamics of the ND virus further.

Furthermore, our study highlights the critical importance of actively supplementing existing genetic sequence databases with new sequences or establishing specialized databases focused on specific pathogens. The integration of molecular genetic data with epizootiological data, as demonstrated here, offers a powerful means of addressing contemporary challenges in veterinary medicine [18]. Such multidisciplinary approaches hold great promise for enhancing our ability to prevent and control infectious diseases [19, 20]. Despite the limited sequence data available in the public domain, we are sufficient to determine the circulation of the vaccine strain of the ND virus on a par with the wild one, which raises concerns about the epizootiological safety of the vaccine strain. Previous research has highlighted the importance of monitoring vaccine strain circulation and its potential impact on disease transmission dynamics [21]. Future research should delve deeper into the dynamics of vaccine strain circulation and its potential impact on disease transmission, thereby informing vaccination strategies and biosecurity measures.

The results of the study showed important information about the genetic variability and phylogenetic relationships of the ND virus. By comparative analysis, it was observed that the Russian sequences formed a cluster with sequences from Turkey and Kazakhstan, which indicates a potential regional similarity of the virus strains. In addition, the analysis demonstrated the presence of a widely used vaccine strain among the analyzed sequences, which raises concerns about its uncontrolled spread and potential impact on epidemiological safety.

Besides that, the integration of molecular genetics and epizootiological data is crucial for the effective solution of urgent problems in the field of veterinary medicine. By comparing genetic and epidemiological information, researchers can gain valuable information about the prevention and control of infectious diseases, including ND. The limitations of the study relate primarily to the sample size. The number of available gene sequences of the ND virus from the RF was relatively small, which could affect the accuracy and representativeness of the analysis.

However, the expansion of sample collection efforts in different regions, countries, and over different periods will provide a more extensive set of data for analysis. This will allow researchers to get a complete picture of the population of the ND virus and its genetic dynamics.

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Author Contributions

All authors contributed equally to the conception, design, and execution of the study. Data analysis and interpretation were carried out collaboratively. The manuscript was collectively written and reviewed by all authors, who also approved the final version for publication.

Conflict of Interest

The authors declare that there is no conflict of interest.

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