



# Distribution of Genetic Variants of the Rabies Virus in the Russian Federation

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## Abstract

Rabies is a social, economically significant disease. According to the World Health Organization, rabies is one of the five economically significant diseases. Domestic and wild animals of all kinds and humans are susceptible to rabies. The disease is registered in various countries of the globe, including Russia. In this article, using the example of the rabies virus, the difference between the quality and availability of epidemiological and molecular genetic data is shown. Using the nucleotide sequences of the rabies virus, the sequences of the N gene (nucleoprotein) and the G gene (glycoprotein) were constructed. Analysis of phylogenetic trees built on the basis of rabies virus sequences showed the presence of homogeneous clusters from a number of regions of the country, with the simultaneous presence of heterogeneous clusters. This fact suggests that in a number of regions of the Russian Federation there are conditions for the isolated circulation of genetic variants of the rabies virus.

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## 1 Introduction

Rabies is an acute disease of warm-blooded animals characterized by damage to the central nervous system. Domestic and wild animals of all kinds and humans are susceptible. The disease is

registered in various countries around the globe [3] Western European countries have received the status of being free from rabies after conducting long-term campaigns for the vaccination of wild carnivores [4, 16].

Currently, rabies is registered in almost all countries of the world and is included in the list of especially dangerous viral diseases. According to the WHO, more than 50,000 people die every year from hydrophobia in the world. The urgency of the problem is also associated with the huge economic costs of prevention and anti-epizootic measures. Annual monitoring of rabies is an important link in the chain of improvement of the epizootic situation. Based on its results, a further strategy for the prevention and control of rabies is built [5, 6, 8].

The rabies virus belongs to the order Mononegavirales, family Rhabdoviridae, genus Lyssavirus, species Rabies lyssavirus [5]. The rabies virus virions are bullet-shaped, about 180 nm long and about 75 nm in diameter. On the outer surface of the viral particle, there are protrusions in the form of spikes 10–12 nm long, which are attached to the bilayer lipid membrane [4]. The genome of the rabies virus is a non-segmented single-stranded negative helical RNA about 12,000 bp in length. o., which encodes five main proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), RNA-dependent RNA polymerase (L). Pseudogene ( $\psi$ -fragment) is located between G- and L-cistrons [9].

Modern science is impossible without the involvement in the analytical work of large amounts of data obtained from various sources. The degree of data availability is very different and depends on many factors, such as the development of the topic, the availability of local and global open databases, and the timely entry of information into databases. In order to improve measures for the prevention and control of infectious diseases, as well as to improve the biosecurity situation in the Russian Federation, data on major animal diseases should be available at least to specialists within the country.

When diagnosing rabies, epizootological and clinical data are taken into account, but the final diagnosis is made only taking into account the results of laboratory diagnostics. Annual epizootic monitoring is an integral part of anti-epizootic and anti-epidemiological measures. Based on these data, one can judge the seasonality and spread of the disease, the effectiveness of preventive measures [3, 5].

Epizootic monitoring of rabies is carried out throughout the Russian Federation. Veterinary specialists use the following methods to diagnose the disease: light microscopy, fluorescent antibody method (MFA), enzyme immunoassay (ELISA), diffuse precipitation reaction (RDP), reverse transcriptase polymerase chain reaction (RT-PCR), bioassay on white mice, virus isolation in cell culture of mouse neuroblastoma CCL-131 or neurinoma Gasser's node of the rat - NGUK-1. These methods are applicable to all members of the Lyssavirus genus [14, 15].

## 2 Method

The analysis carried out consisted of two parts. The first part included an assessment of the rabies epizootic situation in Russia in 2020. It is presented based on the results of the analysis of

materials from annual reports in the form of 4-vet, usually provided by the country's veterinary laboratories to the Federal State Budgetary Institution "Central Scientific and Methodological Veterinary Laboratory" (CSMVL). The combined data are presented on the website of the Rosselkhoznadzor and the World Organization for Animal Health (OIE) [9].

Annual epizootic monitoring of rabies is carried out throughout the Russian Federation. In 2020, 1517 positive results were received. Of these, 47% of cases occur in pets (dogs, cats), 43% in wild animals, 9% in farm animals. Unfavorable points were recorded in 61 constituent entities of the Russian Federation. The tensest epizootic situation has developed in the Central Federal District and the Volga Federal District with 603 and 505 positive cases, respectively [6].

The analysis of the epizootic situation was carried out according to the modern administrative-territorial division of the Russian Federation. Statistical data processing was carried out using Microsoft Excel 2007 software.

The second task of the work was to analyze various genetic variants of the rabies virus from open databases in order to establish the relationship between various strains. Currently, only the international database of genetic sequences GenBank of the National Center for Biotechnology Information (NCBI) can be called fully open to a wide range of researchers from the Russian Federation [7].

Phylogenetic analysis of nucleotide sequences was carried out using the MEGA X program with an open license [18]. The construction method is the maximum likelihood method incorporated in the program algorithms.

Prior to phylogenetic analysis, the sequences were divided into groups according to genetic affiliation. The automatic alignment of the sequences was carried out and incorporated into the MEGA X program according to the Alignment Clustal W algorithm.

### 3 Result and Discussion

According to the information obtained from the official annual veterinary reporting in the 4-vet form, in 2020 the state laboratories of the Russian Federation received more than 11,000 biological materials for rabies testing.

According to CSMVL, 1,517 positive results were received in 2020. The disease was established in 61 subjects of the Russian Federation. Table 1 presents data on the number of examined and diseased animals for 2020.

**Table 1:** Number of species studied and diseased

| Kind of animal        | Researched | Got sick |
|-----------------------|------------|----------|
| Cattle                | 273        | 118      |
| Small cattle          | 71         | 22       |
| Horses                | 23         | 5        |
| Pigs                  | 10         | 1        |
| Dogs                  | 2732       | 434      |
| Cats                  | 2592       | 278      |
| Game and wild animals | 5015       | 648      |
| Fur                   | 51         | 1        |
| Other types           | 440        | 10       |

The largest number of positive results was recorded among domestic animals (712 cases) and among commercial, wild and fur animals (649 cases).

Table 2 presents data on the number of positive results for rabies in federal districts [14].

**Table 2: Number of positive results by animal species in the federal districts of the Russian Federation**

| Federal districts of Russia      | Total | Cattle | Small cattle | Pigs | Horses | Dogs | Cats | Commercial and wild | Fur | Other types |
|----------------------------------|-------|--------|--------------|------|--------|------|------|---------------------|-----|-------------|
| Central Federal District         | 603   | 16     | 9            | 0    | 0      | 171  | 117  | 290                 | 0   | 0           |
| Northwestern Federal District    | 8     | 0      | 1            | 0    | 0      | 1    | 1    | 5                   | 0   | 0           |
| Southern Federal District        | 85    | 11     | 0            | 1    | 1      | 26   | 23   | 23                  | 0   | 0           |
| North Caucasian Federal District | 34    | 10     | 0            | 0    | 0      | 11   | 8    | 5                   | 0   | 0           |
| Volga Federal District           | 505   | 52     | 12           | 0    | 1      | 171  | 112  | 149                 | 0   | 8           |
| Ural federal district            | 135   | 9      | 0            | 0    | 1      | 28   | 14   | 81                  | 0   | 2           |
| Siberian Federal District        | 88    | 8      | 0            | 0    | 0      | 13   | 3    | 63                  | 1   | 0           |
| Far Eastern Federal District     | 59    | 12     | 0            | 0    | 2      | 13   | 0    | 32                  | 0   | 0           |

In 2020, the veterinary laboratories of the Central Federal District received 4,377 samples of pathological material for testing for rabies. As can be seen from Table 2, the bulk of the positive results for rabies is in game animals, as well as dogs and cats in urban conditions. They make up 48 and 47%, respectively. The largest number of positive cases was recorded in Moscow (94), Vladimir (61), Tambov (60), Tver (57), Yaroslavl (56) regions.

In the Northwestern Federal District, 454 samples of pathological material were received for research. 8 positive results were registered for rabies in the Pskov region (5) and the Nenets Autonomous Okrug (3). The main share falls on game animals.

In the Southern Federal District, rabies in urban areas accounts for 57%. The tense situation in the Volgograd region (34 positive results) and the Republic of Crimea (21 positive results).

A difficult epizootic situation has developed in the Volga Federal District. Of the 1930 materials examined, 505 are positive, of which more than 50% are pets, 30% are wild and 13% are farm animals. The largest number of positive results is noted in the Saratov (143) and Penza (114) regions.

In the Urals Federal District, 555 samples of pathological material were submitted for examination, of which 135 were positive. Chelyabinsk (63 cases) and Tyumen (53 cases) regions remain stationary. The largest number of positive results was recorded among wild animals (81), with rabies in urban areas accounting for just over 30%.

In the Siberian Federal District, 600 samples of pathological material were examined. 23 positive results were obtained in the Republic of Khakassia and the Krasnoyarsk Territory, 17 in the Omsk Region and 16 in the Novosibirsk Region. Trouble is associated with a large number of positive samples among wild animals (more than 70% of the occurrence of all positive results).

It should be noted that in the Far Eastern Federal District, epizootic monitoring is carried out with an emphasis on wild fauna. Veterinary laboratories received 1,740 samples of pathological material for the examination, of which 1,320 were from commercial and wild animals. Positive results are less than 4%, of which 32 cases occur in wild animals, 15 in agricultural and 13 in domestic representatives. The largest number of positive responses to rabies was recorded in the Republic of Buryatia and the Trans-Baikal Territory.

For comparison, 581 sequences from the Russian Federation have been added to the international database for all time. The full list is presented in Table 3.

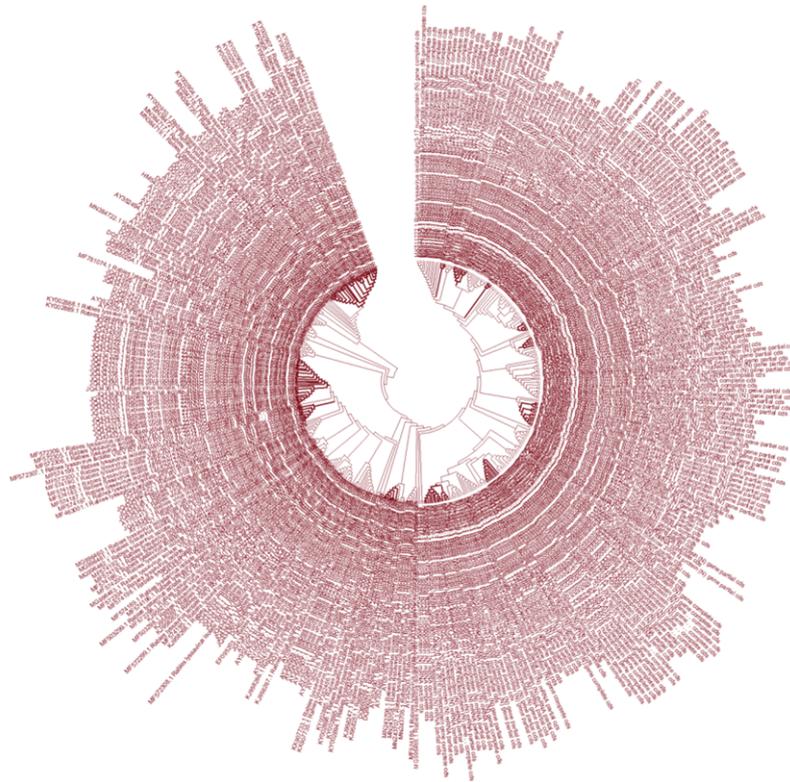
Using the nucleotide sequences of the rabies virus, two phylogenetic trees were built: according to the N (nucleoprotein) gene sequences and according to the G (glycoprotein) gene sequences. The number of other nucleotide sequences is insignificant and they do not play a significant role in the molecular genetic analysis of sequences within Russia.

Analysis of phylogenetic trees built on the basis of rabies virus sequences showed the presence of homogeneous clusters from a number of regions of the country, with the simultaneous presence of heterogeneous clusters. From the point of view of molecular evolution, this fact suggests that in the territory of a number of regions of the Russian Federation there are conditions for the isolated circulation of genetic variants of the rabies virus. At the same time, carriers of the rabies virus are migrating from one region to another.

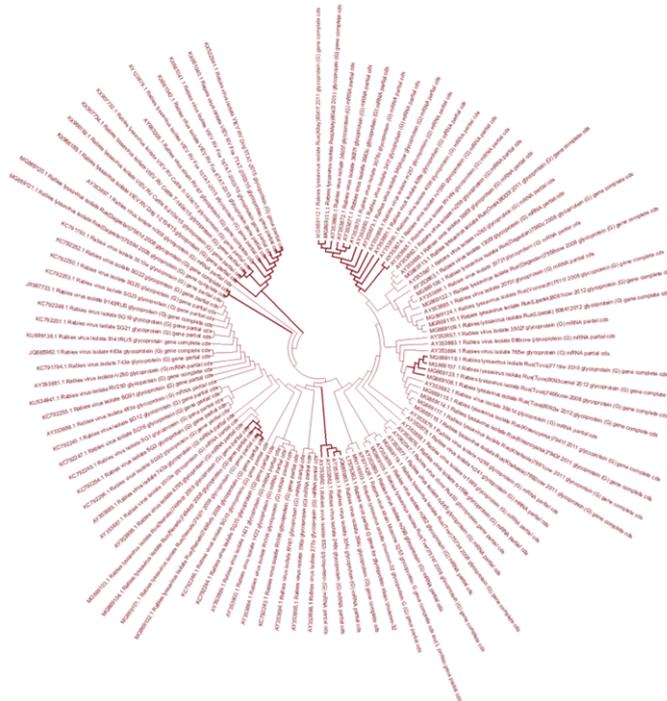
**Table 3:** Complete list of nucleotide sequences of rabies virus genes found in the GenBank database accessed 01.06.2022.

| Gene name           | Number of sequences in the GenBank database |
|---------------------|---|
| <b>Rabies virus</b> |   |
| Total Sequences     | 581   |
| Complete genome     | 18  |
| N protein gene      | 388   |
| G protein gene      | 163   |
| L protein gene      | 1   |
| M protein gene      | 11  |

When added to the analysis of nucleotide sequences obtained in other countries, one can see the distribution of rabies virus gene sequences obtained in Russia over various groups of sequences, that is, the absence of a single phyletic group with sequences from any other countries. These results are confirmed when trees are constructed according to different genes (Figures 1, 2).



**Figure 1:** Distribution of homogeneous and heterogeneous clusters of rabies virus nucleoprotein gene sequences. Bold lines indicate homogeneous clusters from different regions, thin lines indicate heterogeneous ones.



**Figure 2:** Distribution of homogeneous and heterogeneous clusters of rabies virus glycoprotein (envelope) gene sequences. Bold lines indicate homogeneous clusters from different regions, thin lines indicate heterogeneous ones.

An increase in the number of pets, their untimely vaccination or refusal to vaccinate them, the poorly controlled number of homeless animals in cities, and the insufficient work of the veterinary service led to the fact that the urban type of rabies began to dominate over the natural

one. In turn, the trouble among agricultural and domestic carnivores reflects the tense situation in the wild fauna. This confirms the direct correlation between the incidence of rabies in the wild and domestic animals. Epizootic monitoring of rabies is carried out by the veterinary service throughout the Russian Federation. In 2020, the disease was established in 61 subjects. The tensest situation still remains in the Central Federal District (603 cases) and the Volga Federal District (505 cases). Together, these subjects account for 73% of all positive results, of which 38% are domestic animals, 29% are wild and commercial animals. In general, the epizootic and epidemic situation in the Russian Federation remains quite tense. However, monitoring is carried out regularly, the data is constantly updated and is open and available to specialists in various fields.

## 4 Conclusion

From the analysis of molecular genetic data, in contrast to epizootological data, it can be said that the presentation of nucleotide sequences from Russia in an open international database only partially reflects the genetic diversity of viral pathogens in the country. However, it can be seen that genetic diversity is present and tends to increase, noticeable when comparing well-known vaccine strains with other sequences. For a more complete analysis, it is necessary to create either the development or presentation to the general public of Russian databases of nucleotide sequences, since in comparison with the quantity and quality of epizootological information, molecular genetic information on pathogens of infectious diseases in animals loses noticeably.

## 5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

## 6 Acknowledgement

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