











Original Article

Open Access



# Reemergence of arctic-like rabies virus in a rabies-free area in the Amur river basin

Aleksandr D. Botvinkin<sup>1</sup> , Elena M. Poleshchuk<sup>2</sup> , Sergey A. Chupin<sup>3</sup> , Ivan D. Zarva<sup>1</sup> , Ivan V. Meltsov<sup>4</sup> , Vasily A. Yanovich<sup>5</sup> , Nikolay G. Zinyakov<sup>3</sup> , Gennady N. Sidorov<sup>2,6</sup> , Evgeny Andaev<sup>7</sup> , Artem E. Metlin<sup>8</sup> 

<sup>1</sup>Irkutsk State Medical University, Irkutsk 664003, Russia.

<sup>2</sup>Omsk Research Institute for Natural Foci Infections, Omsk 644050, Russia.

<sup>3</sup>Federal Centre for Animal Health, Vladimir 600901, Russia.

<sup>4</sup>Irkutsk State Agrarian University, Irkutsk 664038, Russia.

<sup>5</sup>Pacific State Medical University, Vladivostok 690002, Russia.

<sup>6</sup>Omsk State Pedagogic University, Omsk 644099, Russia.

<sup>7</sup>Irkutsk Research Antiplague Institute of Siberia and Far East, Irkutsk 664038, Russia.

<sup>8</sup>Food and Agricultural Organization in the United Nations, Rome 00153, Italia.

**Correspondence to:** Dr. Aleksandr D. Botvinkin, Irkutsk State Medical University, 1, Krasnogo vosstania Str., Irkutsk 664003, Russia. E-mail: botvinkin\_ismu@mail.ru

**How to cite this article:** Botvinkin AD, Poleshchuk EM, Chupin SA, Zarva ID, Meltsov IV, Yanovich VA, Zinyakov NG, Sidorov GN, Andaev E, Metlin AE. Reemergence of arctic-like rabies virus in a rabies-free area in the Amur river basin. *One Health Implement Res* 2023;3:125-34. <https://dx.doi.org/10.20517/ohir.2023.19>

**Received:** 31 May 2023 **First Decision:** 2 Aug 2023 **Revised:** 23 Aug 2023 **Accepted:** 23 Oct 2023 **Published:** 30 Oct 2023

**Academic Editors:** Guilherme Werneck, Jose M. Martin-Moreno, Martin Pfeffer **Copy Editor:** Pei-Yun Wang **Production Editor:** Pei-Yun Wang

## Abstract

**Background:** A descriptive epidemiological study of the rabies outbreak in the Amur Region of Russia (ARR), which remained rabies-free from 1972 to 2017, was carried out. During 2018-2022, 49 animal rabies cases and one human rabies death were documented in the ARR. This study aimed to detect possible ways of incursion and features of the spatial spread of rabies virus (RABV) in ARR, which was considered rabies-free for 45 years.

**Methods:** After the beginning of the outbreak, 1,450 animal brain samples were tested using a direct fluorescent antibody test. Genetic analysis of RABV isolates was carried out ( $n = 9$ ). GIS was used to analyze the spatial spread of rabies in ARR and adjacent territories.

**Results:** Rabies has been confirmed in red foxes (*Vulpes vulpes*) - 38.8%, raccoon dogs (*Nyctereutes procyonoides*) - 10.2%, wolves (*Canis lupus*) - 14.3%, dogs - 18.4% and farm animals - 18.3% ( $n = 49$ ). The first cases were



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



detected south of the ARR, 30 km from the state border between Russia and China. In 2018-2022, the RABV spread within the forest-steppe landscapes of the Zeya-Bureya Plain northwesterly; the maximum distance from the first recorded event was 192.4 km (Me = 77.6). According to a phylogenetic analysis, the isolates from ARR belonged to the Arctic-like-2 RABV lineage and showed the maximum similarity to the isolates recovered in the province of Heilongjiang (China, 2011, 2018) and the Jewish Autonomous Region (Russia, 1980).

**Conclusion:** Rabies was most likely introduced by wild carnivores from the adjacent territories of Russia or China, located downstream of the Amur River.

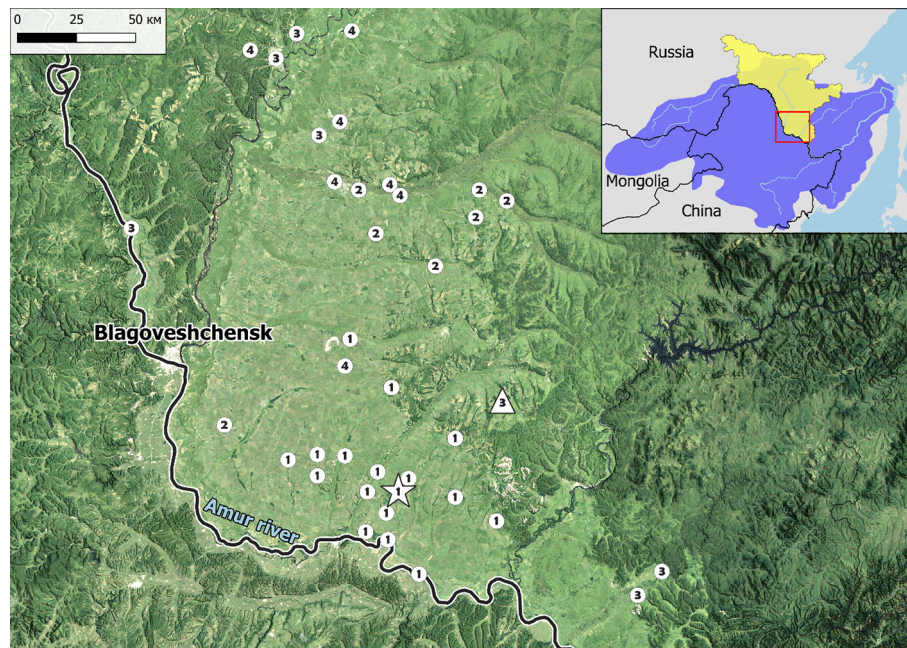
**Keywords:** Rabies, Arctic-like, re-emerging infection, transboundary outbreak

## INTRODUCTION

Rabies is a zoonotic viral infection with an almost 100% case fatality rate, caused by rabies virus (RABV) (order *Mononegavirales*, family *Rhabdoviridae*, genus *Lyssavirus*, species *Lyssavirus rabies*) and other lyssaviruses. Preventive measures can significantly reduce the incidence. According to the Global Burden of Disease Study, the number of human rabies cases globally halved between 1990-2019<sup>[1]</sup>. Many countries have joined the implementation of the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030<sup>[2,3]</sup>. Significant progress has been made in China: from 2007 to 2020, the annual number of human rabies cases has dropped from 3,300 to 202<sup>[4-6]</sup>. Most of these cases were concentrated in the south of the country. Rabies in humans was recorded sporadically in the northern provinces, where wild canids serve as reservoirs of the RABV<sup>[4,7,8]</sup>. However, an uptick in human rabies cases has recently been reported in northern China<sup>[5,9]</sup>. This territory and neighboring territories of Russia and Mongolia lie in the Amur River basin [Figure 1, Supplementary Table 1 and Figure 1].

Human and animal rabies has been historically common in Russia, part of the Amur River basin. Here, rabies penetrates to the north through steppe and forest-steppe areas favorable for such rabies hosts as red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*). This phenomenon contrasts strikingly with the vast territories of Siberia covered by conifer taiga forests, where host populations are scarce, and rabies has been recorded extremely rarely<sup>[10,11]</sup>. In the 1980s, wildlife rabies foci were described in the Russian Far East in lowland areas along the Amur River and around Khanka Lake, where raccoon dog was recognized as a reservoir of RABV<sup>[12,13]</sup>. Despite wildlife rabies, the infection mediated by domestic dogs predominated as the cause of human disease (> 80% of human rabies cases). Only rare human deaths after wolf and raccoon dog bites were reported in the Russian Far East during the previous century<sup>[12]</sup>. RABV variants of the so-called Arctic-like lineage prevail in the adjacent territories of Russia<sup>[14,15]</sup> and China<sup>[16-19]</sup>. The similarity of landscapes, genetic lineages, and natural hosts of RABV led to the conclusion about the transboundary nature of rabies outbreaks in the Amur River basin<sup>[12,16,19]</sup>.

Since the 1980s, the number of human and animal rabies cases and area of rabies-enzootic territories in the Russian Far East have gradually decreased. The area between Lake Baikal and the Jewish Autonomous Region (JAR) became rabies-free. At that time, dog and cat vaccination was enhanced, but the real reasons for rabies disappearance in the rabies-enzootic territories remain unclear. However, in 2014, rabies reemerged in all regions of Russia located to the East of Baikal Lake<sup>[10,20,21]</sup>. The situation in the Amur Region of Russia (ARR) is the most striking illustration of these processes. In 1912, the specialized medical facility (Pasteur Station) was opened in Blagoveshchensk for human post-exposure rabies prophylaxis. Until 1957, 34 human deaths were recorded, mainly after dog bites. In 1972, the last several rabies cases in cattle bitten by wolves were documented<sup>[13]</sup>. Since then, the ARR has been considered rabies-free until 2017. In 2018, only rabies was registered again among wild animals, livestock, and dogs<sup>[10,13]</sup>.



**Figure 1.** Spatial distribution of rabies in the Amur Region of Russia during 2018-2022. Numbers indicate animal rabies cases for specific periods: (1) November 2018-June 2019; (2) July 2019-June 2020; (3) July 2020-June 2021; (4) July 2021-June 2022. The asterisk indicates the first reported rabies case; the triangle indicates the human rabies case. Map insert: blue: Amur River basin; yellow: Amur Region of Russia (ARR); black lines: borders between countries; red square: the area represented on the map.

This study aimed to detect possible ways of incursion and features of the spatial spread of rabies in ARR, which was considered rabies-free for 45 years.

## METHODS

### Epidemiological analysis

Animal rabies records for 2018-2022 collected by the state epidemiological surveillance for zoonotic infections in the ARR were used for retrospective analysis. Only animals with laboratory-confirmed rabies were included in the study ( $n = 49$ ).

### Laboratory diagnosis and confirmation

After the first rabies case was reported, an active surveillance program to detect new cases was established. During 2018-2022, the ARR Regional Veterinary Laboratory examined 1,450 brain samples of wild and rabies-suspected domestic animals. Designated teams of hunters shot and trapped wild canids and other game animals in different areas of ARR. Most collected wild animals did not show clinical signs of rabies or other diseases. The direct fluorescent antibody test<sup>[22]</sup> with FITC-conjugated anti-rabies IgG was used as the primary method of routine diagnosis. Samples positive for RABV antigens were randomly sent for confirmation to the Federal Reference Diagnostic Centers in Vladimir and Omsk cities, where RT-PCR was additionally used<sup>[22]</sup>.

### Phylogenetic study

The list of RABV isolates analyzed phylogenetically is presented in [Table 1](#) and [Supplementary Table 2](#). Isolation of RNA from a 10% animal brain suspension, reverse transcription, amplification of cDNA fragments, and nucleotide sequencing were performed as described previously<sup>[20]</sup>. Phylogenetic analysis of partial RABV nucleoprotein (N) gene sequences (1,110 nt, positions 170-1,279 relative to Pasteur Virus genome, GenBank no M 13215) was performed using the maximum likelihood algorithm implemented in

**Table 1. Rabies virus isolates used for genetic analysis**

No.	Isolate number	GenBank number	Species	Location (coordinates, decimal degrees)	Year
1	2981/2018/Amur	MN384722	Cow	49.68 N, 128.78 E	2018
2	Amur8853 rd	ON246188	Raccoon dog	49.83 N, 128.37 E	2018
3	Amur8855 f	ON246189	Red fox	49.97 N, 129.17 E	2018
4	Amur8854 rd	ON526986	Raccoon dog	49.62 N, 128.65 E	2018
5	Amur8856 f	ON526987	Red fox	50.16 N, 128.80 E	2018
6	Amur8857 f	ON526988	Red fox	49.91 N, 128.37 E	2018
7	Amur8858 hor	ON526989	Horse	49.62 N, 128.65 E	2018
8	Amur8944 w	ON526990	Wolf	49.46 N, 129.00 E	2019
9	Amur8946 w	ON526991	Wolf	49.60 N, 128.77 E	2019

the MEGA X program<sup>[23]</sup>. For comparison, 38 N gene sequences of RABV isolates from various genetic lineages documented in Asia were retrieved from GenBank. Since the isolates of interest belong to the so-called Arctic-like-2 lineage, all the isolates of this group available in GenBank were included in the study, except for highly similar or identical ones. Bootstrap values were determined for 1,000 replicates by the maximum likelihood method.

### Cartography

For cartographic analysis, in addition to the ARR data, we used information on laboratory-confirmed rabies cases in neighboring regions of Russia: the Trans-Baikal Region ( $n = 49$ , 2014-2020)<sup>[21]</sup>, the JAR ( $n = 8$ , 2017-2019), the Khabarovsk Region ( $n = 25$ , 2018-2019), and the Primorsky Region ( $n = 2$ , 2013-2019) [Supplementary Table 3]. Mapping was performed using the QGIS 3.26.3 program based on the Natural Earth electronic landscape-geographic map (<https://www.naturalearthdata.com>) and OpenStreetMap database (<https://www.openstreetmap.org/>). Points on the map were plotted according to the geographical coordinates of where animal rabies cases were detected. The points of detection of genetic variants of the RV in the adjacent territories of China were transferred from the maps in previously published articles<sup>[16-19,24,25]</sup>. The speed of the epizootic spread was estimated by the distance from the point of the first case in 2018 to all other points in annual cycles, starting from July of the current year to June of the following year (resettlement time of a new generation of wild carnivores across the territory). The median (Me), minimum, and maximum distances are presented.

### RESULTS

The first laboratory-confirmed case of rabies in the ARR was documented in a cow on November 10, 2018, 30 km from the state border between Russia and China. The infection was presumably sourced from a fox that ran into the village and attacked dogs and farm animals. As a result of active surveillance, by the end of 2018, 16 rabies cases were detected in red foxes (*V. vulpes*), raccoon dogs (*N. procyonoides*), domestic dogs (*Canis lupus familiaris*), and farm animals [Table 2]. In March 2019, 3 rabies cases were documented in wolves (*Canis lupus*) that attacked humans. One of these wolves was observed by border guards within the guarded border strip in the floodplain of the Amur River. The outbreak was spreading northwesterly. During 2021, most rabies cases were detected in areas distanced by more than 200 km from the first case. Only two cases were registered in the first quarter of 2022 [Figure 1]. In total, during 2018-2022, animal rabies was detected in 13 administrative units of the ARR. In 2020, a woman died with typical symptoms of hydrophobia three months after being bitten by a domestic dog in a rabies-affected area [Figure 1]. The dog died the next day after a bite. Neither human nor dog samples were sent to a laboratory for rabies rule-out. The patient did not receive post-exposure prophylaxis.

**Table 2. Laboratory-confirmed rabies cases in the ARR during 2018-2022**

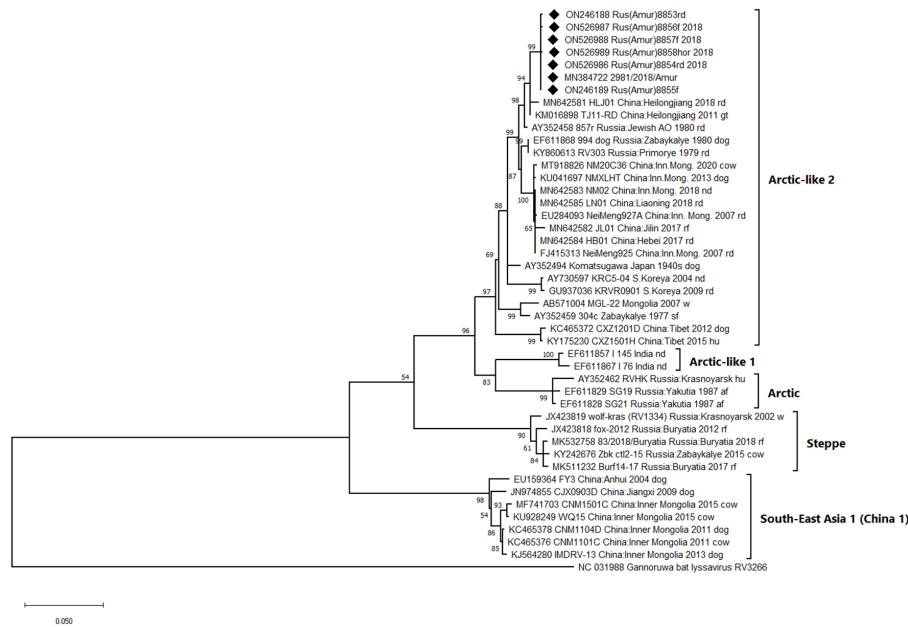
Species	Case number by years					total	Proportion, % (95%CI)
	2018	2019	2020	2021	2022		
Red fox	10	5	2	2	0	19	38.8 (25.2-52.4)
Wolf	0	3	2	2	0	7	14.3 (4.5-24.1)
Raccoon dog	3	1	0	1	0	5	10.2 (1.7-18.7)
Dog	0	1	1	6	1	9	18.4 (7.6-29.2)
Cattle	2	1	0	4	1	8	16.3 (6.0-26.6)
Horse	1	0	0	0	0	1	2.0 (0.0-6.0)
Total	16	11	5	15	2	49	100

ARR: Amur Region of Russia.

The main reservoir of the RABV during this recent outbreak was represented by wild carnivores, which accounted for 53.3% of all reported cases. Most often, especially at the beginning of the outbreak, the infection was detected in foxes [Table 2]. Dog rabies was reported mainly at the end of the analyzed period and mostly among stray dogs.

During 2018-2022, rabies spread within the forest-steppe and agricultural landscapes of the Zeya-Bureya Plain. The successive spread of rabies, mainly in the north and northwest direction from the place of first detection, can be traced on the map [Figure 1]. During the first epizootic cycle (2018-2019), the median of the distance from the primary case to other cases was 23.8 (7-107.2) km; in 2019-2020, it was 112.4 (77.6-128.9) km; in 2020-2021, it was 150.1 (57.1-192.4) km; in 2021-2022, it was 130.7 (57.1-121.4) km. The maximum distance from the first recorded event was 192.4 km for the observation period (Me = 77.6). As the epizootic wave progressed, cases in the initially affected areas decreased.

A full-size N gene nucleotide sequence (1,353 nt) of the RABV isolate from the first case in cows was determined, as well as 1,110 nt N gene fragments of six isolates from red foxes, raccoon dogs, and a horse and 556 nt N gene fragment of the two wolf isolates. The results of the phylogenetic analysis are shown in Figure 2 and Supplementary Table 2. The isolates from wolves (Amur8944w and Amur8946w) were not included in the tree because of the short lengths of their sequenced N gene fragment to prevent a decrease in tree resolution. The compared genome regions of the isolates collected during the first two months after outbreak detection were identical or differed by no more than one nucleotide. Thus, at the beginning of the outbreak, the ARR sequences shared 99.9%-100% identity. The genetic homogeneity of the isolates suggests a single initial source of the infection. It has been determined that isolates from the ARR belonged to the Arctic-like-2 genetic group of RABV and had a high degree of similarity (95.0%-99.4%) with isolates from neighboring regions of Russia and Northern China. They showed the maximum similarity to the TJ11-RD isolate from a goat (Tongjiang city, Heilongjiang province, China, 2011) - 99.4%, the HLJ01 isolate from a raccoon dog from the same province (the exact location is not known, 2018) - 98.6 %, as well as to isolate 857r from a raccoon dog (Leninskoe settlement, JAR, Russia, 1980) - 98.9%. It should be emphasized that the Leninskoye settlement in Russia and the Tongjiang city in China are located approximately 30 km from each other on opposite banks of the Amur River. Thus, this lineage of the RABV circulated in the Middle Amur Plain on both banks of the Amur River since 1980. The isolates also demonstrated 97.6%-98.3% nucleotide identity to Arctic-like-2 RABV sequences recovered in other regions of Russia, Northern China (Hebei, Jilin, Liaoning, and Inner Mongolia), and South Korea from 1977 to 2020. Even the identity of Japanese isolate Komatsugava recovered during the 1940s was as much as 97.2%. The genetic similarity of isolates from the ARR with RABV of the Arctic-like-1, Arctic, Steppe, and South-East Asia 1 (China-1) lineages does not exceed 92%.



**Figure 2.** The phylogenetic tree constructed by analyzing the nucleotide sequences of the 1,110 nt N gene fragment (position in the N gene: 100-1,209) of rabies virus isolates. Legend: The Gannoruwa bat Lyssavirus sequence was used as an outgroup. Square brackets indicate the genetic lineages of the rabies virus. Diamonds mark isolates from this study. The captions for the rest of the isolates indicate GenBank access number, name of the isolate, country, region of detection (if known), year of detection, and host animal species. Abbreviations for animal species: rd: raccoon dog; rf: red fox; sf: steppe fox (corsac); af: arctic fox; dog: dog; cow: cattle; w: wolf; gt: goat; hu: a person; nd: no data. Bootstrap values are presented for critical nodes.

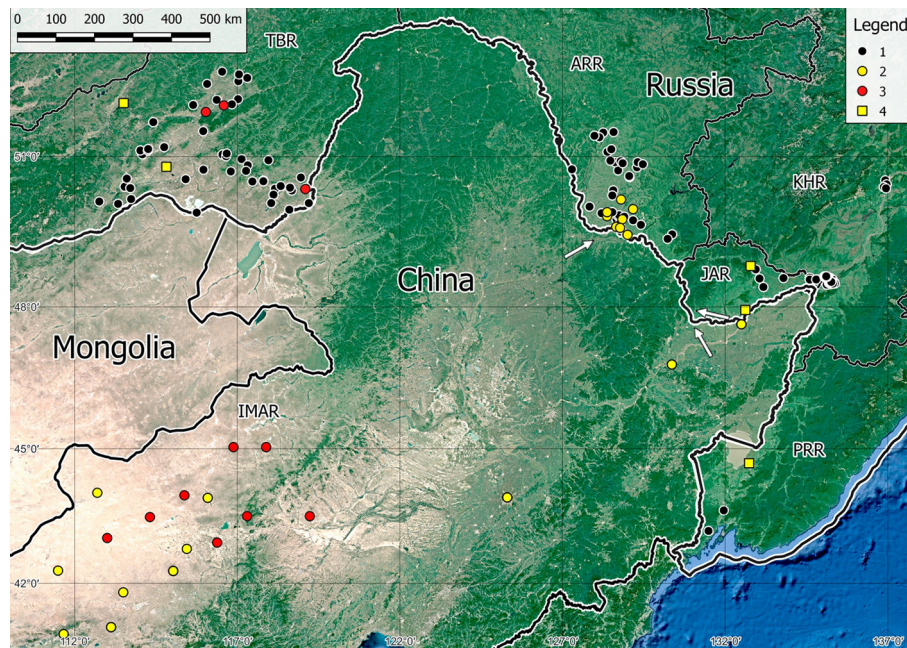
The spatial distribution of rabies in neighboring territories is presented in [Figure 3](#) and [Supplementary Table 3](#).

## DISCUSSION

In Russia, the nearest to the ARR rabies-enzootic territory is in the JAR, 200-300 km to the east, where the infection was historically present in lowland areas, including the Amur River valley<sup>[10,12,13,26]</sup>. During 2017-2018, animal rabies was documented in JAR<sup>[10]</sup>, just before the outbreak in ARR. Later, in 2018-2020, the fox rabies spread down the Amur River valley to Khabarovsk and Komsomolsk-na-Amure cities [[Figure 3](#)]. It appears that viruses of the same lineage were present in adjacent regions of China at least since 2011 (GenBank sequence KM016898; [Figure 2](#)). According to a report from China in 2013, "...several hundred foxes and raccoon dogs have been found dead in Bayannur, the Inner Mongolia Autonomous Region (IMAR), and Tongjiangcity, Heilongjiang, and herdsmen have reported dogs, cattle, goats, horses, and camels falling ill with rabies"<sup>[16]</sup>. In 2006, 2012, 2013, and 2019, human rabies cases were reported in Heilongjiang province<sup>[4,5]</sup>.

In the west, the nearest to the ARR rabies-enzootic area is in the Trans-Baikal Region (TBR) of Russia in the basin of the upper reaches of the Amur River and its tributaries. In the 1970s, animal and human rabies was prevalent in TBR. Several RABV isolates were collected and later identified as Arctic-like<sup>[14]</sup>. From 1983 to 2013, this region was considered rabies-free. Epizootic among foxes reemerged in 2014 because of an introduction of the Steppe genetic RABV lineage<sup>[10,21,27]</sup>. Thus, recent outbreaks in the Trans-Baikal Region of Russia and the ARR are disconnected and unrelated.

Since 2007, raccoon dog and fox rabies have been reported in the IMAR of China<sup>[16,17]</sup>. It has been established that RABV of the Steppe and Arctic-like-2 lineages are circulating here simultaneously; however,



**Figure 3.** Spatial distribution of documented animal rabies cases in the Amur River basin. (1) lineage was not determined; (2) Arctic-like 2 isolates; (3) Steppe isolates. Russian Far East: ARR - 2018-2022; TBR - 2014-2020<sup>[after 21]</sup>; JAR - 2017-2019; KHR - 2018-2019; PRR: adjacent territories of China (2007-2018)<sup>[after 16-19]</sup>; (4) detection of Arctic-like 2 isolates in Russia in 1977-1989<sup>[after 14, the locations were corrected according to original information about the samples collection]</sup>. Black lines: state and regional borders; Arrows: probable directions of rabies virus incursion into ARR. ARR: Amur Region; JAR: Jewish Autonomous Region; KHR: Khabarovsk Region; PRR: Primorsky Region; TBR: Trans-Baikal Region.

the Steppe lineage prevails. Currently, foxes play a pivotal role in the spread of rabies in the IMAR and some other provinces in northern China<sup>[5,18,25]</sup>.

Because of the data collected, two scenarios of RABV incursion into the ARR were suggested. It is most likely that rabies was introduced by wild carnivores from a natural focus located in the middle reaches of the Amur River. Mountain ranges sandwich the narrow valley of the Amur River in a section about 150 km long between the Zeya-Bureya and the Middle Amur Plains. The border between Russia and China lies along the Amur River. Due to the border protection, the floodplain territory, island, and coastal biotopes are little used for economic needs in both countries. The absence of anthropogenic press creates ample opportunities for wildlife, including migrations of carnivores along frozen rivers in winter, and may contribute to the transboundary spread of RABV. Another possible incursion route might occur from the Central Manchurian Plain along the valleys of the right-bank tributaries that flow into the Amur River near the site of the first reported ARR rabies case in 2018 [Figure 3]. However, in available publications, we could not find reports with georeferencing of rabies cases in the bordering area of China.

A retrospective analysis of the outbreak of rabies in the ARR demonstrates the importance of ecological channels and natural barriers for the spread of wildlife rabies. Presumably, the relative geographical isolation of the Zeya-Bureya Plain contributed to the conservation of the rabies-free status of the ARR for a long time. The Zeya-Bureya Plain is separated from similar landscapes in Russia and China by mountain ranges covered with taiga and coniferous-broad-leaved forests (dark green shading on the maps, Figures 1 and 3). The concentration of rabies cases along river valleys and at the junctions of forest and open landscapes is remarkable on this map. The habitats of mesocarnivores in the river floodplains and foothills are preserved even in conditions of intensive use of the plains for crops. It should be emphasized that rabies

had a similar landscape distribution in this area in the middle of the last century<sup>[12,13]</sup>.

Raccoon dogs were highly susceptible to experimental inoculation with the RABV strain isolated in the Russian Far East in 1980 (presumably Arctic-like-2), and all became ill even after a dose of 5 MICLD<sub>50</sub>. At the same time, some foxes did not become ill after a dose of 100 times more<sup>[28]</sup>. In 2007, raccoon dogs became ill during an outbreak of rabies caused by the Arctic-like-2 rabies virus on a fur farm, but rabies was not observed among foxes<sup>[16]</sup>. In the last century, several human rabies cases were associated with raccoon dog bites in adjacent territories of Russia and China<sup>[4,12,28,29]</sup>. Singular human rabies cases after fox bites began to be registered in the Russian Far East only in 2002<sup>[26]</sup> and in China since 2016<sup>[30]</sup>. During recent years, fox rabies has been detected repeatedly in the Amur River basin<sup>[9,10,25]</sup>. Comparison with data from 1949-1978 shows significant changes in ARR's affected species composition. In the past, dogs (48.6%) and farm animals (46.5%) dominated among the registered rabies cases, whereas cats and wild animals (wolf, raccoon dog) accounted for 2.8% and 2.1%, respectively. Foxes were not found in the list of rabid animals in ARR during that period<sup>[13]</sup>.

Thus, the present rabies outbreak in the ARR confirms the increasing role of red foxes as a host of RABV in Eastern Asia. The changes in the abundance of red foxes, raccoon dogs, and wolves in the Amur River basin under the influence of human activity are suggested among the factors that contributed to the activity of rabies epizootics, host shifts, and switches, introduction and re-introduction of the infection into rabies-free territories. Intensive farming, irrigation, sown area expansion, and decreased hunting pressure led to an increasing red fox population<sup>[10,13,21]</sup>. Simultaneously, the population of raccoon dogs in ARR decreased<sup>[13]</sup>. Perhaps we are currently witnessing an establishment of the Arctic-like-2 RABV to red foxes. As suggested by Yu *et al.*, in the past, this genetic lineage of RV evolved in Asia predominantly in dogs, domestic animals, and raccoon dogs<sup>[19]</sup>.

Since the outbreak, the rabies vaccination of dogs, cats, and farm animals in the ARR has increased by five times. Since 2019, oral vaccination of wild carnivores with the Rabistav live rabies vaccine made with the PB-97 strain (Stavropol, Russia) has been performed. It would be premature to expect a quick and lasting effect from these interventions. In 2021, rabies was diagnosed in foxes in Poland, where rabies had not been detected in over 17 years. This finding occurred despite Europe's intensive oral rabies vaccination program since 1993. New animal rabies cases were located 100 km inside the vaccination belt<sup>[31]</sup>.

The median distance from the first case to new cases during the observation period in the ARR was 77.6 km. At the beginning of the fox rabies epidemic in Europe in the middle of the last century, the average speed of epizootic spread was 30-60 km per year<sup>[32,33]</sup>. However, the rate of epizootic spread in the Russian Far East can be much higher since the wolf population remains high in contrast with Europe. Using GPS tracking, the maximum movement of a healthy wolf per day in the Trans-Baikal Territory was 97 km<sup>[34]</sup>. Thus, wolves can contribute to the long-distance introduction of the RABV along the Amur River valley. The regional features of the spatial RABV spread described in the article may help improve rabies prophylaxis and control in the Amur River basin.

## CONCLUSION

An outbreak of rabies in the ARR, registered in 2018 for the first time since 1972, arose because of an introduction of RABV that belongs to the Arctic-like-2 lineage, documented broadly in eastern Asia. Rabies was most likely introduced by wild carnivores from the adjacent territories of Russia or China, located downstream of the Amur River. During the freezing period, the Amur floodplain can serve as an ecological channel for the RABV spread. In 2018-2022, the epizootic expanded for about 200 km northwest within the



forest-steppe landscapes of the Zeya-Bureya Plain. An essential feature of the modern epizootic is the increased role of the red fox in RABV maintenance. International cooperation is essential for effective prophylaxis of the transboundary spread of rabies and its elimination in this region.

## DECLARATIONS

### Acknowledgments

The authors are grateful to the specialists of the veterinary and sanitary-epidemiological services: Samokhvalov SV, Solovey IV, and Yakovleva NV, for their help in data collection.

### Authors' contributions

Conceptualization and first draft of the manuscript: Botvinkin AD

Diagnosis, genetic study, and mapping: Poleshchuk EM, Chupin SA, Zarva ID, Zinyakov NG

Veterinary reports and other data collection: Meltsov IV, Sidorov GN, Yanovich VA

Writing - review and editing: Botvinkin AD, Metlin AE, Andaev E

All authors read and approved the final manuscript.

All authors contributed to the study.

### Availability of data and materials

The gene sequences are available in the GenBank database.

### Financial support and sponsorship

None.

### Conflicts of interests

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2023.

## REFERENCES

1. Gan H, Hou X, Wang Y, et al. Global burden of rabies in 204 countries and territories, from 1990 to 2019: results from the Global Burden of Disease Study 2019. *Int J Infect Dis* 2023;126:136-44. [DOI](#)
2. Minghui R, Stone M, Semedo MH, Nel L. New global strategic plan to eliminate dog-mediated rabies by 2030. *Lancet Glob Health* 2018;6:e828-9. [DOI](#) [PubMed](#)
3. Rupprecht CE, Mani RS, Mshelbwala PP, Recuenco SE, Ward MP. Rabies in the Tropics. *Curr Trop Med Rep* 2022;9:28-39. [DOI](#) [PubMed](#) [PMC](#)
4. Zhou H, Vong S, Liu K, et al. Human rabies in China, 1960-2014: a descriptive epidemiological study. *PLoS Negl Trop Dis* 2016;10:e0004874. [DOI](#) [PubMed](#) [PMC](#)
5. Liu Z, Liu M, Tao X, Zhu W. Epidemic characteristics of human rabies - China, 2016-2020. *China CDC Wkly* 2021;3:819-21. [DOI](#) [PubMed](#) [PMC](#)
6. Miao F, Li N, Yang J, et al. Neglected challenges in the control of animal rabies in China. *One Health* 2021;12:100212. [DOI](#) [PubMed](#) [PMC](#)
7. Yao HW, Yang Y, Liu K, et al. The spatiotemporal expansion of human rabies and its probable explanation in mainland China, 2004-2013. *PLoS Negl Trop Dis* 2015;9:e0003502. [DOI](#) [PubMed](#) [PMC](#)
8. Wang L, Tang Q, Liang G. Rabies and rabies virus in wildlife in mainland China, 1990-2013. *Int J Infect Dis* 2014;25:122-9. [DOI](#)

## PubMed

9. Feng Y, Wang Y, Xu W, et al. Animal rabies surveillance, China, 2004-2018. *Emerg Infect Dis* 2020;26:2825-34. DOI PubMed PMC
10. Poleshchuk EM, Sidorov GN. Comparative analysis of features of epizootiological and epidemic situation and risk of rabies infection in the Russian Federation in early XXI century. *Probl Partic danger infect* 2020;4:16-25. (In Russian). DOI
11. Botvinkin AD, Sidorov GN, Poleshchuk EM, et al. Retrospective evaluation of implementation of long-term forecast on spatial spread of rabies in the Asian part of Russia. *Probl Partic danger infect* 2020;2:13-21. (In Russian). DOI
12. Botvinkin AD, Savitsky VP, Sidorov GN, Yudin VG. Significance of the raccoon dog in the epidemiology and epizootology of rabies in the Far East. *J Microbiol Epidemiol Immunobiol* 1981;58:79-82. (In Russian). Available from: <https://elibrary.ru/item.asp?id=22637494>. [Last accessed on 27 Oct 2023].
13. Botvinkin AD, Zarva I D, Meltsov IV, et al. Rabies re-emergence after long-term disease freedom (Amur Oblast, Russia). *Vet Sci Today* 2022;11:309-18. (In Russian). DOI
14. Kuzmin IV, Botvinkin AD, McElhinney LM, et al. Molecular epidemiology of terrestrial rabies in the former Soviet Union. *J Wildl Dis* 2004;40:617-31. DOI
15. Deviatkin AA, Lukashev AN, Poleshchuk EM, et al. The phylodynamics of the rabies virus in the Russian Federation. *PLoS One* 2017;12:e0171855. DOI PubMed PMC
16. Liu Y, Zhang S, Zhao J, et al. Fox- and raccoon-dog-associated rabies outbreaks in northern China. *Viol Sin* 2014;29:308-10. DOI PubMed PMC
17. Shao XQ, Yan XJ, Luo GL, et al. Genetic evidence for domestic raccoon dog rabies caused by Arctic-like rabies virus in Inner Mongolia, China. *Epidemiol Infect* 2011;139:629-35. DOI
18. Tao XY, Guo ZY, Li H, et al. Rabies cases in the west of China have two distinct origins. *PLoS Negl Trop Dis* 2015;9:e0004140. DOI PubMed PMC
19. Yu X, Zhu H, Bo Y, et al. Molecular evolutionary analysis reveals Arctic-like rabies viruses evolved and dispersed independently in North and South Asia. *J Vet Sci* 2021;22:e5. DOI PubMed PMC
20. Adelshin RV, Melnikova OV, Trushina YN, et al. A new outbreak of fox rabies at the Russian-Mongolian border. *Viol Sin* 2015;30:313-5. DOI PubMed PMC
21. Zarva ID, Pavlov MV, Sorokovoi AA, Botvinkin AD. Application of GIS and earth remote sensing data for the evaluation of the spatiotemporal spread of rabies in eastern trans-baikal region. *Problemy Osobo Opasnykh Infektsii* 2021:100-7. DOI
22. World Health Organization. Laboratory techniques in rabies. 5th ed. In: Abela-Ridder B, Rupprecht CE, Fooks AR, editors. Available from: <https://www.who.int/publications/i/item/9789241515153>. [Last accessed on 27 Oct 2023].
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547-9. DOI PubMed PMC
24. Tao XY, Tang Q, Rayner S, et al. Molecular phylodynamic analysis indicates lineage displacement occurred in Chinese rabies epidemics between 1949 to 2010. *PLoS Negl Trop Dis* 2013;7:e2294. DOI PubMed PMC
25. Feng Y, Wang Y, Hada, et al. Diversity of rabies virus detected in Inner Mongolia, China, 2019-2021. *Transbound Emerg Dis* 2022;69:249-53. DOI
26. Ianovich VA. Epidemiology, epizootology and prevention of rabies in the Jewish Autonomous Region. (In Russian). Available from: <http://www.dslib.net/epidemiologia/jepidemiologija-jepizootologija-i-profilaktika-beshenstva-v-evrejskoj-avtonomnoj.html>. [Last accessed on 27 Oct 2023].
27. Yakovchits NV, Adelshin RV, Zarva ID, et al. Fox rabies outbreaks in the republic of Buryatia: Connections with neighbouring areas of Russia, Mongolia and China. *Transbound Emerg Dis* 2021;68:427-34. DOI
28. Botvinkin AD, Gribanova LYa, Nikiforova TA. Experimental rabies in raccoon dog. *J Microbiol, Epidemiol Immunobiol* 1983;60:37-40. (In Russian). Available from: <https://www.elibrary.ru/item.asp?id=23191761>. [Last accessed on 27 Oct 2023].
29. Sidorov GN, Poleshchuk EM, Sidorova DG. Changes in the role of mammals in the infection of humans with rabies in Russia over the historically foreseeable period in the 16-21 centuries. *Zoological Journal*. 2019;98:437-52. (In Russian).
30. Taxitiemuer A, Tuerdi G, Zhang Y, et al. An investigation of the first case of human rabies caused by a fox in China in May 2016. *Biomed Environ Sci* 2017;30:825-8. DOI PubMed
31. Smreczak M, Orłowska A, Trębas P, Stolarek A, Freuling C, Müller T. Re-emergence of rabies in Mazowieckie Voivodeship, Poland, 2021. *Zoonoses Public Health* 2023;70:111-6. DOI PubMed
32. Steck F, Wandeler A. The epidemiology of fox rabies in Europe. *Epidemiol Rev* 1980;2:71-96. DOI PubMed
33. Freuling CM, Hampson K, Selhorst T, et al. The elimination of fox rabies from Europe: determinants of success and lessons for the future. *Philos Trans R Soc Lond B Biol Sci* 2013;368:20120142. DOI PubMed PMC
34. Kirilyuk VE, Kirilyuk AV, Minaev AN. Wolf's home range and movements in daurian steppe. *Nat Conserv Res* 2019;4:91-105. (In Russian). DOI