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Rabies re-emergence after long-term disease freedom (Amur Oblast, Russia)

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SUMMARY

Retrospective descriptive epizootological study was conducted in the Amur Oblast (Russian Far East), where a rabies outbreak was reported in 2018. The aim of the study was to analyze probable routes of rabies introduction and features of its spatial and temporal spread in the territory that remained free from this infection from 1972 to 2018. In 2018–2021, altogether 1,416 animals were examined for the infection with the rabies virus. Forty-seven animal rabies cases were confirmed; the proportion of wild animals (*Vulpes vulpes*, *Nyctereutes procyonoides*, *Canis lupus*) amounted to 66%. The first cases were detected within 30 km from the state border with China. Nucleotide sequences of the nucleoprotein gene of three rabies virus isolates were determined and their belonging to the Arctic-like-2 genetic lineage was established. Genetically closest rabies virus isolates have been found in Heilongjiang Province (China, 2011, 2018) and Jewish Autonomous Oblast (Russia, 1980). Geographic information systems and open Earth remote sensing data were used to map the rabies cases. After 2018, the epizootic spread within the forest-steppe landscapes of the Zeya-Bureya Plain, where human and animal rabies cases had been earlier reported (until 1972). The front of the epizootic spread in a north-eastern direction at an average speed of 172 (16–302) km during one epizootic cycle. The introduction of the rabies virus was most likely along the Amur River valley from downstream regions of Russia and China that are rabies infected.

Keywords: rabies, spatiotemporal analysis, re-emerging infections, cross-border infections, Arctic-like-2

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Возвращение бешенства после многолетнего межэпизоотического периода (Амурская область, Россия)

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РЕЗЮМЕ

Проведено описательное ретроспективное эпизоотологическое исследование в Амурской области (Дальний Восток России), где в 2018 г. выявлена вспышка бешенства, целью которого было проанализировать возможные пути заноса и особенности пространственно-временного распространения бешенства на территорию, которая оставалась свободной от этой инфекции с 1972 до 2018 г. В 2018–2021 гг. на бешенство были исследованы пробы головного мозга, полученные от 1416 животных. Подтверждено 47 случаев бешенства, доля диких животных (*Vulpes vulpes*, *Nyctereutes procyonoides*, *Canis lupus*) составила 66%. Первые случаи выявлены на расстоянии до 30 км от государственной границы с Китаем. Определены нуклеотидные последовательности гена нуклеопротеина 3 изолятов вируса бешенства и установлена их принадлежность к генетической линии Arctic-like-2. Генетически наиболее близкие к ним изоляты вируса бешенства были выявлены в провинции Хэйлунцзян (Китай, 2011 и 2018 гг.) и Еврейской автономной области (Россия, 1980 г.). Для картографирования случаев бешенства использовали геоинформационные системы и открытые данные дистанционного зондирования Земли. После 2018 г. эпизоотия распространялась в пределах лесостепных ландшафтов Зейско-Буреинской равнины, где заболевания людей и животных регистрировалось в прошлом (до 1972 г.). Фронт эпизоотии распространялся в северо-восточном направлении со средней скоростью 172 (16–302) км за один эпизоотический год (цикл). Занос вируса бешенства наиболее вероятен по долине реки Амур из неблагополучных по бешенству районов России и Китая, расположенных ниже по течению.

Ключевые слова: бешенство, пространственно-временной анализ, возвращающиеся инфекции, трансграничные инфекции, Arctic-like-2

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INTRODUCTION

In spite of low rates of human rabies cases in the Russian Federation, control of this zoonotic infection remains among the topical governmental challenges. Since 2010, on average about 3,300 rabies cases have been annually reported in wild and domestic animals [1, 2]. Natural rabies outbreaks cover huge area, and over the recent decades the infection introduction is reported in the regions previously free from this zoonosis. In some regions of Russia, including Far East, the role of dogs as a source of infection for humans still remains and even increases [1, 3–5]. Dogs are the main rabies reservoir in China, where those virus variants prevail that are actively circulating in the zoonotic foci. Human morbidity is decreasing during the recent years but hundreds after the dog bites are annually reported [6, 7]. In light of these data, additional efforts are required in the eastern part of Asia under the WHO-initiated Global framework for the eradication of dog-mediated human rabies [8].

Rabies used to be widely spread in the Amur Oblast. In 1912, one of the first rabies prevention centers in the Far East was opened in Blagoveshchensk. Since that time, recording of the rabies incidence and animal bite victims have been launched. Before 1957, thirty-four human deaths from rabies were reported against the epidemics mostly in dogs. The latest outbreak was reported in cattle in 1972 presumably due to rabid wolf's attack on the herd [9]. Since then, the Amur Oblast was considered rabies free. In 2018, rabies cases were reported in wild and farm animals in the region after a many-year period of the disease freedom [2, 3].

The study was aimed at the review of possible routes of rabies introduction and its specific spatial and temporal distribution in the territory that remained free from the infection for over 45 years.

MATERIALS AND METHODS

Rabies incidence was subjected to retrospective analysis according to two time periods: 2018–2021 and 1946–1972. After 2018, official data on the animals with laboratory confirmed rabies diagnosis ($n = 47$) as well as outbreak investigation results were cumulated. In order to detect rabies virus circulation in the Oblast in 2018–2021, 1,094 samples from wild animals and 322 samples from domestic and farm ones were tested. Brain of the rabies suspected animals as well as of the animals hunted during the active virological monitoring were tested according to GOST 26075-2013 "Animals. Methods of Laboratory Diagnosis of Rabies". Cartographic analysis of human rabies incidence in 1949–1972 was performed based on the previously published data [9].

Mapping was made using QGIS 3.2.1 software, based on Natural Earth landscape map and Google Earth public data. The dots were put on the map according to the geographic coordinates of the place where animal rabies case was detected. Human cases were mapped according to the place of infection. Cartographic analysis of the current epidemics was made according to the epidemic years (hereafter, cycles) – from July this year and up to June next year [10]. The rate of the epidemic transmission was assessed according to the mean distance from the point where the first case was reported and to all cases detected during this and subsequent epidemic cycles (minimal and maximal values are specified in brackets).

Three rabies virus isolates collected in the region, where the first rabies cases had been detected in 2018, were subjected to the molecular and biological tests. RNA extraction from 10% animal brain suspension, reverse transcription, cDNA fragment amplification and their nucleotide sequencing were performed as earlier described [11]. The phylogenetic analysis of the rabies virus isolates was made using MEGA X software [12] and *maximum likelihood* estimation. For this purpose, N gene sequences deposited in GenBank electronic database were analyzed, including all deposited representatives of genetic lineage Arctic-like-2, some representatives of genetic groups Arctic-like-1 and Arctic as well as members of the group of steppe isolates geographically close to the Amur Oblast. Search and determination of the sequence likelihood degree was determined using BLAST search system[1].

Data on fox, wolf and raccoon dog population were taken from the web-site of the Amur Oblast Administration for Protection, Control and Management of Fauna and its Habitat [13] as updated [12, 14, 15].

RESULTS AND DISCUSSION

The first rabies case in the Amur Oblast was laboratory confirmed in cattle in 30 km from the state border on 10 November 2018 (Shurino, Mikhailovsky Raion). The infection presumably occurred as soon as a fox visited the settlement. Nearly at the same time, on 15 November, videoshooting captured obvious clinical signs in a fox attacking a dog and a car in the administrative center of the Raion (Poyarkovo) on Amur River bank[2]. The fox ran away but the diagnosis left no doubt. Shooting of wild animals was rapidly arranged in the Raion and it resulted in nine rabid foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) detected. Before the end of November, rabies was laboratory confirmed in a horse and cattle in the same Raion. In December 2018, active virological

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[1] BLAST. Available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi (date of access: 20.03.2022).

[2] A fox demonstrating rabies clinical signs was noticed in Amur village (video). Available at: https://www.amur.info/news/2018/11/15/146089 (date of access: 14.02.2022).

Table
Yearly and species distribution of the laboratory confirmed animal rabies cases in the Amur Oblast (2018–2021)

Animal species	Number of rabies cases/year					Share in the total number of cases, % (95%-confidence interval is in brackets)
	2018	2019	2020	2021	Total	
Fox	10	5	2	2	19	40.4 (26.3–54.5)
Wolf	0	3	2	2	7	14.9 (4.7–25.1)
Raccoon dog	3	1	0	1	5	10.7 (1.9–19.5)
Domestic dog	0	1	1	6	8	17.0 (6.2–27.8)
Cattle	2	1	0	4	7	14.9 (4.7–25.1)
Horse	1	0	0	0	1	2.1 (0–6.2)
Total	16	11	5	15	47	100

monitoring demonstrated rabies cases in foxes and in a raccoon dog in three neighboring Raions: Oktyabrsky, Konstantinovsky and Zavitinsky.

In March, 2019, three rabies cases in wolves (*Canis lupus*) in Mikhailovsky Raion received particular concern as the animals ran in the settlement and attacked humans. Frontier guards noticed one of the wolves in the protected border area. Active monitoring allowed for rabies identification in wild animals in three more Raions: Belogorsky, Bureysky and Oktyabrsky. In some settlements the locals came across dogs and foxes demonstrating rabies clinical signs, but such animals either escaped or were killed and burnt on the spot. In late 2019, the diseased foxes and cattle were reported in the Romensky Raion. In this Raion, as well as, in Tambovsky and Belogorsky Raions, the epidemic in wild animals continued until June, 2020. In 2021, the majority of the cases were detected in the Raions located at a considerable distance from the primary outbreak – in Seryshevsky, Svobodnensky and Mazanovsky Raions. However, in 2020–2021 no rabies cases were reported in the Raions that were infected in 2018. The latest case in the reported period was dated back to 22 October 2021. In 2018–2021, rabies was in total detected in 13 administrative raions of the Amur Oblast.

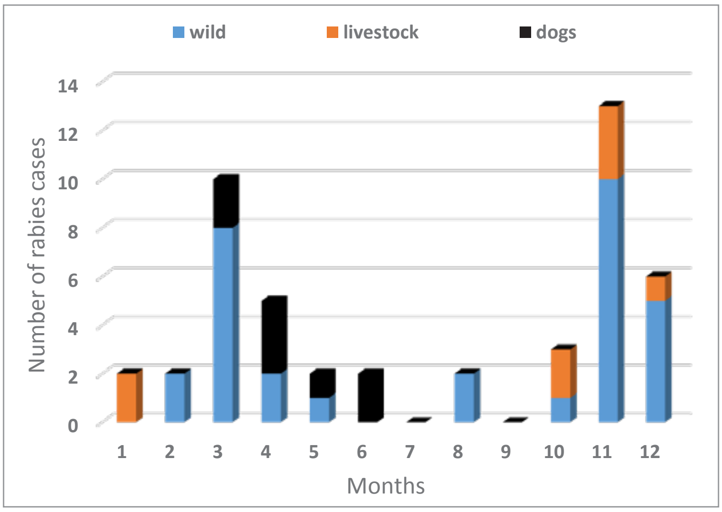

Fig. 1. Seasonal distribution of the confirmed animal rabies cases in the Amur Oblast, 2018–2021

Wild animals were the basic source of the rabies virus and their share amounted to 66% of the diseased animals. Rabies was most frequently detected in foxes, especially in the beginning of the epidemic (Table). Dog rabies was rarely reported, mainly at the end of the reported period and in the urban settlements. Moreover, five of the eight confirmed rabies cases were in dogs lacking the owner. The frequency of the rabies positive samples collected from animals during the active monitoring or from the diseased (dead) animals found in the wild or in the settlements amounted to 4.0% ($n = 326$) in 2018; 1.6% ($n = 576$) in 2019; 3.4% ($n = 117$) in 2020 and 6.7% ($n = 75$) in 2021. The sampling was performed both in the southern forest-steppe areas and in the northern taiga regions. The samples were collected from animals belonging to various systematic groups including mustelids (*Mustelidae*), ursids (*Ursidae*), cervids (*Cervidae*), rodents (*Rodentia*), but rabies positive results were demonstrated only in canines (*Canidae*).

The role of wild carnivorous animals as the reservoirs is supported by the analysis of the seasonal spread of the reported rabies cases. In the beginning of the autumnal increase in the incidence, rabies was reported in wild and domestic animals, and in the second part of the annual epidemic cycle (March – June) dog rabies was detected (Fig. 1). The majority of the reported cases fall on the cold seasons of the year.

N gene nucleotide sequences of rabies virus 2981/2018/Amur isolate recovered from cattle in the first epidemic outbreaks in 2018 were detected (full-size gene, 1,353 bp) as well as N gene nucleotide sequences of the isolates Rus(Amur)8853rd and Rus(Amur)8855f (gene fragment, 1,110 bp) recovered from the raccoon dog and fox, respectively, who were diagnosed as rabid ones in the neighboring settlements in 2018. The resulted nucleotide sequences were deposited to GenBank database under the designations MN384722, ON246188 and ON246189. Their analysis demonstrated identity of the compared genome regions of 2981/2018/Amur and Rus(Amur)8855f isolates, and only one nucleotide was different in the sequence of Rus(Amur)8853rd isolate.

Phylogenetic relationships of the studied isolates and other rabies viruses are demonstrated in Figure 2. The isolates from the Amur Oblast were demonstrated to have the highest relatedness (95.0–99.4%) with the viruses

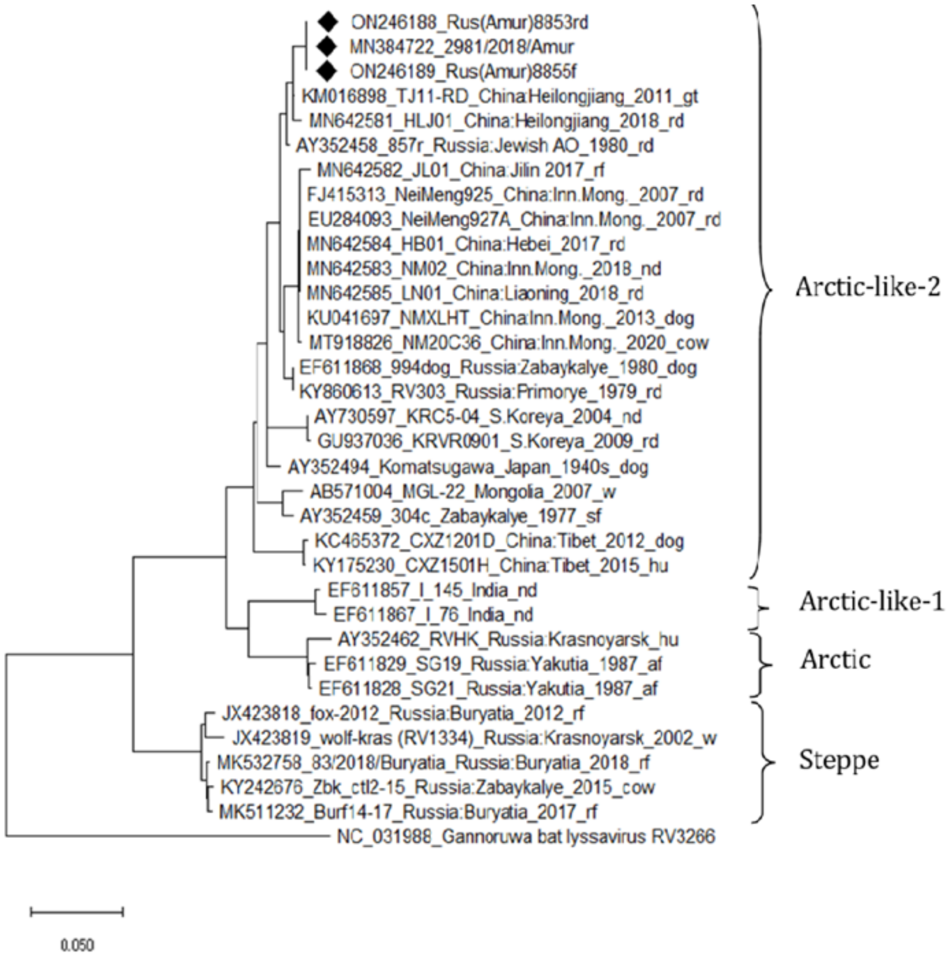

Fig. 2. Phylogenetic tree constructed according to the results of the phylogenetic analysis of nucleotide sequences of 1,110 bp fragment of N gene of the rabies virus isolates (location in N gene: 100–1,209). Gannoruwa bat lyssavirus sequence was used as an outgroup. Rabies virus genetic groups are in curly brackets. Amur Oblast isolates are marked with rhombuses. Designations of the rest of the isolates include: GenBank accession number, name of the isolate, country, region (if known) and year of detection, animal species the isolate was recovered from; rd – raccoon dog; rf – red fox, sf – corsac fox; af – Arctic fox; dog; cow – bovines; w – wolf; gt – goat; hu – human; nd – no data

belonging to Arctic-like-2 genetic lineage. The maximal relatedness was reported with TJ11-RD isolate recovered from the goat (Tongjiang city, Heilongjiang Province, China, 2011) – 99.4%; HLJ01 isolate recovered from the raccoon dog in the same province (precise location is unknown, 2018) – 98.6%, as well as with the Russian isolate 857r recovered from the raccoon dog (Leninskoye, Jewish Autonomous Oblast, Russia, 1980) – 98.9%. It should be emphasized that Leninskoye and Tongjiang mountain are located in approximately 30 km from each other in Amur River valley that is indicative of the persistent circulation of the closely related rabies virus variants in the plain areas in Russia and China along the middle flow of the Amur River. The Amur isolates demonstrate slightly lesser relatedness (98.3%) to the isolates recovered in the Zabaikalsky and Primorsky Krais (1979–1980), and even lesser relatedness (97.6–97.8%) to the isolates recovered in the Chinese provinces Hebei, Jilin, Liaoning and Inner Mongolia from 2007 to 2020. The relatedness to other Arctic-like-2 group members from China, Mongolia, South Korea and Japan amounts to 95.0–97.5%. Even lower genetic relatedness is reported between the Amur Oblast isolates and rabies viruses from the genetic groups Arctic-like-1, Arctic and Steppe, circulating in the neighboring areas – below 92% and 90%, respectively. By now, no viruses of Arctic-like-2 group have been reported in Siberia west to Lake Baikal and in the European part of Russia [3, 4, 16, 17].

Cartographic analysis results trace the consistent rabies spread mostly to the north and north-east from the site, where the first case was detected (Fig. 3). During the first epidemic cycle (2018–2019), the average distance from the primary outbreak to other outbreaks amounted to 49 (16–168) km; in 2019–2020 – 174 (119–201) km; in 2020–2021 – 229 (168–302) km; in the first half of the cycle in 2021–2022 – 237 (190–300) km. Therefore, the speed of the epidemic spread gradually decreased and averaged to 172 (16–302) km in one epidemic cycle. As the epidemic progressed, no disease cases were reported in the initially infected regions any more.

From the beginning of the current century the number of foxes increased and reached its maximum in 2011 (Fig. 4). Over the recent 10–15 years the fox hunting nearly stopped and their behavior changed [14]. Visits

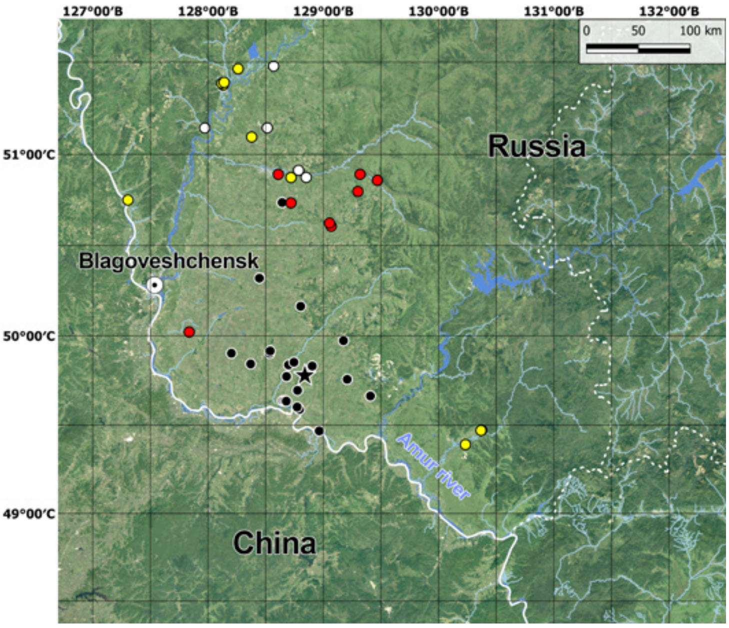

Fig. 3. Dynamics of rabies spatial spread in the Amur Oblast according to the epizootic cycles

● – *epizootic outbreaks before July, 2019;*

🔴 – *from July, 2019 to June, 2020;*

🟡 – *from July, 2020 to June, 2021;*

○ – *from July to December, 2021;*

★ – *first reported epizootic outbreak*

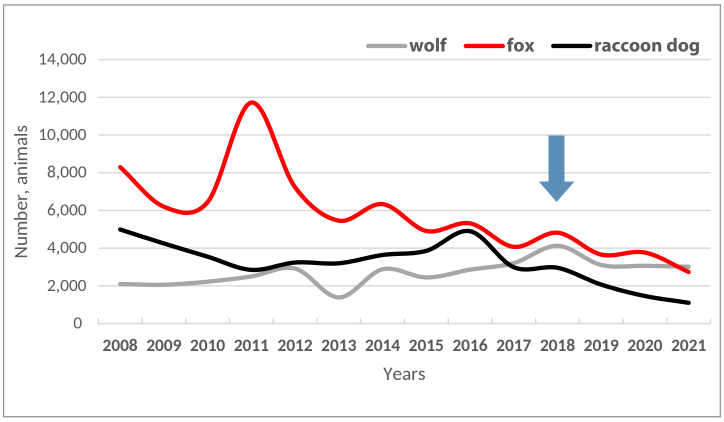

Fig. 4. Dynamics of wolf, fox and raccoon dog population in the Amur Oblast, 2008–2021 (arrow – start of epizootic)

of apparently healthy foxes to the settlements were reported as well as their non-aggressive contacts with humans. After the epidemic onset in 2018, the fox population decreased 1.8-fold and the number of the raccoon-dogs decreased 2.7-fold.

According to the long-term observations, in many areas of the Far East, including Amur Oblast, the number of raccoon dogs decreased along with simultaneous increase of the number of foxes. The ratio of these two species currently changed subsequently to the decrease of the raccoon dog-friendly habitat area due to land drainage and agricultural land expansion. The same processes facilitated growth of the number of foxes. According to the previous investigation results, the highest density of the raccoon dog and fox population is typical for forest-steppe and bottomland-meadow areas in the south of the Oblast: in optimal habitat it reached 7.3–10.4 and over 5.0 animals per 10 km^2 by the end of the century, respectively [14, 18, 19]. The epidemic was detected in the year, when the highest number of the wolf population was reported against the background of its gradual increase (Fig. 4).

In 2018–2021, rabies spread over the Zeya-Bureya plain, which is mostly treeless and ploughed up. There is a link of the reported outbreaks to the Amur, Zeya and other large river valleys (Fig. 5). In the middle of the previous century, the epidemics involving human cases demonstrated similar but wider spread including northern mountain-taiga Raions: Tyndinsky, Skovorodinsky, Mazanovsky (Fig. 6). The epidemics were annually reported from 1948 to 1954 and hereafter the outbreaks were registered in 1957–1958, 1960, 1967–1969 and 1972. Among the diseased animals, dogs and farm animals prevailed (48.6 and 46.5%); cats and wild animals amounted to 2.8 and 2.1%, respectively. However, among the sources of the rabies infection for humans, the percentage of wild animals (wolf, raccoon dog) reached 14.3%. No fox rabies was reported in this period [9].

Absence of rabies cases in humans and animals for 45 years is indicative of the epidemic termination in the Amur Oblast after 1972. In 1976–1977, total of 647 wild animals were tested in the region including 209 wild carnivorous mammals. No rabies virus was however detected [20]. Termination of the rabies virus circulation was facilitated by the relatively isolated geographic position of the Zeya-Bureya plain characterized by unique humid Manchurian type forest-steppes. Bureya ridge and Lesser Khingan separate it from similar Russian and Chinese landscapes located in the middle Amur River and its tributaries. Great Khingan and Yablonovy ridge spurs separate it from the Zabaikalsky Krai steppes and Inner Mongolia. The mountains are covered with taiga and mixed coniferous and deciduous forests (marked with deep green on the maps; Fig. 3, 5, 6).

The first rabies cases detected after the long inter-epidemic period in the settlements located in the south-eastern part of the Oblast near the state border are indicative of the imported origin of the epidemic. The length of the border with China, which passes along the Amur River, is over 1,200 km. The most probable route of the rabies virus introduction into the Amur Oblast can be the Amur River valley. Along 150 km between the Zeya-Bureya and Middle Amur plains it passes through the mountainous area and it is relatively narrow here. Another possible route is from the Central Manchurian Plain (China) along the valleys of the right-hand tributaries of the Amur River, which join Amur not far from Poyarkovo settlement. According to the aerospace survey data, the north-eastern parts of the Lesser Khingan are only partially covered with the forests, and there are significant areas of the agricultural lands (Fig. 5). Insular and littoral biotopes prevail in the Amur River plain bottom in the both countries, which are of little use due to the border regime (Fig. 7). This creates additional possibility for carnivorous mammal migrations after river freezing and can facilitate the transboundary introduction of the rabies virus during ice cover from November to March. Long-distance rabies

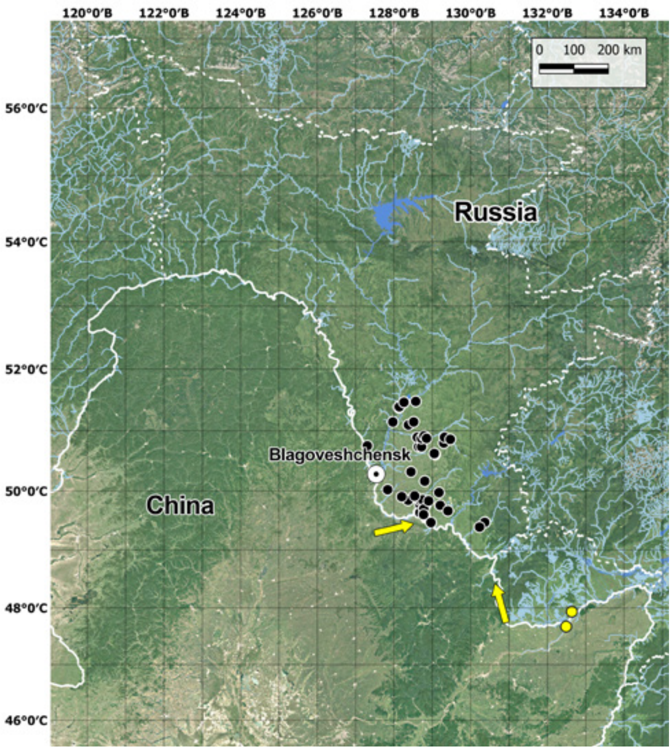

Fig. 5. Possible routes of rabies virus introduction into the Amur Oblast

● – *sites of outbreaks reported in the Amur Oblast in 2018–2020;*

🟡 – *sites of outbreaks reported in the Amur Oblast in 2018–2020;*

arrows – most probable routes of rabies introduction

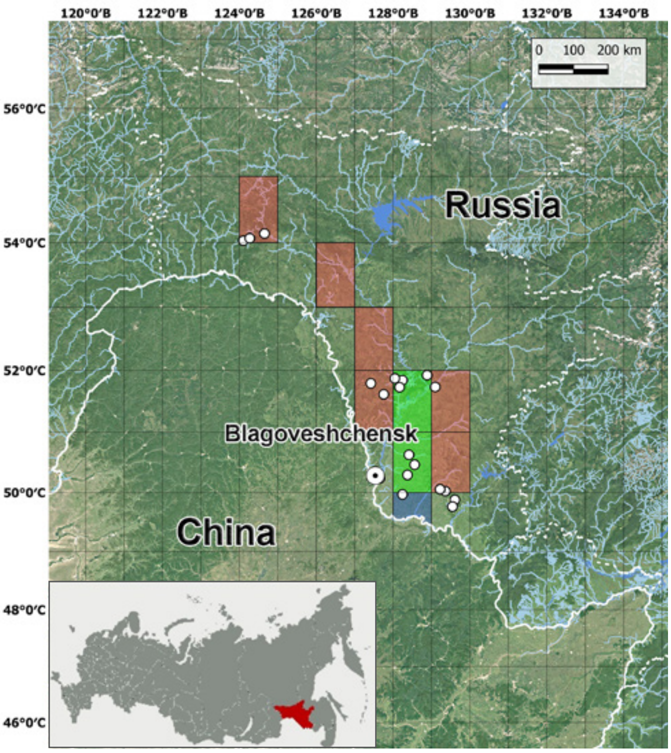

Fig. 6. Rabies spread in the Amur Oblast in 1945–1954 according to [8]

○ – *human rabies cases (according to the site of infection);*

🟩 – *animal rabies was reported for 1–2 years during the whole period;*

🟦 – *animal rabies was reported for 3–4 years;*

🟥 – *animal rabies was reported for 5 years and more;*

sidebar – Amur Oblast in the map of the Russian Federation

introduction into Siberia and Far East is generally associated with the wolves [9, 21], and coincidence of the rabies outbreak onset in 2018 with high number of the wolf population does not seem to be accidental (Fig. 4).

Phylogenetic analysis results support the opinion on the rabies virus spread along the Amur River valley from downstream located Chinese and Russian territories (Fig. 5). The virus was likely to be previously transmitted to longer distances, as before 1983 rabies virus Arctic-like-2 variants circulated in the Zabaikalsky Krai (Fig. 2). Representatives of the Manchurian fauna, including raccoon dogs, are known to enter the Zabaikalsky Krai along the Amur, Arguni and Shilka Rivers [19]. During the period from 1983 to 2014, this region remained rabies free as well as the Amur Oblast. In 2014, the epidemics re-emerged in the south-eastern part of the Zabaikalsky Krai due to introduction of the rabies virus of Steppe genetic lineage from the west [2, 21], and they are not currently directly associated with the epidemics in the Amur Oblast. The rabies virus circulation however continued in the territory located 200–300 km from the Amur Oblast downstream Amur River (Jewish Autonomous Oblast, Heilongjiang Province) [2, 16, 22, 23]. The ranges of the two above mentioned rabies virus genetic linages overlap much further south – in the steppes of Inner Mongolia (China) [22, 23–25].

It should be emphasized that current epidemic was caused by the rabies virus variant previously associated

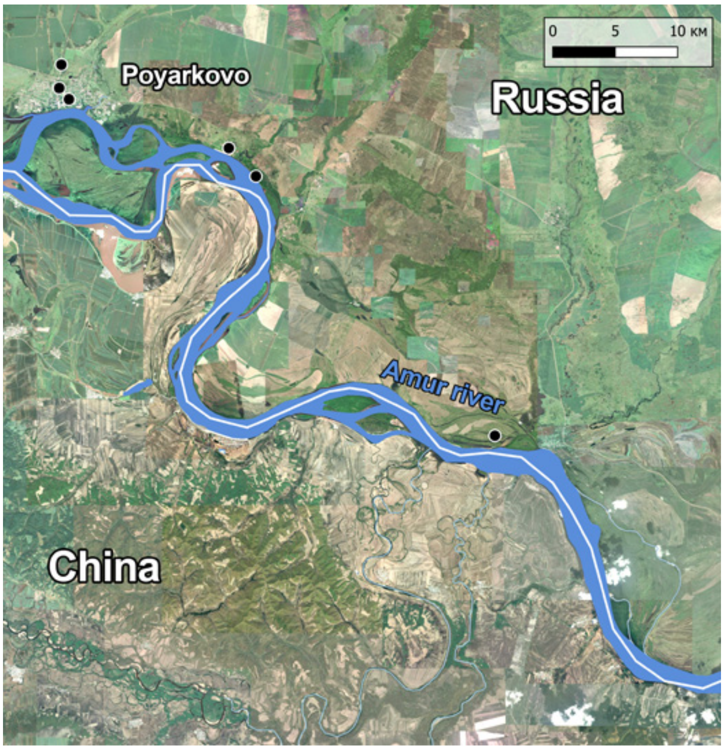

Fig. 7. Airspace image of Russia and China border areas with the sites, where the first rabies cases were reported in the Amur Oblast in 2018

with intense epidemics in dogs in the Zabaikalsky Krai, Primorsky Krai and Amur Oblast that were responsible for the human deaths [5, 21]. It is well known that dogs were and remain the main source of the infection for humans [2, 4, 22]. It was experimentally proved that the virus strain isolated in the Far East in 1980 adapted to raccoon dogs and to lesser extent to foxes. In that time raccoon dogs played a more significant role as a source of infection for humans and domestic animals [26]. Singular disease cases resulted from fox bites were reported in the Far East of Russia only after 2002 [22]. In view of the increased role of foxes in the circulation of Arctic-like-2 genetic lineage in Amur River area, changes of the biological properties of the viruses in the group are possible.

As previously predicted, as soon as introduced, rabies spread over the same regions as in the last century [22, 27]. The factors aiding to the rabies re-emergence in the long-term disease free territories included changes in the amount and migration activity of the foxes, wolves and raccoon dogs in the south of the Far East. Numerous unusually high rises of the numbers of foxes and raccoon dogs in the Amur Oblast are historically known. They were accompanied with mass mortality of the animals due to undetermined reasons [19, 20] and coincided with the large-scale rabies epidemics in dogs [9].

After the epidemic onset in the Amur Oblast the vaccination coverage of dogs, cats and farm animals increased drastically in the Amur Oblast: from 30 thousand animals in 2018 up to 155 thousand animals in 2019–2020. Oral vaccination of wild carnivores with "Rabistav" vaccine has been implemented since 2019 (120–240 thousand doses/year). The data demonstrated in this paper can be used for planning the vaccination strategies and coverage as well as for other rabies control measures. The current situation in the region is scientifically perspective for the examination of the rabies epidemic frequency in the Amur River basin and virus evolution upon change of the basic host (raccoon dog → fox).

CONCLUSION

Rabies epidemic started in the Amur Oblast in 2018 after the introduction of rabies virus Arctic-like-2 variant, which prevails in the east of Asia. Rabies was most likely introduced with the wild animals from the neighboring territories of Russia and China located in the Middle Amur Valley downstream of the Amur River. During freezing period, the Amur overflow land can serve as an ecological channel of the rabies virus transmission. Similar to the last century, the epidemic spread mostly over the open terrains of the Zeya-Bureya valley. The key feature of the current epidemic is drastically increased significance of foxes in the circulation of the rabies virus of Arctic-like-2 genetic lineage. Natural rabies foci associated with this rabies virus variant have transboundary origin.

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