CORONAVIRUSES IN RODENTS AND INSECTIVORES IN ALTAI REPUBLIC

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ABSTRACT

Coronaviruses (family Coronaviridae, genera Alphacoronavirus, Betacoronavirus) are dangerous viral pathogens that have caused three outbreaks of severe respiratory diseases SARS, MERS, COVID-19. In Russia, data on coronaviruses in natural reservoirs are limited, as investigations began only during the COVID-19 pandemic.

The aim of the work. *To study the diversity of coronaviruses among rodents and insectivores in the Altai Republic.*

Materials and methods. *Rodents (n = 67) and shrews (n = 52) were captured in 2022. Samples were analyzed by reverse transcription-polymerase chain reaction followed by sequencing.*

Results and conclusions. *Four samples from rodents (Myodes rutilus, M. glareolus, Apodemus peninsulae, A. agrarius) and two samples from an insectivore (Crocidura sibirica) were positive for coronaviruses, among which three different coronaviruses were detected. Rodent-borne coronaviruses are classified in the genus Betacoronavirus, subgenera Embecovirus, and have shown host associated clustering. The nucleotide sequences of Siberian coronaviruses from rodents were identical for closely related species (M. rutilus and M. glareolus, A. agrarius and A. peninsulae) and close (> 94 % homology) to previously published sequences in each of the groups of carriers found in the territory Novosibirsk region, Europe and China. The coronavirus identified from the insectivore, possibly belonging to a new subgenera of the family Coronaviridae, has also been assigned to the genus Betacoronavirus.*

Conclusion. *Five species of natural carriers of three different coronaviruses were detected in the Altai Republic. A high level of identity of coronaviruses genomes from rodents has been revealed, indicating a relatively low rate of their evolution.*

Key words: *coronavirus, taxonomy, rodents, shrews, Siberia*

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КОРОНАВИРУСЫ, ЦИРКУЛИРУЮЩИЕ СРЕДИ ГРЫЗУНОВ И НАСЕКОМОЯДНЫХ В РЕСПУБЛИКЕ АЛТАЙ

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РЕЗЮМЕ

Коронавирусы (семейство Coronaviridae, роды Alphacoronavirus и Betacoronavirus) относятся к числу опасных вирусных патогенов, вызвавших три вспышки тяжёлых респираторных заболеваний SARS, MERS, COVID-19. В России данные о коронавирусах, циркулирующих в природных резервуарах, ограничены, так как исследования начались только во время пандемии COVID-19.

Цель работы. *Исследование многообразия коронавирусов среди грызунов и насекомоядных в Республике Алтай.*

Материалы и методы. *Грызуны (67 особей) и насекомоядные (52 особи) были отловлены в 2022 г. Образцы проанализированы методом обратной транскрипции – полимеразной цепной реакции и последующим секвенированием.*

Результаты и обсуждение. *Положительными на коронавирусы оказались 4 образца от грызунов (Myodes rutilus, M. glareolus, Apodemus peninsulae, A. agrarius) и 2 образца от насекомоядного (Crocidura sibirica), в которых обнаружены 3 различных коронавируса. Ассоциированные с грызунами коронавирусы отнесены к роду Betacoronavirus, подроду Embecovirus и демонстрировали филогенетическое группирование в соответствии с видом природного носителя. Нуклеотидные последовательности сибирских изолятов коронавирусов от грызунов были идентичными для носителей близкородственных видов (M. rutilus и M. glareolus, A. agrarius и A. peninsulae) и близки (> 94 % гомологии) к ранее опубликованным последовательностям в каждой из групп носителей, обнаруженных на территории Новосибирской области, Европы и Китая. К роду Betacoronavirus отнесён и коронавирус, выявленный от насекомоядного, возможно, относящийся к новому подроду семейства Coronaviridae.*

Заключение. *Обнаружены 5 видов природных носителей 3 различных коронавирусов на территории Республики Алтай. Выявлен высокий уровень идентичности геномов коронавирусов от грызунов, свидетельствующий об относительно низкой скорости их эволюции.*

Ключевые слова: *коронавирус, таксономия, грызуны, насекомоядные, Сибирь*

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INTRODUCTION

Coronaviruses (family *Coronaviridae*, genera *Alphacoronavirus* and *Betacoronavirus*) are among the dangerous viral pathogens that have caused three outbreaks of severe respiratory diseases SARS, MERS, COVID-19 in the last two decades [1–3]. After the SARS outbreak in 2002–2003, epidemiological and etiological studies confirmed that SARS-CoV entered humans via civets [4]. Subsequent field and experimental studies showed that civet was only an intermediate host of SARS-CoV and bats were its natural host [5]. MERS-CoV, which first appeared in the Middle East in 2012, also originates from bats and infects humans via camels [6]. After COVID-19 was first revealed in Wuhan, its etiological agent, a novel coronavirus later named SARS-CoV-2, was not only immediately identified based on genome studies, but also the virus was found to have high homology with a known bat coronavirus identified in 2018 [3, 7, 8]. Additionally, recent studies have revealed that human coronaviruses such as OC43, 229E, HKU1 and NL63 also originate from bats or rodents [9, 10].

Coronaviruses are characterized by high genetic diversity in wild small mammals. Over a long evolutionary history, viruses have not only co-evolved with their hosts, but have also often crossed the interspecies barrier and spread between species and evolved to adapt to new hosts [11]. A very diverse range of coronaviruses has been consistently detected in rodents in recent years, some of which circulate simultaneously in several rodent species or even several subfamilies [12–15]. Recent studies have shown that OC43 and HKU1 coronaviruses, which cause human respiratory diseases, can also be originated from rodents [9, 16]. Along with rodents, small mammals such as shrews, which are also highly genetically diverse, can also be carriers of various coronaviruses. Shrew coronaviruses have recently been discovered to have important evolutionary significance [17].

The problem of coronavirus research is relevant for Russia, since no epidemiological studies were conducted to reveal coronaviruses in natural reservoirs before the pandemic. Only in 2020, SARS-like coronaviruses (Khosta-1 and Khosta-2) were identified in two species of bats of the horseshoe bat genus in the Krasnodar region of the Russian Federation and their full-length genomes were studied [18]. Subsequent studies revealed that the receptor-binding domain of one of the novel coronaviruses is capable of utilising the human ACE2 receptor and poses a potential risk to humans [19]. Another coronavirus was revealed among Pipistrellus bats in the central part of European Russia [20]. The evolutionary history of this novel MERS-like coronavirus suggests recombination of the ancestral genomes from bat and hedgehog. Studies of coronaviruses among rodents and insectivores were first conducted in Siberia (Novosibirsk Region) [21]. Circulation of viral coronaviruses was found in three species of rodents and one species of insectivores. The rodent-associated coronaviruses belonged to the *Betacoronavirus* genus, evidenced phylogenetic grouping according to the natural host species, and showed similarity to related coronaviruses from China and European countries. A new coronavirus species was revealed among insectivores, which belonged to the *Alphacoronavirus* genus.

THE AIM OF THE STUDY

To study the diversity of coronaviruses among rodents and insectivores in the Altai Republic.

MATERIALS AND METHODS

Small mammals were captured on the northern coasts of Lake Teletskoye in the Altai Republic (51° 47´ N, 87° 18´ E). Animals were captured in accordance with the protocol and recommendations for safe work in compliance with methodological guidelines MU 3.1.1029-01, approved on April 6, 2001; cotton swabs were used to collect bat oral swabs; rectum pieces were placed in a solution with RNA Later (QIAGEN, Germany).

RNA isolation was performed using the RIBO-prep kit (Central Research Institute of Epidemiology, Russia) from lavages or rectal slices. 0.2 ml of sterile 0.9% NaCl solution was added to the tubes with lavages before isolation. Samples were screened by two-round reverse transcriptase-polymerase chain reaction (RT-PCR) using RevertAid Premium RNA polymerase (Thermo Fisher Scientific, USA), Hot Start Taq DNA polymerase (Sibenzyme, Russia) according to the protocol and with primers for the conserved region of the coronavirus RNA-dependent RNA polymerase (*RdRp*) gene (fragment 397 nucleotides) that have previously been described [12]. The nucleotide sequences of each amplicon chain were determined using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, USA) on an ABI Prism 310 automatic analyser. A taxonomical identification of coronaviruses and their natural hosts was based on the determination and comparison with the GenBank database of the nucleotide sequences of viral genome fragments and the cytochrome b gene of the hosts' mitochondrial DNA.

Nucleotide sequence alignment was performed using the MUSCLE algorithm in MEGA X software (Mega Software, USA). The maximum likelihood (ML) method with the GRT + G + I evolutionary model was used to construct the phylogenetic tree. Calculations were performed for 500 repeats.

RESULTS AND DISCUSSION

In September 2022, 119 specimens of 10 species of small mammals were captured within the forest area located in the vicinity of the village of Artybash in the Altai Republic (Table 1). All captured animals were tested for the presence of coronavirus RNA.

3.8 % (2/52) of insectivores and 5.9 % (4/67) of rodents were positive for coronaviruses (Table 1). Viral RNA was revealed in 2 out of 11 Siberian shrew (*Crocidura sibirica*), 1 out of 5 bank voles (*Myodes glareolus*), 1 out of 15 red-backed voles (*Myodes rutilus*), 1 out of 20 field mice (*Apodemus agrarius*) and 1 out of 15 Korean field mice (*Apodemus peninsulae*).

Based on the results of phylogenetic analysis, the rodent-borne coronaviruses (*M. rutilus*, *M. glareolus*, *A. peninsulae* and *A. agrarius*) were classified in the subgenus *Embecovirus* of the genus *Betacoronavirus*. The shrew-borne virus detected in *C. sibirica*, was classified in the genus *Betacoronavirus* and is probably a representative of a new subgenus, as it differs significantly from other known coronaviruses (Fig. 1).

The nucleotide sequences of the RdRp gene fragment of new RNA isolates of coronaviruses identified in rodents, have a homology level of 86.7 % between isolates from animals of the genus *Myodes* and *Apodemus*, are identical for closely related species *M. rutilus/M. glareolus* and *A. peninsulae/A. agrarius* and demonstrate close similarity with previously published sequences of coronaviruses found in each of the host groups. Specifically, for the new coronavirus isolates from *M. glareolus/M. rutilus* (RtCoV/Mg-724/RUS/2022 and RtCoV/Mrut-816/RUS/2022), a similarity level of 95.8 % and 93.8–94.9 % was found with the RtCoV/Mrut-288/RUS/2021 RNA isolate identified from *M. rutilus* in the Novosibirsk region and in Germany (D_RMU10_1974/Myo_gla/GER and D_RMU10_1919/Myo_gla/GER), respectively. For Siberian isolates of coronavirus from the Altai Republic from *A. agrarius/A. peninsulae* (RtCoV/Ap-709/RUS/2022 and RtCoV/Aa-818/RUS/2022), a high level of homology was revealed (97.2 % and 98.3–98.6 %, respectively) both with RtCoV / Aa-528/RUS/2021 isolate from *A. agrarius* from the nearby Novosibirsk Region, and with geographically remote strains from China (RtAa/SX2014) and Germany (KS11_0997/Apo_agr/GER/2011), respectively.

The coronavirus identified in two specimens of the Siberian white-toothed shrew (ShrewCov/Cs-711/RUS/2022 and ShrewCov/Cs-764/RUS/2022) differs by more than 28.0 % in nucleotide sequence from other representatives of the genera *Alfacoronavirus* and *Betacoronavirus*. The highest level of difference (45.0 %) was found with coronavirus from another insectivore species, the common shrew (*Sorex araneus*) (ShrewCoV/Sa-314/RUS/2021), circulating in the nearby Novosibirsk region. In contrast to rodent-associated coronaviruses, coronaviruses from insectivores revealed higher genome variability. Specifically, the level of sequence differences obtained from two Siberian white-toothed shrew captured at the same site was 2.0 %. Considering the high level of variation in the conserved *RdRp* gene with representatives of other genera and species, it can be assumed that the RNA isolate from *C. sibirica* is a representative of a new subgenus of the *Betacoronavirus* genus. This requires sequencing the full-length genome and analysing it according to the criteria of the international committee of viral taxonomy [22].

We have studied the diversity of coronaviruses circulating among rodents and insectivores in a natural focus located in the Altai Republic. Co-circulation of several coronavirus species has been shown for the same location, as was previously reported [21]. All the studied animals were captured in the vicinity of the Teletskiy scientific station of the Institute of Systematics and Ecology of Animals SB RAS (Siberian Branch of the Russian Academy of Sci-

TABLE 1

SPECIES COMPOSITION OF INSECTIVORES AND RODENTS TESTED FOR CORONAVIRUS RNAs

Carriers	Type	Coronavirus RNA+/studied
Insectivores	Common shrew (*Sorex araneus*)	0/39
	Taiga shrew (*Sorex isodon*)	0/1
	Laxmann's shrew (*Sorex caecutiens*)	0/1
	Siberian shrew *(Crocidura sibirica)*	2/11
Rodents	Korean field mouse (*Apodemus peninsulae*)	1/15
	Striped field mouse (*Apodemus agrarius*)	1/20
	Root vole (*Alexandromys oeconomus*)	0/3
	Grey-red-backed vole (*Myodes rufocanus*)	0/9
	Red-backed vole (*Myodes rutilus*)	1/15
	Bank vole (*Myodes glareolus*)	1/5

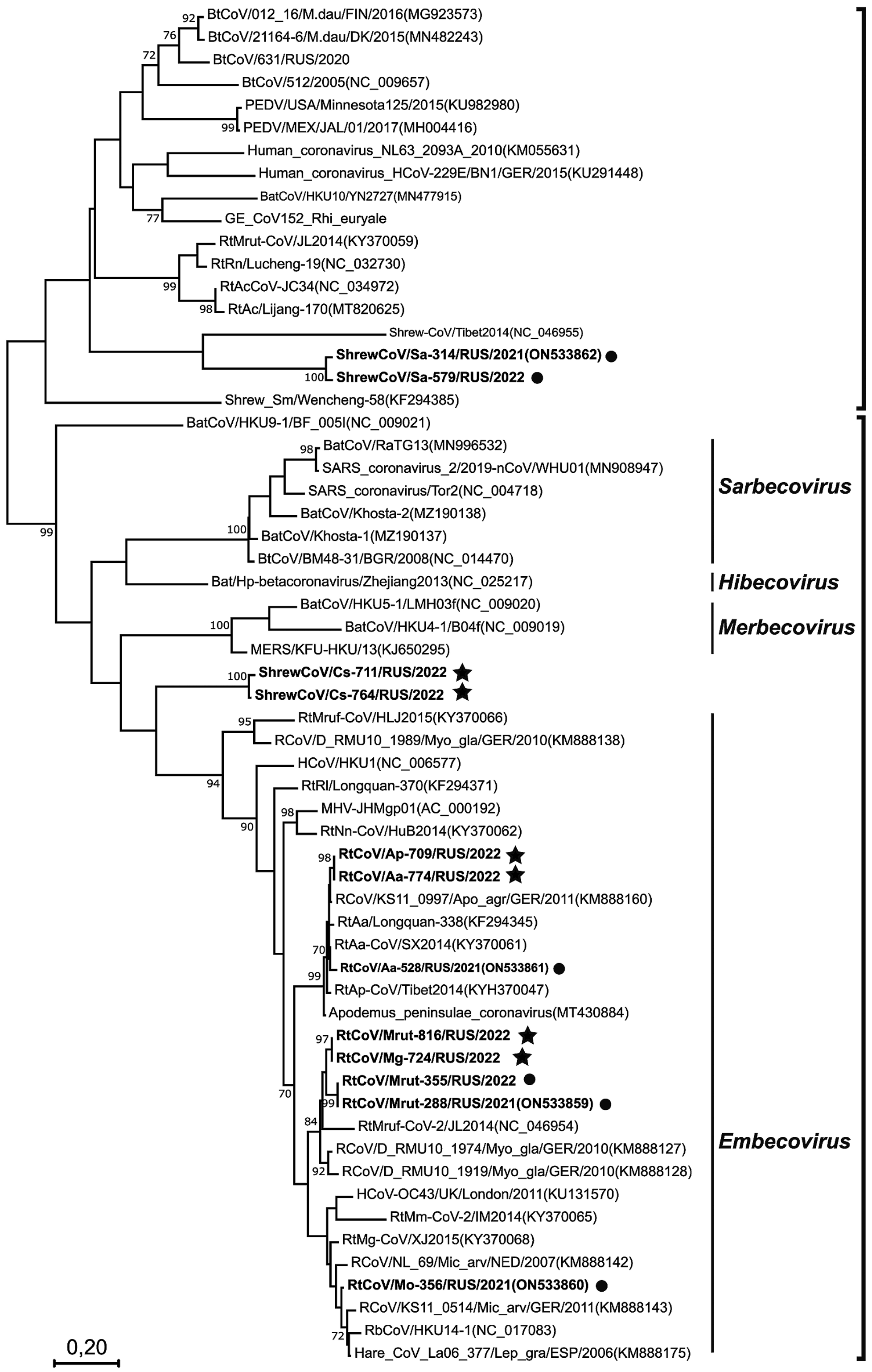

FIG. 1.

Phylogenetic analysis of the nucleotide sequences of the RdRp gene fragment of coronaviruses corresponding to positions 15429-15825 of the HKU-1 isolate (Genbank NC_006577). New coronaviruses obtained from Myodes glareolus (RtCoV/Mg-724/RUS/2022), Myodes rutilus (Rt CoV/Mrut-816/RUS/2022), Apodemus peninsulae (RtCoV/Ap-709/RUS/2022), Apodemus agrarius (RtCoV/Aa-818/RUS/2022) and Crocidura sibirica (ShrewCoV/Cs-711/RUS/2022, ShrewCoV/Cs-764/RUS/2022), were compared with known CoVs belonging to the genera Alphacoronavirus and Betacoronavirus. The tree is constructed using the maximum likelihood method and the GRT + G + I evolution model. Support indices were calculated for 500 repetitions, and support indices (> 70 %) are represented at the respective nodes. The scale bar indicates the number of nucleotide substitutions per site. New isolates are highlighted in bold and asterisk, isolates from the Novosibirsk region are highlighted in bold and circle ences) within a forest area of no more than 0.2 km^2. Co-circulation of two rodent-borne coronaviruses and one coronavirus carried by an insectivorous host, the Siberian shrew, has been demonstrated in a limited area.

Coronaviruses associated with natural carriers belonging to genus *Myodes* and *Apodemus* have previously been reported to be classified into two genera – Alfacoronavirus and Betacoronavirus [15]. New Siberian isolates from rodents classified in the *Embecovirus* subgenus of the genus *Betacoronavirus* were characterised by high (93.8–95.8 % and 97.2–98.3 %) homology with previously known isolates in each group, found over a wide area including Europe (Germany), Siberia (Novosibirsk region) and China, with sequences from a single capture site in the Altai Republic being identical. These findings indicate relatively high stability of coronavirus genomes compared to other RNA-containing viruses associated with rodents. Accordingly, the difference in *RdRp* gene sequences in hantaviruses (family Hantaviridae, genus *Orthohantavirus*) found in the same host species from geographically distant regions of Siberia and Europe was more than 20 % [23]. A higher level of variation in partial nucleotide sequences of the *RdRp* gene was found among coronaviruses from insectivores. The level of sequence difference from two specimens of Siberian shrews was 2.0 %, which is comparable to the level of difference between Seewis hantavirus isolates from the same location [24].

CONCLUSION

Three different coronaviruses were detected among 4 species of rodents and one species of insectivores living together in the natural biotope in the Altai Republic. Coronaviruses from rodents (*M. glareolus/M. rutilus* and *A. agrarius/A. peninsulae*) are classified in the genus *Betacoronavirus* subgenus *Embecovirus*, coronavirus from an insectivore (*C. sibirica*) is classified in the genus *Betacoronavirus* and is assumed to belong to a new subgenus. The genomes of rodent-associated coronaviruses were revealed to be highly stable.

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Conflict of interest
The authors of this article declare no conflicts of interest.

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