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Terrestrial and Subterranean Mammals as Reservoirs of Zoonotic Diseases in the Central Part of European Russia

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Abstract: Russia has a number of historical foci of zoonotic anthropogenic diseases. In Central Russia, the Republic of Mordovia is one of such areas, a region being known to have foci of haemorrhagic fever with renal syndrome (HFRS) and tularemia. It therefore requires continuous monitoring. The role of small terrestrial mammals as reservoirs of zoonoses has been previously proven for the region. The aim of this work is to take an integrated approach to assess the role of terrestrial and subterranean small mammals. Subterranean mammals are often not considered important reservoirs of zoonotic pathogens that cause human morbidity. Among small mammals in the wild environment, the bank vole, the yellow-necked mouse and the house mouse play important roles as vectors of zoonoses. Among wild subterranean mammals, the greater mole rat is important as a vector of tularemia and HFRS. We analyzed homogenized internal organs of these animals (lungs, spleen, kidneys). Of all samples from the greater mole rat, 83% were positive for tularemia antigens and 17% were positive forHFRS. None of the analyzed European moles had antigens of tularemia and HFRS. No double infection with both tularemia and hantavirus was detected in the subterranean mammals. Double infection was found among terrestrial mammals in the bank vole and the forest dormouse.

Keywords: rodents; underground mammals; epidemiology; hantavirus; tularemia; diversity

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1. Introduction

Emerging infectious diseases are carried by animals, and mammals are dominating hosts, therefore biogeographic patterns require research on mammal reservoirs [1]. Transmissions of pathogens and parasites from mammals to humans are dangerous, and their frequencies are increasing [2]. Alien species are important for the spread of zoonotic disease [3], but local mammal species also require attention.

In Russia, mammals of four orders (Rodentia, Lagomorpha, Eulipotyphla and Carnivora) were found infected with tularemia, hemorrhagic fever with renal syndrome (HFRS) and leptospirosis. The circulation of tularemia pathogens was detected in 62, leptospirosis in 55, and hantaviruses in 59 regions of the country [4,5]. The epizootic situation in the country is assessed from a wide range of data on the number and structure of populations of reservoir hosts and vectors of infections, taking into account the seasons of the year, the infection of animals with pathogens of zoonoses, their spread across the territory in various biotopes, and weather. The importance of individual mammal species in the epizootiology of zoonoses varies and depends on many factors. Ecosystems are constantly affected by various environmental factors. This in turn affects the functioning of natural foci and, as a consequence, leads to changes in the role of individual mammal species. In some years, one species may play a leading role as a reservoir, while in other years it may be replaced by other species. Infected animals have been reported among more than

40 species [4–6]. Based on the analysis of the data presented, in 2021, epidemic complications in the form of sporadic cases of the disease tularemia among the unvaccinated population in the following territories were most likely to occur: Central Federal District—in the Oryol, Ryazan and Yaroslavl regions, as well as in Moscow; Northwestern Federal District—in the Arkhangelsk and Leningrad regions, the Republic of Karelia and in St. Petersburg; Volga Federal District—in Tatarstan, Mordovia, Chuvash Republic, Kirov and Orenburg regions; Ural Federal District—in the Khanty-Mansiysk, Yamalo-Nenets Autonomous Districts and the Tyumen region; Siberian Federal District—in the Novosibirsk, Kemerovo, Tomsk and Omsk regions, as well as in the Altai Territory; and the Far Eastern Federal District—in some regions of Kamchatka and Khabarovsk Territories [7].

In Central Russia, one of these territories is the Republic of Mordovia. This region is known for foci of hemorrhagic fever with renal syndrome, tularemia and rabies [8]. Therefore, it requires constant monitoring. Earlier, the role of the bank vole (*Clethrionomys glareolus*), the common vole (*Microtus arvalis*) and the house mouse (*Mus musculus*) as carriers of zoonotic pathogens was proven for the region [9].

Reservoir hosts such as mole-rats and moles harbor microbes that cause the diseases tularemia and HFRS [10–15]. Animals of the genus *Spalax* were carriers of other infections [16]. Greater mole-rat (*Spalax microphthalmus*) in the Rostov region belongs to the representatives of Group I, possessing the properties of susceptibility and infectious sensitivity to tularemia, which determine the possibility and degree of participation in the epizootic process [17]. There are three groups of mammals in Russia in relation to the tularemia pathogen: highly susceptible species (Group I—56 species), highly susceptible but insensitive species (Group II—26 species), low susceptible and almost insensitive species (Group III—19 species) [18].

The European mole (*Talpa europaea*) has been found in 50 regions of the country [19], including Mordovia. Population numbers of that species are low [20,21]. However, it is not a rare species in comparison with other related species from the order Eulipotyphla [22]. *Spalax microphthalmus* was registered in 20 regions of the country [23,24]. In the Volga region it inhabits only some regions on the border of the range. Therefore, the population of this rare species is low. Such a region is the Republic of Mordovia [25].

Lagomorphs and rodents seem to have importance in the transmission of infections [26]. In Mordovia, rodents were the main carriers of zoonotic infections [9,27,28]. Peak densities of rodent populations may trigger tularemia outbreaks.

The aims of this article were: (i) to analyze the role of terrestrial and subterranean small mammals as carriers or zoonotic disease reservoirs, and (ii) to provide data on the occurrence of these diseases in humans. There is a possibility that subterranean mammals *T. europaea* and *S. microphthalmus* are important as reservoirs in the circulation of zoonotic pathogens.

2. Materials and Methods

2.1. Study Area

The material for the work was collected in four regions (north to south): the Republic of Mordovia, the Voronezh region, the Rostov region and the Stavropol region (Figure 1). The Mordovia and the Voronezh regions are both located in the central European part and the other two regions (the Stavropol region and the Rostov region) are located in the southern European part.

The Republic of Mordovia (area 26,200 km^2, 298 km west–east, 140 km north–south) is situated in the central part of the Russian Platform, on the north-western slopes of the Volga Upland, while the western part of the area is in the Oka-Don lowland [29].

The Voronezh region (area 52,216 km^2, 354 km west–east, 278 km north–south) is situated to the south of the Republic of Mordovia [30].

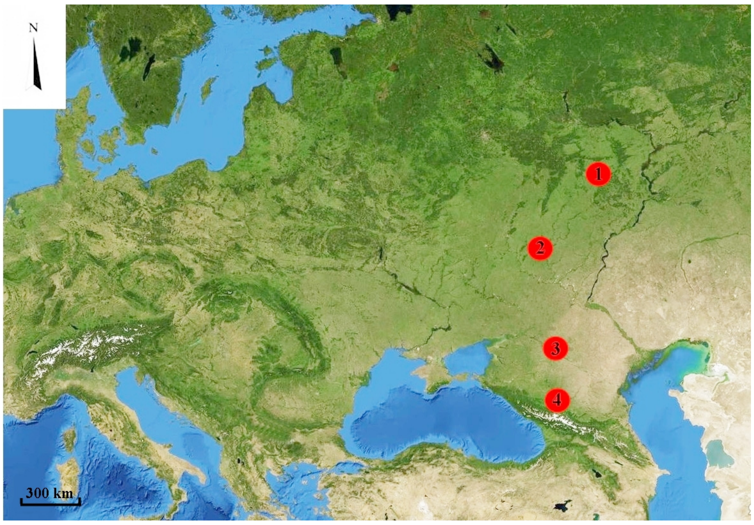

Figure 1. Study area. Regions where material was collected are indicated by numbers: 1—Republic of Mordovia (central point 54.132743 N, 45.125624 E); 2—Voronezh region (50.979972 N, 41.214608 E); 3—Rostov region (46.637851 N. 42.443879 E); 4—Stavropol region (43.836050 N, 42.683688 E). Studies were conducted near the village of Yalga and the city of Saransk (Mordovia), near the village Yellow Ponds (Novokhopersk district, Voronezh region), in the vicinity of the village Proletarsky (Orlovsky district, Rostov region),and 7 km south of the city of Kislovodsk (Predgorny municipal district, Stavropol region, 43.905518 N, 42.715718 E).

The Rostov region (area 100,800 km^2, 455 km west–east, 470 km north–south) is located in the river basin of the Lower Don. The maximum heights of the relief vary at around 250 m above sea level. Basically, the whole area is represented by plains; only from the north the Central Russian Upland is captured a little, and in the west—the eastern part of the Donetsk Ridge. In the southeastern part of the region there is an upland of the Salsko-Manych Ridge. The relief of the region is flat; the predominant natural zone is the steppe; there are few forests—they cover only 5.6 percent of the land fund, while most of the region is occupied by farmland, mainly on highly fertile black soil [31].

The Stavropol Territory (area 66,160 km^2, 370 km west–east, 285 km north–south) mostly is occupied by the Stavropol Upland, which passes in the east into the Terek-Kuma Lowland (Nogai Steppe). In the north, the Stavropol Upland merges with the Kuma-Manych Depression [32].

Since most of the material was collected in Mordovia, we consider it appropriate to describe the biotopes in which the capture of mammals was carried out. The collection of material was carried out in four main different biotopes (stations) of the forest-steppe zone—in forest stations, meadow-field, near-water and urban. Forest biotopes were represented by different types of forests, from mixed to deciduous forests. Meadow-field biotopes were open stations in the form of grass–forb meadows with clumps of shrubs and single trees. Near-water stations were alder thickets with various combinations of tree species and dense tall herbage. The urban biotope was a territory near private one-story houses in the village and the city. Often these areas were overgrown with weeds. Ten lines were set for each biotope at different points. At each point, 7000 trap-days were worked out. The total trapping effort in the four biotopes was 280,000 trap-days.

2.2. Small Mammal Trapping Methods

Captures of terrestrial small mammals were carried out in 2021 and 2022 using traditional methods of trapping lines and trapping ditches [33]. Gero-type traps (manufacturer Stayer Standard, St. Petersburg, Russia) were installed in lines of 25 pieces in different biotopes. Bread soaked in sunflower oil was used as bait. The lines were moved to a new place after the animals stopped falling into traps. Traps were checked daily. *Talpa* *europaea* trapping was carried out using mole traps. *Spalax microphthalmus* were captured using homemade holding traps. A trap was placed for each burrow system. Previously, the hole was dug up and a trap was placed in it. From above, the trap was closed with a board and sprinkled with soil. Capturing only a small number of underground mammals depended on the low population density of *S. microphthalmus* in the study area in Mordovia. Therefore, the capture of underground mammals (Figure 2) was not massive, but selective and random. In other regions, single *S. microphthalmus* were used for analysis.

(a) (b)

Figure 2. Photo of subterranean mammals in Mordovia: (**a**)—*Spalax microphthalmus*, (**b**)—*Talpa europaea*. Photographs by A. Andreychev.

2.3. Molecular and Statistical Analyses

An important stage of research in the analysis of the epizootic situation in the natural focus of infections waslaboratory studies of the organs of animals caught to identify infection with pathogens that are dangerous to humans. The internal organs of mammals (spleen, kidneys, lungs) were used for analysis. All organs were stored at −20 °C and then transported to the laboratory of the Center for Hygiene and Epidemiology of the Republic of Mordovia. Homogenized organs were preliminarily obtained from the internal organs in a mortar with a pestle, and then an electrolyte was added. To identify the antigen of the HFRS pathogen, samples of lung bank voles were studied usingthe indirect method of fluorescent antibodies (MFA) using the polyvalent «Diagnosticum of HFRS» («Diagnosticum of hemorrhagic fever with renal syndrome, cultural, polyvalent for the indirect method of immunofluorescence» produced by FGANU (FNCIRIP named after M.P. Chumakov, RAS, Russia) according to the manufacturer's instructions. The laboratory lacked certified equipment for the identification of species of hantavirus. To identify the tularemia antigen and DNA of the causative agent of tularemia, spleens and kidneys were taken from the mammals. The search for the tularemia antigen was carried out by the serological method using the antibody neutralization reaction (RNAt) from suspensions from animal organs. Real-time polymerase chain reaction (RT-PCR) was used to detect the DNA of the causative agent of tularemia (*Francisella tularensis*) [34,35]. The results were recorded on a photometer for enzyme immunoassay at a wavelength of 492 nm. The duration of the optical density measurement procedure did not exceed 15 min. The results were considered positive if the optical density of the samples was 2 or more times higher than the optical density of the negative control.

Data analysis was performed using MS Excel (Microsoft Corporation, Redmond, Washington, DA, USA). For proportions, 95% confidence interval (CI) was calculated using Quantitative Parasitology software, Qpweb version 1.0.15 (https://www2.univet.hu/qpweb/qp10/index.php, accessed on 30 October 2022) [36]. Comparison of proportions was conducted using a G-test under package RV Aide Memoire [37], implemented via R version 4.0.2 [38] in Rstudio ver.1.3.1093 (https://www.rstudio.com/products/rstudio/older-versions/#rstudio-desktop-131093, accessed on 5 April 2021).

3. Results and Discussion

During two years of research, 440 small mammal individuals were trapped. The dominant species was *C. glareolus* (46.8% ofthe total number, 95% CI = 42.1–51.6%). Five other small mammal species with proportions over five percent of the total number were: striped field mouse, *Apodemus agrarius* (13.6%, CI = 10.6–17.2%); yellow-necked mouse, *Apodemus flavicollis* (8.9%, CI = 6.4–11.9%); house mouse, *M. musculus* (7.9%, CI = 5.6–10.9%); common vole, *M. arvalis*, (6.1%, CI = 4.1–8.8%); and common shrew, *Sorex araneus* (5.5%, CI = 2.6–8.0%).

The proportions of the other species were less than five percent each: Ural field mouse, *Apodemus uralensis* (2.5%, 95% CI = 1.3–4.4%); Norway rat, *Rattus norvegicus* (1.1%, CI = 0.4–2.6%); common hamster, *Cricetus cricetus* (0.7%, CI = 0.1–2.0%); Eurasian water shrew, *Neomys fodiens*, European water vole, *Arvicola amphibius* and forest dormouse, *Dryomys nitedula* (0.5%, CI = 0.1–1.6% each); and harvest mouse, *Micromys minutus*, (0.2%, CI = 0.02–1.3%).

The proportions of the subterranean small mammals were 3.9% (CI = 2.3–6.4%) for *T. europaea* and 1.4% (CI = 0.5–2.9%) for *S. microphthalmus.*

The relative number of small mammals ranged from 4.3 individuals/100 trap-daysin 2021to 8.1 individuals/100 trap-days in 2022.

The laboratory determined that among the samples from the small mammals there were significantly more positive results for tularemia than for HFRS (12.4%, CI = 9.4–15.5% and 7.8%, CI = 5.5–10.7%, respectively, G = 4.59, $p < 0.05$). Mixed infection of these two pathogens was detected in 1.4%, CI = 0.5–3.0%, of investigated individuals. As the same individual can be infected with several pathogens at the same time, this poses a serious threat to public health (Table 1). Similar trends of focality for terrestrial mammals was noted in Mordovia also in previous years [27]. Based on the fact that *C. glareolus* has the main role of a carrier of tularemia (61%, CI = 46.9–74.1% of all individuals were infected), long-term forecasting for the next years is possible based on the trapping results. In the natural environment of Mordovia, *A. flavicollis* was ranked second for presence of tularemia bacteria (27.8%, CI = 16.5–41.6%), while in *A. amphibius*, 3.7% (CI = 0.5–12.8%) of all trapped individuals were infected. The inter-species difference is significant (G = 43.4, $p < 0.0001$).

In many regions of the republic, first of all, in the floodplain areas of the Sura River, where the natural focus of this disease persists for a long time, *C. glareolus* was also the main carrier of the dangerous disease HFRS. The infection rate of *C. glareolus* was 67.6% (CI = 49.5–82.6%). *Apodemus flavicollis* ranked second for presence of HFRS (14.7%, CI = 5.0–31.1%), and *D. nitedula* ranked third (5.9%, CI = 0.7–19.7%). Carriers of zoonotic infections havealso been identified among the subterranean mammals. Of all *S. microphthalmus* analyzed, 83% (CI = 35.9–99.6%) were positive for tularemia antigens. Hantavirus antigen was detected in 17% (CI = 0.4–64%) of *S. microphthalmus* (Table 1). Dual infections with both tularemia and hantaviruses were not detected in the subterranean mammals. Double infection was found among terrestrial mammals in the bank vole and the forest dormouse. It was previously established that the same small mammals can be infected with several pathogens at the same time, therefore representing a serious threat to public health [28]. All captured *T. europaea* were free from both tularemia and HFRS.

A positive relationship between morbidity in the human population and the number of small mammals was revealed for HFRS; 53 cases of human HFRS in 2021 and 126 cases in 2022 corresponded with relative abundance, this being 4.3 and 8.1 individuals per 100 trap-days, respectively. There were no cases of tularemia with in the human population. In the general structure of infectious and parasitic morbidity of the population, HFRS has a significant share.

Table 1. Number and proportions of small mammals involved as reservoirs of tularemia and HFRS, 2021–2022. (*n*—number of specimens, TUL—number (%) of the positive tests for tularemia, HFRS—number (%) of positive tests for HFRS, TUL+HFRS—number (%) of simultaneous detection of positive tests for tularemia and HFRS, M—Mordovia, V—Voronezh, R—Rostov, S—Stavropol).

Species	*n*	TUL	HFRS	TUL + HFRS
Clethrionomys glareolus	206	33 (16.1)	23 (11.2)	5 (2.4)
Microtus arvalis	27	0	0	0
Rattus norvegicus	5	0	0	0
Arvicola amphibius	2	2 (100)	0	0
Dryomys nitedula	2	1 (50)	2 (100)	1 (50)
Cricetus cricetus	3	0	0	0
Apodemus flavicollis	39	15 (38.5)	5 (12.8)	0
Apodemus uralensis	11	0	0	0
Apodemus agrarius	60	0	1 (1.6)	0
Mus musculus	35	0	3 (8.5)	0
Spalax microphthalmus	3 (M)	3 (100)	0	0
Spalax microphthalmus	3 (R,S,V)	2 (66.7) (R,S)	1 (33.3) (V)	0
Micromys minutus	1	0	1 (100)	0
Sorex araneus	24	0	0	0
Talpa europaea	17	0	0	0
Neomys fodiens	2	0	0	0

Natural foci of HFRS functioned throughout the territory of the Republic of Mordovia; the most active of them were detected in the Zubovo-Polyansky district (36 cases of infection, 65.5 per 100 thousand of population, CI = 65.1–65.9). In the Temnikovsky district, eight cases of human infection were registered (57.3 per 100 thousand of population, CI = 56.5–58.1). Other territories were less affected. In the Saransk city district there were 71 cases of HFRS (20.4, CI = 20.3–20.5 per 100 thousand of population), in the Bolshebereznikovsky district and the Torbeevsky district the human infection rate was 16.0, and in the Kovylkinsky district it was 15.6 per 100 thousand of population.

The main mode of infection in humans last year was contact with small mammals and their secretions at home (53 cases). A lower number of cases was related to contact of humans with small mammals and their secretions at work, working in the garden, being in the forest, caring for animals, construction work, hay-making and fishing (Figure 3).

Among the districts of the republic, positive registration of HFRS was noted in the Bolsheignatovsky district (3), Dubensky district (2), Chamzinsky district (2), Ruzaevsky district (1), Ichalkovsky district (3), Zubovo-Polyansky district (2), Bolshebereznikovskydistrict (1), Kovylkinsky district (3), Romodanovsky district (1), Torbeevsky district (1), and Saransk city district(5). Positive findings oftularemia were found in the Romodanovsky district (5), Kovylkinsky district (5), Ichalkovsky district (2), Bolshebereznikovsky district (3), Atyashevsky district (1), Chamzinsky district (2), Zubovo-Polyansky district (3), Bolsheignatovsky district (1), Ruzaevsky district (1), and Saransk city district (5) (Figure 4).

The main factors determining the level of morbidity of the HFRS population are: biological processes in populations of small mammals; infection of the local population as a result of infection by small mammals in the environment; infection of the pendulum—migrating population (city–village–city) in the village when visiting relatives; gardening; animal care; delivery of infected potatoes and others food from the village home and to the market; the use of the territory for recreational purposes; infection in suburban and garden work; and infection in the forest and forest plantations.

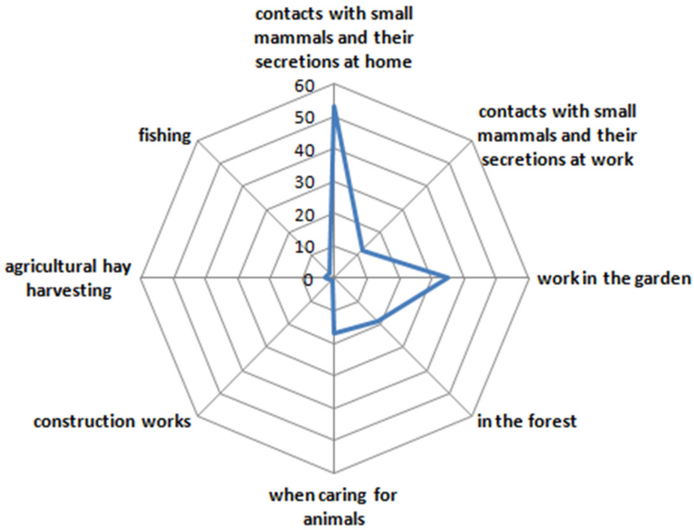

Figure 3. Ways of infection of the population with HFRS during the research period, 2021–2022.

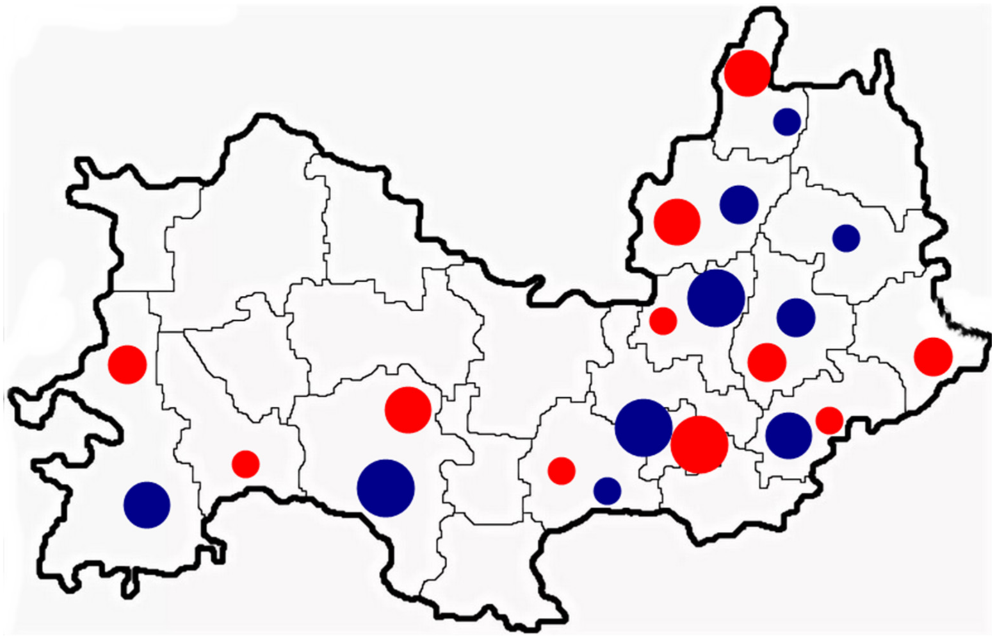

Figure 4. Distribution and magnitude of cases of infection with HFRS (red circles) and tularemia (blue circles) among people of Mordovia.

Based on our results, underground rodents, such as *S. microphthalmus* can be dangerous carriers of zoonotic infections. The same cannot be said for subterranean insectivorous mammals (*T. europaea*). *Spalax microphthalmus* are more susceptible to zoonotic infections from lodgers in their burrows than are *T. europaea*. In previous studies it has been shown that the burrows of these two species are used by other small rodents and insectivores [21]. Thus, interspecific tularemia infection passing from lodgers to *S. microphthalmus* should be considered common, perhaps as an evolutionary trait. In particular, it is already known that the evolution of hantaviruses was accelerated during interspecific animal contacts [39].

Comparing *S. microphthalmus* from different regions for the possibility of being carriers of zoonotic infections, we came to the conclusion that they can be carriers of tularemia in Mordovia's Stavropol region and Rostov region, even though the distance from Saransk to Kislovodsk is more than 1100 km. The likelihood of acting as carriers of hantaviruses is lower, and was confirmed only in the Voronezh region. However, mole rats may not always be carriers of pathogens. For example, in Israel, the Middle East blind mole rat (*Spalax ehrenbergi*) was not infected with microscopic fungi of the genus *Emmonsia* [40]. As for *T. europaea*, they may be not infected, as noted in the Czech Republic [41]. Therefore, the contamination of subterranean mammals requires further research.

Epizootological monitoring of natural foci of infections is of key importance in the epidemiological surveillance of zoonoses. Therefore, comparative assessments of the epizootic activity of subterranean and terrestrial mammals made it possible to confirm the existence of pathogens in species unusual for them. Simultaneous infection of small mammals with several pathogens is not uncommon; it has been confirmed in the other regions of Europe [42–44] and other countries [45]. Co-infection of small mammals with pathogens of different etiologies in the territory of Mordovia indicates a possibility for mixed infection of humans.

The main drawback of this study was the inability of the laboratories in the region to identify HFRS viruses. Rodents are the main host of various hantaviruses such as Dobrava-Belgrade ortho-hantavirus, Tula ortho-hantavirus, Puumala ortho-hantavirus [46] and the new Rusne hantavirus [47]. However, human infections vary in severity and consequences. Therefore, strengthening laboratory capacity in the region would help to improve the quality of public health in the future.

4. Conclusions

There are different groups of animals in the region in terms of their susceptibility to zoonotic infections; however, only a few species may be important reservoirs. Our results show that among small terrestrial mammals in Mordovia, the bank vole, the yellow-necked mouse and the house mouse play an important role in transmission of zoonotic diseases. We found a positive relationship between relative abundance of small mammals and morbidity inthe human population. Among subterranean mammals, tularemia and HFRS were carried only by greater mole rats; European moles were free of these pathogens.

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Institutional Review Board Statement: The study was conducted in accordance with Russian federal law ("On the Animal World". No. 52-FL 24/04/1995, Art. 44 "the Use of the Animal World for Scientific, Cultural, Educational, Recreational and Aesthetic Purposes"; Law the Federal Service for Supervision of Consumer Rights Protection and Human Well-Being No. 296 31/05/2022 "About Monitoring of Viral Transmissible Zooanthroponotic Infections in the Territory of the Russian Federation for the Period up to 2026"). All aspects of trapping and animal handling complied with EU Council Directive 86/609/EEC on the experimental use of animals. Snap trapping was justifiable as we also studied reproduction parameters and collected tissues and internal organs for analysis of pathogens, elemental content and stable isotopes (not covered in this publication).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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