Veterinary Medicine and Science

RODENTS

Investigation of Viral, Bacterial and Parasitic Zoonotic Diseases in Rodents in Turkey

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ABSTRACT

Background: Rodents are reservoir hosts for zoonotic pathogens that cause tropical diseases, many of which have been overlooked.

Objectives: The aim of this study was to investigate the presence of viral lymphocytic choriomeningitis and hantavirus infections, bacterial tularaemia and leptospirosis, and parasitic leishmaniasis and toxoplasmosis in rodents that are likely to carry and spread zoonotic agents, by using molecular methods.

Methods: A total of 498 voles collected from 20 counties of Erzurum province. Conventional PCR was used for pathogen search. PCR-positive samples were subjected to sequence analysis.

Results: Hantavirus (4.8%, 24/498) and tularaemia (0.8%, 4/498) positivity were detected. However, no positivity was detected for other selected pathogens.

Conclusions: Rodents, which are pathogen carriers and potential risk factors, are thought to may act as reservoirs for hantavirus and tularaemia in the study area. A preliminary study has been carried out at the point of detection of these diseases of global importance. The extent of the distribution of the infections, alternative hosts and the consequences of human exposure needs to be clarified through further studies.

1 | Introduction

Zoonotic infections are diseases caused by pathogens that are capable of infecting both animals and humans. As human populations continue to grow and encroach on natural habitats, wildlife habitats are increasingly disrupted, leading to the spread of diseases to human populations. The incidence of diseases originating from wildlife, particularly those associated with changes in geographical areas and climatic conditions, is on the rise. These diseases can infect both humans and domestic animals. The changes in the ecological system contribute to the increase in zoonotic diseases, which can be transmitted from animals to humans, and vice versa. The emergence of diseases, such as plague, leptospirosis and hantavirus infections, has been attributed to the phenomenon of urbanisation (Kurucz et al. 2018). In developing countries, the process of urbanisation is leading to an increase in contact with rodents, which in turn increases the risk of infection. The changes associated with urbanisation bring wildlife closer to humans, thereby increasing the risk of exposure to pathogens (Hassell et al. 2017).

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Rodents represent the most abundant, diverse and widespread order of living mammals in the world (Cófreces 2022). They are well adapted to a wide range of habitats and are undoubtedly the mammals that have most often accompanied humans in their global dispersal. Indeed, rodents are a source of zoonotic pathogens. Nearly 10% of the global rodent population is either a carrier or a reservoir of pathogens of public health importance (Dahmana et al. 2020.; Han et al. 2015). Approximately 90 different diseases are associated with rodents, including more than 30 viral zoonoses, more than 20 bacterial diseases, 20 helminthiases, nearly a dozen protozoa, and four fungal diseases (Cófreces 2022; Yi, Deng, and Guo 2023).

Lymphocytic choriomeningitis virus (LCMV) belongs to the Arenaviridae family and is transmitted by rodents. LCMV infections have been reported in many parts of the world, including Europe, America, Australia and Japan. Various studies in urban areas have shown that between 2% and 5% of the human population have antibodies to LCMV (Peters et al. 2002). LCMV infection can be particularly harmful to pregnant individuals and can cause congenital hydrocephalus, chorioretinitis and mental retardation. The prevalence of LCMV in wildlife in certain regions, including Turkey, is unknown. LCMV can be transmitted by contact with the fresh urine, faeces, saliva or nesting material of infected rodents. Although vertical transmission from infected mother to foetus and rarely through organ transplantation has been reported, person-to-person transmission has not been reported (Amman et al. 2007).

Hantaviruses belong to the Hantaviridae family and their main reservoir is rodents, especially mice (Kuhn and Schmaljohn 2023). Hantavirus can be transmitted to humans in many ways, including directly, through the air, or through food and objects contaminated with rodent secretions. Infections caused by New World hantaviruses (hantavirus cardio-pulmonary syndrome—HCPS) in the Americas and Old World hantaviruses (haemorrhagic fever renal syndrome—HFRS) in Europe and Asia are common (Avsic-Zupanc et al. 2019). These diseases can cause serious illness in humans, with mortality rates ranging from 12% (HFRS) to 40% (HCPS). Many hantavirus infections go undetected and unreported in many countries, and hantaviruses are typically not considered in most cases (Avsic-Zupanc et al. 2019). Hantavirus infection has been reported in Turkey in recent years, especially in the Black Sea region (Kaya et al. 2010). A study on hantaviruses in rodents was also carried out in Erzurum (Polat et al. 2019).

Leptospirosis, which is zoonotic, can cause death and abortion in animals. In *Leptospira* infections, the difference between the *Leptospira* species causing the infection also affects the course of the infection. For this reason, although one animal species may be the main host for the bacteria, another animal species may be an incidental host for the bacteria. The difference in the location of the bacteria during the infection causes the diagnostic method and the samples to be used in the diagnosis to be different. In *Leptospira* infections, the presence of the bacteria in the bloodstream in the acute phase necessitates the examination of blood samples, whereas the colonisation of the bacteria in the kidneys in the chronic phase means that the bacteria should be sought in urine samples. There are many studies on the detection of *Leptospira* in various animal species worldwide (Garcia et al. 2013; Di Azevedo and Lilenbaum 2021Mgode et al. 2015; Suepaul et al. 2010;). Although there are studies on the detection of leptospirosis in livestock and pets in Turkey, the number of studies on mice and rats is very limited (Çetinkaya et al. 2000; Genç et al. 2005).

Tularaemia is a zoonotic infection caused by the *Francisella tularensis* species, and rodents are of great importance in the spread of the infection. The infection, which is mainly found in the northern hemisphere, causes sporadic cases in humans. Infection occurs by the contamination of the skin and mucous membranes of other living beings by the excretions (blood, urine, etc.) of sick animals. As animals, which are natural reservoirs, are often found dead, there are no clinical signs for diagnosis (Çelebi et al. 2006). *F. tularensis*, which is considered a biological weapon because a very small number (10–50) of microorganisms can cause infection, is classified as an infection requiring urgent intervention by the American Center for Disease Control and Prevention (CDC). Hestvik et al. (2015) found *F. tularensis* subsp. *holarctica* positivity in two mice. In 2012, an analysis conducted as a result of sudden animal deaths in a mouse population in Switzerland revealed a *Franciella* positivity rate of 34% by PCR, and an increase in human cases was reported in the region (Origgi et al. 2015). Most studies on tularaemia infections in Turkey have focused on water resources, human cases and domestic animals (Gürcan 2021; Unal-Yilmaz et al. 2014). Although positivity for infection has been sought in humans in Turkey, studies in mice and rats are limited. Unal-Yilmaz et al. (2014) reported positivity by agglutination and PCR in two mice captured in the study of human tularaemia cases in the Thrace region. Studies of tularaemia infection in animals in Turkey are limited to certain regions, and data on animal studies of transmission routes in the eastern Anatolian region are lacking.

Toxoplasmosis is not only important due to its zoonotic nature, but also because it can cause abortion and foetal anomalies in small ruminants. Detection of positive rodents can help us to identify environmental contamination and guide prevention and control methods for the disease. *Toxoplasma gondii*, the causative agent of the disease, is found worldwide, particularly in Europe and North America. It can infect nearly all warm-blooded living creatures, including humans, and can cause serious health problems in immunocompromised individuals, pregnant women, and congenitally infected individuals, as well as in pregnant sheep and goats (Poulsen et al. 2017). Domestic and wild felines are the final hosts of the agent, and many warm-blooded animal species, including cats and humans, serve as intermediate hosts. Feline infections are typically acquired through the ingestion of cystic organs and carcasses of infected intermediate hosts, especially birds and rodents (Fuehrer et al. 2010). Studies on the seroprevalence and molecular characteristics of toxoplasmosis have been carried out in Europe, the Far East and the USA (Dabritz et al. 2008; Hong et al. 2014; Vujanić, Ivović, and Kataranovski 2011). In Turkey, seropositivity has been reported in various domestic animal species, such as cattle, sheep and cats (Can et al. 2014; Leblebicier and Yıldız, 2014; Yücel, Yaman, and Kurt 2014). The only study conducted in Turkey to investigate the presence of *T. gondii* in rodents was by Karatepe et al. (2004), who reported a seropositivity rate of 11.4% in Anatolian field squirrels (*Spermophilus xanthophrymnus*) in Niğde.

Leishmaniasis is a zoonotic disease caused by protozoa of the *Leishmania* lineage, commonly found in tropical, subtropical regions and southern Europe (Alcover, Riera, and Fisa 2021). In Turkey, cutaneous leishmaniasis is most prevalent in the Mediterranean, Central Anatolia, Aegean regions and particularly in the southeastern Anatolia region. *Leishmania major* causes zoonotic cutaneous leishmaniasis in which rodents are the primary reservoir. *L. major* is a pathogen that has emerged in Turkey in 2013 (Ozbilgin et al. 2014) and has not previously been reported in the study area. The effects of global warming, which may have increased the number of *Phlebotomus* species in the country, may be responsible for the appearance of lesions caused by this pathogen (Koltas et al. 2014). Several studies have been carried out around the world to investigate the role of rodents in the *Leishmania* transmission cycle (Akhoundi et al. 2013; Alcover, Riera, and Fisa 2021; Kassahun et al. 2015) There is only one study (Karakuş et al. 2020) in Turkey reporting the presence of *Leishmania infantum*, *L. major* and *Leishmania tropica* in small wild rodents in Turkey.

The province in question is notable for its high rodent population density, both in rural and urban areas. To the best of our knowledge, no such study has been conducted in this region previously. The study aimed to investigate the presence of zoonotic pathogens in rodents that are involved in the transmission of the diseases mentioned above. The selected pathogens are important for both human and animal health because of their ease of transmission and pathological effects in humans, as reported in studies conducted worldwide.

2 | Materials and Methods

2.1 | Study Area and Sampling

The rodent trapping was carried out between February and December 2016, in 49 field sites located in 20 counties of the province of Erzurum (Figure 1). Intensive and active mouse nests in the rural areas of counties were determined and traps were set in these areas. Approximately 70 Sherman traps were set in each of the 49 regions. The traps were baited with peanuts and set in parallel lines 10 m apart. All traps were checked and captured animals were collected daily and then euthanised by cervical dislocation. Sampled animals were tagged with the date and place sampled. The rodents were also identified to genus level using standard morphological criteria (Corbet 1990). The internal organs (liver, spleen, heart, lung and kidney) and brains of these animals were removed and stored in RNA Later solution at −80°C until further processing. Ethical approval to conduct this study was obtained from the Unit Ethics Committee of Atatürk University's Veterinary Faculty (approval no. 2017/21).

2.2 | Tissue Homogenisation

Tissue homogenisation was performed using the Tissue Lyser LT (Qiagen, USA), with a total of 20 mg of tissue fragments placed in 2 mL tubes. The steel beads of the tissue homogeniser were placed on top and homogenised at 4000 rpm for 10 minutes. The same tubes were then placed in a centrifuge and centrifuged at 10,000 rpm for 10 min. At the end of this time, the supernatant was collected and genomic extraction started. The procedure described for viscera was followed for brain tissue.

2.3 | Genomic Extraction

A tissue extraction kit (DNA Genomic Kit, Invitrogen, USA) was used for extraction, with thoracic and abdominal cavity organs together and brain tissue separately. After the one-shot extraction, the PCR reaction was started.

2.4 | cDNA Synthesis

As the viruses (LCMV and hantavirus) under study have RNA genomes, they were converted into complementary DNA before PCR. For this purpose, a reverse transcriptase enzyme and kit (CDNA Synthesis Kit, Thermo Scientific, USA) were used, and the reaction was carried out according to the kit's instructions.

2.5 | PCR

PCR was performed using the primers listed in Table 1 and the optimisation conditions from the relevant reference publications. DNAse–RNase distilled water was used as a negative control. Positive controls were obtained from the laboratory stock.

2.6 | Agarose Gel Electrophoresis

After amplification, 10 μL of PCR products were taken, stained with ethidium bromide, and run on a 1.5% agarose gel and visualised with a Vilber Lourmat (Quantum ST4 1100, Germany) gel imaging system.

2.7 | Sequence Analysis

All samples detected as positive were sent to a commercial company for bidirectional sequencing. All sequence data were edited using Bioedit 7.2.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and Finch TV (http://www.geospiza.com/finchtv), followed by BLAST (http://www.ncbi.nlm.nih.gov/BLAST) analyses and each of the data was validated by comparison with known sequence results in the GenBank database. The sequence results of the samples obtained and verified were recorded in GenBank, and as a result, the isolate data from Erzurum, Turkey were recorded in GenBank.

2.8 | Phylogenetic Analysis

After aligning the sequences obtained in the project with the reference strains collected from the GenBank, a phylogenetic analysis was performed using the MEGA 6.0 program (Tamura et al. 2013). The relationship of the strains obtained in this way with other strains present in the GenBank was determined.

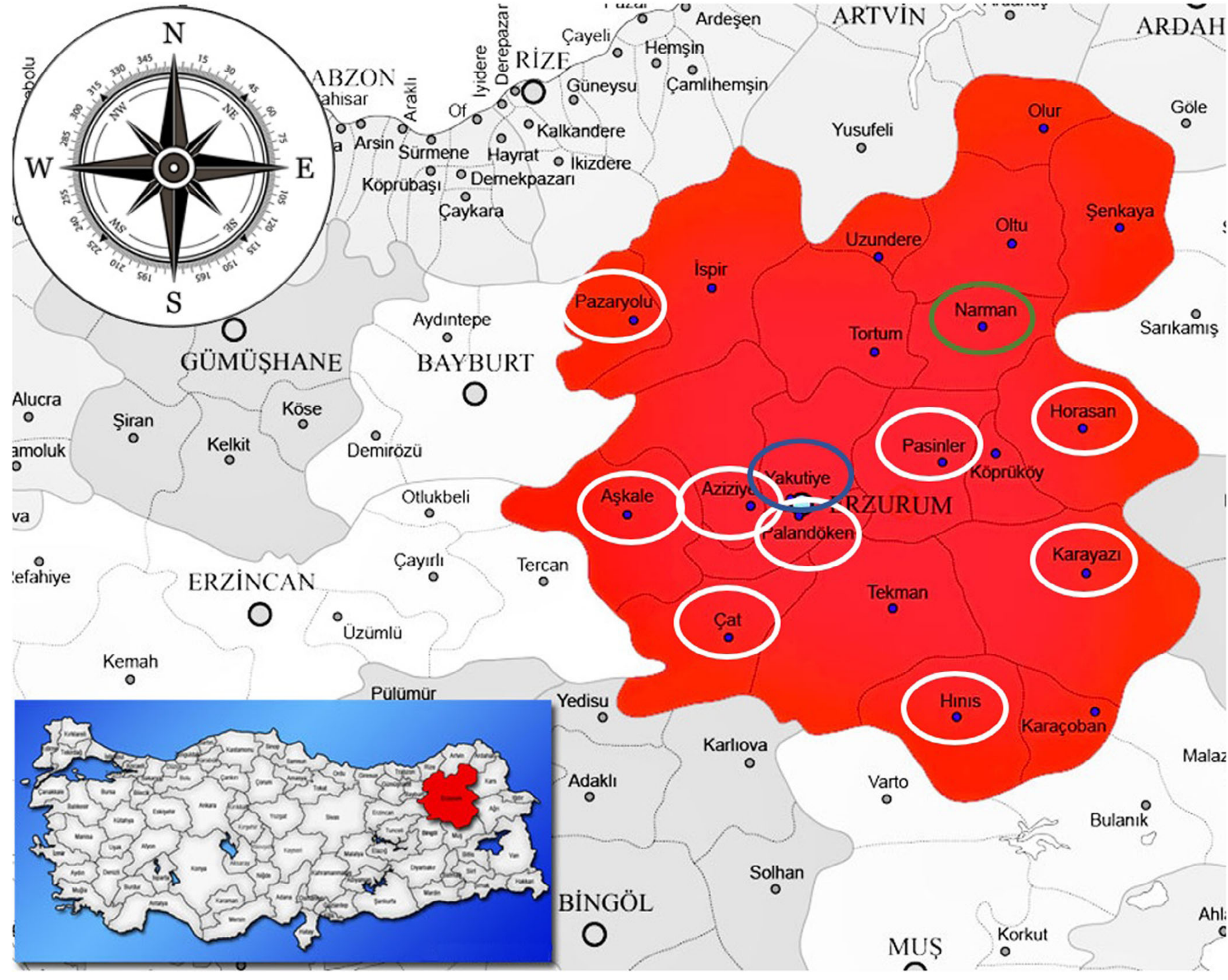

FIGURE 1 | Illustrative map of Turkey and Erzurum province. Regions with positive results are marked with a circle; white circles: hantavirus, green circle: tularaemia, blue circle: both hantavirus and tularaemia.

3 | Results

A total of 498 rodents were used in this study, including 391 *Microtus* spp. (78.5%), 93 *Apodemus* spp. (18.7%), 12 *Mesocricetus* spp. (2.4%) and 2 *Crocidura* spp. (0.4%). Information on the rodents captured, including region, sex and genus, is given in Table 2.

LCMV was not detected in any of the samples tested in the study. Regarding hantavirus, 24 (4.8%, 24/498) rodents were found positive in 10 districts of Erzurum province (Figure 1). Information on hantavirus-positive rodents is given in Table 3, including sample number, region, sex and genus. Sequence analysis showed that the viruses detected in the study at the partial L segment of the virus had 90.6%–100% identity with each other and between 78.8% and 81.5% identity with a reference Tula orthohantavirus (HQ728465). All of the strains detected were *T. orthohantavirus*. However, phylogenetic analysis showed that the Tula orthohantaviruses were divided into two subgroups, mainly Europe and Turkey. It was then found that the Turkish strains were again divided into two small subgroups (Subgroup 1 and Subgroup 2) (Figure 2).

All samples were tested negative for *Leptospira* sp. For tularaemia infection, primarily, the presence of *F. tularensis* subsp. was determined by the *Tul* gene. Tularaemia infection was detected in 4 out of 498 samples. RD1-PCR was used for subspecies differentiation of *F. tularensis*: 921 and 1522 bp bands were accepted as positive for *F. tularensis* subsp. *holarctica* and *F. tularensis* subsp. *tularensis*, respectively. In the RD1-PCR, 4 positive samples yielded a 921 bp band of *F. tularensis* subsp. *holarctica* (Figure 3). Four positive samples were detected in two counties of Erzurum (Figure 1). Information on tularaemia-positive rodents is given in Table 4, including sample number, region, sex and genus. One of the samples (No. = 85) was infected with both Tula orthohantavirus and *F. tularensis* subsp. *holarctica*.

None of the samples were found to be positive for *T. gondii* or *L. major*.

TABLE 1 | The primers, gene regions, sizes of PCR products and references used in the study.

Diseases	Gene	Primers	bp	References
LCMV	RdRp	F:CCACTYTTGTCTGCACTGTCTAT R:CTTTTTGATGCGCAATGGAT	190	Tadin, Tokarz, and Markotić (2016)
Hantavirus	L segment	LF1:ATG TAY GTB AGT GCW GAT GC LR1:AAC CAD TCW GTY CCR TCA TC LF2:TGC WGA TGC HAC NAA RTG GTC LR2:GCR TCR TCW GAR TGR TGD GCA A	First: 452 Second: 390	Klempa et al. (2006)
Toxoplasmosis		Tox4:CGCTGCAGGGAGGAAGACGAAAGTTG Tox5:CGCTGCAGACACAGTGCATCTGGATT	529 bp	Homan et al. (2000)
Leishmaniasis	16s rRNA	R221:GGTTCCTTTCCTGATTTACG R332:GGCCGGTAAAGGCCGAATAG R223:TCCCATCGCAACCTCGGTT R333:AAAGCGGGCGCGGTGCTG	First: 603 Second: 358	Van Eys et al. (1992)
Tularemi	Tul4	4-435 (F):GCTGTATCATCATTTAATAAACTGCTG 4-863 (R):TTGGGAAGCTTGTATCAT GGCACT	410	Wang, Hai, and Zhang (2011)
	RD1	RD1 (F):TTTATATAGGTAAATGTTTTACCTGTACCA RD1 (R):GCCGAGTTTGATGCTGAAAA	900/1100	
Leptospira	16s rRNA	F:GGCGGCGCGTCTTAAACATG R:TCCCCCCATTGAGCAAGATT	330	Merien et al. (1992)
	secY	Gl:CTGAATCGCTGTATAAAAGT G2:GGA AAA CAA ATG GTC GGA AG	285	Gravekamp et al. (1993)

TABLE 2 | Total number of rodents trapped by county, sex and genus.

			Sex		Genus			
No	**County**	***n***	**Male**	**Female**	***Microtus***	***Mesocricetus***	***Apodemus***	***Crocidura***
1	Askale	52	33	19	39	1	12	—
2	Aziziye	31	12	19	29	2	—	—
3	Çat	26	14	12	25	1	—	—
4	Hınıs	23	16	7	20	—	2	1
5	Horasan	14	3	11	14	—	—	—
6	İspir	29	21	8	26	—	3	—
7	Karayazı	22	20	2	21	—	1	—
8	Narman	30	26	4	24	—	6	—
9	Oltu	30	27	3	13	—	17	—
10	Palandöken	43	27	16	38	1	4	—
11	Pasinler	24	8	16	20	4	—	—
12	Pazaryolu	25	19	6	9	—	16	—
13	Tekman	38	25	13	37	1	—	—
14	Yakutiye	111	62	49	102	2	5	2
	Total	498	313	185	417	12	66	3

Note: n, the number of trapped rodents.

TABLE 3 | Information on hantavirus-positive rodents.

No.	Sample No.	County	Sex	Genus	Accession No.
1	19	Aziziye	Male	*Microtus* sp.	MT263470
2	27	Aziziye	Female	*Microtus* sp.	MT263471
3	43	Yakutiye	Female	*Microtus* sp.	MT263472
4	60	Yakutiye	Male	*Microtus* sp.	MT263473
5	85	Yakutiye	Male	*Microtus* sp.	MT263474
6	116	Pasinler	Female	*Microtus* sp.	MT263475
7	132	Aziziye	Male	*Microtus* sp.	MT263476
8	138	Aziziye	Female	*Microtus* sp.	MT263477
9	146	Palandöken	Male	*Microtus* sp.	MT263478
10	202	Çat	Male	*Microtus* sp.	MT263479
11	251	Horosan	Female	*Microtus* sp.	MT263480
12	252	Horosan	Female	*Microtus* sp.	MT263481
13	253	Horosan	Female	*Microtus* sp.	MT263482
14	260	Yakutiye	Male	*Microtus* sp.	MT263483
15	265	Yakutiye	Male	*Microtus* sp.	MT263484
16	274	Yakutiye	Male	*Microtus* sp.	MT263485
17	283	Yakutiye	Male	*Microtus* sp.	MT263486
18	291	Karayazı	Male	*Microtus* sp.	MT263487
19	292	Hınıs	Male	*Microtus* sp.	MT263488
20	389	Pazaryolu	Male	*Microtus* sp.	MT263489
21	390	Aşkale	Female	*Microtus* sp.	MT263490
22	414	Aşkale	Female	*Microtus* sp.	MT263491
23	416	Aşkale	Male	*Microtus* sp.	MT263492
24	441	Aşkale	Female	*Microtus* sp.	MT263493

4 | Discussion and Conclusion

Among the rodent species identified in this study, *Microtus* and *Apodemus* species in particular are well-known reservoirs of a wide range of zoonotic pathogens, including *Campylobacter, Coxiella, Cryptosporidium, Giardia, Trichinella, Listeria*, *Leishmania, Francisella* species and Hantavirus (Jahan, Lindsey, and Larsen 2021.). Species of other identified genus *Mesocricetus* act as reservoirs for *F. tularensis* (Hubálek and Rudolf 2010) and *Leishmania* spp. (Alcover, Riera, and Fisa 2021). The species of the last identified genus *Crocidura* have been identified as reservoirs for a number of pathogens, including *Leptospira, Coxiella burnetti, Hantavirus, F. tularensis* and *T. gondii* (Hubálek and Rudolf 2010; Haring et al. 2023). In the previous studies conducted in Turkey, hantavirus positivity was detected in *Microtus obscurus* (Polat et al. 2019), tularaemia was detected in *Mus macedonicus* and *Apodemus flavicollis* (Unal-Yilmaz et al. 2014), *Toxoplasma gondii* was detected in *S. xanthophrymnus* (Karatepe et al. 2004), and *Leishmania* species were identified in *Apodemus* spp., *Meriones* sp. and *Gerbillus dasyurus* (Karakuş et al. 2020). In the current study, positive results for hantavirus and tularaemia were found in the genus *Microtus. Microtus* voles are ecologically diverse and are the dominant herbivorous small mammals in many northern hemisphere habitats (Jaarola et al. 2004.). Turkey is also rich in terms of species number, with some endemic species throughout Turkey (Yiğit, Çolak, and Sözen 2016).

LCMV has been studied worldwide in rodents. Although there are many studies on humans, studies on the reservoir, that is carrier rodents, are limited. In general, studies are conducted on various factors, including LCMV, in endemic or sub-endemic areas of the country where the study is conducted. Tadin et al. (2016) conducted a study on 242 rodents in eight different regions in Croatia but could not find LCMV positivity. Knust et al. (2014) conducted a study in the United States and detected IgG positivity in 382 out of 1820 rodents and virus positivity by RT-PCR in 13 mice. N'Dilimabaka et al. (2015) conducted a study on domestic mice in Gabon, collecting 797 mice. They claimed that the strain detected in the phylogenetic analysis of the viruses they found was closely related to North American isolates and could be a risk factor for acute encephalitis cases. Yama, Cazaux, and Britton-Davidian (2012) targeted the northwest region of France and screened 821 mice for LCMV. They detected positivity in domestic mice and concluded that there is a regionally distinct strain. There is only one study on LCMV infection in Turkey. In this serological study, Laakkonen et al. (2006) collected 330 rodents from Trabzon, Rize, and Izmir provinces and detected LCMV antibodies in eight (2.4%) rodents. No antigenic study on LCMV

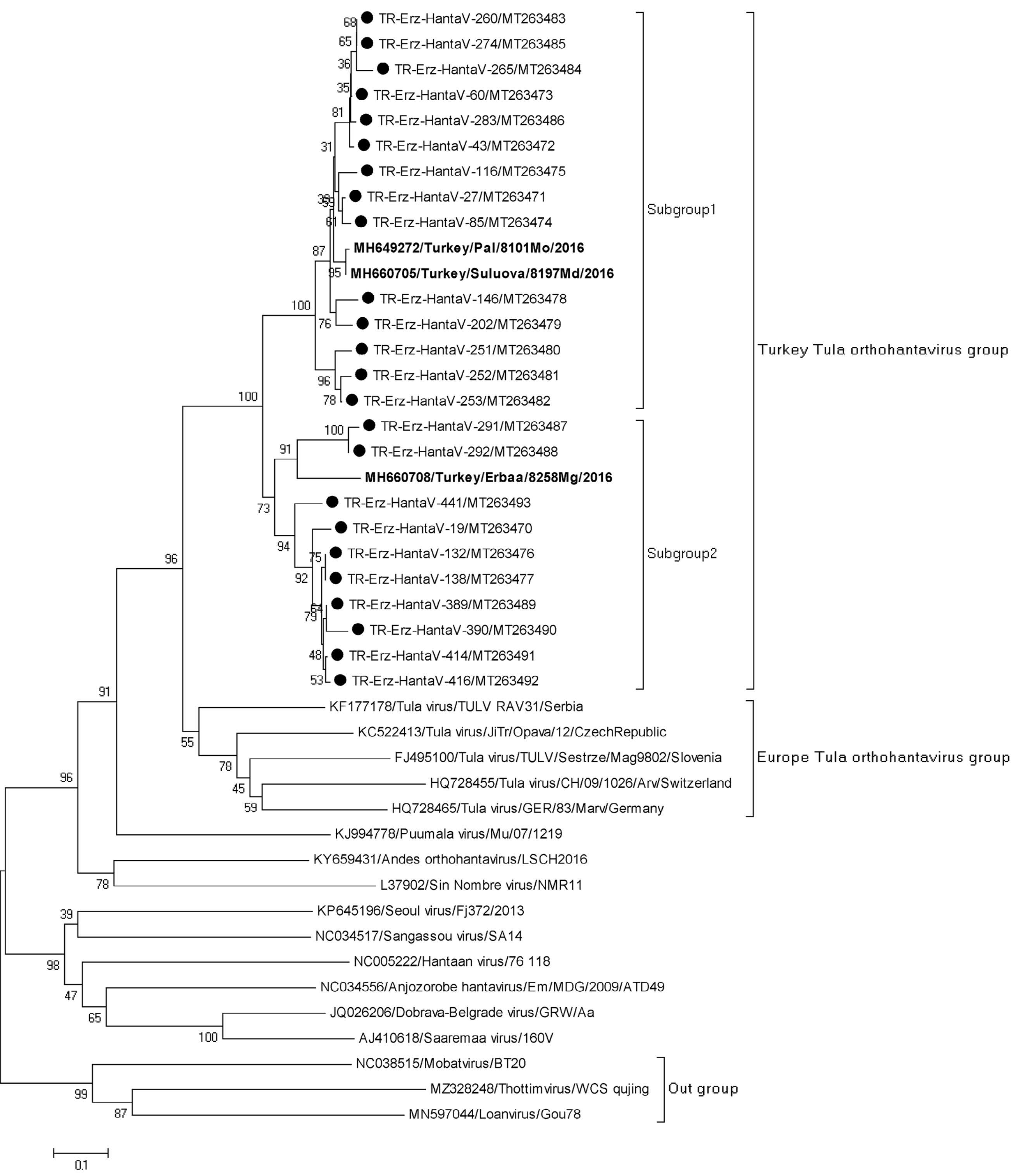

FIGURE 2 | Maximum likelihood analysis of the hantaviruses partial segment L coding sequences (390 bp). Tree is constructed using Tamura 3-parameter model for 500 replications. Bootstrap values higher than 60 are shown. Global viruses are represented by GenBank accession number, abbreviation, isolate/strain identifier and country of origin. In this study, Turkish Tula orthohantaviruses are shown with black circle and other Turkish Tula orthohantaviruses are shown bold. Mobat virus, Thottim virus and Loan virus are included as outgroups.

was found in Turkey. This is the first rodent study specifically for LCMV, but no virus has been detected in the study.

Another pathogen identified in this study is Tula orthohantavirus. Originally identified in the Tula region of central Russia, TULV has been shown to be a widespread orthohantavirus in Eurasia and has been reported in Austria, Belgium, the Czech Republic, Croatia, France, Germany, Kazakhstan, Poland, Serbia, Slovakia and Switzerland (Schmidt et al. 2016). Current information on the pathogenicity of TULV and its impact on

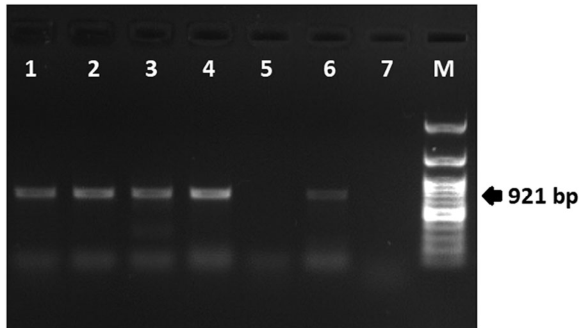

FIGURE 3 | *Francisella tularensis* subsp. *holartica* RD1 gene PCR image (M: 100 bp DNA marker, 1 (sample 54), 2 (sample 58), 3 (sample 85), 4 (sample 119): positive field samples, 5: negative field sample, 6: positive control (provided by the Refik Saydam Hygiene Institute, Ankara, Turkey), 7: negative control (DNAase–RNAase free water).

TABLE 4 | Information on tularaemia-positive rodents.

No	Sample No	County	Sex	Genus
1	54	Yakutiye	Male	*Microtus* sp.
2	58	Yakutiye	Female	*Microtus* sp.
3	85	Yakutiye	Male	*Microtus* sp.
4	119	Narman	Female	*Microtus* sp.

human health is limited. Although previously thought to be a non-pathogenic virus, some reports have identified human exposure to and symptomatic infections caused by TULV. Antibodies specific to the virus were found in healthy blood donors in the Czech Republic and in forestry workers and an HFRS patient in Germany (Klempa et al. 2006; Mertens, Hofmann, and Petraityte-Burneikiene 2011; Vapalahti, Lundkvist, and Kukkonen 1996). Additionally, TULV RNA has been found in the blood of an immunocompromised individual with pulmonary-renal syndrome from the Czech Republic, and in a patient with no significant medical history of HFRS from France (Reynes et al. 2015; Zelená, Mrázek, and Kuhn 2013). These studies clearly demonstrate that symptomatic human infections occur in regions where the virus is circulating. Further research is therefore needed to assess in detail the relationship and implications of virus distribution, alternative rodent hosts and potential human exposure.

Phylogenetic analysis of Turkish Tula orthohantaviruses revealed their differences from previously characterised TULV strains using different approaches and models. Interestingly, a separate TULV clade has emerged that shares common ancestry with certain strains found in the Czech Republic, Serbia, Slovenia, Switzerland and Germany, despite being distantly related to virus strains of European origin. Therefore, it can be argued that this phylogeny supports the existence of two distinct groups, namely European and Turkish. Turkish strains are further divided into two subgroups (Subgroups 1 and 2) at the level of their L segment. A similar observation was made by Polat et al. (2019) in a previous study conducted in Erzurum province. Although they only detected positivity in four samples from Erzurum, which is a very limited sample size, this current study identified 24 strains, indicating the potential variability of Turkish strains. Polat et al. (2019) also reported the emergence of a new lineage between Asian and European lineages, providing the first evidence of Tula orthohantavirus circulation and its potential reservoirs in Anatolia. Therefore, further research is needed to provide a detailed description of the rodent hosts, including alternative hosts, and the consequences of human exposure to TULV and other infections in Erzurum.

The role of rodents in the epidemiology and transmission of *Leptospira* infections is well known. Rodents effectively transmit infection by carrying different *Leptospira* serovars. *Leptospira* serovars in different geographical areas may be specific to different animal species as well as to different rodent species. It has been found that infection rates in rodent hosts vary according to their geographical location. Among rodents, the highest positivity for *Leptospira* infection was found in rats, followed by forest mice and house mice. However, no positivity was found in field mice (Çetinkaya et al. 2000). Leptospirosis positivity has also been observed in rodents that share living space with humans. Although the infection is present in nature, the differences in detection have been explained by researchers as a result of the consistent absence of *Leptospira* species in nature, particularly because the urinary excretion of the bacteria is intermittent (Warnasekara, Srimantha, and Kappagoda 2022). They also reported that the positivity rates varied depending on the tissue and organ sampled and that different results could be obtained depending on the extraction method, the type of PCR used and the numerical density of the bacteria. It is also generally accepted that many variables, such as the distance of the sampling area from the city centre, the climatic structure of the area, the status of existing rivers and whether different animals live in the area, affect the epidemiology of *Leptospira*. It has also been reported that the tropical-subtropical climate of the region and the humidity are effective in the occurrence of the infectious agents (Biscornet et al. 2021). In the present study, *Leptospira* positivity could not be detected in the tissue and organ samples examined by DNA extraction and PCR analysis. It was considered that the negative result could be due to the distance of the animal sampling areas from the city centre and the extraction method and PCR technique used in the analysis.

Tularaemia, first identified in humans in Turkey in 1936, is an infection that has been studied mainly in humans, domestic animals and environmental samples. Most studies on the detection of infection have emphasised that the increasing rodent population increases the risk of infection in humans, the environment and animals (Akalın, Helvacı, and Gedikoğlu 2009; Şimşek and Çankaya 2019; Gürcan 2021). Although it is noted that the majority of positive human cases are seen in people who consume plants and vegetables, it has been reported that the consumption of spring water in the environment (Kılınç et al. 2007) and changes in precipitation due to climate change influence the spread of this infection (Balci et al. 2014). An evaluation of studies investigating the presence of tularaemia infection in rodents found that PCR tests for *F. tularensis* in rodent samples from different countries showed a positivity of 1.2% in Germany, 1.9% in Switzerland and no positivity in the Czech Republic (Jeske et al. 2019; Mihelčić et al. 2018; Origgi et al. 2015).

In a review of tularaemia studies in rodents between 1992 and 2012, different results were obtained depending on the diagnostic methods used, and it was reported that positivity rates determined by PCR varied between 1.1% and 23% in different European countries (Germany, France, Bulgaria, and Sweden) (Hestvik et al. 2015). In a study conducted in Iran, *F. tularensis* positivity was found to be 1.91% using the PCR method in 140 animals consisting of different rodent species (Mostafavi et al. 2018). In Sweden, Broman et al. (2011) reported two *F. tularensis* subsp. *holarctica* positivity in 97 rodents, with higher positivity found in water from which mice were collected. The researchers also reported the seasonal pattern of tularaemia infection, showing that infection-related positivity is more common from May to September (Origgi et al. 2015). In Turkey, Unal-Yilmaz et al. (2014) detected 10.5% positivity in mice using RT-PCR in their analysis of tularaemia in the Thrace region, whereas Kaygusuz et al. (2010) found negative results in all animals in their screening of 42 mice in the Central Anatolia region. In this study, DNA extraction and PCR testing from rodent tissue and organ samples yielded four positive results for *F. tularensis* subsp. *holarctica*, and no positivity was found for *F. tularensis* subsp. *tularensis*. This result was found to be consistent with studies determining *F. holartica* positivity (Broman et al. 2011; Jeske et al. 2019; Hestvik et al. 2015; Mayer-Scholl et al. 2014). The researchers found that *F. tularensis* positivity is variable. There are differences between studies due to factors such as the subtypes of the bacterium in Tularaemia infections, the seasonal and geographical occurrence of the bacterium in addition to rodents, as well as parameters such as the distance of rodent habitats from human and domestic animal habitats, and the methods and sensitivities used in the laboratory, which affect infection positivity (Merien et al. 1992). In the present study, the positivity of four samples for *F. holartica* subsp. *holartic*a indicates that water analyses should be carried out together with human infections in the areas where the samples were taken.

The seroprevalence of toxoplasmosis in rodents in the world has been reported to be between 2.3% and 60% (Afonso et al. 2007; Hong et al. 2014), whereas the prevalence has been reported to be between 0.17% and 83.3% (Hong et al. 2014; Vujanić, Ivović, and Kataranovski 2011). In the only study conducted in Turkey on the presence of *T. gondii* in rodents, Karatepe et al. (2004) reported a seropositivity rate of 11.4% in Anatolian ground squirrels (*S. xanthophrymnus*). In studies conducted on human toxoplasmosis in Erzurum province, Yiğit et al. (2000) reported 0.4% IgM and 24% IgG positivity, and Akaltun et al. (2018) reported IgG positivity rates of 9.2%–37.2% in children and adolescents. Animal studies have reported seropositivity of 4.58% in sheep (Aktas et al. 2020) and 62% in donkeys (Balkaya et al. 2011). However, in this study conducted in rodents in Erzurum using molecular methods, *T. gondii* positivity could not be detected. The lack of positivity in rodent samples in the study area where the presence of the disease in humans and animals has been demonstrated can be explained by the insufficient sample size and the fact that rodent samples are usually taken from distant points of their habitat. For rodents to become infected, they need to come into contact with cystic tissue or *Toxoplasma* oocysts. The possibility that the sampled rodents could not reach these infective forms is considered to be one of the reasons for the negative result.

The presence of *L. major* in rodents has been reported in countries such as Iran (Pourmohammadi, Mohammadi-Azni, and Kalantari 2017), Tunisia (Othman et al. 2018) and Pakistan (Khan et al. 2020). In Turkey, the incidence of *L. major* infection, which was previously unknown, has increased in recent years due to the influx of immigrants (Ozkeklikci et al. 2017). Autochthonous cases of *L. major* have also been reported in the Mediterranean, southeastern Anatolia, eastern Anatolia and Aegean regions of Turkey (Ozbilgin et al. 2014). However, this study did not yield positive results. The presence and prevalence of the pathogen, vectors and reservoir hosts, as well as ecological factors, play an important role in the epidemiology of the disease. Given the high level of human migration from endemic regions to Erzurum, a region where *Phlebotomus* species typically do not thrive due to unsuitable climatic conditions, where there have been no reports of *L. major*, this study was designed to investigate the possibility of vector populations settling and developing in this region due to climate change resulting in milder weather conditions. Although the negative results suggest that *L. major* was not present in the rodent population in the region and that climate change may not be conducive to the establishment and development of vector populations, the small sample size and the possibility of sampling from areas where the human-vector-rodent cycle does not occur could also explain the results.

To our knowledge, this study is the first to demonstrate natural infection of rodents with hantaviruses and tularaemia. Although six infections were tested, no positive results were obtained for LCMV, *Leptospira* sp., toxoplasmosis and Leishmania sp. However, this study will raise an awareness of the presence and spread of such common infections in rodents and will provide a basis for predicting common infections in the human population and preparing public health interventions. In areas where infections are detected in rodents, the presence of the pathogens in humans or animals should be tested.

Author Contributions

Mehmet Ozkan Timurkan: formal analysis, investigation, methodology, supervision, writing–original draft. **Esin Guven:** formal analysis, investigation. **Seyda Cengiz:** formal analysis, investigation. **Hakan Aydin:** formal analysis, investigation. **Ridvan Kirman:** formal analysis, investigation. **Hamza Avcioglu:** investigation.

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Ethics Statement

Ethical approval was received from the Unit Ethics Committee of Atatürk University (Date: 20.12.2017, Decision No: 2017/21).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in Gen-Bank, National Centre for Biotechnology Information (NCBI) at https://www.ncbi.nlm.nih.gov. under the Accession Number MT263470-MT463493.

Peer Review

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