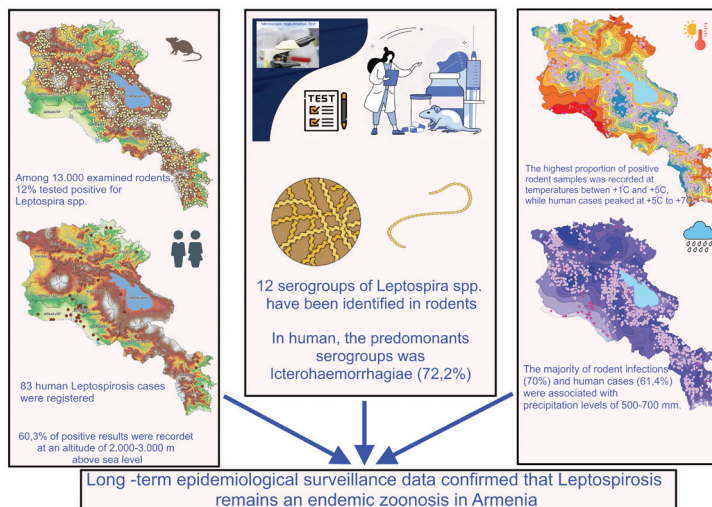




ORIGINAL ARTICLE

Rodent Reservoirs of Leptospirosis: Serogroup Distribution and Spatial Analysis

Graphical abstract



Authors

Arsen Manucharyan, Alvard Avanesyan, Marine Yakhshyan, Gayane Melik Andreasyan, Ruben Danielyan and Stepan Atoyan

Correspondence

arsen.manucharyan.1976@gmail.com (AM)

Brief statement

This study demonstrated that leptospirosis in Armenia is sustained by two interconnected transmission systems: a natural cycle dominated by wild rodents, particularly the common vole (*Microtus obscurus*), and a synanthropic cycle associated with rats in human settlements. Spatial and serological analyses revealed that natural foci are concentrated in mountain ecosystems, whereas human infections occur primarily in populated areas and are associated with the *Icterohaemorrhagiae* serogroup. These findings provide an evidence base for improving national surveillance, ecological monitoring, and targeted prevention strategies.

Highlights

- Long-term surveillance data (2003–2024 for humans; 2015–2024 for rodents) confirmed that leptospirosis remains an endemic zoonosis in Armenia.
- Among 13,000 examined rodents, 12.1% tested positive for *Leptospira* spp., and 97.3% of positive findings occurred in the common vole (*Microtus obscurus*).
- Natural foci were concentrated in mountain-steppe and high-altitude zones, and 60.3% of positive rodent findings were recorded at 2,000–3,000 meters above sea level.
- Analysis of climatic factors revealed a clear relationship between the presence of leptospires and environmental conditions, in which moderate temperatures and precipitation contribute to the preservation and spread of leptospires.
- Twelve *Leptospira* serogroups were identified in rodents, among which Ballum, Hebdomadis, Autumnalis, Pyrogenes, and Javanica predominated.
- A total of 83 human leptospirosis cases were registered, primarily in densely populated anthropogenic landscapes.
- In humans, the *Icterohaemorrhagiae* serogroup predominated (72.2%), thus indicating that rats have a major epidemiological role as synanthropic reservoirs.
- The findings support integrated surveillance, rodent control, and targeted prevention programs for agricultural and high-risk populations.

Rodent Reservoirs of Leptospirosis: Serogroup Distribution and Spatial Analysis

Arsen Manucharyan^{1,*}, Alvard Avanesyan¹, Marine Yakhshyan¹, Gayane Melik Andreasyan¹, Ruben Danielyan¹ and Stepan Atoyan¹

Abstract

Objective: Human cases of leptospirosis, an endemic zoonotic disease in the South Caucasus, are reported almost every year. In Armenia, the infection is widely distributed in wildlife, particularly rodents, and is considered a priority zoonosis because of its public health importance and outbreak potential.

Methods: This study analyzed annual reports and archival records from the Armenian National Center for Disease Control and Prevention. The dataset included field and laboratory data on *Leptospira*-positive rodents collected between 2015 and 2024, as well as officially registered human cases from 2003 to 2024. Rodent trapping, sampling, and laboratory testing were conducted according to standardized protocols. All data were entered into a georeferenced digital database and analyzed with a geographic information system to assess spatial patterns.

Results: Common voles accounted for 97.3% of *Leptospira*-positive rodents, whereas six other rodent species accounted for the remaining 2.7%. Most positive detections (60.3%) occurred at elevations of 2,000–3,000 meters. Among the 12 identified serogroups, Ballum, Hebdomadis, Autumnalis, Pyrogenes, and Javanica predominated and together represented 57% of detections. From 2003 to 2024, 83 human cases were reported, 72.2% of which involved Icterohaemorrhagiae.

Conclusion: Our results indicated distinct natural and synanthropic transmission systems. The findings emphasize needs for targeted surveillance, rodent control, and spatial risk mapping to decrease human infection risk.

Key words: leptospirosis, mapping, rodents, serogroups

*Corresponding authors:

E-mail: arsen.manucharyan.1976@gmail.com (AM)

¹National Center for Disease Control and Prevention, Ministry of Health of the Republic of Armenia, 12 Mkhitar Heratsi, 0025 Yerevan, Armenia

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INTRODUCTION

Leptospirosis is among the most widespread and ecologically complex zoonotic infections worldwide, and it poses persistent public health and veterinary challenges, particularly in regions with diverse landscapes and intensive human–animal interaction [1]. The disease is caused

by pathogenic spirochetes of the genus *Leptospira* (family Spirochaetaceae), which occupy an intermediate biological position between bacteria and protozoa, and are capable of long-term survival in moist environmental conditions [2].

Leptospirosis affects diverse domestic and wild animals, including livestock, carnivores, rodents, and birds, many of

which act as reservoir hosts [3]. Rodents, particularly small murine species, play central roles in the maintenance and dissemination of *Leptospira* spp. in natural ecosystems. Rodents often remain asymptomatic lifelong carriers and shed leptospires into the environment through their urine, thus contaminating soil and water sources, and sustaining natural foci of infection [1]. Human infection occurs primarily through direct or indirect contact with environments contaminated by infected animal excreta. Consequently, leptospirosis is widely recognized as an occupational disease affecting agricultural workers, veterinarians, slaughterhouse employees, sewage workers, and others exposed to contaminated water or soil [4]. The clinical spectrum of human leptospirosis ranges from mild, influenza-like illness to severe, potentially fatal forms characterized by jaundice, renal failure, pulmonary hemorrhage, and multi-organ dysfunction [5].

In Armenia, leptospirosis is a recognized endemic zoonosis whose documented history spans more than seven decades. The first human case was reported in 1948 in the Shirak region, and was followed by sporadic cases and localized outbreaks in the Armavir and Tavush regions during the latter half of the 20th century. Between 1986 and 2002, 95 human cases were officially registered in the country [6]. Although most reported cases in Armenia involve mild disease [7], the persistence of infection among animal reservoirs indicates an ongoing transmission risk.

Armenia's diverse topography, including semi-desert lowlands, foothills, mountain-steppe zones, and high-altitude ecosystems, creates favorable ecological conditions for the formation and maintenance of natural foci of leptospirosis. These landscapes support a wide variety of rodent species, many of which are closely associated with agricultural activities and pastoral land use. Annual epizootiological monitoring conducted by the National Center for Disease Control and Prevention (NCDCP) of Armenia has demonstrated continued circulation of *Leptospira* spp. among wild rodents, thus underscoring their epidemiological importance.

Although rodents are recognized as critical reservoir hosts, comprehensive long-term analyses integrating epizootiological, epidemiological, and spatial data remain limited in the region. In particular, information on the geographical distribution of infected rodent populations, the diversity of circulating *Leptospira* serogroups, and their relationship with registered human cases remains insufficient. However, integrated analyses are essential for understanding transmission dynamics, identifying high-risk areas, and developing targeted prevention and control strategies.

This study was aimed at assessing the prevalence of *Leptospira* spp. among rodent reservoirs in the Republic of Armenia; determining the geographical and altitudinal distribution of infected rodents and dominant serogroups; conducting a spatial analysis of registered human leptospirosis cases in relation to circulating serogroups; and identifying key environmental and ecological factors influencing the distribution of rodent

reservoirs and *Leptospira* infection. By integrating long-term surveillance data with geographic information system (GIS) analysis, we sought to map areas with the highest epidemiological importance and clarify the roles of rodents in the epizootiology of leptospirosis in Armenia.

MATERIALS AND METHODS

Data collection

All rodent samples included in this study were collected by the authors during routine epizootiological monitoring conducted between 2015 and 2024, and were subsequently documented in the annual reports of the NCDCP. In total, 13,000 rodents were captured and examined. Laboratory-confirmed records of *Leptospira* spp. derived from these samples were retrieved from the NCDCP database and included in the analysis.

Field epizootiological surveys targeting reservoirs and vectors of zoonotic and vector-borne infections were performed annually from April to October across multiple regions of Armenia during the study period (2015–2024). Sampling sites were selected according to the principal habitats of reservoirs and vectors associated with natural-foci zoonotic diseases.

Data on human leptospirosis cases were obtained from the official statistical reporting forms for infectious diseases in the Republic of Armenia and complemented by records from the national epidemiological surveillance system. A total of 83 confirmed human cases reported between 2003 and 2024 were included in the study. The dataset comprised demographic and epidemiological variables, including age, sex, place of residence, clinical outcome, and, when available, the identified *Leptospira* serogroup.

The diagnosis of human leptospirosis was established according to clinical presentation, epidemiological history, and laboratory confirmation, in accordance with national guidelines and World Health Organization recommendations.

Sample collection

Rodent sampling and dried blood spot collection

Rodents were captured with standard trapping techniques in accordance with NCDCP protocols, and methodological guidelines for trapping and burrow excavation. Animals were handled according to established biosafety and ethical standards.

Dried blood spot (DBS) samples were used for the serological detection of *Leptospira* spp. with the microscopic agglutination test (MAT) to determine the presence of specific antibodies. Blood samples were collected from peripheral vessels (tail vein or artery) with sterile instruments. A drop of whole blood was applied onto sterile filter paper (e.g., Whatman) to obtain DBSs approximately 5–10 mm in diameter. Samples were air-dried at room temperature for 2–4 hours, then individually stored in

labeled envelopes containing desiccant and transported to the laboratory. Before analysis, DBS samples were stored at +4°C or −20°C.

Human blood sampling

Blood samples from patients with suspected leptospirosis were collected in healthcare facilities by trained medical personnel following standard aseptic technique. Venous blood (3–5 mL) was obtained via venipuncture from the antecubital vein with sterile disposable needles and vacuum tubes.

Collected samples were allowed to clot at room temperature and were subsequently centrifuged to obtain serum. Serum samples were transferred into sterile cryovials and stored at +4°C for short-term storage or −20°C until further laboratory testing.

Laboratory testing

Serological analysis was performed with the MAT, which is considered the gold standard for leptospirosis diagnosis. The test was used to detect antibodies to various *Leptospira* serovars. A panel of 12 *Leptospira* serotypes cultured for 6–10 days was used for the MAT. For rodent samples collected via DBSs, the “pressed drop” method was applied [8]. Human cases were considered confirmed

when diagnostic antibody titers were detected, or a four-fold increase in MAT titers was observed in paired serum samples.

Data analysis and mapping

All rodent and human case data were entered into a georeferenced digital database. Geographic coordinates of rodent capture sites and human case locations (at the settlement level) were used for spatial analysis. Mapping and spatial visualization were performed with a GIS to assess the spatial distribution of infected rodents, circulating *Leptospira* serogroups, and human leptospirosis cases. Elevation data were derived from digital elevation models and were associated with rodent sampling points and human case locations to analyze altitudinal patterns of leptospirosis circulation.

RESULTS

Spatial distribution of *Leptospira* in rodent populations

The data included 1,580 positive results among rodents during the study period. Of these, 60.3% were recorded at elevations between 2,000 and 3,000 meters above sea level, corresponding to the high-mountain and mountain-steppe zones of Armenia. Annual laboratory testing

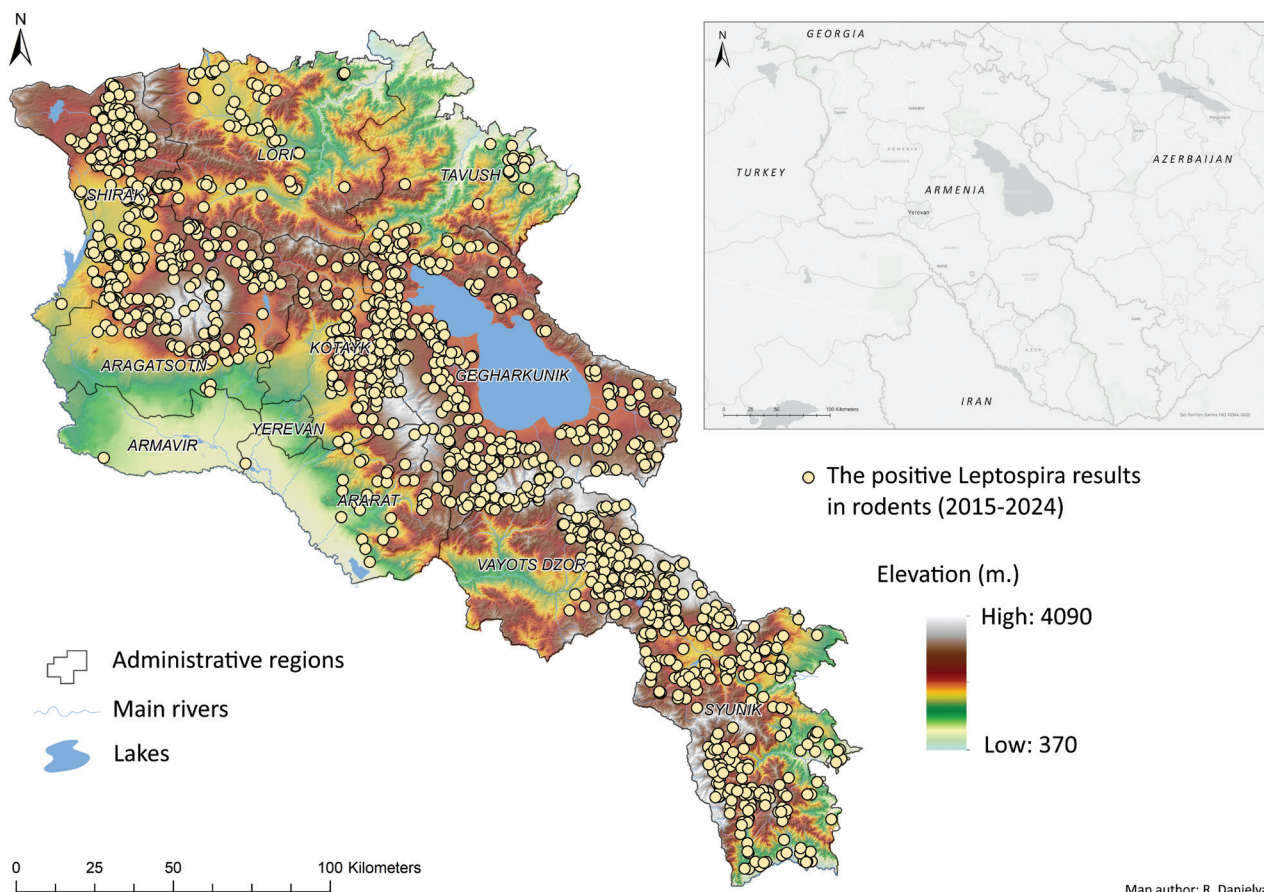


FIGURE 1 | Map of the spatial distribution of leptospires in rodents in Armenia.

of field samples recorded 65–256 positive results for leptospires among rodents each year (Fig 1).

Rodent species composition and infection rates

Among all rodents with positive results, 97.3% were recorded in the common vole (*Microtus obscurus*), whereas the remaining 2.7% were recorded in six other rodent species (Table 1). A total of 12.1% of all examined rodents tested positive for leptospires, among which 11.8% were common voles. These results indicated that rodents, particularly the common vole, are important carriers of *Leptospira* spp. These findings contribute to the understanding of the eco-epidemiology of leptospirosis in Armenia.

Diversity and distribution of *Leptospira* serogroups in rodents

During the study period, 12 *Leptospira* serogroups were identified in rodents. Among them, the serogroups Ballum, Hebdomadis, Autumnalis, Pyrogenes, and Javanica predominated and accounted for 57% of the total (Table 2).

TABLE 1 | Rodent species and numbers of individuals with confirmed *Leptospira* positivity.

N	Rodent species	Number of positive individuals	Number of examined individuals
1	<i>Microtus obscurus</i>	1,538	8,737
2	<i>Microtus socialis</i>	21	1,621
3	<i>Sylvaemus uralensis</i>	12	1,113
4	<i>Meriones vinogradovi</i>	4	631
5	<i>Mus Abboti</i>	3	711
6	<i>Rattus rattus</i>	1	50
7	<i>Meriones persicus</i>	1	137
Total		1,580	13,000

Host-serogroup relationships

The rodent species positive for leptospires were observed at elevations between 600 and 1,400 meters above sea level, corresponding to the foothill and mountain-steppe zones (Fig 2). Table 3 shows the distribution of *Leptospira* serogroups among the identified rodent species. Most positive findings were recorded in *Microtus obscurus*, which exhibited the highest diversity and frequency of detected serogroups, including Ballum (202), Hebdomadis (186), Autumnalis (172), Pyrogenes (166), and Javanica (154). The serogroup Icterohaemorrhagiae was detected exclusively in *Microtus obscurus* (97 cases). Other rodent species, including *Microtus socialis*, *Sylvaemus uralensis*, *Meriones vinogradovi*, *Mus abboti*, *Rattus rattus*, and *Meriones persicus*,

TABLE 2 | Distribution of *Leptospira* serogroups among rodents.

N	<i>Leptospira</i> spp. serogroups in rodents	Number of positive specimens	Percentage of total positive individuals
1	Ballum	211	13.4
2	Hebdomadis	192	12.3
3	Autumnalis	178	11.2
4	Pyrogenes	171	10.8
5	Javanica	159	10.1
6	Canicola	144	9.2
7	Australis	124	7.8
8	Grippotyphosa	110	6.9
9	Icterohaemorrhagiae	97	6.1
10	Bataviae	75	4.7
11	Tarassovi	62	3.9
12	Pomona	57	3.6
Total		1,580	100

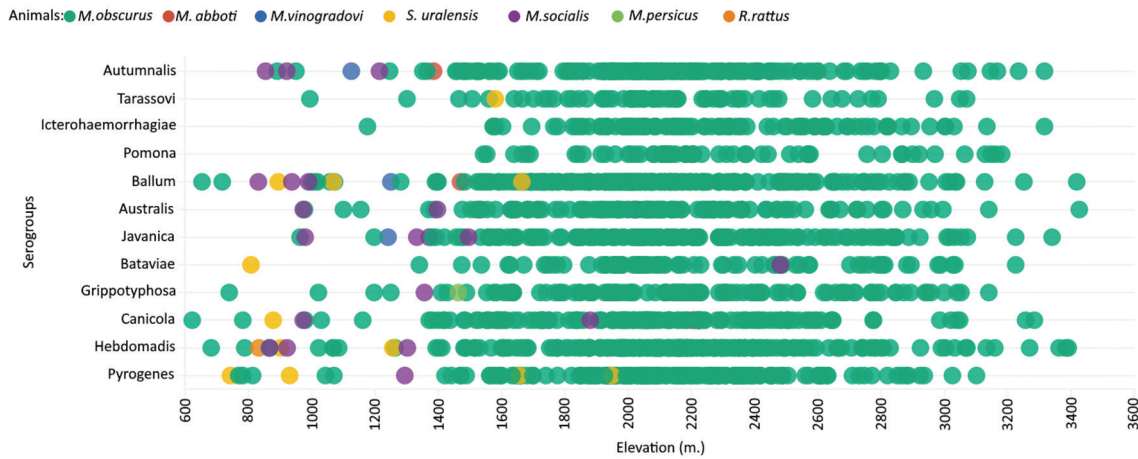


FIGURE 2 | Altitudinal distribution of rodent species and their identified *Leptospira* serogroups.

TABLE 3 | Distribution of *Leptospira* serogroups among distinct rodent species.

Rodent species	Leptospira spp. serogroups											
	Ballum	Hebdomadis	Autumnalis	Pyrogenes	Javanica	Canicola	Australis	Grippotyphosa	Icterohaemorrhagiae	Bataviae	Tarassovi	Pomona
<i>Microtus obscurus</i>	202	186	172	166	154	140	122	108	97	73	61	57
<i>Microtus socialis</i>	4	3	3	1	4	2	2	1	0	1	0	0
<i>Sylvaemus uralensis</i>	3	2	0	4	0	1	0	0	0	1	1	0
<i>Meriones vinogradovi</i>	1	0	1	0	1	1	0	0	0	0	0	0
<i>Mus abboti</i>	1	1	1	0	0	0	0	0	0	0	0	0
<i>Rattus rattus</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Meriones persicus</i>	0	0	0	0	0	0	0	1	0	0	0	0

TABLE 4 | Identified positive leptospirosis cases and serogroups in humans.

N	<i>Leptospira</i> spp. serogroups in humans	Number of positive specimens	Percentage of total positive individuals
1	Icterohaemorrhagiae	60	72.2
2	Hebdomadis	7	8.4
3	Autumnalis	4	4.8
4	Javanica	4	4.8
5	Canicola	3	4
6	Australis	2	2.3
7	Grippotyphosa	2	2.3
8	Pyrogenes	1	1.2
Total		83	100

showed considerably lower frequencies and a more limited distribution of *Leptospira* serogroups. Overall, the results indicated that rodents of the genus *Microtus*, particularly *Microtus obscurus*, were the primary reservoirs of the detected *Leptospira* serogroups in the studied rodent populations.

Human leptospirosis cases and serogroup distribution

The number of confirmed leptospirosis cases in humans from 2003 to 2024 was 83 (Table 4). Among the eight identified serogroups, Icterohaemorrhagiae predominated, accounting for 72.2% of cases. A map of the spatial distribution of registered human leptospirosis cases in Armenia from 2003 to 2024 was created (Fig 3). According to spatial analysis data, human cases were found in the semi-desert, subtropical, and mountain-steppe landscape zones of Armenia. Most human leptospirosis cases (85%) were recorded in areas with high settlement density.

Climatic associations with *Leptospira* distribution

Analysis of climatic factors revealed clear associations between *Leptospira* occurrence and environmental conditions (Table 5).

Spatial distribution of *Leptospira* in relation to climate

The highest proportion of positive rodent samples (55.3%) was recorded at temperatures between +1°C and +5°C, whereas human cases peaked (56.6%) at +5°C to +7°C (Fig 4A). Similarly, most rodent infections (70%) and human cases (61.4%) were associated with precipitation levels of 500–700 mm (Fig 4B). These findings suggested that moderate temperature and precipitation conditions favor *Leptospira* persistence and transmission.

Clinical and demographic characteristics of human cases

Most patients were diagnosed with the icteric (jaundice) form of leptospirosis (Weil's disease), which is most

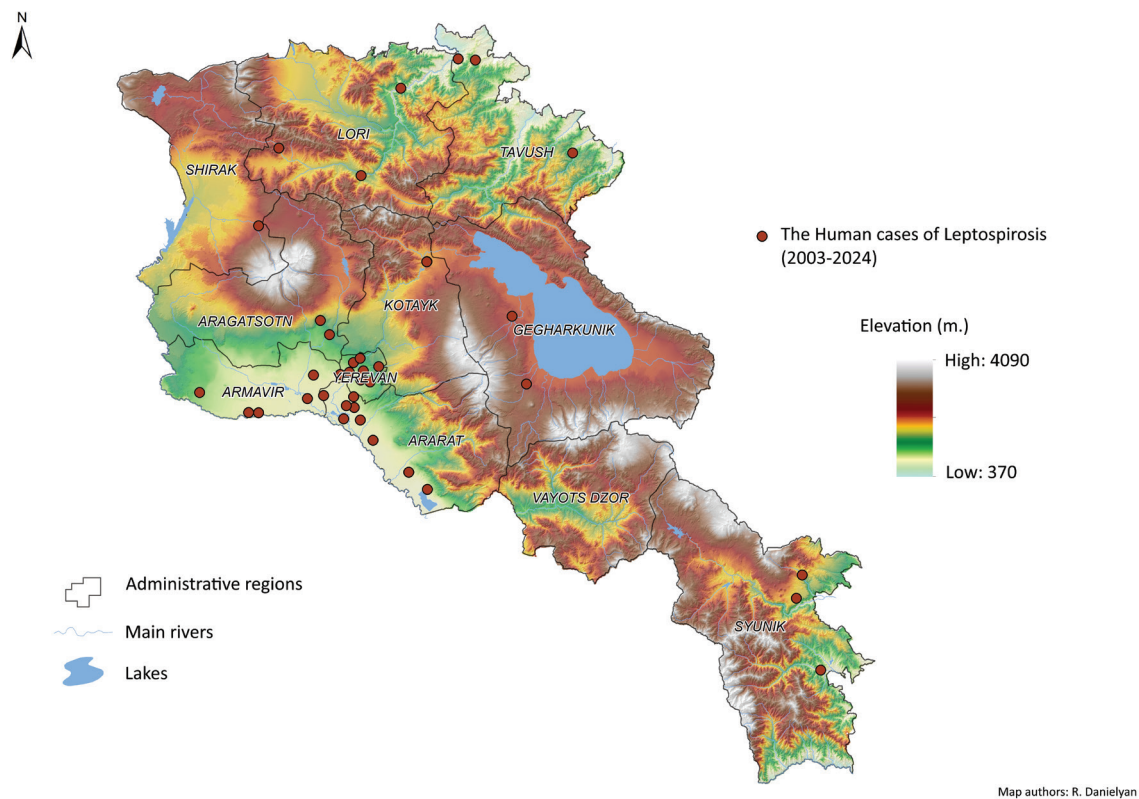


FIGURE 3 | Spatial distribution of human leptospirosis cases, 2003–2024.

TABLE 5 | Associations of *Leptospira* with temperature and precipitation in Armenia.

<i>Leptospira</i> in rodents				Leptospirosis in humans			
Temperature zones (°C)	–5 to –1	+1 to +5	+9 to +13	Temperature zones (°C)	+1 to +3	+5 to +7	+9 to +11
Number of positive <i>Leptospira</i> results in rodents	238	874	468	Number of positive leptospirosis tests in humans	14	47	22
Percentage of registered <i>Leptospira</i> in rodents	13.2	55.3	29.6	Percentage of registered human cases of leptospirosis	16.9	56.6	26.5
Precipitation zones (mm)	200–400	500–700	800–1,000	Precipitation zones (mm)	200–400	500–700	800–1,000
Number of positive <i>Leptospira</i> results in rodents	137	1,106	337	Number of positive leptospirosis tests in humans	17	51	15
Percentage of registered <i>Leptospira</i> in rodents	8.7	70	21.3	Percentage of registered human cases of leptospirosis	20.5	61.4	18.1

frequently associated with the Icterohaemorrhagiae serogroup. Clinical manifestations included fever, chills, myalgia, headache, hemorrhages on the skin and mucous membranes, epistaxis, thrombocytopenia, and moderate liver involvement.

The age of patients with leptospirosis ranged from 8 to 70 years, and the mean age was 40 years. A total of 91.5%

(76) of patients were men, and 8.4% (7) were women. The interquartile range (Q_3 (51) – Q_1 (27)) was 24.

DISCUSSION

The results confirmed that rodents are the principal reservoir hosts of *Leptospira* spp. in Armenia. Among 13,000

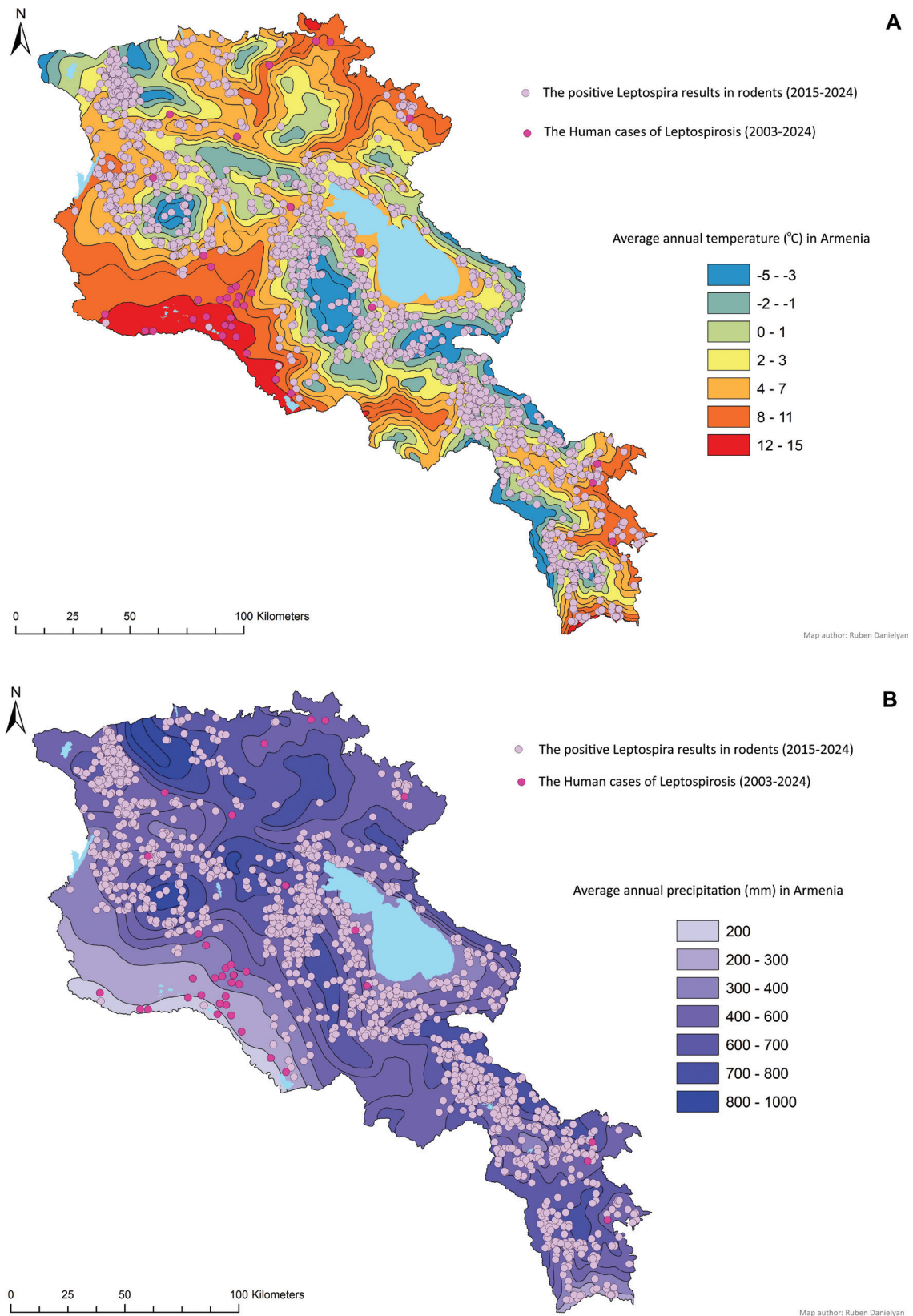


FIGURE 4 | (A) Average annual temperature (°C) in Armenia, with data on *Leptospira* positivity among rodents and cases of leptospirosis among humans. (B) Average annual precipitation (mm) in Armenia, with data on *Leptospira* positivity among rodents and cases of leptospirosis among humans.

examined animals, 12.1% tested positive, and most infected individuals (97.3%) were common voles (*Microtus obscurus*). This marked predominance highlighted the central role of this species in maintaining natural foci of leptospirosis.

The high prevalence of infection in voles was closely associated with their ecological distribution. Most positive findings (60.3%) were recorded at elevations of 2,000–3,000 meters, corresponding to mountain and mountain-steppe ecosystems. These observations indicated that the natural foci of leptospirosis in Armenia are primarily in high-altitude landscapes, where environmental conditions favor both host abundance and pathogen persistence.

Climatic analysis further supported this pattern. Most *Leptospira*-positive rodents were detected in zones characterized by moderate temperatures (+1°C to +5°C) and precipitation levels of 500–700 mm. Similarly, human cases were most frequently observed in slightly warmer conditions (+5°C to +7°C) within the same precipitation range. These findings emphasize the importance of local climatic factors in shaping pathogen distribution and transmission dynamics.

High serological diversity was observed among rodent populations, among which 12 *Leptospira* serogroups were detected. The predominance of Ballum, Hebdomadis, Autumnalis, Pyrogenes, and Javanica (57% of all cases) reflected the multicomponent structure of natural foci and the circulation of multiple serovars within rodent communities. Despite the major role of *M. obscurus*, spatial analysis revealed enhanced species diversity of infected rodents at elevations of 600–1,400 meters, thus suggesting that landscape heterogeneity and ecological variability contribute to reservoir complexity.

During 2003–2024, 83 human cases of leptospirosis were recorded. Although the incidence remained relatively low, spatial analysis demonstrated a strong association with anthropogenic environments, including semi-desert, subtropical, and mountain-steppe zones, as well as areas with high population density (85% of cases). This pattern underscores the roles of human activity, sanitation conditions, and environmental exposure in determining infection risk.

Notable differences in serogroup structures were observed between rodents and humans. Whereas rodent populations exhibited diverse serogroups, human infections were dominated by the Icterohaemorrhagiae serogroup (72.2%). However, this serogroup accounted for only 6.1% of rodent isolates and was detected exclusively in *Microtus* spp. (Table 3). This divergence might be explained by eco-epidemiological differences in reservoir hosts. *Leptospira icterohaemorrhagiae* is maintained primarily in rats (*Rattus* spp.) [9], which are closely associated with human settlements. Although few positive rats were detected in this study, their epidemiological importance remains high, as supported by regional and global data on urban leptospirosis.

Our findings therefore suggest the coexistence of two partially independent epidemiological systems: a natural

cycle maintained predominantly by voles and other wild rodents, and a synanthropic cycle involving rats. The lack of a direct correspondence between dominant serogroups in wildlife versus humans highlighted the importance of considering both systems in surveillance and control strategies.

The socio-demographic profiles of affected humans (91.5% male; mean age 40 years) were consistent with established patterns of occupational exposure. Activities such as agriculture, irrigation, and contact with livestock facilities or open water sources are likely to increase infection risk. The detection of only a single fatal case aligned with the mortality levels reported in settings with accessible healthcare and timely diagnosis.

The regional context further reinforced the relevance of this study. Leptospirosis cases are reported each year in Georgia, and less frequently in Armenia and Azerbaijan [7]. The inclusion of this disease among the priority zoonoses in Armenia underscores its epidemiological and veterinary-sanitary importance [10, 11]. Moreover, the detection of *Leptospira* spp. in bats of the family Vespertilionidae suggests a broader ecological niche of the pathogen than previously recognized; therefore, further investigation of additional reservoir hosts is warranted [12].

Overall, these findings highlight a need for integrated surveillance approaches targeting both natural and synanthropic reservoirs. Expanded epizootiological monitoring of rodent populations, particularly rats in urban and rural settings, should be prioritized, alongside improvements in sanitation and targeted awareness programs for high-risk groups. Given the high prevalence of infection in mountain-steppe and high-altitude zones, where agro-industrial activities are concentrated, preventive measures should particularly focus on agricultural workers.

The compiled dataset and analytical framework in this study provide a robust foundation for future eco-epidemiological studies. Such work would enable more precise risk assessment, improve understanding of pathogen circulation within complex ecosystems, and support the development of evidence-based strategies for leptospirosis prevention and control.

CONCLUSION

Leptospirosis in Armenia is maintained through both natural (vole-dominated) and synanthropic (rat-associated) transmission cycles. *Microtus obscurus* is the primary reservoir in high-altitude ecosystems, and *Leptospira Icterohaemorrhagiae* drives most human infections. These findings highlight the need for integrated surveillance and targeted control measures focusing on rodent reservoirs and high-risk anthropogenic environments.

DATA AVAILABILITY STATEMENT

Supplementary data and materials supporting the findings of this study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Laboratory and field investigations were reviewed and approved by the Scientific Council of the National Center for Disease Control and Prevention (protocol No. 154/2021). All stages of the study were conducted in accordance with applicable ethical standards, biosafety requirements, and approved methodological guidelines for research involving humans, animals, and biological materials.

AUTHOR CONTRIBUTIONS

All authors participated in developing and planning the research. Author 1 contributed to conceptualization (lead), writing the original draft (lead), and formal analysis (lead). Author 6 contributed to writing, review, and editing. Authors 3 and 4 assisted with review and editing (equal). Author 2 conceptualized the study (supporting). Author 5 contributed to map creation and spatial analysis. All authors have read and approved the final article.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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