Leptospirosis in Zakarpattia Oblast (2005–2015)

Olga Markovych,1 Victoria Tymchyk,2 and Iryna Kolesnikova3

Abstract

Background: Leptospirosis occurs sporadically and as outbreaks throughout Ukraine and is a nationally reportable disease. Zakarpattia oblast, in the southwestern region of the country, is endemic for disease. This study examines changes in epidemic patterns from 2005 to 2015.

Materials and Methods: Suspected cases from health care services were identified based on clinical presentation and serological samples were collected. Patient sera were tested by microscopic agglutination test (MAT) against serovars of Leptospira spp. belonging to 13 serogroups. Small mammals were also collected, sampled, and tested near locations of suspected exposures. Changes in leptospirosis incidence in Zakarpattia oblast were characterized over an 11-year period.

Results: A total of 420/2079 possible human cases were identified as having leptospirosis and 401/420 were confirmed by MAT. There was no annual trend in prevalence. Incidence increased from 2005 to 2009, peaked in 2010 (6.24 cases/100,000), and by 2015, there were only sporadic cases (0.88/100,000). The predominant serogroups were Icterohaemorrhagiae, Hebdomadis, and Grippotyphosa of Leptospira spp. The dominant serogroups shifted during the study from predominantly Icterohaemorrhagiae to Grippotyphosa in later years. A total of 2820 small mammals were assayed for Leptospira spp. Apodemus agrarius, Rattus norvegicus, and Mus musculus were the most common species sampled (76.1% of all captures). Among small mammals, antibodies were found mostly for Icterohaemorrhagiae, Pomona, and Grippotyphosa serogroups, and were detected in 276 samples (9.79% ± 0.56%). The dominant serogroups of Leptospira spp. isolated from mammals and patients changed cyclically, but the common human serogroups tended to differ from that seen in the concurrent mammal populations.

Conclusions: Patients with leptospirosis in this endemic region decreased more than fivefold during the past decade. Leptospira infections in small mammals remained common across multiple species ranging from 5% to 14%.

Keywords: leptospirosis, rodents, diagnostics, epidemiology, Ukraine

Introduction

Leptospirosis, a disease caused by bacterial infection with various pathogenic serovars from genus Leptospira, occurs globally in numerous environments, typically associated with rural and agricultural populations (Vinetz 2001). However, infection can also occur among urban poor when large populations of synanthropic species serve as reservoirs (Vinetz et al. 1996). The disease, if recognized early, is treatable with antibiotics. However, the diverse range of signs and symptoms makes it challenging to diagnose. In addition, laboratory diagnostics are oftentimes unavailable at the level of community-based services. Diagnostic methods include PCR, which is common for early diagnosis of leptospirosis (Vinetz et al. 1996), and serological tests such as microscopic agglutination test (MAT) (Vinetz 2001).

In various regions of the world, leptospirosis does not require national mandatory reporting. For example, in Canada and the European Economic Area (EAA) countries, notification is not required (European Centre for Disease Prevention and Control 2007). In the United States, leptospirosis was reinstated as a nationally notifiable disease since January
14 Cynopteri Cynopteri Vleermuis 3868
13 Pyrogenes Pyrogenes Salinen
12 Ballum Ballum Muz-127
11 Bataviae Djatzi HS-26
10 Australis Bratislava Jez Bratislava
9 Autumnalis Autumnalis Akijami A
8 Javanica Poi Poi

Human data

Study area

The study is a summary of data about trends and changes in Leptospira infection during 11 years in Zakarpattia oblast, a region of Western Ukraine, where the disease is endemic. Zakarpattia oblast is located on the western boundary of Ukraine with Romania, Hungary, Poland, and Slovakia. The Carpathian Mountains cover almost two-thirds of the region, while the rest is lowlands. The region is predominantly (63%) rural, with more than half covered by forests, and the climate is moderately continental. Zakarpattia includes regions with foci for leptospirosis, the most commonly reported disease, as well as other zoonotic diseases, including hemorrhagic fevers and Lyme borreliosis.

Materials and Methods

Study area

The study is a summary of data about trends and changes in Leptospira infection during 11 years in Zakarpattia oblast, a region of Western Ukraine, where the disease is endemic. Zakarpattia oblast is located on the western boundary of Ukraine with Romania, Hungary, Poland, and Slovakia. The Carpathian Mountains cover almost two-thirds of the region, while the rest is lowlands. The region is predominantly (63%) rural, with more than half covered by forests, and the climate is moderately continental. Zakarpattia includes regions with foci for leptospirosis, the most commonly reported disease, as well as other zoonotic diseases, including hemorrhagic fevers and Lyme borreliosis.

Human data

Suspected human cases were identified at local health care facilities based on clinical signs and symptoms as well as histories of potential animal exposures. Sera from patients suspected of leptospirosis were collected throughout the oblast and tested at the Especially Dangerous Infections (EDI) Laboratory of Zakarpattia Oblast Laboratory Center of the State Sanitary and Epidemiological Service (SSES) of Ukraine (currently named the Zakarpattia Oblast Laboratory Center of the Ministry of Health of Ukraine). This is the unique laboratory in the oblast authorized to perform these tests. Results were reported to the health care facilities; positive results were reported to SSES rayon (subterritorial) institutions for follow-up epidemiological investigation, and the Ministry of Health was notified.

Table 1. Panel of Leptospira Reference Strains Used in the Microscopic Agglutination Test, According to the Respective Serovars and Serogroups (Methodological Recommendations 2002)

| Nr. | Serogroup     | Serovar       | Reference strain |
|-----|---------------|---------------|------------------|
| 1   | Icterohaemorrhagiae | Copenhageni | M-20             |
| 2   | Grippotyphosa  | Grippotyphosa | Moskva V         |
| 3   | Pomona         | Pomona        | Pomona           |
| 4   | Canicola       | Canicola      | Hond-Utrecht IV  |
| 5   | Tarassovi      | Tarassovi     | Perepelcin       |
| 6   | Hebdomadis     | Cabura        | Cabura           |
| 7   | Sejroe         | Polonica wolffi | 493 Poland 3705a |
| 8   | Javanica       | Poi           | Poi              |
| 9   | Autumnalis     | Autumnalis    | Akijami A        |
| 10  | Australis      | Bratislava    | Jez Bratislava   |
| 11  | Bataviae       | Djatzi        | HS-26            |
| 12  | Ballum         | Ballum        | Muz-127          |
| 13  | Pyrogenes      | Pyrogenes     | Salinen          |
| 14  | Cynopteri      | Cynopteri     | Vleermuis 3868   |

*aSerovar Polonica wolffi of Sejroe serogroup was not available in the diagnostic kit of Leptospira strains of Especially Dangerous Infection laboratory during the study period.

Paired sera were screened initially at 1:5 and 1:50 dilutions. In case of positive reaction at these titers, we diluted the serum to 1:10, 1:100, 1:200, and less (the method is described in the Methodological Recommendations 9.1.1.098-02 Anti-Epidemic Measures and Laboratory Diagnostics of Leptospirosis approved by the Decree of the Chief State Sanitary Doctor of Ukraine No. 39 of 11 December 2002 [Methodological Recommendations 2002]). For negative control, we mixed physiological solution and Leptospira in equal volumes. Antibody titers of 1:100 were considered positives in combination with clinical and epidemiological data of the case. Fourfold increase of antibody titers in paired sera was a doubtless proof of an acute infection. MAT was performed against 13 Leptospira spp. serovars that were available in EDI Laboratory of Zakarpattia Oblast Laboratory Center of the SSES to identify the presumptive serogroup of infective serovar (Table 1).

Mammal data

Small mammals were collected as part of active case surveillance in follow-up to human cases as well as part of ongoing surveillance of wild animal sources of infection in areas that historically had been identified as leptospirosis foci near population centers. A diverse array of habitats (farmland, forest, shrubs, perennial grasses, and population centers) and landscapes and geographical zones of the oblast—mountain, foothill, and lowland—were surveyed.

Wild terrestrial mammals were collected with small snap traps used for “mouse-sized” mammals, while larger snap traps were used for “rat-sized” mammals. If animals were found alive in traps, to comply with bioethics requirements, euthanasia was performed according to national standards. Captured animals were transported to the EDI laboratory for testing to determine blood antibodies with MAT in the same way as it was described for human samples, except there was only a single sample from each animal. During rodent necropsy, we collected blood from heart cavity on filtration paper 1×1 cm, dried them out, and prepared “dry blood drops.” We extracted the “dry blood drop” in 0.5 mL of physiological solution (dilution 1:10) to prepare next dilutions.

334 MARKOVYCH, TYMCHYK, AND KOLESNIKOVA
On average, 100–120 surveys by workers of the EDI laboratory bio group and SSES territorial institutions were organized annually. During a survey transect, 80–100 traps were set approximately every 5 meters. Traps were checked, animals collected, and the traps were reset one to two times, over night, per survey. Throughout a year, 1500–1800 trap-days were expended. Historically, epizootological surveys were conducted in each district (= rayon) twice a year—in spring and during summer-autumn period (June–October). The size and number of traps in each biozone depended upon the size of the district and the species of mammals expected to be collected, and were determined by EDI laboratory biologist.

Statistical analysis of the data was performed using Excel software to calculate basic descriptive statistics and differences in rates.

Results

A total of 420 suspected cases of leptospirosis were identified by the health care surveillance system out of 2079 people presenting for evaluation (20.2%), and 401 of these (95.5%) were confirmed by MAT. Leptospirosis incidence fluctuated widely across years, but there was a general downward trend since the start of the study. Cases were more frequent during the first half of study (2005–2009) ranging from 36 to 51 cases, and peaked in 2010 (77 cases) before declining to 17–29 cases annually (2011–2015) (State Service of Ukraine for Emergency Situations (SSUES) 2016). The incidence ranged from 0.88 to 6.24 per 100 thousand, mirroring changes in the crude national rate. Between 2005 and 2009, Icterohaemorrhagiae predominated among identified human cases. In 2010, this serogroup was abruptly displaced when 31 laboratory-confirmed cases of Grippotyphosa (from 76 cases) were identified. Grippotyphosa had not been previously reported in humans from the region, but it remained the predominant serogroup for 4 subsequent years. During the last 2 years of study, the diversity of serogroups increased with nearly equal occurrence of Tarassovi, Icterohaemorrhagiae and Pomona. During this time, several serogroups that had previously been rarely encountered (Autumnalis, Ballum, Javanica, and Cynopteri) became more commonly reported in humans (accounting for 26.5% of cases; Fig. 2a).

Previous experience has shown that small mammals were leading sources of leptospirosis (Badra 2008, Zmudzki et al. 2016). A total of 2820 small mammals were collected and tested by MAT (Table 3). The preponderance (84.5%) of individuals captured belonged to four species: Apodemus agrarius, Rattus norvegicus, Mus musculus, and Apodemus sylvaticus. The remaining six species (including the single individual of the European mole, Talpa europaea) were less commonly sampled.

Antibodies to Leptospira spp. were detected in 276 animals by MAT (9.79% ± 0.56%). Samples were collected from 10 species with a preponderance of A. agrarius, R. norvegicus, and M. musculus (75.71%). Among the examined small mammals, the carriers of pathogenic Leptospira spp. were animals from natural habitats (A. agrarius, Apodemus flavicollis, Sorex araneus, A. sylvaticus, Myodes glareolus, Microtus arvalis, and Dryomys nitedula) as well as synanthropic small mammals (R. norvegicus and M. musculus). Generally,

### Table 2. Annual Incidence of Leptospirosis in Zakarpattia Oblast (2005–2015) Compared with National Statistics

| Years | No. of people diagnosed | Morbidity per 100,000 people | No. of people with MAT-positive results/clinical dx of leptospirosis in Oblast | No. of people studied in Oblast | Rate per 100,000 population in Oblast |
|-------|-------------------------|------------------------------|-----------------------------------------------------------------|-------------------------------|-------------------------------------|
| 2005  | 679                     | 1.40                         | 49/51                                                          | 274                           | 4.1                                 |
| 2006  | 490                     | 1.04                         | 49/50                                                          | 266                           | 4.0                                 |
| 2007  | 674                     | 1.44                         | 46/49                                                          | 222                           | 3.9                                 |
| 2008  | 530                     | 1.10                         | 48/49                                                          | 221                           | 3.9                                 |
| 2009  | 442                     | 0.96                         | 33/36                                                          | 179                           | 2.9                                 |
| 2010  | 632                     | 1.37                         | 76/77                                                          | 284                           | 6.24                                |
| 2011  | 310                     | 0.68                         | 22/23                                                          | 154                           | 1.85                                |
| 2012  | 317                     | 0.70                         | 28/29                                                          | 135                           | 2.12                                |
| 2013  | 358                     | 0.79                         | 16/17                                                          | 119                           | 1.38                                |
| 2014  | 474                     | 1.04                         | 26/28                                                          | 124                           | 2.23                                |
| 2015  | 301                     | 0.70                         | 8/11                                                           | 101                           | 0.88                                |
| Total number | 5207                     | Average rate 1.02 | 401/420                                                                        | 2079                           | Average rate 3.05                     |

MAT, microscopic agglutination test.
when a species of small mammal was well represented in the collections (>50 animals), the prevalence of infected animals tended around 10–15%, with the exception of *M. musculus* where the prevalence was about half that level (Table 4). There was a significant difference in the proportion of positive animals by species ($\chi^2 = 39.26; p < 0.001$). The “excess” of positive *A. agrarius* and the dearth of positive *M. musculus* accounted for more than 80% of the deviation from independence of infection among species. Despite this, evidence for infection in multiple species was common.

Most of the serogroups identified in small mammals included Icterohaemorrhagiae, Pomona, and Grippotyphosa of *Leptospira* spp. During the study period, changes in the dominant serogroups were observed among the small mammals, as much as was observed in humans. However, the changes in small mammal serogroups did not mirror the changes seen in humans across years. Thus, for the period from 2005 to 2009, the dominant serogroups in rodents were Icterohaemorrhagiae (44.6%) and Pomona (34.4%). From 2010 to 2012, Pomona became dominant (50.9% of positives). From 2013 to 2015, Grippotyphosa became the dominant serogroup (53.2% of all positives). Moreover, during this period, rarely encountered serogroups (Australis, Ballum, Bataviae, and Autumnalis) were identified in 17 cases (27.42%) (Fig. 2b).

While serogroups Icterohaemorrhagiae, Hebdomadis, Pomona, Canicola, Grippotyphosa, Autumnalis, Ballum, and Tarassovi of *Leptospira* spp. were reported in humans cases and studied rodents, the prevalence differed between the two groups within years (Fig. 2a, b). Thus, small mammals remain to be the main source of pathogenic *Leptospira* spp. for humans, but additional factor(s) are associated with the likelihood of transmission to the humans on a yearly basis.

**Discussion**

Leptospirosis is an infectious disease of global importance, which occurs both in urban regions of industrialized and developing countries, as well as in rural regions. Importation of leptospirosis is possible due to expanding international connections, tourism, and recreation (Bharti et al. 2003, Pappas et al. 2008).

Nearly the entire territory of Ukraine is enzootic for leptospirosis. Since 2008, a decreasing morbidity trend has been reported. Overall, the national-level mean incidence was 1.02 per 100,000 population. However, the incidence generally declined from 2005 to 2015, from 1.44 per 100,000 population (674 cases) in 2007 to 0.68 per 100,000 population (310 cases) in 2011. During the same time, leptospirosis morbidity in European countries typically was lower than in Ukraine (Pappas et al. 2008).

Zakarpattia oblast shares borders with Romania, Hungary, Poland, and Slovakia in the West, and with Ivano-Frankivsk and L’viv oblasts of Ukraine in the East. Zakarpattia is a territory with moderate continental climate, with sufficient and excessive moisture, moderately hot summers, and mild winters (Pop 2011). There are geographical, landscape, zoological, and parasitological conditions that influence various natural focal diseases, including leptospirosis. The human population density in the oblast is above the average for the country, and a large proportion of the population (63%) lives in rural areas. High leptospirosis incidence is often reported in regions with high proportions of surface fresh water (Wasinski et al. 2013). The river system in Zakarpattia is the densest in Ukraine (1.7 km/km²), which is influenced by high humidity and mountain relief. Increasing water levels in spring and early summer, and recurrent flooding

**FIG. 1.** Case-fatality rates during the study period among patients with leptospirosis in Zakarpattia and nationally.
are typical for Zakarpattia, which can have disastrous impacts (Markovych 2010). In 2010, for example, flooding caused by prolonged heavy rain in June–July inundated villages in lowland rayons of Zakarpattia. During this period, leptospirosis incidence peaked (Table 2).

Flooding is one of the aspects of climate change that may lead to increased morbidity of infectious diseases, including leptospirosis. Higher leptospirosis morbidity after floods also was reported in the Czech Republic, and in Poland after the floods in 1997–2002 (Desai et al. 2009; Wasin´ ski et al. 2013). Although in temperate climatic zones, leptospires survive in the environment for a much shorter time than in tropical countries, increased floods may drive the reemergence of disease.

During the study period, 420 leptospirosis cases were recorded in the oblast, and the incidence was three times higher than the average incidence for Ukraine (Table 2). The case-fatality rate in the oblast also was elevated compared to national statistics (Fig. 1). The predominant serogroups in humans were Icterohaemorrhagiae, Hebdomadis, and Grippotyphosa of Leptospira spp. with a shift in the predominant serogroups during the study (Fig. 2a).

The most important natural sources of infection are various small rodent species. Studies conducted in Croatia showed that 7.0% to 29.9% of small rodents carried Leptospira (Majetić et al. 2014). In the Czech Republic, serological studies of 4634 samples from wild mammals found 12%
### Table 3. Results of Epizootiological Study of Zakarpattia Oblast in 2005–2015

| Species       | Apodemus agrarius | Rattus norvegicus | Apodemus flavicollis | Sorex araneus | Apodemus sylvaticus | Myodes glareolus | Microtus arvalis | Mus musculus | Dryomys nitedula | Talpa europaea |
|---------------|-------------------|-------------------|----------------------|---------------|---------------------|-----------------|-----------------|--------------|----------------|--------------|
| Positive test no. | Number studied | Positive test no. | Number studied | Positive test no. | Number studied | Positive test no. | Number studied | Positive test no. | Number studied | Positive test no. | Number studied |
| 2005          | 7                | 89                | Pom.               | 10             | 157                | Ict.            | 5               | 27           | Hebd.          | 0            | 7              | —               |
| 2006          | 17               | 82                | Pom.               | 25             | 145                | Ict.            | 0               | 0            | 0              | 0            | 0              | —               |
| 2007          | 15               | 64                | Pom.               | 10             | 126                | Ict.            | 2               | 20           | Gripp.         | 3            | 36              | Ict., Pom., Pyr. |
| 2008          | 4                | 80                | Pom.               | 4              | 86                 | Ict.            | 0               | 6            | 0              | 0            | 18             | Gripp.         |
| 2009          | 11               | 80                | Pom.               | 15             | 54                 | Ict.            | 0               | 9            | 1              | 13           | Can.           | 34              |
| 2010          | 13               | 69                | Pom.               | 9              | 87                 | Ict.            | 0               | 9            | 0              | 0            | 38             | —               |
| 2011          | 14               | 72                | Pom.               | 4              | 34                 | Gripp.          | 0               | 30           | 0              | 6            | 3              | 12              |
| 2012          | 1                | 7                 | Gripp.             | 3              | 10                 | Pom.            | 0               | 0            | 0              | 0            | 3              | 3               |
| 2013          | 0                | 21                | —                  | 1              | 10                 | Gripp.          | 0               | 0            | 0              | 0            | 7              | 3               |
| 2014          | 16               | 31                | Gripp.             | 0              | 4                  | —               | 4               | 10           | Gripp.         | 2            | 6              | Can.           |
| 2015          | 6                | 29                | Gripp.             | 0              | 3                  | —               | 0               | 6            | 4              | 14           | Gripp.         | —               |
| Total         | 104              | 724               | Pom.               | 81             | 716                | Ict.            | 11              | 117          | Gripp.         | 8            | 86             | Gripp.         | 20              | 249          | Gripp.         | 15           | 198            | Pom.           | 35            | 695            | Gripp.        | 0            | 2              | 0              | 1            |

Annual numbers of individual small mammal species, from ten species sampled, with positive result by MAT.
positive, and of these, 99% had antibodies against serovars belonging to Grippotyphosa serogroup. This serogroup is maintained within various small rodent species in Europe, including common voles (Microtus arvalis), muskrat (Ondatra zibethicus), European hamster (Cricetus cricetus), wood mouse (A. sylvaticus), and the yellow-necked mouse (A. flavicollis) (Zmudzki et al. 2016).

Studies in Switzerland on the prevalence of Leptospira infections in urban populations of rodents showed, depending on the species, 10–20% positive results (Adler et al. 2002). In Germany, a study on Rattus from 16 urban regions revealed 19% of kidney samples contained DNA of Leptospira spp. (Mayer-Scholl et al. 2012). Serological surveys of rats trapped in Wrocław, Silesia (Poland), found antibodies to serovars Icterohaemorrhagiae, Canicola, Hebdomadis, and Sejroe (Wincewicz et al. 2001).

The Zakarpattia fauna contains ~80 species of mammals and more than 45% of mammals are rodents. Small rodent populations have sharp seasonal increases and declines. In the spring, their number is not large; while population peaks in the autumn (Pop 2011). Among tested rodent species, A. agrarius, R. norvegicus, and M. musculus were most common (75.7% of all captures). Positive results were found both in the natural areas and among synanthropic rodents. During the study period, the predominant serogroups changed (Fig. 2b). According to researchers, recurrent change of the dominant Leptospira serogroup has been recognized. For example, the predominant serogroups in cattle from the Lower Silesian province were most frequently recognized. Serological surveys of rats trapped in Zakarpattia Oblast of Ukraine decreased more than fivefold during the past decade. Leptospira infections in small mammals were common across multiple species ranging from 5% to 14% in eight species with sufficiently sampled individuals. Antibodies of serogroups of Leptospira spp. detected in patients matched those of studied rodents, but differed in proportions of occurrence. This suggests that, while the small mammals were likely sources of infection, the contact with various species may impact the exposure in this endemic region. Thus, changes in the patterns of infection in the small mammal community might drive changes in the exposure risk to different serogroups in adjacent human populations.

### Acknowledgments

The author would like to acknowledge the United States Department of Defense, Defense Threat Reduction Agency (DTRA), Cooperative Biological Engagement Program (CBEP), for their assistance and financial support in publication of this article. While DTRA/CBEP did not support the research described in this publication, the Program supported the article publication. The contents of this publication are the responsibility of the author and do not necessarily reflect the views of DTRA or the United States Government.

Sincere thanks to Dr. Greg Glass, University of Florida, for his mentoring, invaluable help, and support in writing this article. We also wish to express our appreciation to two anonymous reviewers whose comments greatly improved the article.

### Author Disclosure Statement

No competing financial interests exist.

### References

Adler H, Vonstein S, Deplazes P, et al. Prevalence of Leptospirosis spp. in various species of small mammals caught in inner-city area in Switzerland. Epidemiol Infect 2002; 128:107–109.

Badra BM. Leptospirosis as Metropolis Zoonthroposis: Etiological Structure, Epizootiological and Epidemiological Peculiarities, Diagnostics and Prophylaxis. Saint Petersburg: Thesis Abstract, 2008.

Bharti AR, Nally JE, Ricaldi JN, Matthias MA, et al. Leptospirosis: A zoonotic disease of global importance. Lancet Infect Dis 2003; 3:757–771.

Centers for Disease Control and Prevention (CDC). Technical information for leptospirosis. 2018. Available at https://www.cdc.gov/leptospirosis/health_care_workers/index.html.

Desi S, Treeck U, van Lierz M, Espelage W, et al. Resurgence of field fever in a temperate country: An epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. Clin Infect Dis 2009; 48:691–697.

European Centre for Disease Prevention and Control. The First European Communicable Disease Epidemiological Report.

---

### Table 4. Summary of Prevalence Results for Serologic Studies of Rodents for 2005–2015

| Species                          | Apodemus agrarius | Rattus norvegicus | Apodemus flavicollis | Sorex araneus | Apodemus sylvaticus | Microtus arvalis | Myodes glareolus | Mus musculus |
|---------------------------------|-------------------|-------------------|----------------------|---------------|---------------------|------------------|-----------------|--------------|
| Total no. of studied rodents    | 724               | 716               | 117                  | 86            | 249                 | 198              | 32              | 695          |
| Positive test no.               | 104               | 81                | 11                   | 8             | 20                  | 15               | 2               | 35           |
| Percentage of positive tests    | 14.36±1.3         | 11.31±1.18        | 9.40±2.7             | 9.30±3.13     | 8.03±1.72           | 7.58±1.88        | 6.25±4.28       | 5.04±0.83    |

There is a statistically significant association ($\chi^2 = 39.26; p < 0.001$) between small mammal species and the likelihood of positive Microscopic Agglutination Test results.
Majetić Š, Galloway R, Ruzić Sabljic E, Milas Z, et al. Epi-
zootiological survey of small mammals as Leptospira spp. reservoirs in Eastern Croatia. Acta Trop 2014; 131:111–116.

Markovych V. Epidemiological aspects of emergency situations natural genesis (based on the model of floods in Transcarpathian region of Ukraine). Dissertation. Kyiv 2010; 4:49–50.

Mayer-Scholl A, Luge E, Hammer J, Dremsek P, et al. Study on the Leptospira prevalence in rats in major German cities. Proceedings of the European Meeting of Leptospirosis Eurolepto, Dubrovnik, Croatia, May 31–June 2, 2012, 2012.

Methodological Recommendations 9.1.1.098-02 Anti-Epidemic Measures and Laboratory Diagnostics of Leptospirosis approved by the Decree of the Chief State Sanitary Doctor of Ukraine No. 39 of 11 December 2002. Ministry of Health of Ukraine, Kyiv 2002:43–49.

Pappas G, Papadimitriou P, Siozopoulou V, Christou L, et al. The globalization of leptospirosis: Worldwide incidence trends. Int J Infect Dis 2008; 12:351–357.

Pop S. Nature-Protected Areas of Transcarpathian Region. Uzhgorod: Karpaty, 2011:14–18.

Sidelnikov YN. Non-transmission Natural-Foci Infections of the Far East. Khabarovsk: Publishing Center of IPKSZ, 2006: 24.

State Service of Ukraine for Emergency Situations (SSUES). Analytical review of technogenic and natural safety in Ukraine for 2015. Kyiv, 2016:174–175. Available at http://cdn.sns.gov.ua/ua/Analitichniy-oglyad-stamu-technogennoyi-ta-prirodnoyi-bezpeki-v-Ukrayini-za-2015-rik.html.

Vinetz JM. Leptospirosis. Curr Opin Infect Dis 2001; 14:527–538.

Vinetz JM, Glass GE, Flexner CE, Mueller P, et al. Sporadic urban leptospirosis. Ann Intern Med 1996; 125:794–798.

Wasinski B, Dutkiewicz J. Leptospirosis—Current risk factors connected with human activity and the environment. Ann Agric Environ Med 2013; 20:239–244.

Winczewicz E, Klimentowski S, Smielewska-Łoś E, Jopek Z, et al. Bacterial and mycotic infections in wild rats from various environments. Med Weter 2001; 57:402–407.

Zmudzki J, Jabłoński A, Arent Z, Żebek S, et al. First report of Leptospira infections in red deer, roe deer, and fallow deer in Poland. J Vet 2016; 60:257–260.

Address correspondence to:
Olga Markovych
Laboratory of Especially Dangerous Infections
Department of Biological Factors Studies
Zakarpattia Oblast Laboratory Center of the Ministry of Health of Ukraine
96 Sobranecsa Str.
Uzhhorod 88000
Ukraine
E-mail: o.markovych@i.ua