Part 1. Veterinary medicine

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THE RESEARCH OF CIRCULATION OF PATHOGENIC LEPTOSPIRA AMONG POPULATIONS URBAN BROWN RATS IN KYIV, UKRAINE

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Summary. Urban brown rats (*Rattus norvegicus*) are frequently implicated in the spread of *Leptospira* spp. Urban brown rats (n = 116) were trapped in different places near the lakes and ponds in Holoseevsky and Obolon districts of Kyiv and tested for *Leptospira* spp. using an agglutination test (MAT), which was conducted with 21 *Leptospira*'s serological groups.

It is identified the circulation of pathogenic *Leptospira* among urban brown rats population of Kyiv, Ukraine. In total, 65 (56%) rats were positive by MAT. The antibodies against serogroups *Grippotyphosa, Mini* and *Ballum* were dominant in positive reactions.

Keywords: Leptospirosis, urban brown rats (*Rattus norvegicus*), antibodies, microscopic agglutination test (MAT), Kyiv, Ukraine

Introduction. Leptospirosis is a major emerging infection with a worldwide distribution (Levett, 2001). It is a systemic disease of humans and domestic animals (Adler and la Peña Moctezuma, 2010). Regarded globally as a zoonosis because it is acquired by humans from contact with animals or from water contaminated with the urine of infected animals, it is presumed to be the most widespread zoonotic disease in the world (Adler and la Peña Moctezuma, 2010; Levett, 2001).

Leptospirosis is caused by leptospira species, which has over 20 serogroups and more than 250 serovars and distributed worldwide (Bharti et al., 2003).

The natural reservoir of *Leptospira* is wild vertebrate animals, mostly mammals (Turner, 1967). Today we know that a wide variety of animals sheds *Leptospira* into the environment and thus puts others, including human, at risk of infection. An incomplete list of wild mammals known to be infected includes such orders as Rodentia (rats, mice, voles, gerbils, coypus), Insectivora (hedgehogs, shrews), Carnivora (dogs, foxes, mongooses, jackals, civets, skunks, raccoons), Marsupialia (bandicoots, opossums), Chiroptera (bats), Artiodactyla (deer), and Lagomorpha (hares, rabbits). Among the domestic animals, dogs, cattle, swine, and goats are frequently affected by this organism (Stoenner, 1976; Stoenner and MacLean, 1958). In addition, birds (Frezza, Sindoni, and Tredici, 1968), snakes, turtles, lizards (Lindtner-Knific et al., 2013), frogs (Gravekamp et al., 1991), and fish (Maestrone and Benjamenson, 1962) may be rare sources of infection.

Wild mammals, and in particular rodents, are the primary reservoirs of the infection; whereas domestic animals such as cattle, dogs and pigs may act as carriers for several months (temporary carrier) while rodents usually remain carriers throughout their life (permanent carrier). Rodents are therefore considered to be the major reservoir of infection (Bharti et al., 2003).

Two commensal (*L. cum mensa*) species are common inhabitants on farms worldwide: the house mouse (*Mus musculus*) and the brown rat (*Rattus norvegicus*). Both species originated from Asia, from where they spread over the world along with the development of agriculture, which provided shelter and supplies of food. They are underground dwellers, omnivorous and can breed year-round when conditions are optimal (Hanney, 1975; Nowak, 1999).

Generally, the urban rodent problem is caused by three species: the Norway rat (*Rattus norvegicus*), the roof rat (*Rattus rattus*), and the house mouse (*Mus musculus*). Although the Norway rat is known also as the brown rat and the roof rat as the black rat, these names are misleading because the colors of both species range from light brown to black. These three rodents, of all rodent pests (nutria, cotton rat, and the like),

are most often identified with rodent-borne diseases and economic loss through destruction and contamination (Clinton, 1969).

The rat was the first incriminated as a source of human infection and was long thought responsible for most human illness. Thus, the *Leptospira Icterohaemorrhagiae*, was first reported from a human with Weil's disease in 1916 and then from wild brown rats (*Rattus norvegicus*) in 1917 (Inada et al., 1916; Noguchi, 1917).

In 1990–1991 were reported, that most, if not all, rat populations are infected with *Leptospira Icterohaemorrhagiae* at a prevalence of 50–70% (Golding, 1990; Waitkins, 1991).

Nowadays rats are the primary carriers of *Leptospira* serovars *Icterohaemorrhagiae* and *Copenhageni* (i.e., the *Icterohaemorrhagiae* serogroup) and serogroup *Ballum* (Aviat et al., 2009; Ko, Goarant, and Picardeau, 2009; Levett, 2001). These serovars are the major in leptospirosis of human (Ko, Goarant, and Picardeau, 2009), dogs (Cerri et al., 2003) and pigs (Cisneros Puebla et al., 2005).

The brown rats are regarded as the carriers of other serovars which are dangerous for human and animals (Babudieri, 1958; Keenan et al., 2009.

Rats are colonial and territorial animals that promote intra-colony interactions (Barnett, 1963). In the urban environment, limited available space leads to the formation of colonies with a small home range, and inter-colony contacts may occur. Therefore, the urban rat population more likely functions as a meta-population, as described by Viana et al. (2014), in which the sub-unit is the rat colony. Determining rat meta-population behavior with regard to *Leptospira* strain distribution would provide evidence of rat interactions resulting in rat infections.

Over the last twelve years, studies of rodent infection in the vicinity of index cases have associated rats with human infection in both rural (Ganoza et al., 2006; Johnson et al., 2004) and urban settings (Jansen et al., 2005; Pezzella et al., 2005; Vinetz et al., 1996).

For example, five studies published during the period 2003–2014 the report on the prevalence of *Leptospira* infection among urban rats to range between 11.1% (N = 592) in Vancouver, Canada (Himsworth et al., 2013), 16% (N = 127) in Tokyo, Japan (Koizumi et al., 2009), 48% (N = 23) in Santa Fe, Argentina (Vanasco et al., 2003), 65.3% (N = 201) in Baltimore, USA (Easterbrook et al., 2007), and 63% to 83% (N = 226) in Salvador, Brazil (Costa et al., 2014).

The aim of this study is to investigate a large number of blood sera samples from wild brown rats living in urban areas to determine the current leptospira status, both in terms of its prevalence and the range of serogroups and serovars that were carried out.

Materials and methods. *Strains:* Twenty-one pathogenic *Leptospira* spp. strains were genotyped. These strains were part of the bacterial collection of the Leptospirosis Laboratory of Farm Animals with the Museum of Microorganisms of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine.

Rodents trapping: The investigation was carried out in two districts of Kyiv during the period from May 2014 to September 2015. Overall 116 rodents were collected, with various types of traps: sticky traps 'Catch Expert' and trap metal mesh. Traps were set on the banks of reedy lakes and ponds in parks and recreation areas consistently with the observations of rodent signs, droppings, nests, and burrows, and were controlled every day. On arrival at the laboratory, trapped Rodents were immediately anesthetized with ethyl ether and numbered, and the record of species and places of trapping was prepared.

Study sites: Sera from 116 urban brown rats, which were caught in the parks and public recreation areas in Holoseevsky and Obolon districts of Kyiv during 2014–2015, had been studied.

In Holoseevsky district, urban brown rats were restored in the following locations: Holoseevsky Park (cascade Horihuvatsky Pond and Holosiivsky Pond, Didorivka Lake), park 'Feofania' (Palladinski Lakes), ponds near the residential complex 'Towers-2', Mysholovski Lakes, and Kytayevski artificial ponds.

In Obolon district, rodents were caught near Verbne, Jordan, and Kirilivske Lakes, and the cascade of Opechensky Lakes.

Serological test (MAT): The research was carried out by microagglutination test (MAT) using antigens of 21 *Leptospira* serogroups which are listed in Table 1.

Table 1 – List of strains used for research

№	Serogroup	Serovar	Strain
1	*Javanica*	javanica	*Veldrat Bataviae 46*
2	*Bataviae*	*djatzi*	*HS 26*
3	*Mini*	*szwajizak*	*Szwajizak*
4	*Sejroe*	*polonica*	*493 Poland*
5	*Hebdomadis*	*kabura*	*Kabura*
6	*Tarassovi*	tarassovi	*Perepelicyni*
7	*Pomona*	*pomona*	*Pomona*
8	*Grippotyphosa*	*grippotyphosa*	*Moskva V*
9	*Canicola*	*canicola*	*Hond Utrecht IV*
10	*Icterohaemorrhagiae*	*copenhageni*	*M 20*
11	*Louisiana*	louisiana	*LSU*
12	*Shermani*	*shermani*	*LT 821*
13	*Panama*	*panama*	*CZ 214 K*
14	*Semaranga*	*patoc*	*Patoc 1*
15	*Celledoni*	*whitcombi*	*Whitcomb*

16	*Australis*	erinaceieuropaei	*Jez 1*
17	*Autumnalis*	*autumnalis*	*Akiyami A*
18	*Cynopteri*	*cynopteri*	*Vleermuis 3868*
19	*Pyrogenes*	*pyrogenes*	*Saline*
20	*Ballum*	*ballum*	*Mus 127*
21	*Australis*	bratislava	Jez-bratislava

MAT was used in dilutions: 1:50, 1:100, 1:500, 1:2,500. Antibody titers 1:50 and above were considered as positive.

The study of blood sera of wild rodents was conducted in the Laboratory of Leptospirosis with the Museum of Microorganisms of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine and in the Laboratory of Special Danger Infections of the Kyiv Regional Laboratory Center of the State Sanitary-and-Epidemiologic Service of Ukraine.

Results. During 2014–2015, 116 samples of blood sera from urban brown rats were investigated, 65 samples of them (56.0%) were positive.

In Holoseevsky district over the period, 79 samples of blood sera from urban brown rats (68.1% of the total) have been analyzed (Table 2).

As shown in Table 2, the urban brown rats contamination by pathogenic leptospira in the analyzed period was lower in 2014 (45.0%). In 2015, the rate was 62.7%. We see that in two years there is a significant percentage of mixed reactions, representing respectively 66.7% and 83.8%. This fact contributes to significant increase in the total number of positive reactions (mono + mixed).

In 2014, 20 samples of blood sera were investigated and 9 received reactions (45.0%) were positive in MAT. Antibodies to serogroup *Grippotyphosa* (31.6%) and *Icterohaemorrhagiae* (26.3%) of the total number of positive reactions were often diagnosed in urban brown rats this year. In significantly lesser amount of cases the serological groups *Pomona* (15.8%), *Cynopteri* (10.5%) and *Ballum* (10.5%) were registered. The smallest number of animals reacted positively to leptospirosis serogroup *Mini* (5.3%) (Table 2).

During 2015, 59 samples were analyzed and 37 positive reactions (62.7%) were received. The dominant serogroup, as in 2014, was *Grippotyphosa* — 17.3% of positive reactions. In 2015, antibodies to Leptospira of serological group *Mini* was diagnosed in 17.3% of samples while in 2014 — in 5.3% of samples. Other serological groups — *Ballum* (12.7%) and *Pomona* (11.0%) — were found in smaller quantities.

In 2015, the percentage of positive reactions to serogroup *Icterohaemorrhagiae* decreased by 18.6% compared to previous year; the following serological groups was recorded for the first time: *Celledoni* — 5.5% and *Australis* — 5.5% (serovar *bratislava*), *Javanica* — 5.0%, *Shermani* — 4.0% and *Australis* — 3.1% (serovar *erinaceieuropaei*). The lowest etiological role was played by *Sejroe* (0.9%), *Hebdomadis* (0.9%), and *Pyrogenes* (0.9%) serogroups.

Table 2 – The results of serological studies of blood sera samples of urban brown rats caught in Holoseevsky district, Kyiv

Years			**2014**	**2015**	**Total**
Studied samples of blood sera			20	59	**79**
Positive results		total	9	37	**46**
		%	45.0	62.7	**58.2**
Monoreactions, %			33.3	16.2	**19.6**
Mixed reactions, %			66.7	83.8	**80.4**
Positive results (mono + mixed)			19	220	239
Antibodies to leptospira serogroups	*Javanica*	total	—	11	**11**
		%	—	5.0	**4.6**
	Mini	total	1	38	**39**
		%	5.3	17.3	**16.3**
	Sejroe	total	—	2	**2**
		%	—	0.9	**0.8**
	Hebdomadis	total	—	2	**2**
		%	—	0.9	**0.8**
	Pomona	total	3	24	**27**
		%	15.8	11.0	11.3
	Grippotyphosa	total	6	38	**44**
		%	31.6	17.3	**18.4**
	Icterohaemorrhagiae	total	5	17	**22**
		%	26.3	7.7	**9.2**
	Shermani	total	—	9	**9**
		%	—	4.0	**3.8**
	Celledoni	total	—	12	**12**
		%	—	5.5	**5.0**
	Australis	total	—	7	**7**
		%	—	3.1	3.0
	Cynopteri	total	2	18	**20**
		%	10.5	8.2	**8.4**
	Pyrogenes	total	—	2	**2**
		%	—	0.9	**0.8**
	Ballum	total	2	28	**30**
		%	10.5	12.7	**12.6**
	Australis (bratislava)	total	—	12	**12**
		%	—	5.5	**5.0**

Over the period, in parks and places of public entertainment in Obolon district 37 wild rodents (31.9% of the total) were trapped and their blood sera samples were analyzed (Table 3).

Table 3 – The results of serological studies of blood sera samples of urban brown rats caught in Obolon district of Kyiv

Years			2014	2015	Total
Studied samples of blood sera			17	20	**37**
Positive results		total	9	11	20
		%	53.0	55.0	**54.0**
Monoreactions, %			55.6	9.1	**30.0**
Mixed reactions, %			44.4	90.9	**70.0**
Positive results (mono + mixed)			9	44	**53**
	Mini	total	2	10	**12**
		%	22.2	23.3	**22.6**
	Pomona	total	1	6	7
		%	11.1	13.6	**13.2**
	Grippotyphosa	total	3	10	**13**
		%	33.4	23.3	**24.6**
	Celledoni	total	—	1	**1**
		%	—	2.3	**1.9**
	Cynopteri	total	1	7	**8**
		%	11.1	16.2	**15.1**
	Ballum	total	2	10	**12**
		%	22.2	23.3	**22.6**

As shown in Table 3, during the whole period of research, the leptospirosis infection in urban brown rats was relatively at the same level (53.0% in 2014 vs. 55.0% in 2015). The percentage of monoreactions sharply reduced by 46.5% (from 55.6% in 2014 to 9.1% in 2015).

During 2014, we have studied 17 samples of blood sera and received nine positive reactions in MAT (53.0%). Leptospira serogroup *Grippotyphosa* was dominant (33.4%). At the second place — *Mini* (22.2%) and *Ballum* (22.2%). The lowest percentage of positive reactions was recorded for serological groups *Pomona* (11.1%) and *Cynopteri* (11.1%).

During 2015, 20 samples were analyzed and 11 positive reactions (55.0%) were received. Among them, prevalent serogroups were the following ones: *Mini* (23.3%), *Grippotyphosa* (23.3%), and *Ballum* (23.3%). Serogroups *Cynopteri* (12.5%) and *Pomona* (13.6%) were registered in significantly lesser amount of cases. This year, antibodies to serogroup *Celledoni* (2.3%) were diagnosed for the first time.

In general, 15 monoreactions were registered in urban brown rats from both areas for two years (Obolon district — 6, Holoseevsky district — 9). The etiological structure for their serogroups is shown in Fig. 1.

As shown in Fig. 1, the monoreactions were detected only to four leptospira serological groups.

In the samples of blood sera of wild rodents, which were trapped in Holoseevsky district of Kyiv, antibodies to serogroups *Grippotyphosa* (44.5%) and *Ballum* (33.3%) prevailed; *Mini* (11.1%) and *Pomona* (11.1%) serogroups were recorded less frequently.

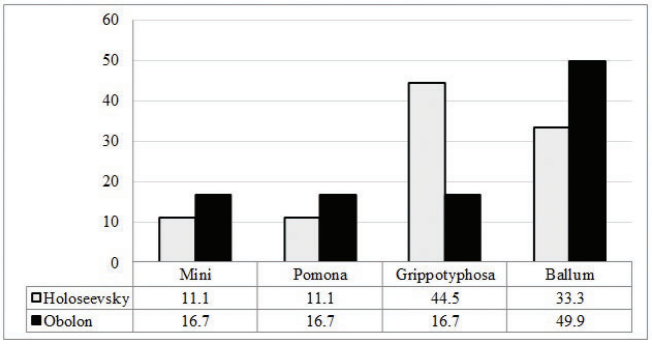

Figure 1. The etiological structure of monoreactions

Among the samples from Obolon district, the serogroup *Ballum* was dominant — it was registered almost half of positive cases (49.9%) of monoreactions. The other half is made up of serological groups *Mini* (16.7%), *Pomona* (16.7%), and *Grippotyphosa* (16.7%).

Thus, based on the analysis of the results, we see both mixed and monoreactions in the samples of blood sera from urban brown rats trapped in Holoseevsky and Obolon districts of Kyiv during 2014–2015. Amount them, serogroups *Mini*, *Grippotyphosa*, and *Ballum* were prevalent. The percentage of positive reactions to each serogroup in the total number of animals reacting shows in Fig. 2.

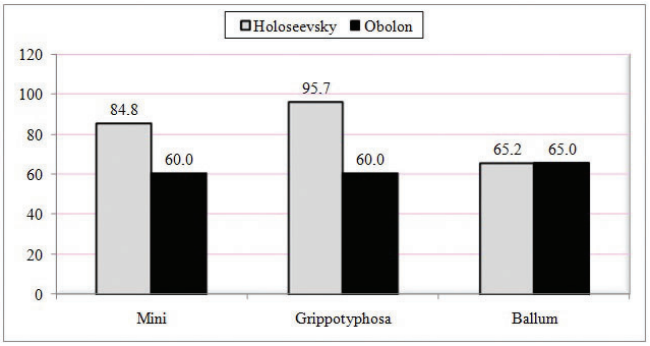

Figure 2. The percentage of each serogroup in the total number of positive reactions

After analyzing Fig. 2, it can be argued that all three serological groups play an important role in the etiology of leptospirosis of urban brown rats in Kyiv. In Obolon district, these groups play almost the same etiological role (12–13 cases of 20 positively reacting animals). In Holoseevsky district *Grippotyphosa* and *Mini* were dominating (44 and 39 cases of 46 positively reacting animals). At the same time, serogroup *Ballum* was diagnosed in 65.2%.

Discussion. Leptospirosis is the most wide spread zoonosis worldwide, which is present in all continents

except Antarctica (Adler and la Peña Moctezuma, 2010) and evidence for the carriage of *Leptospira* has been found in over 150 mammalian species (Ko, Goarant, and Picardeau, 2009).

Animals, including humans, can be divided into maintenance hosts and accidental (incidental) hosts. The disease is maintained in nature by chronic infection of the renal tubules of maintenance hosts (Babudieri, 1958). A maintenance host is defined as a species in which infection is endemic and is usually transferred from animal to animal by direct contact. The most important maintenance hosts are small mammals, which may transfer infection to domestic farm animals, dogs, and humans. The extent to which infection is transmitted depends on many factors, including climate, population density, and the degree of contact between maintenance and accidental hosts. Different rodent species may be reservoirs of distinct serovars, but rats are generally maintenance hosts for serovars of the serogroups *Icterohaemorrhagiae* and *Ballum*, and mice are the maintenance hosts for serogroup *Ballum* (Levett, 2001).

On the whole, we have studied 116 samples of the blood sera from urban brown rats: Holoseevsky district — 79, Obolon district — 37. In both areas, the percentage of positively reacting rodents is rather high (58.2% and 54%) that correlates with studies of urban brown rats in other countries (Costa et al., 2014; Easterbrook et al., 2007; Himsworth et al., 2013; Koizumi et al., 2009; Vanasco et al., 2003).

Our research on the samples of blood sera from urban brown rats in Kyiv showed the presence of diagnostic titers of *Leptospira* antibodies to the 14 serogroups in Holoseevsky district and to 6 — in Obolon district of 21 serological groups used for the study. Serogroups *Grippotyphosa*, *Mini*, and *Ballum* were dominating in the structure of positive reactions in both districts as well as in most countries (Levett, 2001).

In Holoseevsky district, antibodies to serogroups *Pomona* (11.3%), *Icterohaemorrhagiae* (9.2%), *Cynopteri* (8.4%), *Celledoni* (5%) and *Australis* (5%) (serovar *bratislava*) were less common. At the same time, in Obolon district serogroups *Cynopteri* (15.4%) and *Pomona* (11.5%) were recorded.

As we see, *Icterohaemorrhagiae* serological group was found only in the samples of blood sera from Holoseevsky district and played a minor etiologic role, despite the fact that urban brown rats are considered as its main carriers (Aviat et al., 2009; Golding, 1990; Inada et al., 1916; Ko, Goarant, and Picardeau, 2009; Levett, 2001; Noguchi, 1917; Waitkins, 1991).

Antibodies to other serological groups, such as *Javanica, Sejroe, Hebdomadis, Shermani, Australis* (serovar *erinaceieuropaei*), and *Pyrogenes*, had been registered less often.

The percentage of mixed reactions is significantly higher than monoreactions in both areas (80.4% vs. 19.6% in Holoseevsky district; 70% vs. 30% in Obolon district).

Antibodies to serological groups were found in MAT in various diagnostic titers: from 1/50 to 1/2,500. Their structure in positive reactions was the fallowing: 1/50 — 14.7%, 1/100 — 30.1%, 1/500 — 40.8%, and 1/2,500 — 14.4%.

The circulation of the pathogenic leptospira among the population of urban brown rats in Kyiv was indicated by the analysis of the research data and by systematization of the data.

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