

## THE ROLE OF MICROMAMMALS IN THE MAINTENANCE OF LEPTOSPIROSIS FOCI IN THE REPUBLIC OF MOLDOVA

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**Abstract.** *The study was conducted between 2006 and 2008 using the zoological and ecological-epizootological methods. In the study period 22 micromammal species (15 rodents and 7 insectivores) were registered on the territory of the republic. Out of these species 18 were caught in traps in all studied zones, including Chisinau city. The study revealed the intense circulation of leptospirosis causative agent in 2 of 3 geographical zones of the republic: the northern (7.9%) and central-Chisinau city (2.8%). In epizootic circulation of leptospirosis on the territory of the Republic 12 MM species from 18 studied are involved. High leptospirosis contamination rates were identified in species with major epidemiologic potential-*R. norvegicus* (32.7%), *A. sylvaticus* (21.8%) and *A. agrarius* (20.9%). The relevant serological groups belong to *L. gryppotyphosa*-40.9%, *L. icterohaemorrhagiae*-34.5%, which are frequently determined in outbreaks of human leptospirosis. On the republic territory there are biocenotic microcomplexes with favourable conditions for the development and maintenance of pathogen agent, but the intense anthropogenic activities place them closer and closer to localities, where there is permanently necessary to accomplish combating measures of the reservoir and epidemiologic surveillance.*

**Keywords:** *leptospires, leptospirosis, biotope, natural outbreaks, epizootological study, micromammals.*

**Rezumat. Rolul micromamiferelor în menținerea focarelor de leptospiroze în Republica Moldova.** *Cercetările au fost efectuate în 2006-2008 cu utilizarea metodelor zoologice și ecologo-epizootologice. În perioada de studiu pe teritoriul republicii au fost înregistrate 22 specii de micromamifere (15 rozătoare și 7 insectivore). Dintre acestea 18 specii au fost capturate cu capcane în toate zonele studiate, inclusiv municipiul Chișinău. Studiul a evidențiat circulația intensă a agentului cauzal al leptospirozei în 2 din 3 zone geografice a republicii: de nord (7,9%) și centrul municipiului Chișinău (2,8%) cu antrenarea în circuitul epizootic a 12 specii de mamifere mici din cele 18 investigate pe teritoriul republicii. Cote înalte de contaminare cu leptospire au fost identificate la specii cu potențial epidemiologic major *R. norvegicus* (32,7%), *A. sylvaticus* (21,8%) și *A. agrarius* (20,9%). Grupele serologice relevante aparțin *L. gryppotyphosa*-40,9%, *L. icterohaemorrhagiae*-34,5%, frecvent determinate în focarele de leptospiroză umană. Pe teritoriul republicii există microcomplexe biocenotice cu condiții favorabile pentru dezvoltarea și menținerea agentului patogen, însă activitățile antropogene intense le apropie tot mai mult de localități, unde sunt necesare măsuri permanente de combaterea a rezervorului și supravegherea epidemiologică.*

**Cuvinte cheie:** *leptospire, leptospiroză, biotop, focar natural, cercetare epizootologică, micromamifere.*

### INTRODUCTION

Leptospirosis is a zoonosis with global distribution that affects human population from the continents with tropical and temperate climate, being presently identified as an important emerging infectious disease in many countries (LEVETT, 2001; RICALDI & VINETZ, 2006). This disease is known as a global public health problem, because of its increasing incidence in both developing and economically advanced countries.

Leptospirosis reservoir includes a great number of wild animal species, especially small mammals and domestic animals (cattle and swine), which after infecting with the pathogenic agent of leptospirosis survive and disseminate the pathogenic agent with the urine in the environment during a long period (MAGDEI et al., 1998; VIEIRA et al., 2006; VINETZ et al., 2005; SHLYAKHOV, 1986).

In nature, practically all mammal species can be indicated as a leptospirosis source, but the most important are the rodents and the insectivores that represent the true living environment, particularly favourable for leptospirosis hosting, reproduction and dissemination (WHO, 2003).

In the Republic of Moldova, a potential risk of infection is maintained on the territories where leptospirosis carrier status was registered in wild animals and in domestic animals diseases or where the status of asymptomatic carriers was registered. These territories are considered as leptospirosis foci and are classified in natural, anthropogenic and mixed ones. According to the data published in the Republic of Moldova two foci types can be mentioned: natural and anthropogenic (COTOFANA et al., 1987; PRISACARI, 1993).

The natural leptospirosis outbreaks can be usually traced in swampy zones, floated meadows, near streams or stagnant water basins. In these outbreaks the pathogen agent circulates within the population of wild animals (BELOUS et al., 1989; PRISACARI, 1993; SHLYAKHOV, 1986). The micromammal (MM) species from Rodentia and Insectivora orders, the spreading area of which includes wet biotopes, constitute the infection reservoir: the rat (*Rattus* sp.), mice and vole species (*Mus* sp., *Apodemus* sp., *Arvicola* sp., *Microtus* sp.). The limits of these outbreaks are determined by landscape-geographical and climatic conditions (humidity, temperature etc.), as well as by faunistic ones (diversity of vertebrate populations as hosts for leptospirosis pathogenic agent) (BHARTI et al., 2003; LEVETT, 2001; PRISACARI, 1986).

The anthropogenic outbreaks are not characterized by landscape-geographic peculiarities of the territory and can occur in rural localities, as well as in urban ones. The formation of these outbreaks is determined by the appearance of infected or leptospirosis carrier animals in farms, enterprises, etc. The sick animals eliminate the leptospire in urine thus contaminating the environment components (water, forage plants, grass etc.) through which the healthy animals are infected. The infection of animals in anthropogenic foci is registered all over the year, while in the case of animals kept in pasturage the infection occurs during pasturing.

The intense anthropogenic activity in the Republic of Moldova from the last decades contributed to the considerable reduction of the territories in which natural outbreaks were registered. The intensification of anthropogenic activity such as large swamp area drying up and their use, partial land cultivation with formation of mosaic aspect of cultivated and abandoned sectors led to the involvement of a large number of people contacting with the elements of natural biotypes.

Still, in the Republic there remain meadow-swamp, forest and steppe biotopes that allow the existence of stable zoonoses outbreaks including leptospirosis.

The recent results of ecological-epizootological studies confirm the existence of mixed outbreaks in our country at present, determined by the absence of large areas inaccessible for man. Practically all the country territories, including the forests and wet ecosystems are subject of anthropogenic transformations, fact that excludes the possibility of strict delimitation of natural and artificial outbreaks.

## MATERIAL AND METHODS

To accomplish the study different territories were selected in order to determine the evolution and peculiarities of leptospirosis event at present. With this purpose, 380 various biotopes in different ecosystems from all the zones of Moldova were studied. The study was conducted between 2006 and 2008 using the following methods: zoological and ecological-epizootological. Small mammals were caught using Hero snap traps. After sacrificing the trapped mammals, blood was collected from heart. The serological examination was done using microagglutination test (MAT). MAT was performed on all samples using a panel of 15 serovars of *Leptospira*: *L. icterohaemorrhagiae*, *L. pomona*, *L. hebdomadis*, *L. grippotyphosa*, *L. australis*, *L. canicola*, *L. iavanica*, *L. ballum*, *L. autumnalis*, *L. tarassovi*, *L. batavia*, *L. cynopteri*, *L. sejroe sejroe*, *L. hardjo*, *L. wolffi*.

As a whole 19,920 traps/day were performed and 3,691 MM were caught. 3,473 specimens were examined in laboratory; the rest of MM could not be determined and examined due to their damaged conditions. Nevertheless, these specimens were taken into account while determining seasonal, annual and multiannual population density indices.

During species identification the following statistical parameters of corporal biometric indices were calculated: body length (LCORP), tail length (LCD), tarsus length (LTARS) in millimetres and body weight (G) in grams. To characterize the biotopic distribution of the species the frequency index was calculated (BALOGH, 1958):

$F = \frac{100p}{P}$ , where  $P$  - number of samples,  $p$  - samples in which the species is present, and the index of species

dominance (abundance) after the formula  $D = \frac{100 n}{N}$ , where  $n$  - individual number of certain species in sample,  $N$  -

total individual number. Trappability index:  $C_c = \frac{100 n}{N}$  where  $n$  - number of traps that have caught animals,  $N$  - total number of traps. All indices are expressed in percents.

## RESULTS AND DISCUSSION

In the study period 2006-2008, on the territory of the republic there were registered 22 MM species (15 rodents and 7 insectivores). Out of these species 18 MM species were caught in traps in all the studied zones, including Chisinau city (Table 1).

Table 1. Species diversity and number of micromammals registered in different zones of the Republic of Moldova.  
 Tabel.1 Diversitatea speciilor de micromammalii întâlnite în diferite zone a Republicii Moldova.

Nr	MM species	Northern zone	Central zone	Southern zone	Chisinau	Total
1.	<i>Dyromys nitedula</i>	0	5	1	0	6
2.	<i>Muscardinus avellanarius</i>	4	1	0	1	6
3.	<i>Clethrionomys glareolus</i>	130	147	34	25	336
4.	<i>Arvicola terrestris</i>	7	7	6	1	21
5.	<i>Microtus arvalis</i>	43	35	38	26	142
6.	<i>Microtus subterraneus</i>	3	16	0	1	20
7.	<i>Apodemus uralensis</i>	86	82	249	9	426
8.	<i>Apodemus sylvaticus</i>	158	137	191	258	744
9.	<i>Apodemus flavicollis</i>	140	175	217	66	598
10.	<i>Apodemus agrarius</i>	410	220	88	71	789
11.	<i>Mus musculus</i>	118	70	134	15	337

12.	<i>Micromys minutus</i>	3	10	8	4	25
13.	<i>Rattus norvegicus</i>	76	9	11	4	100
14.	<i>Sorex minutus</i>	17	18	9	15	59
15.	<i>Sorex araneus</i>	16	28	10	1	55
16.	<i>Crocidura suaveolens</i>	13	2	4	0	19
17.	<i>Crocidura leucodon</i>	1	0	4	0	5
18.	<i>Neomys anomalus</i>	0	1	1	0	2
	Total individuals	1225	963	1005	497	3691

The species that were determined by direct field observations or as a result of vital activity traces and were not included in table 1 are the following: squirrel (*Sciurus vulgaris*), mound building mouse (*Mus spicilegus*), hedgehog (*Erinaceus europaeus*) and mole (*Talpa europae*).

In 12 species of 18 MM recorded in the country, leptospirosis positive results were registered, which suggests high species diversity involved in the epizootic process (Table 2). From the total number (3,473 MM) of the studied MM in 3.2% were established leptospirosis positive results. Among the species with *Leptospira*, some species were dominant and subdominant in various biotopes.

Table 2. Micromammal species diversity with leptospirosis positive results.  
Tabel 2. Diversitatea speciilor de micromamalii cu rezultate pozitive de leptospiroză.

Nr.	MM species	caught MM	studied MM	infected MM	% of MM species with <i>Leptospira</i> out of the total no of positive results
1.	<i>Dyromys nitedula</i>	6	6	-	-
2.	<i>Muscardinus avellanarius</i>	6	6	1	0.9
3.	<i>Clethrionomys glareolus</i>	336	307	2	1.8
4.	<i>Arvicola terrestris</i>	21	21	2	1.8
5.	<i>Microtus arvalis</i>	142	132	3	2.7
6.	<i>Microtus subterraneus</i>	20	16	-	-
7.	<i>Apodemus uralensis</i>	426	415	2	1.8
8.	<i>Apodemus sylvaticus</i>	744	733	24	21.8
9.	<i>Apodemus flavicollis</i>	598	593	10	9.1
10.	<i>Apodemus agrarius</i>	789	703	23	20.9
11.	<i>Mus musculus</i>	337	298	5	4.5
12.	<i>Micromys minutus</i>	25	25	-	-
13.	<i>Rattus norvegicus</i>	100	98	36	32.7
14.	<i>Sorex minutus</i>	59	45	1	0.9
15.	<i>Sorex araneus</i>	55	48	1	0.9
16.	<i>Crocidura suaveolens</i>	19	19	-	-
17.	<i>Crocidura leucodon</i>	5	5	-	-
18.	<i>Neomys anomalus</i>	2	2	-	-
	Total MM	3691	3473	110	100.0

The obtained data confirm that in one species more *Leptospira* serogroup can be detected (Table 3). In harvest field mouse (*A. agrarius*) species, it was registered the highest diversity of leptospire species (*Leptospira icterohaemorrhagiae*, *L. pomona*, *L. gryppotyphosa*, *L. ballum*). In the case of house mouse (*M. musculus*) and yellow necked mice (*A. flavicollis*), 3 *Leptospira* serogroups were revealed, while in rat (*R. norvegicus*) and water vole (*A. terrestris*) - 2 *Leptospira* serogroups.

Table 3. The etiological structure of leptospirosis in micromammals.  
Tabel 3. Structura etiologică a leptospirozelor la micromamalii.

Nr	MM Species	caught MM	studied MM	infected MM	Serogroups					
					<i>L. gryppotyph</i>	<i>L. icterohaem</i>	<i>L. ballum</i>	<i>L. pomona</i>	<i>L. autumnalis</i>	<i>L. javanica</i>
1.	<i>M. avellanarius</i>	6	1	16.7	1	-	-	-	-	-
2.	<i>C. glareolus</i>	307	2	0.7	2	-	-	-	-	-
3.	<i>A. terrestris</i>	21	2	9.5	1	1	-	-	-	-
4.	<i>M. arvalis</i>	132	3	2.3	3	-	-	-	-	-
5.	<i>A. uralensis</i>	415	2	0.5	2	-	-	-	-	-
6.	<i>A. sylvaticus</i>	733	24	3.3	22	-	-	2	-	-
7.	<i>A. flavicollis</i>	593	10	1.7	5	-	-	4	1	-
8.	<i>A. agrarius</i>	703	23	3.3	6	4	1	12	-	-
9.	<i>M. musculus</i>	298	5	1.7	2	1	-	2	-	-
10.	<i>R. norvegicus</i>	98	36	36.7	-	32	-	-	-	4
11.	<i>S. minutus</i>	45	1	2.2	-	-	-	-	-	1
12.	<i>S. araneus</i>	48	1	2.1	1	-	-	-	-	-
	Total	3399	110		45	38	1	20	1	5

It is necessary to mention that in some species, for example in *Rattus norvegicus*, the highest proportion among leptospire belongs to *L. icterohaemorrhagiae*, which constitute 32.7% from the total number of studied MM. This fact proves the importance of *R. norvegicus* species in *L. icterohaemorrhagiae* maintaining in the environment. The *L. icterohaemorrhagiae* serogroup was detected in 3 more species from the 12 studied.

The tableau analysis of leptospirosis in MM in 2006-2008 (Fig. 1) revealed the presence of 6 leptospire serogroups (*L. icterohaemorrhagiae*, *L. pomona*, *L. gryppotyphosa*, *L. javanica*, *L. batavia*, *L. autumnalis*).

In the epizootic process 12 MM species caught in the northern zone of the country are involved (Table 4). In 7.9% of studied MM, 4 leptospire serogroup were detected from the 6 ones recorded on the territory of our country. Out of the total number of infected MM the highest infection rate-47.4% was recorded in *R. norvegicus* species, followed by *A. terrestris* (28.6%). Among the MM species with positive results out of total number with *Leptospira* the highest ratio was recorded by *R. norvegicus*, *A. agrarius* and *A. sylvaticus*, the last two species being dominant in the mentioned areas.

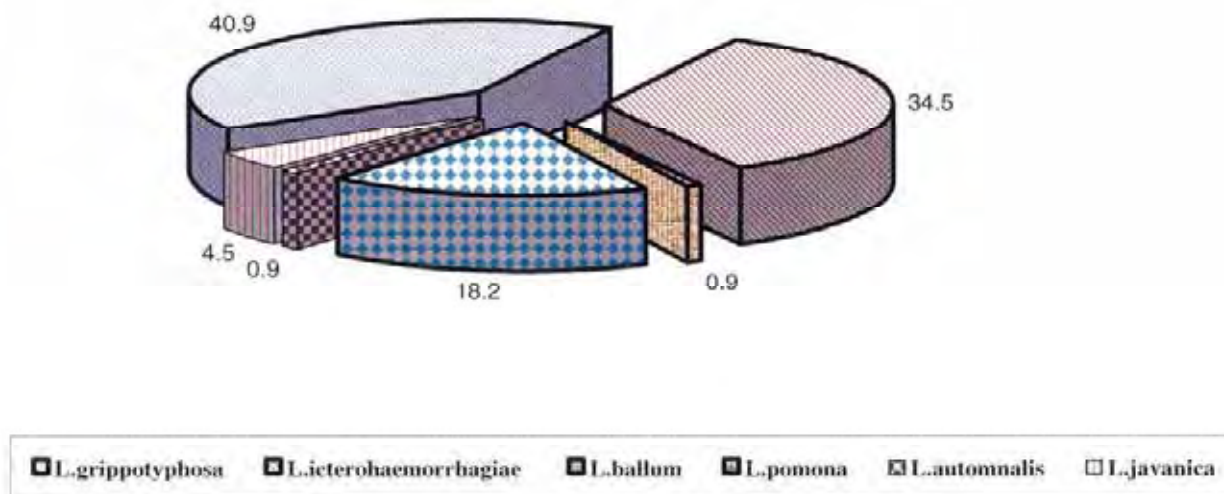


Figure 1. The leptospirosis representation in MM.

Figura 1. Rezentarea leptospirelor la MM.

It is necessary to mention that the highest significance was registered in *L. gryppotyphosa*-40.9% and *L. icterohaemorrhagiae*-34.5% serogroups.

These data confirm the relatively intense circulation of leptospirosis agent among various populations of MM species in the northern zone that maintain the risk of environmental objects contamination and leptospirosis contact by humans.

In laboratory conditions 1,910 MM individuals were investigated from the central and southern zones. There were not detected positive results at leptospirosis in these zones of the Republic of Moldova.

Table 4. Results of leptospirosis epizootological studies in the northern zone.  
 Tabel 4. Rezultatele cercetărilor epizootologice la leptospiroză în zona de nord.

MM diversity	studied MM	No of positive results	% of infected MM	% of MM species with <i>Leptospira</i> out of total no of positive results	serogroups			
					L. gryppotyph.	L. icterohaem	L. pomona	L. javanica
<i>M. avellanarius</i>	4	1	25.0	1.0	1	-	-	-
<i>C. glareolus</i>	130	2	1.5	2.1	2	-	-	-
<i>A. terrestris</i>	7	2	28.6	2.1	1	1	-	-
<i>M. arvalis</i>	43	3	7.0	3.1	3	-	-	-
<i>M. subterraneus</i>	3	-	-	-	-	-	-	-
<i>A. uralensis</i>	86	2	2.3	2.1	2	-	-	-
<i>A. sylvaticus</i>	158	17	10.8	17.5	17	-	-	-
<i>A. flavicollis</i>	140	9	6.4	9.3	5	-	4	-
<i>A. agrarius</i>	410	18	4.4	18.6	3	4	11	-
<i>M. musculus</i>	118	5	4.2	5.2	2	1	2	-
<i>M. minutus</i>	3	-	-	-	-	-	-	-
<i>R. norvegicus</i>	76	36	47.4	37.1	-	32	-	4
<i>S. minutus</i>	17	1	5.9	1.0	-	-	-	1
<i>S. araneus</i>	16	1	6.3	1.0	1	-	-	-
<i>C. suaveolen.</i>	13	-	-	-	-	-	-	-
<i>C. leucodon</i>	1	-	-	-	-	-	-	-
Total	1,225	97	7.9	-	37	38	17	5
Serogroup rate (%)	-	-	-	-	38.1	39.2	17.5	5.2

In Chisinau city the ecological-epizootological studies in various biotypes were accomplished only in 2008. The capture rate in the first semester proved to be 3.4% (Table 5).

The average indexes of MM number in the winter-spring period in different biotypes (agrocoenoses, urban biotopes, wet biotopes and forest biotopes-forest shelter belts, recreational sectors) represented 3.6% in February and 4.4% in March versus multiannual average of the index (8.8% in the last 8 years). In forest biotopes this index was 2.9%, in wet biotypes-7.3% and in agrocoenoses with scrub and tree vegetation it constituted 6.0%. Among the registered MM species caught in Chisinau city the dominant species proved to be the red vole (*C. glareolus*) with 48.3%, and the subdominant species-the yellow necked mouse (*A. flavicollis*) with 17.1%. During the first half of the year 7 MM species were registered, in the second one-13 MM species. The mean trappability rate in the second semester was 39.4%. The dominant species was the wood mice with 54.8%, the subdominant ones were the harvest field mice (*A. agrarius*)-14.8% and the yellow necked mouse (*A. flavicollis*)-12.9% from the total number of caught MM.

Table 5. Results of leptospirosis epizootological studies in Chisinau city in 2008.  
Tabel 5. Rezultatele cercetărilor epizootologice la leptospiroză în orașul Chișinău în anul 2008.

MM species	1 <sup>st</sup> semester		2 <sup>nd</sup> semester			serogroups			
	No of caught MM	No of studied MM	No of caught MM	No of studied MM	No of positive results	<i>L. gryppotyph.</i>	<i>L. batavia</i>	<i>L. pomona</i>	<i>L. autumnalis</i>
<i>M. avellanarius</i>	-	-	1	1	-	-	-	-	-
<i>C. glareolus</i>	14	14	11	11	-	-	-	-	-
<i>A. terrestris</i>	-	-	1	1	-	-	-	-	-
<i>M. arvalis</i>	-	-	26	26	-	-	-	-	-
<i>M. subterraneus</i>	-	-	1	1	-	-	-	-	-
<i>A. uralensis</i>	5	5	4	4	-	-	-	-	-
<i>A. sylvaticus</i>	3	3	255	255	7	5	-	2	-
<i>A. flavicollis</i>	6	6	60	60	1	-	-	-	1
<i>A. agrarius</i>	2	2	69	69	5	3	1	1	-
<i>M. musculus</i>	-	-	15	15	-	-	-	-	-
<i>M. minutus</i>	-	-	4	4	-	-	-	-	-
<i>R. norvegicus</i>	-	-	4	4	-	-	-	-	-
<i>S. minutus</i>	1	1	14	14	-	-	-	-	-
<i>S. araneus</i>	1	1	-	-	-	-	-	-	-
Total MM	32	32	465	465	13	8	1	3	1
Total traps/days	955	-	1,180	-	-	-	-	-	-
Rate of caught MM (%)	3.4	-	39.4	-	-	-	-	-	-
Species no	7	-	13	-	-	-	-	-	-
No of species carriers of leptospire	-	-	-	-	13	2	2	3	2
Rate of species carriers of leptospire (%)	-	-	-	-	2.8	-	-	-	-
Serogroup rate (%)	-	-	-	-	-	61.5	7.7	23.1	7.7

The laboratory studies revealed the presence of leptospire in 2.8% of MM investigated samples. 4 serogroups were emphasized: *L. gryppotyphosa*-61.5%, *L. pomona*-23.1%, *L. batavia* and *L. autumnalis*-7.7%.

Comparing the obtained dates with previous results (PRISACARI, 1993) the rates of infected MM decrease from North to South and specimens with leptospirosis were found in all geographical zones (PRISACARI, 1993). In our study, there were not detected positive results at leptospirosis in southern zones. We can conclude that similar data were obtained about the intense circulation of leptospirosis agent among various populations of MM species in the northern zone; this fact maintains the risk of environmental objects contamination and leptospirosis contact by humans.

## CONCLUSIONS

The study revealed the intense circulation of leptospirosis causative agent in 2 of 3 geographical zones of the republic: the northern (7.9%) and central-Chisinau city (2.8%). In epizootic circulation of leptospirosis on the territory of the Republic 12 MM species from 18 studied are involved.

High leptospirosis contamination rate were identified in species with major epidemiologic potential-*R. norvegicus* (32.7%), *A. sylvaticus* (21.8%) and *A. agrarius* (20.9%)

The relevant serological groups belong to *L. gryppotyphosa*-40.9%, *L. icterohaemorrhagiae*-34.5%, which are frequently determined in outbreaks of human leptospirosis.

On the republic territory there are biocoenotic microcomplexes with favourable conditions for the development and manifestation of the pathogen agent and the intense anthropogenic activities place them closer and closer to localities, where permanent epidemiologic surveillance is necessary to be accomplished.

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