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BURLACU VICTORIA

BIO-ECOLOGICAL PECULIARITIES AND THE ROLE OF SMALL MAMMALS (MAMMALIA: RODENTIA, INSECTIVORA) IN MAINTENANCE OF LEPTOSPIROSIS FOCI ON THE TERRITORY OF THE REPUBLIC OF MOLDOVA

165.02 – Zoology

Summary of the doctoral thesis in biological sciences

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The thesis was developed in the laboratory of Terrestrial vertebrates of Institute of Zoology and National Agency for Public Health

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The thesis defense will take place on 17 January 2023 at 10.00, in the meeting of the Doctoral Commission within the Doctoral School of Biological, Geonomic, Chemical and Technological Sciences within the national consortium administered by the State University of Moldova, Biology block, meeting room 352, Academiei Str. 1, Chisinau (http: //www.usm.md).

The doctoral thesis and the abstract can be consulted at the National Library of the Republic of Moldova, the "Andrei Lupan" Scientific Library (Institute), the USM Library, on the ANACEC website (http://www.cnaa.md) and on the USM website (http://usm.md/).

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CONTENT

CONCEPTUAL GUIDELINES OF RESEARCH

The actuality of the subject. The fauna of small mammals on the territory of the Republic of Moldova includes species with high density, high adaptive potential in the conditions of anthropogenic and climatic changes, which are spread in different habitats and types of ecosystems and includes 23 species of rodents and 7 species of insectivores [10, 11]. Due to the ability to adapt to any changes in the environment, the small mammals represent an important link in the circulation of many pathogens such as bacteria, viruses, parasites etc., being involved in the transmission of zoonotic diseases with natural focality, including leptospirosis. Some authors point out that small mammals can simultaneously be reservoir hosts for several pathogens with natural focality, which represent an insufficiently addressed problem with clinical, diagnostic, epidemiological implications [1, 3, 16, 17, 18].

Currently, leptospirosis is recognized as an emerging global public health problem, being addressed in an interdisciplinary context related to biomedicine, ecology, veterinary medicine, public health, in social and economic aspects [2, 15, 19, 20, 25]. The ubiquitous spread of leptospirosis in the world is associated with a wide range of susceptible animal species that can be reservoirs for pathogenic leptospires, but the main reservoir in nature remains rodents and insectivores – true living environments particularly favorable for hosting, multiplying and eliminating the leptospires [21, 27]. In the Republic of Moldova, leptospirosis is one of the most frequent zooanthroponoses with a natural focality, the first cases of the disease in humans being attested since 1963 [5, 14, 30].

Description of the situation in the field of research and identification of research problems. Natural foci of leptospirosis, as a rule, are detected in wet biotopes: marshy areas, floodplains, near watercourses or stagnant water basins, and their limits are determined by landscape-geographical, climatic and biotic conditions – the diversity of small mammal populations as reservoirs that ensure the viability of the causative agent of leptospirosis in nature [2, 7]. Geographical location, climatic conditions, biodiversity of the Republic of Moldova create favorable conditions for the formation and functioning of leptospirosis foci in different territories of the country, which are determined by the coexistence of the pathogen and the reservoir of infection in a certain biotope of the ecosystem, being also influenced by the action of natural and anthropogenic factors [3, 7, 30]. The intense anthropogenic activity in the republic in the last decades has contributed to the considerable reduction of the territories of natural foci. At the same time, the clean-up of some swampy areas and their exploitation, the partial processing of the land with the formation of insular sectors of processed land, led to the involvement of a larger number of people who come into contact with the elements of natural biotopes, thus

increasing the risk of contracting the disease. The specialized literature highlights that anthropogenic transformations in the country's ecosystems, including those with forests and wet biotopes, exclude the possibility of strict delimitation of natural foci of leptospirosis from anthropogenic ones [7, 30], socio-economic, hygiene and behavioral aspects being important in the context of anthropogenic foci. The mentioned facts determine the need to further study the general principles of formation and functioning of leptospirosis foci and establish the epidemic potential. In specialized research, special attention is paid to the influence of climatic and anthropogenic changes upon the populations of small mammals in various types of ecosystems and the evolution of the epizootic process through leptospirosis in small mammals. The realization of an original and complex study through the faunal and medical prism of small mammal communities in order to obtain updated epizootological and ecological information, highlighting the epidemiological significance and risks for public health, and for the argumentation of complex measures to control zoonotic diseases, highlights the importance, the novelty and the actuality of this research.

Scientific research methodology. As methodological and theoretical-scientific support served the works of Averin et al. (1979), Chicu et al. (2012), Murariu (2000), Popescu and Murariu (2001) etc. [6, 9, 13, 23]. Fieldwork was carried out according to standard accepted methodologies, reflected in the works of Novikov, Murariu, Chicu et al. [6, 9, 13, 26]. Epizootological research was carried out in the administrative territories with stationary multi-year monitoring points, where states of carriage with the pathogen *Leptospira* spp. in small mammals and cases of leptospirosis in humans were recorded. The small mammals were investigated for leptospiran presence [4, 6].

Resulting from the exposed problem, the **purpose of the research** is to elucidate the biological and ecological peculiarities of the species of the Rodentia and Insectivora orders to determine the small mammals with major epidemiological potential and their importance in the formation and maintenance of leptospirosis foci.

The following **objectives** have been set: 1) The faunal, taxonomic and ecological study of small mammal communities in natural and anthropized ecosystems in the north, center and south of Republic of Moldova; 2) Highlighting the species of small mammals infected with leptospires and involved in the epizootic process of leptospirosis; 3) Studying the etiological structure of leptospires in the population of small mammals; 4) Elucidation of the role of small mammals in the functionning of natural foci of leptospirosis on the Republic of Moldova territory.

Scientific novelty and originality. Complex researches of small mammal communities in natural and anthropogenic ecosystems were carried out in the northern, central and southern

areas of the Republic of Moldova. The bio-ecological peculiarities of 22 species of small mammals were highlighted and their influence on the evolution of the epizootic process in the population of small mammals was determined. The administrative territories with natural foci of leptospirosis were identified and mapped, and the risks for the health of the population associated with the anthropization process of natural ecosystems were highlighted. The results of the study elucidated the diversity of small mammal species involved in the epizootic process of leptospirosis, their distribution in natural and anthropogenic biotopes according to the geographical areas of the country. The significance of small mammals as a primary reservoir for 8 leptospira serogroups was established, and potentially epidemiological important species were determined. The obtained results contribute to the completion of knowledge regarding the diversity and structure of small mammal communities that actively maintain the epizootic process and contribute to the formation of leptospirosis foci, the spectrum of leptospire serogroups circulating in the country's ecosystems and the particularities of the formation of natural foci under current conditions and present scientific evidence for national programs to control zoonotic diseases.

The scientific problem solved consists in the elucidation of the role of small mammals (reservoirs) in the functioning of natural foci of leptospirosis, the level of infection of small mammals with *Leptospira* spp. in the investigated ecosystems, which will allow the forecasting of the epizootological situation and the development of rigorous measures to prevent the risk of the spread of leptospirosis in the Republic of Moldova.

Theoretical significance. New data on the structure of small mammal communities in natural foci of leptospirosis have been obtained. The obtained results expand the knowledge regarding the fauna and ecology of small mammals, as well as their epizootological and epidemiological role in the functioning of leptospirosis foci in the Republic of Moldova.

The applicative value of the work. The role of small mammals in the functioning of natural foci of leptospirosis has been elucidated. Useful materials were developed that can be included in health education programs in the field of control and preventing zoonotic diseases. The results contribute to the development of semi-annual and annual epizootological forecasts, the improvement of the national legal framework in the field of zoonotic diseases control.

Implementation of scientific results. The results of the study were implemented in the practical activity of the National Agency for Public Health, the epidemiological surveillance section of highly contagious, zoonotic and parasitic diseases, within the territorial Public Health Centers. The research results are used and implemented in the didactic process, in the

preparation of bachelor's and master's theses in high education institutions with biological and ecological profile.

Approval of scientific results. The results of the research were presented and approved at national and international scientific events.

Publications on the topic of the thesis. The results of the studies were presented in 54 scientific papers (4 as unique author), including: 5 articles in national scientific journals, 10 articles in international journals (3 with impact factor) and one methodic quidance.

The volume and structure of the thesis. The thesis is written in Romanian, computer-edited, traditionally compartmentalized and includes: title page, copyright page, summaries in Romanian, English and Russian, table of contents, introduction, keywords, 4 chapters, conclusions and practical recommendations, bibliographic references, annexes, statement regarding the assumption of responsibility, the author's CV. The thesis is presented on 135 pages of text compiled in the Word editor, it is illustrated with 24 tables, 42 figures. The thesis is based on 209 bibliographic references.

Keywords: small mammals, ecological peculiarities, biotopică distribution, pathogen agent, *Leptospira* spp., natural foci, zoonozes.

The content of the thesis

In **Introduction** the actuality and importance of the problem, the necessity of the scientific research carried out are argued. The current situation in the field is reflected and the purpose and objectives of the study are formulated. The elements of scientific novelty and originality of the thesis, the solved scientific problem, the theoretical and applied value of the research results, the implementation of the scientific results and their approval are shown.

SUMMARY OF THE CHAPTERS

1. HISTORY OF SMALL MAMMAL (MAMMALIA: INSECTIVORA, RODENTIA) AND LEPTOSPIROSIS FOCI RESEARCH

This chapter includes a synthesis of the theoretical bases and practical experiences of specialists in the field of ecology, biology and epizootological surveillance of leptospirosis foci and reservoir species of small mammals. The main benchmarks in the field of research at the national, European and world level are exposed. Important scientific studies, carried out in the last decade, regarding the existing methodology in the world in this chapter, the results obtained by different researchers are described. The current data from the specialized literature regarding the study of the diversity of small mammal species and their distribution area, the importance of monitoring natural foci of leptospirosis in the health system are generalized. The purpose and

objectives of the thesis are argued through the in-depth analysis of recent bibliographic reference sources of internationally renowned scientists [2, 5, 7, 8, 10, 11, 25, 28, 29, 30].

2. MATERIALS AND METHODS OF STUDY

2.1. The physical-geographical characteristic of the studied territory. Selection of territories for the epizootological study. In this subchapter, the physical-geographic characteristics of the Republic of Moldova and of the study points, where research was carried out and the material was collected during the study period, are presented. The ecosystems of small mammals collecting included several types of natural biotopes, where the natural vegetation are largely preserved (forests, forest edges, some swamp habitats) and anthropogenic ones (agrocenoses, forest curtains, recreation area, settlements). The collecting of small mammals was carried out in 28 territorial administrative units from the north, center and south of the Republic of Moldova, thus covering the entire territory of the country (figure 2.1.1). The material for the study was accumulated systematically, semi-annually, annually during the period 2003 - 2020, the research being carried out on the faunal diversity, ecological and biological aspects of small mammals from the order Rodentia and the order Soricomorpha in different natural and anthropogenic ecosystems of the Republic of Moldova.

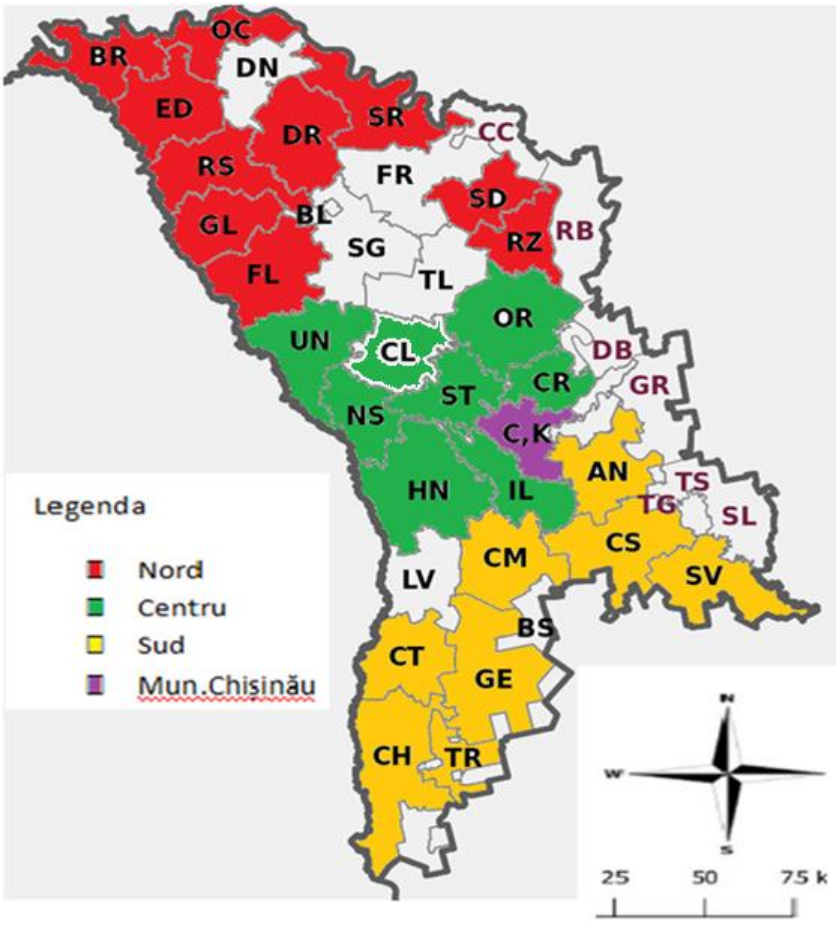


Figure 2.1.1. Distribution map of the investigated administrative territories

In order to identify natural foci of leptospirosis, collecting of small mammals were carried out in territories with stationary multi-year monitoring points, where leptospira carriage states were recorded in wild animals and territories where cases of human illness were recorded [4, 6].

2.2. Material and methods of study. Faunal material was collected annually according to standard, generally accepted methods. Hero snap traps were used, the method most frequently applied in the field that allows in a relatively short time to obtain data on the structure, population density, species predilection for certain stations etc. The traps were installed in 25 50 or 100 pieces, either in a line or randomly, the distance between them being on average 5 m and left for 1-2 nights. Traps were checked once a day, in the early hours of the morning. They were provided with bait – pieces of bread crust soaked in unrefined sunflower oil [6, 12, 24].

All collected individuals were determined to species, except for the sibling species of gen. *Microtus* (*M. arvalis* and *M. rossiaemeridionalis*), the age structure (juvenile, subadult, adult), sex and reproductive status was also established. The identification of the taxonomic affiliation of small mammals was made on the basis of morphological characters and identification keys [9, 13]. In the laboratory, the small mammals were dissected in order to study their reproductive activity. In pregnant or lactating females, embryos or the number of black dots on the uterine horns were counted to assess the prolificacy and reproductive success of the species.

For the detection of leptospires in small mammals in the microbiologic laboratory of the National Agency for Public Health, the serological method was applied - the microagglutination and lysis reaction. This technique includes the use of a set of leptospira strains of international reference (*Icterohaemorrhagiae, Pomona, Hebdomadis, Grippotyphosa, Australis, Canicola, Javanica, Ballum, Autumnalis, Tarassovi, Bataviae, Cynopteri, Pyrogenes*), recommended and accepted by World Health Organization. At the moment, the microagglutination and lysis reaction is the most accessible and frequently used laboratory method [4].

For the analysis and evaluation of the data regarding the multi-year morbidity due to leptospirosis, the statistical reports (form no. 2) "Regarding infectious and parasitic diseases" were analyzed; annual national Reports with reference to the surveillance, prevention and control of communicable diseases; informative notes on the epizootological and epidemiological situation in zooanthroponosis; the semi-annual and annual epizootological forecasts for the period 2003-2020.

Based on the data obtained for the years 2003-2020, the ecological analysis was carried out by calculating the capture (trappability) index (Ic), relative abundance (A) and frequency (F)

indexes. Synthetic ecological indexes were used: ecological significance index (W), Simpson dominance index, as well as Shannon, Margalef, Alpha, Berger-Parker diversity indices. The analysis and statistical processing of the material, the graphic presentation were carried out according to the contemporary methods of interpreting the results obtained by using Microsoft Word, Excel, Statistica and BioDiversity Pro programs.

3. BIO-ECOLOGICAL PECULIARITIES OF SMALL MAMMAL COMMUNITIES (RODENTIA, INSECTIVORA) IN THE REPUBLIC OF MOLDOVA

3.1. Faunistic diversity of small mammals on the territory of the Republic of Moldova.

Within the accomplished research in the preiod 2003 - 2020 there have been collected 18056 individuals from 22 species. 5 species belong to order Soricomorpha, Gregory, 1910: *Sorex araneus* Linnaeus, 1758, *Sorex minutus* Linnaeus, 1766, *Neomys anomalus* Cabrera, 1907, *Crocidura leucodon* (Hermann, 1780), *Crocidura suaveolens* (Pallas, 1811) and 17 species – to order Rodentia, Bowdich, 1821: *Myoxus glis* (Linnaeus, 1766), *Dryomys nitedula* (Pallas, 1779), *Muscardinus avellanarius* (Linnaeus, 1758), *Rattus norvegicus* (Berkenhout, 1769), *Mus musculus* Linnaeus, 1758, *Mus spicilegus* Petenyi, 1882, *Apodemus sylvaticus* (Linnaeus, 1758), *Apodemus flavicollis* (Melchior, 1834), *Apodemus uralensis* (Pallas, 1811), *Apodemus agrarius* (Pallas, 1771), *Micromys minutus* (Pallas, 1771), *Cricetulus migratorius* Pallas, 1773, *Cricetus cricetus* (Linnaeus, 1758), *Clethrionomys glareolus* (Schreber, 1780), *Arvicola terrestris* (Linnaeus, 1758), *Microtus* sp. (Schrank, 1798), *Microtus subterraneus* (de Selis-Longchamps, 1836), other 2 species (*Erinaceus roumanicus* Barret-Hamilton, 1900, *Talpa europaea* Linnaeus, 1758) being recorded after direct observations.

The multi-year average index of capture of small mammals in the period 2003-2020 was 21.45%, for the first and second semesters it was 15.05% and 26.05%, respectively. The number distribution of small mammals in the three areas was different, and it was found that in the northern area the multi-year average capture index of small mammals was the highest compared to the central and southern areas and constituted 25.55%, for I and II semesters were 20.99% and 28.59%, respectively. In the central area, the multiannual average capture index of small mammals was - 20.43% and in the southern area - 18.34%. In terms of semesters, higher indices were recorded in the southern area in the first semester - 13.09%, in the central area -12.13%, and in the second semester higher values were in the central area - 25, 54%, in the southern area – 23.54%.

During the study period, there was a seasonal but also an annual variation in the number of small mammal species. In the years 2005, 2009 and 2014, the highest annual capture index of

small mammals was recorded, constituting 28.82%, 26.53% and 29.51%, respectively, the lowest values - in 2019-2020 (about 14%) (figure 3.1.1). For the first semester, the highest capture index of small mammals was recorded in 2009 with 24.15%, in 2014 – 22.65%, lowest values - in 2003 (6.27%). In the second semester, the highest values were reported in 2005, 2014 and 2017, constituting about 34%, lowest values - in 2019 (15.65%).

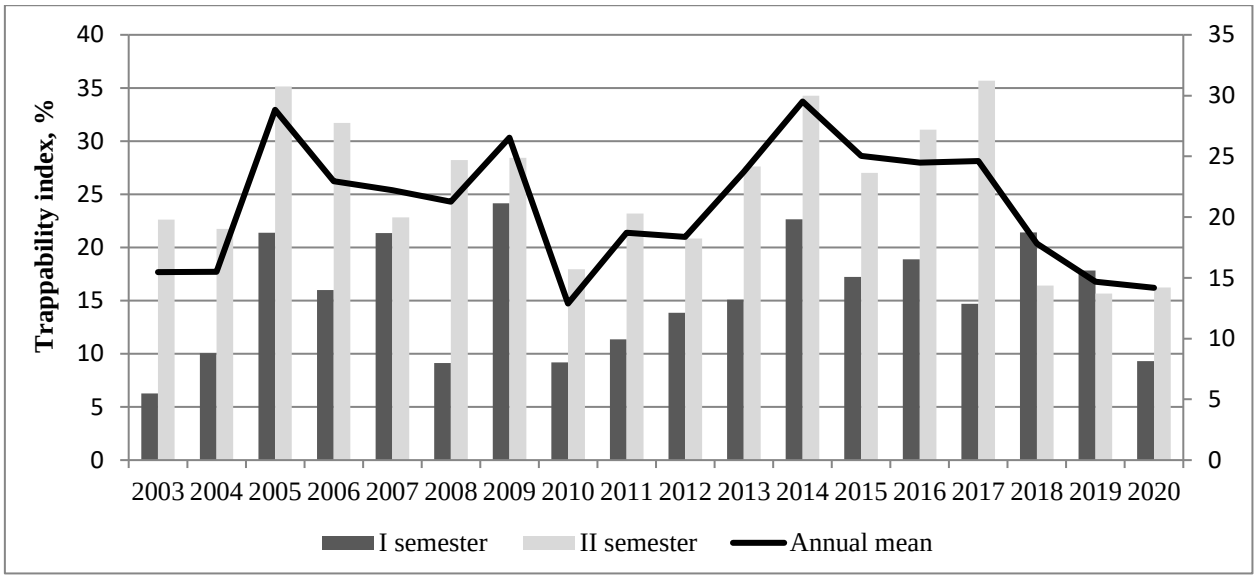


Figure 3.1.1. Multiannual dynamics of small mammals from the Republic Moldova

During the research period the numer and the abundance of small mammal species varied from year to year depending on the climatic conditions and the study period. In 2003, the highest species number of small mammals were recorded (19 species), in 2006-2008, faunal diversity consisted of 18 species, in 2004, 2005, 2013, 2015 and 2017 – 17 species, the lowest number of species - in 2020 (10 species). During the study years, a total of 12955 individuals of the *Apodemus* genus were collected – the largest number with more than 70% of the total individuals collected. The population number of *C. glareolus* and *Microtus* sp. was 2031 and 1083 collected individuals, respectively. The rodent species *M. musculus* (374 individuals) and *R. norvegicus* (148 individuals) form synanthropic populations and are of major epidemiological importance as a source and reservoir of leptospires. Among the insectivorous mammals, the population number of *S. araneus* was the highest, constituting about 52% (322 individuals) of the total of collected shrews (619 individuals), followed by *S. minutus* 27.2% (168 individuals), *C. suaveolens* 10.5% (65 individuals), *C. leucodon* 7.8% (65 individuals) and *N. anomalus* 2.42% (15 individuals).

3.2. Structure of small mammal communities in studied biotopes of the Republic of Moldova. The structure of small mammal communities in the studied biotopes during the years 2003-2020 was analyzed, the distribution of individuals being reflected in table 3.2.1. A total of

3213 individuals from 14 species were collected in the forest biotope. The most numerous being the individuals of *A. flavicollis* 36.69%, followed by *C. glareolus* with 25.43%, *A. sylvaticus* 19.98% and for *A. agrarius* it is 11.80%. Among the shrews, only the species *S. araneus* had a higher abundance, constituting 1%, while the other species constituted below 0.62% each.

At the forest edge 3255 individuals of 20 species of small mammals were collected. The dominant species was *A. flavicollis* with an abundance of 31.86%, followed by *A. sylvaticus* 20.18%, *A. agrarius* 19.85% and *C. glareolus* 13.73%. The shrew species had low abundance.

Table 3.2.1. Ecological indices of small mammal communities in the biotopes of the Republic of Moldova in the years 2003-2020

Nr.	Species	Biotope							Relative abund. %	Fre-quency, %	Ecological significance	
		Forest	Forest edge	Forest belt	Agro.	Paludous	Locality	Recreation area			%	W
1	S. minutus	0.62	0.34	0.56	0.90	1.71	1.46	0.90	0.93	100.0	0.93	W1
2	S. araneus	1.00	0.61	1.37	1.30	3.73	1.62	1.99	1.79	100.0	1.79	W2
3	N. anomalus	0.03	0.03	0.05	-	0.24	-	0.10	0.08	71.43	0.06	W1
4	C. leucodon	0.03	0.06	0.76	0.46	0.22	0.16	0.30	0.27	100.0	0.27	W1
5	C. suaveolens	0.06	0.25	0.41	0.46	0.53	0.65	0.30	0.36	100.0	0.36	W1
6	M. glis	-	0.06	-	-	0.04	-	-	0.02	28.57	0.01	W1
7	D. nitedula	0.19	0.12	0.20	-	0.02	-	0.10	0.09	71.43	0.06	W1
8	M. avellanarius	0.22	0.12	-	0.09	0.13	-	0.40	0.13	71.43	0.10	W1
9	A. uralensis	3.49	7.77	12.19	11.59	7.71	9.72	6.17	8.18	100.0	8.18	W3
10	A. sylvaticus	19.98	20.18	26.56	24.28	19.14	33.23	22.09	21.92	100.0	21.92	W4
11	A.flavicollis	36.69	31.86	19.96	11.41	12.31	13.29	26.37	21.68	100.0	21.68	W4
12	A. agrarius	11.80	19.85	15.74	20.77	28.16	12.80	19.00	19.93	100.0	19.93	W4
13	M. musculus	-	0.22	1.98	1.56	3.67	12.80	2.59	2.05	85.71	1.76	W2
14	M. spicilegus	-	1.87	6.04	11.27	0.98	-	0.50	3.43	71.43	2.45	W2
15	M. minutus	-	0.37	-	0.29	1.33	-	0.10	0.46	57.14	0.26	W1
16	R. norvegicus	-	-	-	0.03	2.80	3.08	0.20	0.82	57.14	0.47	W1
17	C. migratorius	-	0.03	0.10	0.06	-	-	-	0.03	42.86	0.01	W1
18	C. cricetus	-	0.03	0.36	0.03	-	-	0.10	0.06	57.14	0.03	W1
19	C. glareolus	25.43	13.73	7.31	3.24	8.42	5.51	9.75	11.28	100.0	11.28	W4
20	A. terrestris	-	-	-	0.03	0.87	-	0.10	0.23	42.86	0.10	W1
21	Microtus sp.	-	2.33	6.40	12.22	7.60	5.35	8.06	6.00	85.71	5.14	W3
22	M. subterraneus	0.47	0.15	-	-	0.36	0.32	0.90	0.26	71.43	0.19	W1
Total specii		14	20	16	18	20	13	20	22			

In forest shelter belts 1969 individuals of 16 small mammal species were collected. The dominant species was *A. sylvaticus* with an abundance of 26.56%, *A. flavicollis* with 19.96% and *A. agrarius* with 15.74%. *S. araneus* had an abundance of 1.37%, and *S. minutus* – 0.56%. The

species *C. leucodon* only in this biotope had a fairly high abundance – 0.76%, compared to other biotopes, while *N. anomalus* and *C. suaveolens* less than 0.4%.

In agrocenoses 3452 individuals from 18 species were collected. The dominant species was *A. sylvaticus* with 24.28%, followed by *A. agrarius* with 20.77%, while *A. uralensis*, *A. flavicollis* and *M. spicilegus* had similar abundance of about 11%.

In paludous biotopes 4499 individuals from 20 species were collected. The dominant species were *A. agrarius* with 28.16%, *A. sylvaticus* with 19.14% and *A. flavicollis* with 12.31%, in *C. glareolus* and *Microtus* sp. the abundance was 8.42% and 7.60%, respectively. *M. musculus* had an abundance of 3.67% and *M. spicilegus* – 0.98%. The shrews *S. araneus* and *S. minutus* had the highest values of relative abundance in this type of biotope, constitutind 3.73% and 1.71%, respectively.

In localities 617 individuals from 13 species were collected. The dominant species was *A. sylvaticus* with 33.23%, followed by *A. flavicollis* and *A. agrarius* with 13.29% and 12.80%, respectively. The synanthropic species *M. musculus* and *R. norvegicus* had the highest abundance - 12.80% and 3.08%, respectively, compared to the other studied biotopes. The shrews *S. araneus* and *S. minutus* had a rather high abundance, constituting 1.62% and 1.46% respectively, for *C. suaveolens* the abundance was the highest 0.65%, and *C. leucodon* – only 0.16% .

In recreationa sectors 1005 individuals from 20 species were collected. Dominant were *A. flavicollis* with 26.37%, *A. sylvaticus* 22.09% and *A. agrarius* with 19%, *C. glareolus* and *Microtus* sp. had the abundance of 9.75% and 8.06%, respectively. *S. araneus* had the abundance of 1.99%, while other shrew species – below 0.90% each.

Ecological indexes. The analysis of the relative abundance of individuals of each species, their frequency and ecological significance was performed (table 3.2.1). During the study period high abundances were highlighted in *A. sylvaticus* 21.92%, *A. flavicollis* – 21.68% and *A. agrarius* – 19.93%, followed by *C. glareolus* 11.28%, *A. uralensis* 8.18% and *Microtus* sp. - 6%. Low abundance was recorded for the other species – less than 4% each.

The most frequent species were *A. uralensis, A. sylvaticus, A. flavicollis, A. agrarius* and *C. glareolus,* as well as the S. *minutus*, *S. araneus*, *C. leucodon* and *C. suaveolens,* registered in all studied biotopes (table 3.2.1). High frequency values were registered in *Microtus* sp., *M. musculus* (85.71%), *M. spicilegus*, *M. subterraneus, D. nitedula, M. avellanarius* and *N. anomalus* (71.43%). The lowest frequency was recorded in stenotopic species *M. glis* – 28.57%.

Depending on the index of ecological significance, the analyzed small mammal species fell into 4 classes: W_1 (accidental) – 13 species (*S. minutus*, *N. anomalus, C. leucodon, C.*

suaveolens, M. glis, D. nitedula, M. avellanarius, M. minutus, R. norvegicus, C. migratorius, C. cricetus, A. terrestris, M. subterraneus); W_2 (accessorial) – 3 species (*S. araneus, M. musculus, M. spicilegus*); W_3 (characteristic) – 2 species (*A. uralensis*, *Microtus* sp.) and W_4 (constant) – 4 species (*A. sylvaticus, A. flavicollis, A. agrarius, C. glareolus*).

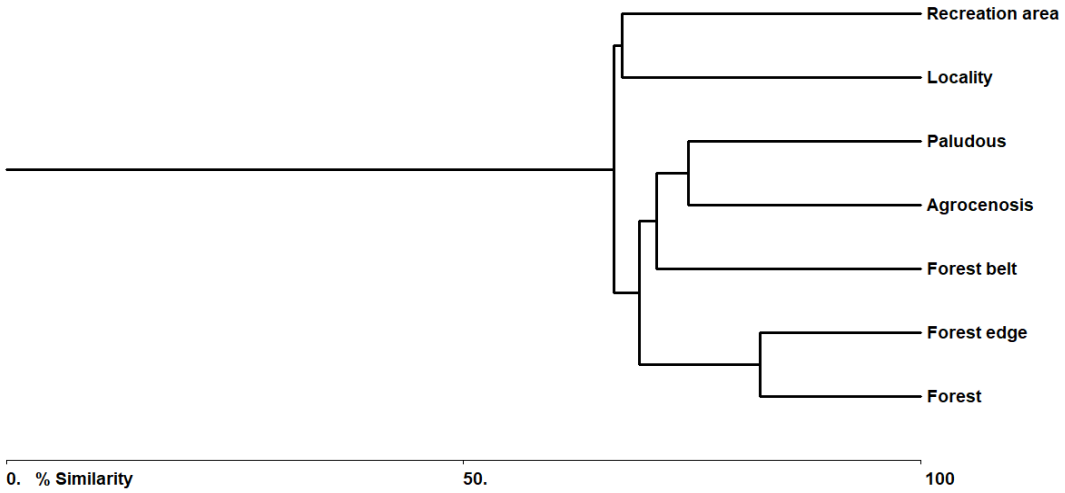
Diversity of small mammal species in studied biotopes. Diversity analysis was performed according to Shannon, Simpson, Margalef, Berger-Parker and Alpha indices (table 3.2.2). Each index can provide different interpretation of the diversity of small mammal communities in a given biotope. The values of the diversity indexes depend on the number of species and on the number of individuals and their distribution by species. The Shannon index indicates the highest diversity (0.78) in localities where individuals are relatively evenly distributed and one species is dominant (*A. sylvaticus* – 33.23%), the lowest diversity was recorded at the edge of the forest (0.595), where the share for 2 species (*A. sylvaticus, A. flavicollis*) was over 52%. The Simpson index indicates diversity by the high abundance of a single species in the community. Thus, the highest index was recorded in the forest (0.254), where *A. flavicollis* dominated (36.69%), the lowest in the marsh biotope (0.154). The Margalef index is an index of species richness and indicates the uniform distribution of individuals by species, being maximum in localities (7,526), where few species were reported, but with small variations in abundance, the minimum index was recorded in the marsh biotope (5.749). The Alpha index is the highest in the recreation area (3.544), where most species with the smallest population were recorded. The Berger-Parker index is based on sample size and richness and was maximum in forest (0.367).

Table 3.2.2. Diversity indexes of small mammal communities in the Republic of Moldova in 2003-2020

Index	Forest	Forest edge	Forest belt	Agro.	Paludous	Locality	Recreation area
Shannon	0.602	0.595	0.731	0.702	0.728	**0.78**	0.664
Simpson	**0.254**	0.207	0.163	0.158	0.154	0.176	0.175
Margalef	5.988	5.979	6.375	5.935	5.749	**7.526**	6.995
Alpha	1.734	2.844	2.387	2.492	2.707	2.331	**3.544**
Berger-Parker	**0.367**	0.319	0.266	0.243	0.282	0.332	0.264
Total species	14	20	16	18	20	13	20
Total individuals	3213	3255	1969	3452	4499	617	1005

***Similarity of small mammals communities in the studied biotopes*.** The analysis of the similarity of the communities of small mammals from various types of studied biotopes was performed and the dendrogram of the similarity was performed, which depends on the abundance of each species, the diversity and the total number of individuals collected. According to the Cluster analysis, we observe that some biotopes are similar to each other according to the

qualitative and quantitative structure of small mammal communities (figure 3.2.1). A separate cluster with the highest similarity is formed by the forest biotope and forest edge, which constitutes approximately 82%, due to the dominance of *A. flavicollis* and *A. sylvaticus* species, as well as the rich shrew fauna. Another cluster formed by agrocenosis and wet biotopes with a similarity of about 71-74%. Less similar to the other biotopes is the group formed by the localities and the recreation area with a similarity of over 66% and a similarity of less than 60% with the other biotopes. Thus, the group of natural forest ecosystems and that of heavily anthropized ecosystems have the least faunal similarity.



Figures 3.2.1. Dendrogram of the similarity of small mammal communities in the studied biotopes of the Republic of Moldova in 2003-2020

3.3. Reproductive peculiarities of dominant species of small rodents in ecosystems with high anthropogenic impact. In the urban and rural ecosystems of the central area in the autumn period, the ratio between sex and age groups of the dominant rodent species shows that for the species *A. sylvaticus* subadult males predominate (32.3%), slightly less than the share of adult females (31, 3%) and the lowest – in adult males (17.5%) (table 3.3.1). In the *A. agrarius* population subadult males prevail (51.3%), subadult females number were almost 2 times lower (26.3%). The share of adult individuals was insignificant, at the same time males turned out to be approximately 2 times less (7.9%) compared to females (14.5%). In the species *A. flavicollis* the population structure is different. Thus, the dominant group of this species were adult males (30.7%), the shares in the population of subadult males (27.7%) and the population of adult females (26.1%) were practically similar. The ratio of subadult females was the lowest (15.5%). The ratio between sex and age groups of the *M. musculus* species was different from that of other species, in the population the ratio between adult and subadult males was equal, including subadult females (27.2% each). The least were adult females (18.4%). In the species *M. spicilegus*, the dominant demographic group turned

out to be subadult males (39.9%), followed by adult males (26.7%), the ratio of adult and subadult females was similar (16.7%). The ratio between sex and age groups in *M. rossiaemeridionalis* was different, subadult males predominated (50.5%), followed by adult females (23.3%), and the share of adult males and subadult females was equal and smaller (13.1%). A more uniform distribution of the demographic groups was recorded in *C. glareolus*, with the predominance of subadult males (35.8%), while adult males, adult and subadult females were registered in equal proportions (21.4% each).

Table 3.3.1. Sex ratio and age structure of dominant rodent species during autumn in urban and rural ecosystems

Species	♂♂ (%)		♀♀ (%)	
	adult	subadult	adult	subadult
A. sylvaticus	17.5	32.3	31.3	18.9
A. agrarius	7.9	51.3	14.5	26.3
A. flavicollis	30.7	27.7	26.1	15.5
M. musculus	27.2	27.2	18.4	27.2
M. spicilegus	26.7	39.9	16.7	16.7
M. rossiaemeridionalis	13.1	50.5	23.3	13.1
C. glareolus	21.4	35.8	21.4	21.4

The reproduction proces of the dominant rodent species during the autumn period have the following peculiarities: the spermatogenesis was absent in half of the adult males of *A. agrarius*, *A. flavicollis* and *M. rossiaemeridionalis* (table 3.3.2). All the subadult males were non-reproductuve, only one subadult *A. agrarius* male showed the presence of spermatogenesis. Most adult females in October-November did not breed. Fewer breeding females were found in the population of *A. sylvaticus* and *A. flavicollis* (11.5% each), and in *A. agrarius* females – 16.7%. In *M. rossiaemeridionalis* the reproducing females constituted 1/3 of the total number of adult females. In *A. sylvaticus* reproductive population, predominate the females pregnant for the first time (75%), the others (25%) were pregnant for the second time. All females from the *A. agrarius* and *A. flavicollis* populations reproduced for the first time (100%), being represented by females born in the summer of the current year. In *M. rossiaemeridionalis* about 1/3 of reproductive females were at the second pregnancy (28.6%). All subadult *A. flavicollis* females were non-reproductive, and the number females of the same category in *A. sylvaticus* and *A. agrarius* was practically similar. A quarter of the total number of subadult *M. rossiaemeridionalis* females were found to be ready for reproduction, but none of the females were pregnant. Most of the subadult females in the populations of the 4 dominant species were

non-reproductive, while in 3 species between 25% and 40% of the females were sexually mature (table 3.3.2).

Table 3.3.2. Reproductive activity of the dominant rodent species in urban and rural ecosystems in autumn

Species	♂♂ (%)		♀♀ (%)					
	Adult	Sub-adult	Adult				Subadult	
	Sp.+	Sp.-	Sex. mature	Reprod.	I Gest.	II Gest.	Non-reprood.	Sex. mature
A. sylvaticus	29	100	88.5	11.5	75.0	25.0	66.0	34.0
A.agrarius	50	97,4	83.3	16.7	100.0	0	60.0	40.0
A.flavicollis	50	100	88.2	11.8	100.0	0	100.0	0
M.rossiaemeridionalis	50	100	66.7	33.3	71.4	28.6	75.0	25.0

Note: Sp.+ spermatogenesis present, Sp.- spermatogenesis absent, Gest. - gestation

The most favorable biotopes for rodents in heavily anthropized areas are the forests, forest edges, paludous biotopes, meadows, weedy sectors, orchards and parks, while the agrocenoses with cereal crops are the poorest from a faunal point of view. The dominant species in most biotopes are *A. sylvaticus*, *A. agrarius*, in forests – *A. flavicollis*, in agrocenoses and meadows – *M. spicilegus* and *M. arvalis*. The species *A. sylvaticus, A. flavicollis* and *A. agrarius* are species with characteristic or constant ecological significance in all the studied biotopes, being the most tolerant towards anthropogenic factors. Thus, the fauna of small rodents is well represented in the recreational and urban ecosystems of Chisinau city, which denotes the adaptation of species to anthropogenic conditions, as well as the existence of many habitats that correspond to the ecological needs of rodents in urban environment [28, 29].

4. EPIZOOTOLOGICAL CHARACTERISTICS OF LEPTOSPIROSIS FOCI IN THE REPUBLIC OF MOLDOVA

Leptospirosis is a naturally occurring zoonosis caused by spirochetes of the genus *Leptospira* that affects animals and humans worldwide. Disease agents with a natural focality circulate in nature without human involvement in this process. A natural foci can be any natural ecosystem, in the component of which there is the presence of a population of pathogenic agents. The formation and functioning of leptospirosis foci is determined by the coexistence of the causative agent (*Leptospira* spp.) and the reservoir of agents (different species of small mammals) in a certain biotope of the ecosystem and is influenced by the action of natural and anthropogenic factors [8, 22] .

4.1. Study of the circulation of the causative agent of leptospirosis in small mammal communities in the Republic of Moldova. The research was carried out during 2003-2020 in 28 administrative territories in all three areas of the republic. Foci of leptospirosis were registered in 11 territories, including Glodeni, Ocnița, Briceni, Fălești, Șoldănești, Drochia, Ialoveni, Căușeni, Ștefan Vodă, Cahul and Chișinău districts.

In the research of small mammals in order to establish specific antibodies against *Leptospira* spp., 15685 specimens of small mammals from 22 species were examined in the microbiologic laboratory of the National Agency for Public Health. The average level of leptospira infection in small mammals was 1.62%. The presence of leptospires in small mammals was determined annually in different periods of the year (seasonal), except for the years 2003 and 2013. The highest share of infection (4.9%) with leptospires of small mammals was reported in 2008, in 2006 – 4.06% and 2014 – 3.88% (figure 4.1.1). The regression analysis of the dependence of the annual number of small mammals and the share of individuals infected with leptospires showed a significant positive correlation (r=0.5182, p=0.0276).

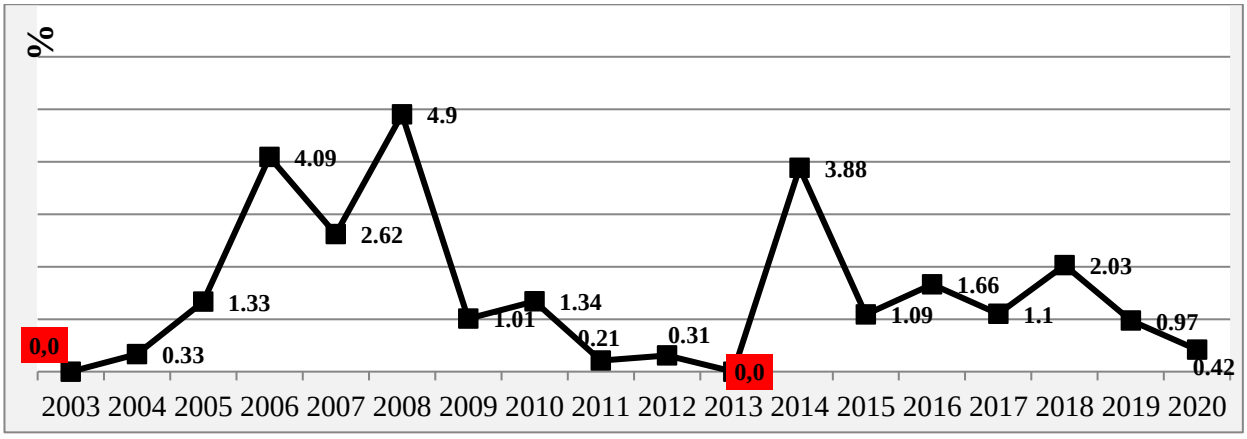


Figure 4.1.1. Dynamics of positive serological results for the presence of leptospires in the populations of small mammals in the Republic of Moldova

In natural foci of leptospirosis the average level of leptospire infection in small mammals was 4.27%, in the first semester – 3.07%, and in the second semester – 4.49%. The level of infection shows seasonal variations, a significant correlation being found between the monthly number of small mammals and the share of individuals infected with leptospires (r=0.5796, p=0.0482). The analysis of the data shows that the pathogen *Leptospira* spp. is more frequently found in small mammals in the paludous biotope where it accounted for 44.31% of the total number of positive samples, in forest – 18.82%, in agrocenoses – 18.43%, in localities – 6.67%, at forest edge – 6.27%, in forest belt – 3.13% and in the recreation area – 2.35% (figure 4.1.2). A significant correlation was established between the number of individuals per biotope and the share of those infected with leptospires (r=0.7954, p=0.0324), as well as between the number of

species involved in the epizootic process of leptospirosis and the share of infected individuals in the biotope (r=0.9821, p =0.00008).

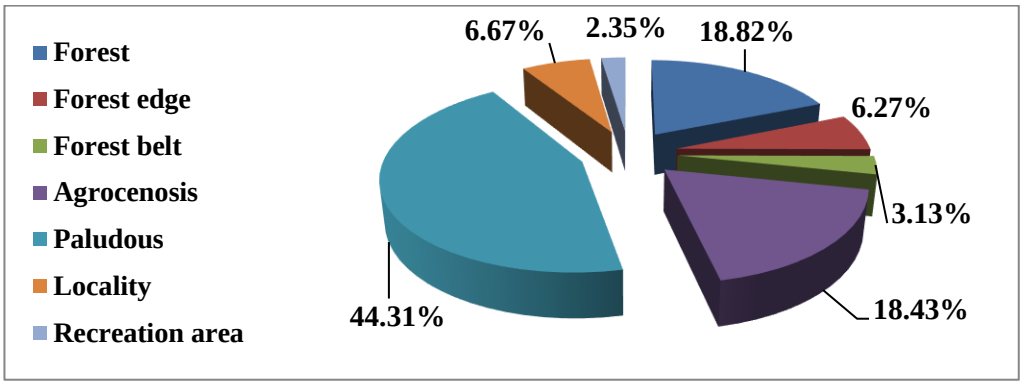


Figure 4.1.2. Circulation of leptospires in small mammal population in various types of biotopes in 2003-2020

15 species of small mammals were recorded with positive serological results for leptospirosis (*S. araneus, S. minutus, C. leucodon, M. avellanarius, R. norvegicus, M. musculus, M. spicilegus, A. sylvaticus, A. flavicollis, A. uralensis, A. agrarius, C. glareolus, A. terrestris, M. subterraneus, Microtus* sp.), constituting a share of 68.2% of the total number of investigated species (22 species). The analysis of the territorial distribution of small mammals determined with positive serological results for leptospirosis elucidated that the highest share is determined in the northern area - 86.27%, followed by the central area with 9.41% and the southern area - 4.31% (figure 4.1.3). The regression analysis between the number of individuals infected with leptospires and the total number of small mammals in the north, center and south showed a significant correlation (r=0.7925, p=0.0063).

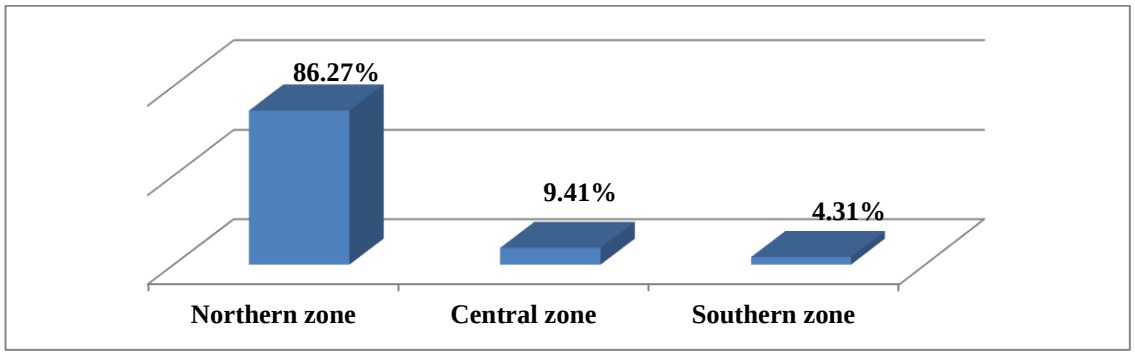


Figure 4.1.3. Indices of positive serological results for the presence of *Leptospira* spp. specific antibodies in small mammals from the Republic of Moldova in 2003-2020

8 serogroups of leptospires have been detected in the listed small mammal species: *Icterohaemorrhagiae, Grippotyphosa, Pomona, Bataviae, Autumnalis, Javanica, Ballum* and *Canicola*. In 2008, the greatest diversity of serogroups (n=5) was detected in small mammals, the largest share belongs to the leptospires *Grippotyphosa* - 67.3% and *Pomona* - 27.3%, while

Javanica, *Batavia* and *Autumnalis* were less common – 1.8% each. In 2006, 2007 and 2015, a diversity of 4 serogroups was recorded, the highest share went to serogroups *Icterohaemorrhagiae* (about 37%) and *Grippotyphosa* (33.3%). In 2014, when 3 serogroups were registered, the highest share was registered *Pomona* leptospires – 91.3%. In the years 2004, 2005, 2009, 2010, 2011, 2012, 2016-2020, 1-2 serogroups were isolated, with the highest share being *Icterohaemorrhagiae*, *Grippotyphosa* and *Pomona*, constituting values from 25% - 100% and which substituted each other in different periods of time.

In the species *A. agrarius* the greatest diversity of leptospire serogroups was detected (5 serogroups), in the structure of which *Pomona* dominated, constituting 69.7%. In the species *A. uralensis* and *A. flavicollis*, 4 serogroups of leptospires were detected. In *A. uralensis* the dominant species was the *Grippotyphosa* serogroup with 40%, in *A. flavicollis* - the *Pomona* serogroup with a share of 70%. Reservoirs for 3 serogroups of leptospires are *M. musculus*, *R. norvegicus* and *A. terrestris*, recording the highest share for *Grippotyphosa* – 50% (*M. musculus*) and *Icterohaemorrhagiae* – with 82.8% in *R. norvegicus* and 50% in *A. terrestris*. In the blood serum of the rodent species *M. avellanarius*, *M. spicilegus* and *M. subterraneus*, only one serogroup of leptospires was detected – Grippotyphosa, and the shrew species that were collected in smaller number *S. araneus*, *S. minutus* and *C. leucodon* are reservoirs for 4 leptospira serogroups: *Icterohaemorrhagiae*, *Grippotyphosa*, *Pomona* and *Javanica*. It should be noted that the *Grippotyphosa* serogroup is hosted by the largest variety of small mammal species, reservoirs being 12 species, *Pomona* is hosted by 10 species and *Icterohaemorrhagiae* by 7 species, *Javanica* by 4 species, and *Ballum*, *Bataviae*, *Autumnalis* and *Canicola* – only by one species of small mammals (figure 4.1.4).

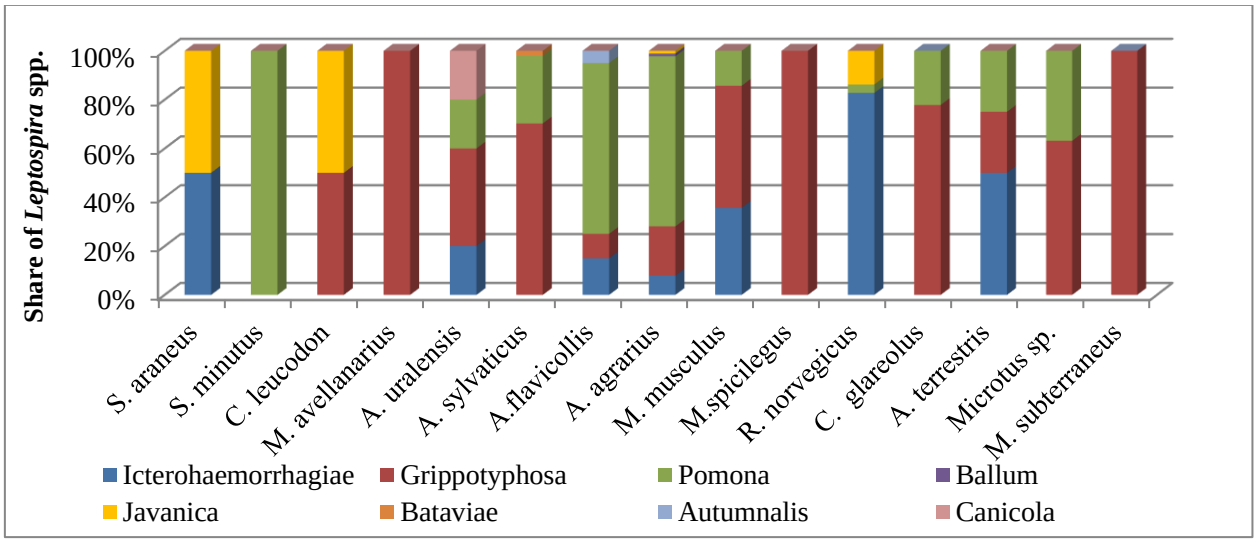


Figure 4.1.4. Distribution of leptospires in different species of small mammals in 2003-2020

Among 8 serogroups of leptospires determined in the population of small mammals, the largest share belongs to *Pomona*, with 41.96% of the total positive samples, followed by *Grippotyphosa* with 36.86%, *Icterohaemorragiae* – 16.86%, and *Javanica*, *Ballum*, *Bataviae*, *Autumnalis* and *Canicola*, which are less common, represent values between 0.39% and 2.75% (figure 4.1.5).

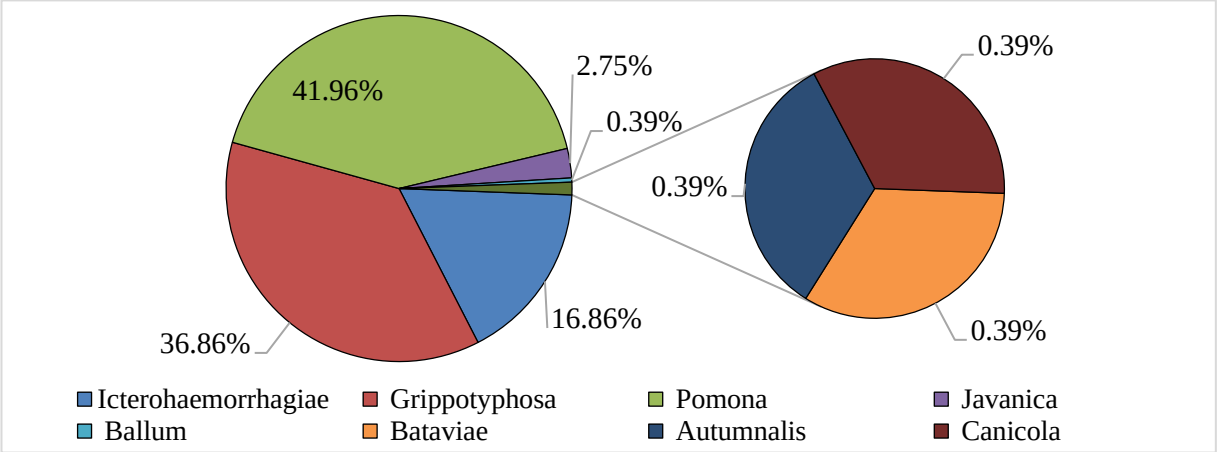


Figure 4.1.5. Proportion of leptospira serogroups determined in the population of small mammals in 2003-2020

According to the data of the study, it appears that the species *A. agrarius* (Ar= 19.93%) had the highest share of infection with leptospires, constituting 34.90% of the total positive for leptospirosis samples, being followed by *A. sylvaticus* (Ar= 21.92%) with 18.43%, and *R. norvegicus* (Ar= 0.82%) – with 11.37% (figure 4.1.6). The share of positive serological results for leptospirosis between 7.1%-7.8% was recorded in the species *A. flavicollis* (Ar=21.68%), *C. glareolus* (Ar=11.28%) and *Microtus* sp. (Ar=6%). In *A. uralensis* (Ar=8.18%) and *M. spicilegus* (Ar=3.43%) the infection rate was 2% and 0.8%, respectively, the given species are not reported as the most abundant but may contribute to circulation and preservation of leptospires mainly in agricultural biotopes where they are registered as constant species.

In the forest, paludous biotope and localities 5 serogroups de leptospire out of 8 registered on the territory of the country were highlighted, in agrocenosis – 3 serogroups, and at the forest edge, in forest belts and the recreation area – 2 serogroups. In all the investigated biotopes the *Grippotyphosa* serogroup was detected, which is maintained in nature by 12 species of small mammals: *C. leucodon, M. avellanarius, A. uralensis, A. sylvaticus, A. flavicollis, A. agrarius, M. musculus, R. norvegicus , C. glareolus, A. terrestris, Microtus sp.* and *M. subterraneus* and its highest share was in the forest (45.8%) and the recreation area (83.3%). The *Pomona* serogroup was reported in 6 biotopes, the highest equal share was recorded at the edge of the forest and forest belt (62.5% each) and agrocenosis (61.7%), while in the locality it was

dominant (41.2%). Host for the *Pomona* serogroup are 10 species, of which *A. flavicollis, A. agrarius, M. musculus, C. glareolus, A. terrestris* and *Microtus* sp. dominate. The serogroup *Ichterohaemorrhagiae* was isolated from 7 species collected from 5 biotopes, the highest share was in the paludous one (28.3%), where it is maintained by the *R. norvegicus* and *A. agrarius* populations. The host composition for the other serogroups is comparatively smaller, maintained by 1-4 species of small mammals.

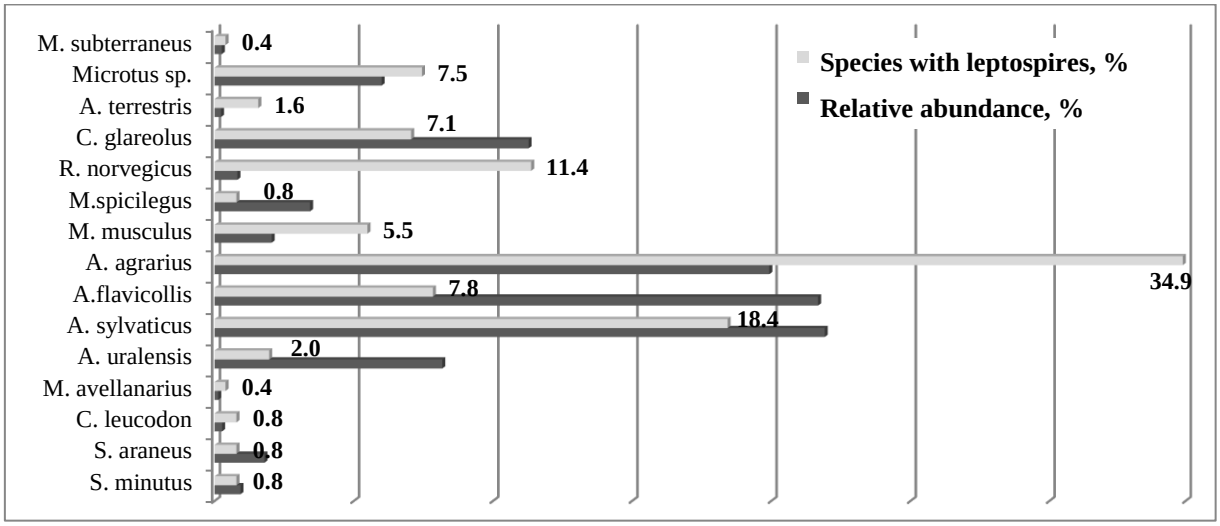


Figure 4.1.6. Relative abundance and proportion of infected small mammals with *Leptospira* spp. in 2003-2020

In the northern area in 6 administrative territories (Glodeni, Ocnița, Briceni, Drochia, Șoldănești, Fălești) foci of leptospirosis were recorded in the study period. Positive serological results for leptospirosis were detected in 15 species of small mammals (*S. araneus, S. minutus, C. leucodon, M. avellanarius, R. norvegicus, M. musculus, M. spicilegus, A. uralensis, A. sylvaticus, A. flavicollis, A. agrarius, C. glareolus, A. terrestris, M. subterraneus, Microtus* sp.). The infected small mammals were hosts for 6 serogroups of leptospires, the dominant being *Pomona* (42.27%) and *Grippotyphosa* (38.18%), followed by *Icterohaemorrhagiae* (17.27%), while *Javanica, Ballum* and *Canicola* constituted less than 1.36%. The obtained results confirm a considerable circulation of leptospirosis pathogen with the involvement of 15 small mammals species in the epizootic process, thus there is a risk of massive contamination of environmental objects and contracting leptospirosis by humans during the practice of various outdoor activities.

In the center area foci of leptospirosis were registered in 2 administrative territories – Ialoveni district and Chisinau municipality. In the Ialoveni district positive serological results for leptospirosis were detected in 4 species of small mammals: *S. araneus, A. agrarius, R. norvegicus* and *M. musculus*. In the species *S. araneus* and *A. agrarius*, the leptospires serogroup *Grippotyphosa* was determined, and in *A. agrarius, M. musculus* and *R. norvegicus* – the

serogroup *Icterohaemorrhagiae*. In the *species A. agrarius* both serogroups of leptospires were determined. In Chisinau municipality positive serologicical results for leptospirosis were found in 3 small mammal species: *A. sylvaticus*, *A. flavicollis* and *A. agrarius*, that are reservoirs for 4 leptospire serogroups (*Grippotyphosa*–61,53%, *Pomona*-23,07%, *Bataviae*–7,69%, *Autumnalis* – 7,69%). In *A. sylvaticus* 3 serogroups were determined (*Grippotyphosa, Pomona*, *Bataviae),* in *A. agrarius* – 2 (*Grippotyphosa*, *Autumnalis*), in *A. flavicollis* – the serogroup *Pomona*.

Thus, in the central area 6 species of small mammals (S. *araneus, A. agrarius, A. sylvaticus, A. flavicollis, M. musculus, R. norvegicus*) were involved in the epizootic process, which were hosts for 5 serogroups of leptospires (*Icterohaemorrhagiae, Grippotyphosa*, *Pomona*, *Bataviae, Autumnalis*).

In the southern area foci of leptospirosis were registered in 3 administrative territories: Cahul, Căușeni and Ștefan Vodă. 6 species of small mammals were involved in the epizootic process (*A. uralensis, A. agrarius, A. flavicollis, M. musculus*, *R. norvegicus, A. terrestris*) being reservoirs for a single serogroup of leptospires – *Icterohaemorrhagiae*. At the same time, the number of reservoir species can be expanded as well as leptospire serogroups due to the ecological peculiarities of small mammals and the causative agent of leptospirosis (virulent, persistent in a host organism).

4.2. Aspects of leptospirosis surveillance in the Republic of Moldova. Currently, the potential risk of human infection remains in the territories where leptospires carriage states have been registered in wild animals, and in domestic animals – manifestation of diseases or asymptomatic carriage states. Thus, it is obvious the importance of the operation of an early warning system at the national level that allows the early detection of risk situations and ensuring the mutual exchange of information between the veterinary and public health authorities in a routine regime and their collaboration in order to prevent and control leptospirosis. In the Republic of Moldova, detection, laboratory confirmation and reporting of leptospirosis cases in humans are carried out based on the standard case definition, which includes clinical, laboratory and epidemiological criteria. The notification of the disease case is carried out in the first 24 hours after the detection, monthly and annually the cases are reported based on Form No. 2 "Statistical report on some infectious and parasitic diseases registered in the Republic of Moldova". The data on leptospirosis cases are reported in the annual statistics from 1963. As part of the investigation of human leptospirosis foci, epizootological research is carried out in the field and laboratory investigation of samples collected from the environment.

During the research period, 137 cases of leptospirosis were reported (2003 – 5 cases, 2004 – 5, 2005 – 10, 2006 – 14, 2007 – 13, 2008 – 18, 2009 – 6, 2010 – 13, 2011 – 5, 2012 – 6,

2013 – 4, 2014 – 19, 2015 – 3, 2016 – 1, 2017 – 5, 2018 – 7, 2019 – 3). Statistical data show that cases of leptospirosis are recorded every year, except for 2020 when no cases were reported.

The territorial distribution of leptospirosis cases is quite wide, this disease being registered in 23 administrative territories, including territories on the left bank of the Dniester (figure 4.2.1). The analysis of the territorial distribution of the reported cases of illness made possible to establish that 128 cases were registered on the right bank of the Dniester and 9 cases were reported in the districts on the left of the Dniester.

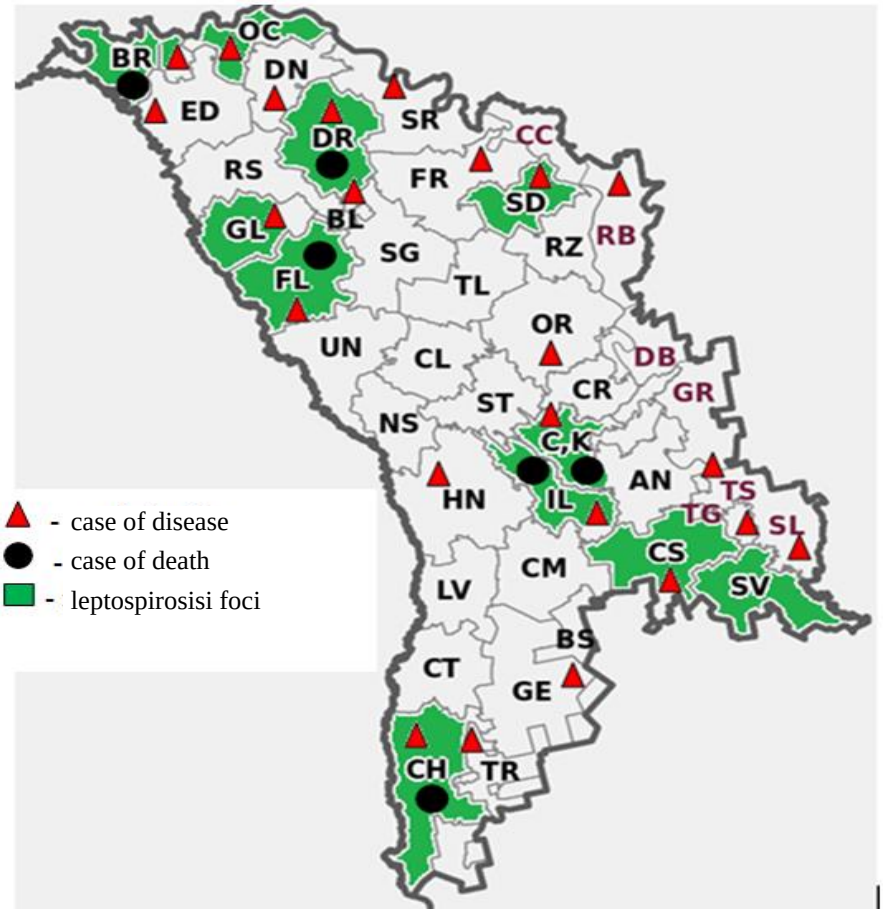


Figure 4.2.1. Distribution map of natural foci of leptospirosis, cases of disease and deaths by leptospirosis in the Republic of Moldova in 2003-2020

The epidemic process of leptospirosis was characterized by predominantly affecting the population in rural areas (70.07%) and by the age structure 95.6% of adults were affected. The diseases caused by leptospirosis were determined by 7 serological groups of *Leptospira* spp. The particularities of the epidemic process are largely due to the causative agent, presented by the leptospira *Icterohaemorrhagiae* in 48.40% and *Grippotyphosa* in 29% of cases, maintained in nature by the rodent populations. The serogroups *Pomona* and *Javanica* are also involved in the epidemic process, being identified as the etiological agent in 6.45% of disease cases each. The share of serogroups *Sejroe*, *Autumnalis* and *Canicola* in the etiological structure of the reported

disease cases is 3.23% each. During the study period 15 cases of death were registered, of which 9 cases were in the northern area (Drochia, Briceni, Făleşti districts); 5 cases in the central area (Chisinau municipality, Ialoveni district) and one case in the southern area (Cahul district).

The research on leptospirosis foci has shown that human infection mainly occurs in natural foci following exposure to contact with external environmental factors contaminated with the pathogen during bathing, fishing in open water pools contaminated by rodents and other animals, using drinking water from unsanitary sources, food products not protected from contamination, mowing in wet biotopes and in other circumstances accompanied by the presence of rodents and conditions for transmission of the pathogen. Within the selective epizootological investigations of disease foci, depending on their particularities, the presence of small mammal species *A. sylvaticus, R. norvegicus, M. musculus, A. uralensis, A. agrarius, C. glareolus, A. flavicollis* and *Microtus* sp. were registerd and positive serological results being determined for leptospire serogroups *Grippotyphosa, Icterohaemorrhagiae, Pomona* and *Javanica*.

GENERAL CONCLUSIONS

The results obtained in correlation with the purpose and objectives formulated in the doctoral thesis "Bio-ecological peculiarities and the role of small mammals (Mammalia: Rodentia, Insectivora) in maintaining leptospirosis foci on the territory of the Republic of Moldova", led to the formulation of the following general conclusions:

As result of research carried out between 2003 and 2020, the small mammal fauna of the Republic of Moldova consists of 24 species, taxonomically classified into 3 orders and 6 families. 6 species belong to the order Soricomorpha (*Talpa europaea, Neomys anomalus, Sorex araneus, S. minutus, Crocidura leucodon, C. suaveolens*), order Erinacemorpha one species (*Erinaceus roumanicus*) and order Rodentia – 17 species (*Muscardinus avellanarius, Dryomis nitedula, Myoxus glis, Apodemus sylvaticus, A. agrarius, A. flavicollis, A. uralensis, Rattus norvegicus, Mus musculus, M. spicilegus, Micromys minutus, Microtus* sp., *M. subterraneus, Clethrionomys glareolus, Cricetulus migratorius, Cricetus cricetus, Arvicola terrestris*).

Following the ecological analysis, it was found that the species *A. sylvaticus* and *A. flavicollis* are dominant, with a total abundance of 21.92% and 21.68%, respectively, followed by *A. agrarius* with an abundance of 19.93%, *C. glareolus* with 11, 28%, *A. uralensis* with 8.18% and the frequency of 100%, and *Microtus* sp. with an abundance of 6% and a frequency of 85.71%. The other species have an abundance of less than 3.43% and a frequency of 28-71%. The diversity analysis according to the Shannon index denotes the highest diversity in localities (0.78), according to the Simpson index – in the forest (0.254), for the Margalef index – in localities 7.526, the Alpha index recorded the highest value in the recreation area (3.544) and

Berger-Parker - in the forest (0.367). The greatest similarity was recorded between the forest biotopes and the forest edge - 82%.

The species *Apodemus sylvaticus, A. flavicollis, A. agrarius, Clethrionomys glareolus, A. uralensis* and *Microtus* sp. present the following bio-ecological peculiarities as reservoir species: they are dominant or subdominant in various types of biotopes, they have the highest share in small mammal communities, they have the most numerous populations on the territory of the republic, they have an increased reproductive potential, especially in anthropized ecosystems, they are widely distributed in all studied biotopes with frequencies of 80-100%.

The study carried out in 28 administrative territories of the Republic of Moldova established the presence of leptospirosis foci in 11 territories (Ocnița, Șoldănești, Fălești, Glodeni, Briceni, Drochia, Ialoveni, Chișinău municipality, Ștefan Vodă, Căușeni, Cahul), mainly found in the northern area (54.5%) of the country, followed by the southern (27.3%) and center (18.2%). The natural foci of leptospirosis in the northern area are characterized by the greatest diversity of small mammal species (n=15) and serogroups of *Leptospira* spp. (n=6) involved in the epizootic process, in the central area – the foci are maintained by 6 species of small mammals and 5 serogroups of *Leptospira* spp., and in the southern area – of 6 species and a single serogroup of *Leptospira* spp.

The obtained results highlight the importance of 15 species of small mammals (*Sorex araneus, S. minutus, C. leucodon, M. avellanarius, A. uralensis, A. sylvaticus, A. agrarius, A. flavicollis, Mus musculus, M. spicilegus, Rattus norvegicus, Clethrionomys glareolus, Arvicola terrestris, Microtus* sp., *M. subterraneus*) determined as reservoir hosts of leptospires, which ensure the intense circulation of the etiological agent of leptospirosis in all the studied biotopes in the northern, central and southern areas of the republic with the formation and maintenance of natural leptospirosis foci. High rates of infection with Leptospira spp. were detected in the following species of small mammals: *A. agrarius* – 34.90%, followed by *A. sylvaticus* with 18.43%, *R. norvegicus* – 11.37%, *A. flavicollis* – 7.84%, *Microtus* sp. – 7.45%, *C. glareolus* – 7.06% and *M. musculus* – 5.49%.

The species *A. flavicollis, A. agrarius, A. sylvaticus, C. glareolus, R. norvegicus, Microtus* sp. and *M. musculus* are determined as potentially important species from epidemiological point of view due to the increased level of portage of a wide range of leptospira serogroups and to their ecological peculiarities.

It was confirmed that the small mammals are rezervoirs for 8 leptospira serogroups, mainly being found the serogroup *Pomona* with 41.96%, followed by *Grippotyphosa* – 36.86%, *Icterohaemorrhagiae* – 16.86%, *Javanica* – 2.75%, the serogroups *Bataviae, Autumnalis, Ballum*

and *Canicola* –0.39% each. The serogroup *Grippotyphosa* is hosted by 12 reservoir species, *Pomona* – by 10 species and *Icterohaemorrhagiae* – by 7 reservoir species. The relevant serological groups are the leptospires *Icterohaemorhagiae* and *Gryppotyphosa*, which are frequently determined (48.40% and 29% of cases, respectively) in leptospirosis foci in humans.

It has been found that a single species of small mammal can be the reservoir for 4-5 serogroups of leptospires. The species *A. agrarius* is the reservoir for most serogroups of *Leptospira* spp. (n=5), in the structure of which *Pomona* dominated (69.7%). *A. uralensis* and *A. flavicollis* are reservoirs for 4 serogroups, in which *Grippotyphosa* (40%) and *Pomona* (70%) dominates, and the species *M. musculus, R. norvegicus* and *A. terrestris* are reservoirs for 3 serogroups, with a high share of *Grippotyphosa* and *Icterohaemorrhagiae* (over 50%).

The research has established that in natural foci of leptospirosis the average level of infection with leptospires in small mammals was 4.27%. The pathogen *Leptospira* spp. is more frequently found in the paludous biotopes (44.31%), forest (18,82%) and agrocenoses (18.43%), followed by the localities (6.67%), the forest edge (6.27%), the forest belts (3.13%) and the recreation area (2.35%). The anthropogenic activities in paludous biotopes and agrocenoses, such as fish farming, fishing, bathing, agricultural works increase the risk of contracting the causative agent of leptospirosis in the human population.

The persistence of natural foci of leptospirosis, the intense anthropization of ecosystems that favor direct or indirect contact of small mammals with the human population, the manifestation of epizootics in small mammal populations have contributed to the occurrence of leptospirosis cases in humans, mainly in the northern (69.34% cases) and central (17.52%) areas of the republic, the serogroups of leptospires detected in patients being to a large extent common with those recorded in rodents.

PRACTICAL RECOMMENDATIONS

1. Continuation of multiannual research and monitoring of small mammal populations, potential carriers of the pathogen agent, of biotic and abiotic factors within the national system of epidemiological surveillance of communicable diseases for the early identification of changes in the status and basic elements (reservoir, pathogen agent, biotope) of natural foci of leptospirosis under conditions of intense climate and anthropogenic changes.

2. The use of the scientific results obtained and the maps developed during the study for the foundation of national programs for the prevention and combating of zoonotic diseases, the improvement of leptospirosis surveillance at national and territorial level, the planning of actions with the aim of combating leptospirosis at the objects with increased epidemiological risk.

3. Carrying out information, education and communication activities with the general population and in risk groups regarding the menaces associated with various activities in natural and anthropogenic ecosystems (recreation, agreement, tourism, fishing, agricultural works etc.) and measures to prevent infection with leptospirosis.

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ADNOTARE

Burlacu Victoria. ”Particularitățile bio-ecologice şi rolul mamiferelor mici (Mammalia: Rodentia, Insectivora) în menținerea focarelor de leptospiroză pe teritoriul Republicii Moldova”, teză de doctor în științe biologice, Chișinău, 2022.

Teza constă din introducere, 4 capitole, concluzii generale şi recomandări, bibliografie din 209 titluri, 135 pagini de text de bază, 42 figuri, 24 tabele. Rezultatele obținute sunt publicate în 54 lucrări științifice.

Cuvinte cheie: mamifere mici, particularități ecologice, repartizare biotopică, agent patogen, *Leptospira* spp., focar natural, zoonoze.

Domeniul de studiu: 165.02-Zoologie

***Scopul lucrării*:** Elucidarea particularităţilor biologice şi ecologice ale speciilor din ordinele Rodentia şi Insectivora pentru determinarea mamiferelor mici cu potenţial epidemiologic major și importanței acestora în formarea și menținerea focarelor de leptospiroză.

***Obiectivele*:** Studiul faunistic, taxonomic și ecologic al comunităților de mamifere mici în ecosistemele naturale și antropizate din zona de nord, centru și sud a țării; evidențierea speciilor de mamifere mici infectate cu leptospire și implicate în procesul epizootic la leptospiroză; studierea structurii etiologice a leptospirelor în populația mamiferelor mici; elucidarea rolului mamiferelor mici în funcționarea focarelor naturale de leptospiroză pe teritoriul republicii.

Noutatea și originalitatea științifică. Au fost realizate cercetări complexe ale comunităților de mamifere mici în ecosisteme naturale și cele antropice în zonele de nord, centru și sud a țării. Au fost evidențiate particularitățile bio-ecologice a 22 specii de mamifere mici și stabilită influența acestora asupra evoluției procesului epizootic. Au fost identificate și cartate teritoriile administrative cu focare naturale de leptospiroză și evidențiate riscurile pentru sănătatea populației asociate cu procesul de antropizare a ecosistemelor naturale. Rezultatele au elucidat 15 specii de mamifere mici implicate în procesul epizootic la leptospiroză, răspândirea lor în biotopurile naturale și antropogene, speciile potențial importante din punct de vedere epidemiologic, semnificația lor ca rezervor de bază pentru 8 serogrupuri de leptospire.

Problema științifică soluționată constă în elucidarea rolului mamiferelor mici în funcționarea focarelor naturale de leptospiroză, nivelul de infectare a mamiferelor mici cu *Leptospira* spp. în ecosistemele cercetate, ce va permite prognozarea situației epizootologice și elaborarea măsurilor pentru prevenirea riscului de răspândire a leptospirozelor în republică.

Semnificația teoretică. Au fost obținute date noi privind structura comunităților de mamifere mici din focarele naturale de leptospiroză. Rezultatele obținute extind cunoștințele privind fauna și ecologia mamiferelor mici, precum și rolul lor epizootologic și epidemiologic în funcționarea focarelor de leptospiroză din Republica Moldova.

Valoarea aplicativă a lucrării. A fost elucidat rolul mamiferelor mici în funcționarea focarelor naturale de leptospiroză. Au fost obținute rezultate care pot fi incluse în programele de educație pentru sănătate în domeniul prevenirii zooantroponozelor și care contribuie la elaborarea pronosticurilor epizootologice, perfecționarea cadrului legal în sănătate publică.

Implementarea rezultatelor științifice. Rezultatele studiului au fost implementate în activitatea practică a Agenției Naționale pentru Sănătatea Publică, secția supravegherea epidemiologică a bolilor extrem de contagioase, zoonoze și parazitoze; în cadrul Centrelor de Sănătate Publică. Rezultatele sunt utilizate și implementate în procesul didactic, la realizarea tezelor de licență și de masterat la instituțiile de învățământ cu profil biologic și ecologic.

АННОТАЦИЯ

Бурлаку Виктория. «Био-экологические особенности мелких млекопитающих (Mammalia: Rodentia, Insectivora) и их роль в поддержании очагов лептоспироза на территории Республики Молдова», кандидатская диссертация по биологическим наукам, Кишинев, 2022.

Диссертация состоит из введения, 4 глав, общих выводов и рекомендаций, библиографии из 209 наименований, 135 страниц основного текста, 42 рисунков, 24 таблиц. Результаты опубликованы в 54 научных статьях.

Ключевые слова: мелкие млекопитающие, экологические особенности, биотопическое распространение, возбудитель, *Leptospira* spp., природный очаг, зоонозы.

Область исследования: 165.02-Зоология

***Цель диссертации*:** выяснить биологические и экологические особенности видов отрядов Rodentia и Insectivora для определения эпидемиологического потенциала и их значения в формировании и поддержании очагов лептоспироза.

***Задачи*:** фаунистическое, таксономическое и экологическое изучение сообществ мелких млекопитающих в природных и антропургических экососистемах северной, центральной и южной части республики; выявление видов мелких млекопитающих зараженных лептоспирами и вовлеченных в эпизоотический процесс при лептоспирозе; изучение этиологической структуры лептоспир в популяции мелких млекопитающих; выяснение роли мелких млекопитающих в подержании природных очагов лептоспироза на территории республики Молдова.

Научная новизна и оригинальность. Было проведено комплексное исследование сообществ мелких млекопитающих в природных и антропургических экосистемах в северных, центральных и южных районах страны. Выделены особенности 22-х видов мелких млекопитающих и установлено их влияние на эволюцию эпизоотического процесса. Нанесены на карту административные территории где выявлены природные очаги лептоспироза, также выделены риски для здоровья населения связанные с процессом антропизации природных экосистем. Результаты выявили разнообразие мелких млекопитающих вовлеченных в эпизоотический процесс лептоспироза, распределение в природных и антропогенных биотопах, их потенциальную эпидемиологическую значимость, значение как основного резервуара для 8-ми серогрупп лертоспир.

Разрешённая научная проблема заключается в выяснение роли мелких млекопитающих в функционировании природных очагах лептоспироза, уровень инфицированности мелких млекопитающих лептоспирами в исследованных экосистемах что позволит прогнозировать эпизоотологическую ситуацию и разработать мероприятия по предупреждению риска распространения лептоспирозов в республике.

Теоретическая значимость. Получены новые данные о структуре сообществ мелких млекопитающих из природных очагах лептоспироза. Результаты расширяют знания о фауне, экологии мелких млекопитающих, а также о их эпизоотологической и эпидемиологической роли в функционировании очагов лептоспироза в республике.

Практическая значимость исследования. Выяснена роль мелких млекопитающих в функционировании природных очагах лептоспироза. Результаты могут быть включены в программах санитарного просвящения в области профилактики зоонозных заболеваний и способствуют разработке эпизоотологических прогнозов, совершенствованию законодательства общественного здоровья.

Внедрение научных результатов. Полученные результаты использовались в практическую деятельность Национального Агентства Общественного Здоровья, Отдел Эпидемиологический надзор за особо опасными инфекциями, зоонозы и паразитозы; в Центрах общественного здоровья. Результаты исследований используются и внедрены в учебный процесс, при выполнении бакалаврских и магистерских диссертаций в учебных заведениях биолого-экологического профиля.

ANNOTATION

Burlacu Victoria. „Bioecological peculiarities and the role of small mammals (Mammalia: Rodentia, Insectivora) in maintaining leptospirosis foci on the territory of the Republic of Moldova", PhD thesis in biological sciences, Chisinau, 2022.

The thesis consists of introduction, 4 chapters, general conclusions and recommendations, bibliography of 209 titles, 135 pages of basic text, 42 figures, 24 tables. The obtained results are published in 54 scientific works.

Key words: small mammals, ecological peculiarities, biotopic distribution, pathogen agent, *Leptospira* spp., natural foci, zoonozes.

Field of study: 165.02-Zoology.

The purpose of the paper: Revealing of the biological and ecological peculiarities of the species of the Rodentia and Insectivora orders for the determination of small mammals with major epidemiological potential and their importance in the formation and maintenance of leptospirosis foci.

***Objectives*:** Faunal, taxonomic and ecological study of small mammal communities in natural and anthropogenic ecosystems from the north, center and south of the republic; highlighting the species of small mammals infected with leptospires and involved in the epizootic process of leptospirosis; studying the etiological structure of leptospires in the population of small mammals; elucidating the role of small mammals in the functionning of natural foci of leptospirosis on the territory of the republic.

Scientific novelty and originality. Complex researches of small mammal communities in natural and anthropogenic ecosystems were carried out in the northern, central and southern areas of the country. The bio-ecological peculiarities of 22 species of small mammals were highlighted and their influence on the evolution of the epizootic process was established. The administrative territories with natural foci of leptospirosis were identified and mapped, and the risks for the health of the population associated with the anthropization process of natural ecosystems were highlighted. The results elucidated 15 species of small mammal involved in the epizootic process in leptospirosis, their distribution in natural and anthropogenic biotopes, the main potentially epidemiological important species, their significance as a basic reservoir for 8 serogroups of leptospires.

The solved **scientific problem** consists in elucidating the role of small mammals in the functioning of natural foci of leptospirosis, the level of infection of small mammals with Leptospira spp. in the investigated ecosystems, which will allow the forecasting of the epizootological situation and the development of measures to prevent the risk of spreading leptospirosis in the republic.

Theoretical significance. New data on the structure of small mammal communities in natural foci of leptospirosis have been obtained. The obtained results expand the knowledge regarding the fauna and ecology of small mammals, as well as their epizootological and epidemiological role in the functioning of leptospirosis foci in the Republic of Moldova.

The applicative value of the work. The role of small mammals in the functioning of natural foci of leptospirosis has been elucidated. The results can be included in health education programs in the field of zooanthroponosis prevention and will contribute to the development of epizootological prognoses, the improvement of the legal framework in public health.

Implementation of scientific results. The results of the study were implemented in the practical activity of the National Agency for Public Health, the epidemiological surveillance section of highly contagious, zoonotic and parasitic diseases; within the Public Health Centers. The results are used and implemented in the didactic process, in the preparation of bachelor's and master's theses at educational institutions with a biological and ecological profile.

BURLACU VICTORIA

BIO-ECOLOGICAL PECULIARITIES AND THE ROLE OF SMALL MAMMALS (MAMMALIA: RODENTIA, INSECTIVORA) IN MAINTENANCE OF LEPTOSPIROSIS FOCI ON THE TERRITORY OF THE REPUBLIC OF MOLDOVA

165.02 – Zoology

Summary of the doctoral thesis in biological sciences

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