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HELMINTH FAUNA OF MYOMORH RODENTS (RODENTIA, MYOMORHA) IN THE CENTRAL CHERNOZEM STATE NATURE RESERVE

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Abstract
Objective of research. Helminth fauna of rodents was investigated in the Central Chernozem State Nature Reserve (Kursk Region, Russia) in 2012-2014.
Results and discussion. In total 499 individuals of twelve species from four families of rodents were examined and total prevalence by helminths was 46,3%. Thirty two species of helminths were recorded, including one species of trematodes, thirteen species of cestodes and eighteen species of nematodes. Three species of nematodes: *Heligmosomoides polygyrus*, *Syphacia frederici*, *Heligmosomum costellatum* and one species of cestodes: *Catenotaenia henttoneni* were in the dominant helminth group. Low value of helminth species richness in *Myodes glareolus* is detected and we suggest possible explanation for this pattern.
Keywords: helminths, rodents, fauna, Central Chernozem State Nature Reserve.

Rodents are the largest group of mammals, encompassing 2277 species, or approximately 42% of worldwide mammalian biodiversity [51]. Among mammals in Central Chernozem region (Kursk, Belgorod, Voronezh, Lipetsk, Tambov regions) rodents are the most diverse and abundant group. They host helminth parasites, some of which are zoonotic, e.g. the most important in Palearctic *Echinococcus multilocularis, Capillaria hepatica, Trichinella spiralis, Pararodentolepis nana, Hymenolepis diminuta*.

The helminth fauna of rodents has been studied in the Central Chernozem region by a few authors: in the Voronezhsky State Nature Reserve in the Voronezh Region by Romashov B.V., Romashova N.A., in the Khopyorsky State Nature Reserve in the Voronezh Region by Rukhlyadeva M.N., Rukhlyadev D.P., Romashov V.A., Romashov B.V., Romashova N.A., in the State Nature Reserve "Voroninsky" in the Tambov Region by Romashova N.B., Trapeznikov D.A. [34–38]. However, data for the territory of the Kursk Region include only one article about one species of cestode *Rodentolepis straminea* from *Cricetus cricetus* [6] and report about trichinella larvae from *Rattus norvegicus* and *Microtus arvalis + M. rossiaemeridionalis* [49].

Therefore, the aim of the present study was to investigate and analyze the helminth fauna of rodents from the Central Chernozem State Biosphere Nature Reserve named after professor V.V. Alyokhin (referred to below as CCSNR), located within the Kursk Region, the natural forest-steppe ecosystem, which has never been investigated in this aspect.

Materials and methods

The study area, CCSNR is located in the European part of Russia, in the south of the Central Russian Upland. The reserve consists of six sites located within Kursk Region. Its total area is 52,87 km^2. The Streletsky and Kazatsky sites (51°34′N, 36°24′E) are located at altitude of 178-262 m above sea level; Zorinsky site (51°11′N, 36°24′E) – at 169-200 m, and the Psyol Flood Land (51°11′N, 36°19′E) at 155-167 m above sea level in the basins of the Seym and Psyol rivers belonging to the Dnieper river system. Two sites of CCSNR: Barkalovka (51°33′N, 37°39′E) and Bukreyevy Barmy (51°30′N, 37°18′E) are located at altitudes 163 – 238 m above sea level in the basin of the Oskol River belonging to the Don River system. The Streletsky and Kazatsky sites consist of meadow steppes and oak forests (*Quercus robur*). The Zorinsky site is composed of open lands with sphagnum bogs and forests, represented by oak, birch (*Betula pendula*, *B. pubescens*) and aspen groves (*Populus tremula*) surrounded by fallows and meadows. The Psyol Flood Land consists of water bodies, wetlands, forests, represented by alder (*Alnus glutinosa*), willow (*Salix alba*, *S. fragilis*) and oak groves. The Bukreyevy Barmy and Barkalovka sites consist of oak forests and meadow steppes on the chalk hills [47]. The Streletsky site has a human settlement. The approximate distance between the Streletsky and Kazatsky sites is 18 km, Streletsky and Zorinsky, Psyol Flood Land sites – 45 km, Streletsky and Bukreyevy Barmy – 90 km, Streletsky and Barkalovka – 135 km, Bukreyevy Barmy and Barkalovka – 25 km.

The sampling of rodents took place at all six sites of CCSNR: at the Streletsky site in spring, summer and autumn of each year, at the other sites irregularly. Rodents were captured, using standardized procedure that allows to compare the relative density of rodents, in 2012-2014 [22]. Trap lines of 50 or 100 traps (snap traps), separated by 5-m intervals were deployed during 1, 2 or 3 nights, for a total of 4700 trap nights (4150 at the Streletsky site, 550 at the other sites). Traps were set up at the Streletsky site in the following habitats: forest, unmown steppe, yearly mown steppe, periodically mown steppe, pasture. Traps were set up in Kazatsky in forest and unmown steppe, in the Psyol Flood land in the willow grove near the Psyol river, in Zorinsky in the aspen-oak grove and in the meadow near sphagnum bogs. In Bukreyevy Barmy traps were set up in the forest and in unmown steppe, in Barkalovka in the grove near the stream. Additionally, we used for investigation carcasses of rodents found dead in the territory of CCSNR and animals caught by local people in their houses in the settlement. Systematic position, Latin names of rodents are given according to [31].

The animals were dissected according to [21]. The body cavity, digestive tract (esophagus, stomach, small intestine, caecum and colon), liver, kidneys, heart, trachea and lungs were examined for the presence of helminths. Besides, diaphragm and intercostal muscles of 92 individuals were investigated for the possible presence of trichinella larvae. All material was isolated, rinsed and preserved in 70% ethanol.

Nematodes were cleared in solution, consisted of equal volumes of lactic acid, glycerol and water. Cestodes and trematodes were stained in hematoxylin or acetocarmine, differentiated with acidified ethanol, dehydrated in graded series of ethanol solutions (70, 80, 90, 95 and 100%), cleared in clove oil and mounted in Canada balsam.

Identification of helminths was carried out according to identification keys [12, 14, 16, 18, 24, 40, 41, 45] and articles with species descriptions [9, 13, 15, 17, 19, 27–29, 43, 46].

Four kinds of specificity are defined according to Euzet, Combes (1980) [see 8]: oioxenous parasite – highly specific to a host species; stenoxenous parasite – specific at the level of host genus; oligoxenous parasite – specific at the level of a host family; euryxenous parasite – infests a broad range of non-related hosts. Stenoxenous and oioxenous helminthes are combined in one group.

Eight ecological groups of helminths are defined according to [48]: I group – helminths, which larvae are in eggs, hosts become infected by ingesting the infective eggs; II group – helminths, which free-living larvae are in terrestrial biocoenosis (usually on plants), hosts become infected by ingesting the infective larvae; III group – helminths, which free-living larvae are in aquatic biocoenosis (usually on plants), hosts become infected by ingesting the infective larvae; IV group – helminths, which larvae locate in aquatic invertebrates, hosts become infected by eating the

intermediate hosts; V group – helminths, which larvae locate in terrestrial invertebrates, hosts become infected by eating the intermediate hosts; VI group – helminths, which larvae locate in aquatic vertebrates, hosts become infected by eating the intermediate hosts; VII group – helminths, which larvae locate in terrestrial vertebrates, hosts become infected by eating the intermediate hosts; VIII group – all stages develop in one host.

We performed hierarchic grouping of rodent helminth assemblage according to [7]. Prevalence in samples of each helminth species was calculated among all individuals of rodents. Samples of species were compared, using their prevalence with the help of unconditional test [32]. Statistical significance was established at p=0,05 and samples of species were set in groups: dominants, subdominants, rare and very rare. Species samples which showed no significant difference between neighbor species samples were set in groups – "intermediate".

Prevalence, 95% confidence interval (CI) and comparison of prevalence were calculated, using statistical package Quantitative Parasitology 3.0 [39].

Results

A total of 499 individuals of twelve rodent species from four families were collected and investigated for helminth parasites (Table 1). Sibling species of genus *Microtus* recorded in the CCSNR *Microtus arvalis* and *M. rossiaemeridionalis* were set in one sample.

Table 1.

List of rodent species collected in the sites of CCSNR

Rodent species	Total number	Sites					
		S	K	P	Z	BB	B
Sminthidae							
Sicista severtzovi Ognev, 1935	5	5	-	-	-	-	-
Sicista strandi Formosov, 1931	2	2	-	-	-	-	-
Spalacidae							
Spalax microphtalmus Gueldenstaedt, 1770	2	2	-	-	-	-	-
Muridae							
Sylvaemus uralensis Pallas, 1811	168	157	5	5	-	1	-
Sylvaemus flavicollis Melchior, 1834	17	11	2	2	1	1	-
Micromys minutus Pallas, 1771	6	4	-	2	-	-	-
Apodemus agrarius Pallas, 1771	84	71	2	1	5	4	1
Mus musculus Linnaeus, 1758	3	3	-	-	-	-	-
Cricetidae							
Myodes glareolus Schreber, 1780	123	92	7	-	8	-	16
Microtus arvalis Pallas, 1778+ *M. rossiaemeridionalis* Ognev, 1924	83	66	13	-	3	1	-
Cricetulus migratorius Pallas, 1773	6	4	2	-	-	-	-
Total	499	417	31	10	17	7	17

S – Streletsky site, K – Kazatsky site, P – Psyol Flood land, Z – Zorinsky site, BB – Bukreyevy Barmy, B – Barkalovka.

Numerically dominant rodent species in forest habitats at the sites of CCSNR are *Sylvaemus uralensis*, *Myodes glareolus*, numerically dominant rodent species in steppe habitats are *S. uralensis*, *Apodemus agrarius, M. arvalis + M. rossiaemeridionalis*.

The total prevalence of rodents by helminths was 46,3% (CI=41,85–50,78). The total helminth prevalence for the numerically dominant rodent species was 43,9% (CI=34,97–53,14) for

M. glareolus, 67,5% (CI=56,3–76,36) for *M. arvalis + M. rossiaemeridionalis*, 50,0 % (CI=42,2–57,8) for *S. uralensis*, 20,2% (CI=12,25–30,42) for *A. agrarius*.

In total 32 species of helminths were recorded, although not all helminths were identified to species level (Table 2).

Table 2.

List of helminth species collected from rodents in CCSNR

Helminth species	g	u	f	a	im	m	g	a	m	m	se	st
Trematoda												
Plagiorchis elegans	V											
Cestoda												
Paranoplocephala omphalodes												
Anoplocephaloides dentata												
Anoplocephalinae sp.												
Catenotaenia henttoneni												
Catenotaenia sp.												
Skrjabinotaenia lobata												
Pseudocatenotaenia matovi												
Hymenolepis apodemi												
Hymenolepis sp.												
Rodentolepis straminea												
Nomadolepis merionis												
Hydatigera taeniaformis larvae												
Paruterinidae spp. larvae												
Nematoda												
Trichocephalus muris												
T. arvicolae												
T. spalacis												
Heligmosomum costellatum	I											
Heligmosomoides polygyrus	I											
H. sp.	I											
Syphacia obvelata												
S. frederici												
S. stroma												
S. agraria												
S. petrusewiczi												
S. nigeriana												
S. mesocriceti												
S. vandenbrueli												
S. sp.												
Aspiculuris tetraptera												
Mastophorus muris												
Physaloptera myotis												

Eg – ecological group, Su - *Sylvaemus uralensis*, Sf - *Sylvaemus flavicollis*, Aa - *Apodemus agrarius*, Mim - *Micromys minutus*, Mm - *Mus musculus*, Mg - *Myodes glareolus*, Ma - *Microtus*

arvalis + M. rossiaemeridionalis, Cm - *Cricetulus migratorius*, Sm - *Spalax microphtalmus*, Sse - *Sicista severtsovi*, Sst - *Sicista strandi*

Specificity of helminths from rodents and species richness, which is the number of helminth species found in each host species are shown in Table 3.

Table 3.

Helminth species richness of each species of rodents and specificity of their helminths

Host species	N	C	T	Oioxenous and Stenoxenous	Oligoxenous	Eurixenous	Helminth species richness
S. severtzovi	1	1	-	1	-	1	2
S. strandi	-	1	-	1?	-	-	1
S. microphtalmus	1	-	-	1	-	-	1
S. uralensis	4	6	-	5	4	1	10
S. flavicollis	3	3	-	4	1	1	6
M. minutus	1	-	-	1	-	-	1
A. agrarius	1	3	1	1	2	2	5
M. musculus	4	-	-	1	1	2	4
M. glareolus	2	1	-	2	1	-	3
M. arvalis + M. rossiaemeridionalis	4	5	-	3	4	2	9
C. migratorius	2	1	-	2	-	1	3
Total	18	13	1	20	4	7	32

N– nematodes. C – cestodes, T - trematodes

Murid rodents harbor equal number of helminth species (mean = 5,2) to cricetid rodents (mean=5) at the sites of CCSNR. The highest number of helminth species was found in *S. uralensis* (n=9) and *M. arvalis + M. rossiaemeridionalis* (n=9). The helminth fauna of *A. agrarius* consists of 5 species. The helminth fauna of *M. glareolus* is composed of very small number of species – 3.

Nematodes were predominant among the helminth fauna with 18 species, followed by cestodes with 13 species, and trematodes with 1 species only. The most prevalent group is oioxenous and stenoxenous helminths (20 species), followed by eurixenous (7 species) and oligoxenous (4 species) groups.

The dominant species in helminth assemblage of rodents at the sites of CCSNR are 4 species – 3 species of nematodes and 1 species of cestodes: *H. polygyrus*, *S. frederici*, *H. costellatum* and *C. henttoneni* (Table 4). Nevertheless, no one of them has high value of total prevalence in helminth assemblage of rodents.

Table 4.

Helminth assemblage of rodents, ranked in accordance with helminth species prevalence

Type of species	Helminth species	Prevalence, %	95 % CI
Dominant	*H. polygyrus*	8,8	6,47 – 11,67
	S. frederici	8,6	6,3 – 11,44
	H. costellatum	8,4	6,13 – 11,21
Intermediate	*C. henttoneni*	6,6	4,59 – 9,17
Subdominant	*S. petrusewiczi*	4,6	2,94 – 6,84
Intermediate	*A. dentata*	3,4	1,99 – 5,4
	T. arvicolae	3,4	1,99 – 5,4

	P. omphalodes	3,0	1,69 – 4.91
	H. taeniaformis	2,6	1,39 – 4,42
Rare	*H. apodemi*	2,0	0,96 – 3,66
	S. agraria	1,8	0,82 – 3,4
	R. straminea	1,8	0,82 – 3,4
	S. nigeriana	1,8	0,82 – 3,4
	S. lobata	1,8	0,82 – 3,4
Intermediate	*S. stroma*	1,6	0,69 – 3,14
Very rare	Other species	≤ 0,4	

Helminths of rodents from CCSNR are included in four ecological groups. The most numerous group is I group, consisting of 15 species (50%): 13 species of nematodes and 2 species of metacestodes. Secondary in number of species is V group, consisting of 11 species (37%): 9 species of cestodes and 2 species of nematodes. II group consists of 3 species (10%) – nematodes from family Heligmosomidae. IV group consists of 1 species of trematodes (3%).

Discussion

Low total helminth prevalence among rodents from CCSNR was observed. Helminth species richness of rodents consists of 32 species, with the most numerous group – nematodes (n=18). A species group of genus *Syphacia* forms half of the number of nematode species.

Species of genus *Syphacia* are parasites of rodents from superfamily Muroidea [20] which is represented by Muridae, Cricetidae and Spalacidae families in Palearctic. Nematodes from genus *Syphacia* have not been detected in rodent species from Spalacidae. *Syphacia spp*. are strict-specific parasites, most of them host specific at the genus level (stenoxenous). Hence, we suppose that species richness of nematodes from genus *Syphacia* in the region depends on rodent species richness from families Cricetidae and Muridae in that region, mostly at the genus level. Here, we investigated rodent species from 7 different genera of families Muridae and Cricetidae, in which we found 9 species from genus *Syphacia*. Rodents from genus *Sylvaemus* host 2 species of genus *Syphacia*: *S. frederici* and *S. stroma*. In *M. arvalis + M. rossiaemeridionalis* two species were found: one typical parasite of genus *Microtus* in Palearctic – *S. nigeriana* and the other one (*Syphacia sp.*), which we assume as occasional parasite for *M. arvalis + M. rossiaemeridionalis* and specific parasite of unexplored in our study rodent species. Rodents from the other genera of families Muridae and Cricetidae have one specific species of genus *Syphacia*.

Cestodes are less common than nematodes and include 13 species. Rodent species, which have most number of cestode species, are *S. uralensis* (6 cestode species from 9 helminth species) and *M. arvalis + M. rossiaemeridionalis* (5 cestode species from 9 helminth species). Together they have 9 species from 13 cestode species.

Low numbers of trematode species (1 species) can be explained by small area of water bodies in the reserve and small sample effort from sites with water bodies.

We report *Hymenolepis apodemi, Nomadolepis merionis* for the first time in Europe, *Physaloptera myotis, Trichocephalus arvicolae,* for the first time on the territory of Russia, *Syphacia frederici* for the first time on European part of Russia, *Catenotaenia henttoneni*, *Syphacia mesocriceti, S. vandenbrueli* for the first time on the territory of the Central Chernozem region.

Three helminth species: *P. elegans (=P. muris), H. taeniaformis, S. obvelata* are of zoonotic importance and were detected by human [44 Appendix].

Nematode *Physaloptera myotis* was described from mouse-eared bat *Myotis oxygnathus* in Hungary [43]. Nevertheless, this species was recorded from rodents in Moldavia: *Dryomys nitedula* Pallas, 1778 and *S. flavicollis* [41]. Detection of this nematode in *S. severtzovi* may be due to the birch mice diet, large part of which consists of invertebrates, mostly of insects [42], which nematodes from family Physalopteridae use for intermediate hosts [1].

Probably due to the higher abundance of birds of prey and/or owls in Bukreyevy Barmy, metacestodes Paruterinidae spp., which definitive hosts can be birds from Falconiformes and/or Strigiformes, were detected only in that site.

We can conclude that among helminth species of rodents from CCSNR the predominant group is specific parasites of rodents, consisting of oioxenous and stenoxenous helminths (20 species or 62,5%). If we consider oligoxenous and euryxenous species as generalists (11 species), and oioxenous and stenoxenous species as specialists, specialists are still the predominant group.

The most numerous in helminth community of rodents are I and V ecological groups, which are in line with the other studies carried out on rodent helminth fauna in Russia [25, 36]. Nevertheless, two helminth species from II group are the dominant species in helminth community of rodents. Mostly, species from these three groups are typical parasites of rodents (stenoxenous and oligoxenous).

Interestingly, *M. glareolus* shows low value of helminth species richness among the other numerically dominant rodent species investigated here. Appeared to be parasitized by a high number of helminth species in other localities of Russia [4, 23, 25, 36] and Europe [see 11 for a comprehensive list, 2, 3, 30, 33], here the helminth community of *M. glareolus* composed of three species, and one species of them was recorded in one individual only. Previously low helminth species richness of *M. glareolus* was recorded only in some studies in Great Britain and islands surrounding it [see 11 for a comprehensive list]. Besides, the most remarkable feature is the absence of nematodes from family Heligmosomidae in our samples: *Heligmosomum mixtum* and *Heligmosomoides glareoli* – typical parasites of the genus *Myodes*.

This pattern may be related to the population structure of *M. glareolus* in forest-steppe area and to the forest fragmentation in CCSNR and Kursk region. Moreover, each forest in the reserve is small in size – from 0,3 to 5 km^2 and population structure of *M. glareolus* in forest-steppe area and in the territory of CCSNR is pulsative tending to be insular [10, 50]. Hence, populations of *M. glareolus* locate in forests and subsequently population size may depend on the forest size.

Species-poor parasite communities were reported previously for insular populations of *S. sylvaticus* and *M. glareolus* in Europe and there was significant effect of island area size on the helminth species richness [11, 26]. Additionally, it was suggested that host population fragmentation with decrease of population size and with great distances between population fragments could lead to the extinction of parasites [5]. It can be cautiously suggested that those reasons above could make fragmented populations of *M. glareolus* in CCSNR relatively isolated and similar in some features to insular populations on small islands, which could lead to the decrease of helminth species richness and extinction of some parasite species.

On the other hand, *A. agrarius* – the numerically dominant species in steppe areas and agricultural landscapes in the Kursk region has poor helminth species richness and the lowest general prevalence by helminths (20,2%) among numerically dominant rodent species. From recorded five species two were detected only once: metacestodes Paruterinidae spp. and trematode *P. elegans*.

To cast more light on these patterns, such as poor helminth species richness of *M. glareolus* and poor helminth species richness and low helminth prevalence of *A. agrarius*, future studies will have to be carried out, using larger samples of rodents from distant sites of CCSNR (Barkalovka, Bukreyevy Barmy, Psyol Flood land, Zorinsky sites) and other localities of the region.

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ГЕЛЬМИНТОФАУНА МЫШЕОБРАЗНЫХ ГРЫЗУНОВ (RODENTIA, MYOMORHA) ЦЕНТРАЛЬНО-ЧЕРНОЗЕМНОГО ЗАПОВЕДНИКА

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Реферат

В 2012–2014 гг. были проведены исследования гельминтофауны грызунов Центрально-Черноземного заповедника (Курская область, Россия). Двенадцать видов грызунов из четырех семейств (499 особей) были заражены 32 видами гельминтов, из них 1 видом трематод, 13 видами цестод, 18 видами нематод. Доминирующую группу составили 3 вида нематод: *Heligmosomoides polygyrus, Syphacia frederici, Heligmosomum costellatum* и 1 вид цестод: *Catenotaenia henttoneni*. Установлено низкое видовое богатство гельминтов у рыжей полевки, нами предложено возможное объяснение этой особенности.

Ключевые слова: гельминты, грызуны, фауна, Центрально-Черноземный заповедник.