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Research Article

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SOME RESULTS OF THE HELMINTHOLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS OF INSECTIVORES (INSECTIVORA: ERINACEIDAE) AND RODENTS (RODENTIA: SCIURIDAE) ON THE TERRITORY OF THE URBAN FOREST AREA OF MEGAPOLIS UNDER CONDITIONS OF ANTHROPOGENIC PRESSURE

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ABSTRACT

The results of helminthological and bacteriological studies of rodents (squirrels) and insectivores (hedgehogs) of the urban biotope are presented. Causes of helminthiases outbreaks were studied. The possibility of the use of information about helminth fauna and the invasion intensity of micromammals by helminths as indicators of anthropogenic pollution are pointed. The possible measures for fighting and prevention of the helminthiases of micromammals inhabiting in the urban forest area of Moscow are proposed.
Keywords: *Squirrels, hedgehogs, parasite fauna, invasion intensity, helminthological studies, bacteriological studies, forest parks area, megapolis, anthropogenic pollution.*

INTRODUCTION

Today is considered certain that parasitism is an ecological phenomenon in natural biocenoses, one of the forms of interspecies relationships [1]. Parasites are an important mechanism participating in maintaining the qualitative and quantitative composition of ecosystems, as well as the spread of helminthiases and tension of helminthiasis foci - one of the factors regulating the number of host species [2-3]. Meaning of parasitological data for development of biology has reached such a level that they become an integral part of the knowledge of many biological disciplines. Factual data of parasitology are the subject of investigations both fundamental directions of biology and applied branches of medicine, veterinary medicine, agronomy, ecology with independent service tasks [4]. Thus, the composition of the parasite fauna, the dynamics and intensity of helminth infection are the necessary and indispensable component of the environmental monitoring of terrestrial biocenoses, which is widely being carried at present in all natural areas, not excluding the territory of the megapolises [5] especially in connection with the growing anthropogenic pressure.

In natural ecosystems the manifestation of the pathogenicity of the parasite appears as a result of the reduction of host immunity and relates to disturbances of the number of functions of the host organism. Hidden infestation becomes clinically significant when the high quantity of helminths is combined with adverse environmental conditions [4]. Thus, parasites, on the one hand - a natural component of natural ecosystems, on the other - a factor of the biological pollution of environment [6,7]. Parasites are become the factor of biological pollution in the case when a natural ecosystem is subjected to intensive anthropogenic pressure, adverse factors have a powerful influence, the principle of natural selection in populations aimed at the preservation of low virulent forms of the parasites and increase of resistance of the host organism is violated [4]. This is particularly true for the urban ecosystems of megapolises.

Thus, in numerous studies it was found that in the present conditions on the background of human impact on natural ecosystems both the ecological status and the dynamic balance of parasitic systems are changing [6-8]. In its turn, rodents and insectivores are the necessary and natural faunal component of virtually all natural areas not excluding urban complexes. Having the wide trophic and chorologic connections, high reproductive potential and metabolic level they are primarily respond to any disturbances in natural ecosystems also by changing in the qualitative and quantitative composition of the helminth fauna and by the occurrence of clinically expressed helminthiases. These animals play an important role in the circulation of many species of helminths, including those with epidemiological and epizootic significance [8,9]. The relevance of this kind of works is shown by the fact that the study of micromammals helminthiases allows to evaluate the health of the animals, to develop science-based environmental protection measures and protection measures against the invasive and infectious diseases of small mammals in the territory of the urban biocenoses.

The purpose of this study was to establish the species composition of the helminths of hedgehogs and squirrels in the territory of Moscow urban forest areas which play a key role in the occurrence of clinically significant helminthiases, to analyse the causes of the formation of helminthiases outbreaks in the studied area, to assess the extensiveness and intensity of invasion, to identify the possible role of bacterial agents in the appearance of the pathologies of hedgehogs and squirrels in natural biotopes and in captive content leading to spontaneous death, to pre-evaluate the effectiveness of anthelmintic drugs on the basis of praziquantel and fenbendazole.

Materials and Methods

Studies were conducted on the territory of the forest park Kuzminki of the South-East Municipal District of Moscow. In the conditions of captive content spontaneous death of squirrels with the manifestations of adynamia, cachexia, dehydration and the spontaneous discharge from the anus of the large number of small white segments of helminths with almost round shape was noted. Occasionally, close to the nearby places of permanent habitat of hedgehogs, the corpses of these animals with the noticeable cachexia and expressed anemia of the mucous membranes of the oral cavity were found.

The corpses of three squirrels (2 Eurasian red squirrels (*Sciurus vulgaris*) and 1 Teleut squirrel (*Sciurus vulgaris exalbidus*) - introduced species from the Far East) and four common or European hedgehogs (*Erinaceus europaeus*) were the material for the research. Animals were subjected to incomplete helminthological autopsy by the method of K.I. Skryabin in the modification of B.V. Romashov (2003) [10]. Yet another specimen of the hedgehog was subjected to intravital helminthoscopic researches (the excretion of cestodes segments with faeces was observed). From the floor of the enclosure for squirrels the impersonal samples of faeces for helminth ova detection using flotation method of G.A. Kotelnikov and V.M. Hrenov were also collected [11].

In addition, sowings from the parenchymal organs (liver, kidney, spleen) and bone marrow (tubular bone) of dead animals on culture media (beef-extract agar, beef-extract broth, Levine EMB agar, bismuth sulphite agar, Kitt-Tarozzi medium and Sabouraud agar) and further bacteriological studies of isolated cultures (Gram-stained smears microscopy, sowings on Hiss media), agglutination assay and bioassay on white mice weighing 16-18 g were conducted.

Results and Discussion

Helminths were found in all animals after helminthological autopsy (Fig. 1-4). In the stomach and the anterior part of the small intestine of hedgehogs nematodes were found, in all parts of the small intestine - cestodes. The squirrels also had numerous cestodes in small intestine. The intensity of the infestation of hedgehogs was on average 700 cestodes and 25 nematodes. We could not calculate the exact amount of cestodes in squirrels because they were fragmented, but the intensity of infestation was also extremely high. During helminth ova detection in the samples of squirrels faeces the cestodes eggs (suborder *Anoplocephalata*) of a characteristic shape with the presence of the pear-shaped apparatus surrounding oncosphere were found in all cases.

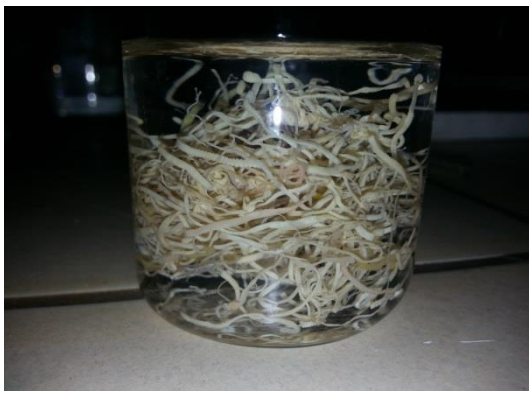

Fig. 1. Cestodes of genus *Dilepis* from the single specimen of hedgehog (original)

Fig. 2. Nematodes *Physaloptera clausa* from the stomach of hedgehog (original)

Fig. 3. Fragmented cestodes from squirrels (original)

Fig. 4. Parts of cestodes strobilae after spontaneous excretion of the female hedgehog

Determination of the species (genus) of detected helminths was performed using literary sources, reference books and classifiers [12-13]. Cestodes of the dead hedgehogs belonged to the order *Hymenolepidata*, family *Dilepididae*, genus *Dilepis*. They have characteristic morphological features: scolex with pronounced rostellum, a long neck (Fig. 5), in the mature proglottid the sack of eggs breaks down into individual capsules containing the eggs of cestode type [14]. Cestodes of family *Dilepididae* are the widespread component of the helminth fauna of insectivorous mammals in all natural zones [15,16]. Spontaneous excretion with faeces of the large proglottids of the cestodes of suborder *Hymenolepidata* another species was observed in one of the hedgehogs. The clinical condition of this animal (young female hedgehog) was satisfactory. Systematic affiliation of these cestodes was determined up to family *Hymenolepididae*. Presumably they relate to the genus *Hymenolepis*: elongated narrow proglottids of craspedote type, unilateral genital openings, not interspersed, single genital apparatus, eggs within the proglottid have a thin transparent outer shell and form shapeless dense clusters [14] (Fig. 4,6,7). Segments excreted by groups that made up the fragments of strobila (Fig. 4). Some species of the cestodes of this family are characterized by variable morphology, holarctic distribution, lack of strict specificity to the definitive hosts, i.e. they are polyxenous

parasites. A.A. Makarikov with co-authors [17] established that the main sign of species diagnostics for *Hymenolepididae* is the form and the size of a cirrus and character of its weapon (Fig. 6).

Fig. 5. The head end of the cestode of genus *Dilepis* (original) (4×10)

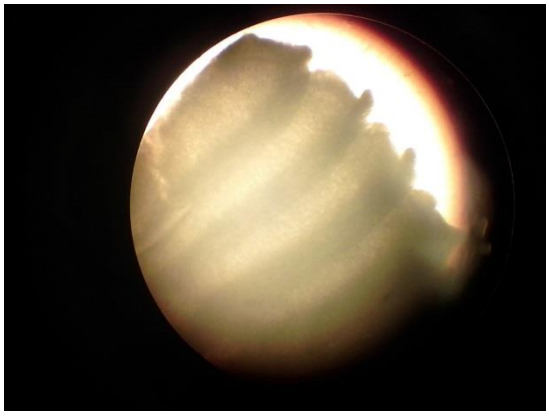

Fig. 6. Side edge of the craspedote type strobila of cestode genus *Hymenolepis* with cirruses (4×15) (original)

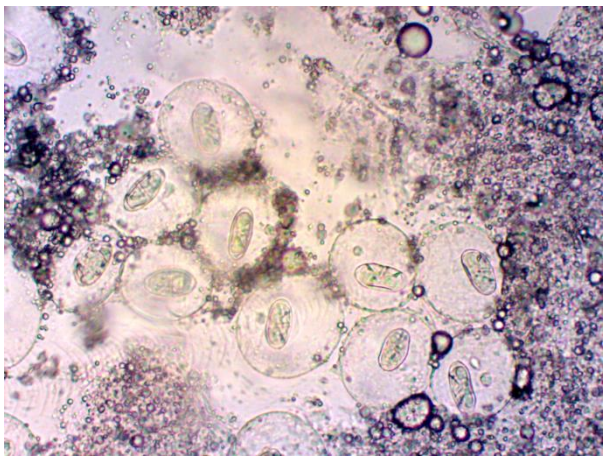

Fig.7. Eggs in a mature proglottid of the cestode of genus *Hymenolepis*, spontaneously excreted by the hedgehog (10×10) (original)

Cestodes *Catenotaenia dendritica* (suborder *Anoplocephalata*, family *Catenotaeniidae*) which were found in squirrels have a branched sack of eggs in mature segment, scolex - unarmed with powerful suction cups. These cestodes are also typical representatives of the helminth fauna of *Sciuridae* rodents [14,18,19] (Fig. 8,9,10).

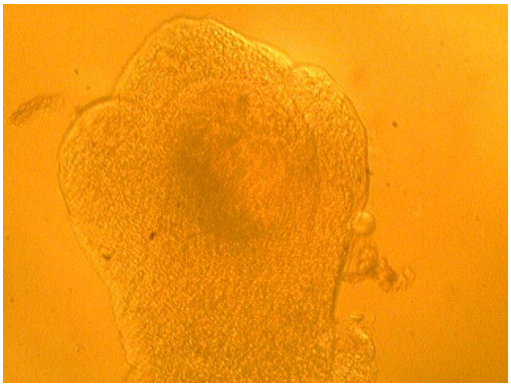

Fig. 8. Scolex of *C. dendritica* (4×10) (original)

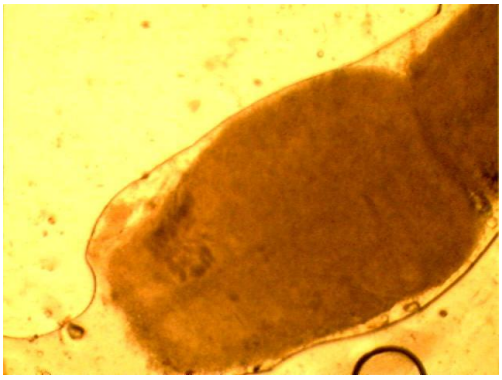

Fig. 9. Mature proglottid of cestode *C. dendritica* (4×10) (original)

Fig. 10. Hermaphroditic proglottid of *C. dendritica* (4×10) (original)

Male nematodes of the genus *Physaloptera*, which were found in hedgehogs, have preanal vesicle at the rear end of the body which is an accrete rounded tail wings extending along the body freely (Fig. 11) and stalked preanal and postanal papillae (Fig. 12). The females have a pointed tail (Fig. 13). In the distal part of the proglottid - are typical for Physalopteridae eggs with developing larvae (Fig. 14). They are also found in the faeces of cadaver material from hedgehogs.

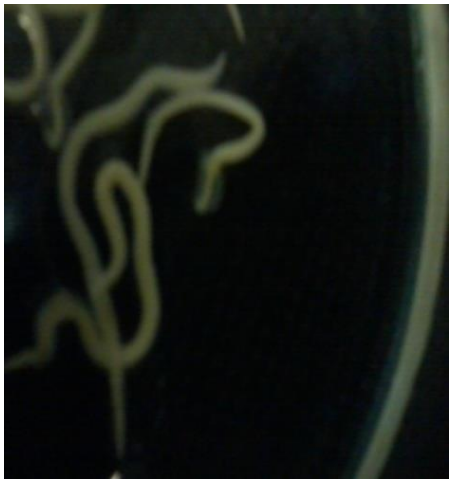

Fig. 11. The male nematode of the genus *Physaloptera*. Preanal vesicle is visible (original)

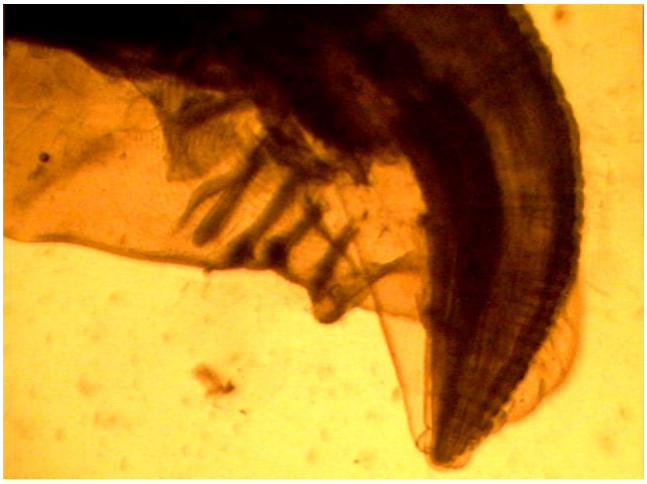

Fig. 12. The tail end of male *P. clausa* (4×10) (original)

Fig. 13. The tail end of female nematode *P. clausa* (4×10) (original)

Fig. 14. Egg of the nematode of genus *Physaloptera* (8×10) (original)

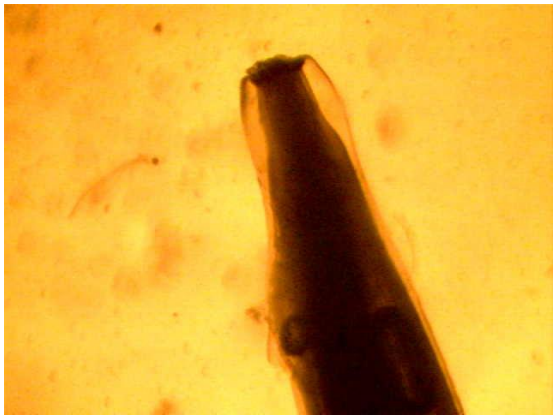

Fig. 15. The head end of nematode *P. clausa* from the stomach of hedgehog (4×10) (original)

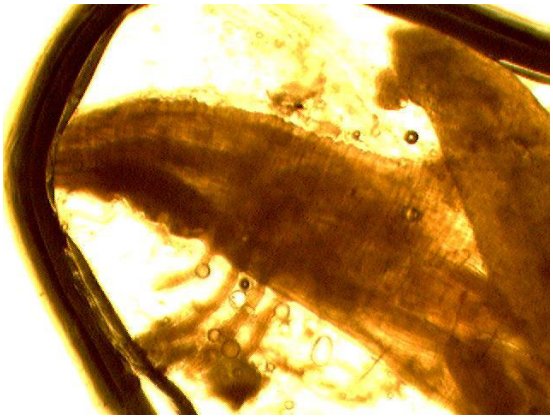

Fig. 16. The splitting of the tail end of the male *P. clausa* (4×10) (original)

These features are typical for nematodes of the suborder *Spirurata*, family *Physalopteridae* [12,20]. Discovered by us *Physaloptera clausa* is common and frequently occurring species in hedgehogs [19,21,22] (Fig.15).

According to the literature, in natural conditions in hedgehogs (family *Erinaceidae*) and squirrels (family *Sciuridae*) helminths of different taxonomic groups were found. So, in hedgehogs at least 6 species of helminths which are specific to it were found [21,22]. The species composition of helminths is linked to trophic and chorologic features of hedgehog (dwelling in forest habitats and feeding on small vertebrates and invertebrates, which are the intermediate, additional and paratenic hosts of biohelminthes). Helminths of squirrel rodents are also represented by at least 5 monoxenous and heteroxenous species. Mostly vegetative diet and close contact with the forest litter here are of great importance [23].

Thus, the typical representatives of the helminth fauna of hedgehogs and squirrels were found in all cases. However, usually such intensity of infestation, which leads to death, does not occur in natural conditions, and a kind of balance between parasites and hosts is maintained and invasions are mainly asymptomatic.

During the study some interesting data about the changes in the morphology of helminths that may have the character of gross mutations were also received: for example, we have identified structural abnormalities - bifurcation of the tail end of the male nematode *Physaloptera clausa* (Fig. 16), bifurcation of the rostellum of cestode *Dilepis*. Perhaps, these anomalies are the result of the impact of the existing adverse environmental conditions of megapolis. However, these data are preliminary and require further investigation, verification and confirmation.

Next stage of work was to clarify the possible role of bacterial agents in the etiology of the disease. During the bacteriological research of 2 liver and kidney samples from squirrels and 3 samples from hedgehogs the cultures of family *Enterobacteriaceae* with characteristic morphological, tinctorial and cultural properties were isolated and identified on the basis of biochemical properties and agglutination reaction as the *Escherichia coli* (Gram-negative rods with rounded ends, facultative anaerobe forming a uniform turbidity on the beef-extract broth, a colony in the S-shape on the beef-extract agar, magenta colonies with metallic shine on Endo's medium, weak growth of green colonies on bismuth sulfite agar, fermented glucose, sorbitol, inositol, lactose, formed indole) and *Proteus vulgaris* (polymorphic gram-negative rods with peritrichous flagella, facultative anaerobe forming on beef-extract agar creeping growth in the form of a bluish veil and uniform turbidity with white sediment in the beef-extract broth, fermented glucose, maltose, sucrose, D-xylose, formed indole).

For carrying out bioassay white mice were subcutaneously infected by the cultures of isolated enterobacteria in a dose of 500 million microbe cells (two mice for one culture). Within 3 days after infection, diseases and the deaths of mice was not observed. There was no growth of culture from the bone marrow and spleen of the corpses of these animals. Considering the lack of growth and pathogenicity in the bioassay, it can be stated that the infectious factor in the studied disease of hedgehogs and squirrels is excluded.

As measures against the clinically manifested helminthiases of squirrels in captivity, deworming of animals that remained in the enclosure (7 animals) was carried out with drug based on fenbendazole (20 mg/kg) and praziquantel (10 mg / kg), individually orally for 4 consecutive days. For these purposes complex anthelmintic drug in the form of suspension, recommended as a remedy for helminthiases of rodents, was used. The clinical state of the animals that received the drug visually improved, physical activity increased, appetite improved. In control helminth ova detection studies after 14 days of treatment in faecal samples the eggs of helminths were not found. In the case of the forced deworming of hedgehog infested by *Hymenolepididae*, the same complex anthelmintic drug was also applied, but the spontaneous excretion of the segments of cestodes was continued. At the same time parasitism of cestodes has no effect on the clinical condition of the infected female hedgehog placed in the captivity conditions: appetite and activity were normal, the full hibernation with normal fatness was observed in the fall. Subsequently, monodrug of praziquantel in the dose of 20 mg/kg was applied to it twice.

DISCUSSION OF THE RESULTS

Small mammals of the orders of rodents and insectivores in the cities live in the urban forest zones, in protected natural areas. These animals are often the objects of captive content, are easily tamed, have no fear of human, contact with him, visiting sites for feeding, and their quantity in limited areas may become too high. Such a violation of ecological balance leads to the appearance among their populations of the diseases outbreaks of infectious and invasive nature. Marked high intensity of infestation means the formation of the stationary foci of helminthiases, as was observed in the studied urban forest zone. The formation of such foci was the result of the dramatic increase of contamination in environment by invasive source, its accumulation in the soil, litter, and, respectively, in the intermediate (or paratenic) hosts of helminths that live here, by eating which the infestation of rodents and insectivores (definitive hosts) occurs. In our conditions, it is observed in the usual feeding places that often visited for a long time by animals as well as in forest enclosures for their maintenance. Violation of a kind of equilibrium in relations between parasites and hosts has led to an increase in the intensity of infestation, accompanied by the expressed clinical manifestations of helminthiases.

It should be noted that for all identified helminths insectivores or rodents are the definitive hosts in the populations of which is carried out the main circulation of the parasites. On the background of the accumulation of invasive elements in the environment, parasitic systems may include other mammalian species as well as man. In particular, in certain environmental conditions, for example at the junctions of natural and anthropogenic ecosystems, rodents can serve as "reservoirs" of heteroxenous helminth species,and such foci should be categorized as permanent regulatory factors as opposed to infections [8]. In our casethe infestation of dogs, which walk in the forest park, by the nematodes of family *Physalopteridae* and by the cestodes of family *Hymenolepididae* is theoretically possible, and for a man the representatives of the cestodes of genus *Hymenolepis* can be potential dangerous [9].

As a preventive measure, is recommended to carry out deworming quarterly. Coverage of the enclosures should be periodically disinfected, refuge houses - disinfected with an obligatory change of bedding. Putting new animals in cages should be carried out after compulsory quarantine with implementation of preventive deworming, also animal welfare standards should be kept depending on the area of the enclosure, preventing overcrowding. It is also possible to offer the periodic changing of the places of animals feeding, soil digging at the areas of feeding for reducing contamination by invasive source.

CONCLUSIONS

In this studied case, human impact on the natural biotopes of rodents and insectivores is occur, which led to the disruption of the natural balance of relations between parasites and hosts which resulted in the sharp increase of the intensity of the invasion of small mammals living in a megapolis by pathogenichelminthsand to the clinical manifestations of helminthiases.

Two kinds of cestodes belonging to the suborder *Hymenolepidata*, families *Dilepididae* and *Hymenolepididae* was found in hedgehogs, cestodes of suborder *Anoplocephalata*, family *Catenotaeniidae* -insquirrels. Hedgehogs were also parasitized by nematodes of suborder *Spirurata*, family *Physalopteridae*, species *Physaloptera clausa*. Discovered helminths are typical for hedgehogs and squirrels in natural biotopes.

There is no significant role of bacterial agents in pathology development in connection with the negative bioassay result and lack of growth from bone marrow.

It should be noted that, for example, gastric nematodes *Physaloptera clausa* found in hedgehogs are heteroxenous and they also parasitize in small predatory animals [12,14], therefore, they may be the source of the infestation of domestic cats and dogs, and cestodes from the family *Hymenolepididae* can infect the human [8,9].

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