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HUMAN PARASITES IN THE AMUR RIVER: THE RESULTS OF 2017–2018 FIELD STUDIES

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Foodborne diseases are an important health problem worldwide. In the Far East of Russia, the greatest epidemiological significance is found for representatives of four trematode genera, including Clonorchis and Metagonimus. In 2017, it was discovered that a new species of Metagonimus suifunensis, previously described as M. yokogawai, was distributed in the Primorsky Krai and the Khabarovsky Krai. In 2017-2018 the infection of the first and second intermediate hosts by Clonorchis sinensis u Metagonimus spp. was assessed in the Amur River basin. New results for these parasites from the Khabarovsky Krai, comparison with data for the Primorsky Krai and discussion in connection with the human activity in the Far East region are reported in current research. A possible location with the presence of a disruptive selection for Metagonimus spp. was found in Khabarovsky Krai. In addition to Clonorchis sinensis and Metagonimus spp., new information on the distribution was obtained for another trematode species in Khabarovsky Krai.

Keywords: *Clonorchis sinensis, Metagonimus spp., human parasites, Russian Far East.*

Introduction

Foodborne diseases is an important health problem worldwide. In the Russian Far East (FE), the greatest epidemiological significance is found for representatives of four trematode genera, including *Clonorchis* (Opisthorchiidae) and *Metagonimus* (Heterophyidae). *Clonorchis sinensis* parasitizes in the human liver causing proliferative processes in the bile duct tissue and leading to pathological changes in the organ. This parasite can cause cancer and is officially included in the list of biological carcinogens [2]. Representatives of *Metagonimus* are intestine parasites of humans and animals. High invasion intensity can also lead to a severe course of the disease. In 2017, it was discovered that a new species, *Metagonimus suifunensis*, previously described as *M. yokogawai*, was distributed in the Primorsky Krai [4] and the Khabarovsk Krai (unpublished data). In 2017-2018, within the framework of the field studies supported by the Russian Science Foundation (project RSF No. 17-65-00004), the infection of the first and second intermediate hosts by *Clonorchis sinensis* и *Metagonimus* spp. was assessed in the Amur River basin.

First intermediate hosts of *C. sinensis* in the southern part of the FE are snails from the Bithynidae family (genus *Parafossarulus*), while snails of the Semisulcospiridae family are the hosts of *Metagonimus* and many other trematode species. It is important to note that there are some problems with taxonomic identification of the hosts mentioned above. For example, Strong and Köhler [5] indicated that a description of genus "*Parajuga*" (Semisulcospiridae) does not distinguish any anatomical features of this group of mollusks, and on the basis of the genetic data obtained in their study they indicated that this genus is not considered valid. In another work based on genetic data, Köhler [3] reduces one of the representatives of the genus "*Parajuga*" to *Koreoleptoxis amurensis*. According to our own preliminary molecular data, the taxonomy of these host species requires additional studies. Moreover, the presence of a large number of species in the genus previously known as *Juga* ("*Parajuga*") is in doubt. Therefore, it is designated conditionally that the snails, from which the cercariae of parasites were obtained, belong to two genera, *Parafossarulus* for *C. sinensis* and *Koreoleptoxis* for *Metagonimus* spp. Second intermediate hosts of *Clonorchis sinensis* и *Metagonimus* spp. are Cyprinidae fish. In current research data for the Khabarovsky Krai are described.

Materials and Methods

The first and second intermediate hosts of parasites were collected from 13 localities of the

Khabarovsky Krai. Only emitted cercariae were analyzed. The snails were not crushed to search for sporocysts and rediae of parasites. The naturally infected Cyprinidae fish were fed to rats and (or) ducklings. Euthanasia of laboratory animals was carried out in accordance with the Committee on the Ethics of Animal Experiments of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Russia (Permit Number 3 of 2 June 2011). Since the difficulties in identifying *Metagonimus* representatives based on morphological data [4, 6], have been noted previously, cercariae, metacercariae and adult flukes of parasites were collected for subsequent identification at the molecular level.

Results and Discussion

Yagodnoe village (the Amur River). 243 specimens of *Koreoleptoxis* snails were collected, from which four were infected. Cercariae of *Metagonimus* spp. and *Nanophyetus salmincola* (Nanophyetidae) were detected. Fish were not examined.

The Machtovaya River. 17 of 73 collected *Koreoleptoxis* snails were infected by cercariae from different genera: *Pygidiopsis* (Heterophyidae), *Plagioporus* (Opecoelidae), *Sanguinicola* (Sanguinicolidae), *Echinochasmus* (Echinostomatidae), as well as stilet cercariae of various genera. 28 fish were examined and metacercariae were not obtained.

Komsomolsk-on-Amur city (the Amur River). 341 samples of *Koreoleptoxis* snails were examined. Nine infected snails were obtained, from which eight emitted *Metagonimus* cercariae, another one had *Echinochasmus milvi* cercariae. Fish were not examined.

Komsomolsk-on-Amur city (Mylki Lake). The snails were not collected. Infected by opisthorchiid metacercariae fish were fed to rats and duckling. No trematodes were detected in the rats, while adult *Metorchis* flukes were obtained in the gallbladder of duckling. Intestine of the duckling had adult flukes from the Cyathocotylidae family.

The Gur River. 218 specimens of *Koreoleptoxis* snails were examined, from which 55 emitted cercariae of different genera: *Metagonimus*, *Pygidiopsis*, *Echinochasmus*, *Nanophyetus*, *Centrocestus* (Heterophyidae), stilet cercariae of various genera. The most of infected snails (30 specimens) had *E. milvi*. One snail had indeterminate furkocercariae. The naturally infected fish were fed to rats and ducklings. No rats were infected. Two ducklings had adult *Metagonimus* flukes in the intestine.

The Anuj River. 40 of 301 examined *Koreoleptoxis* snails were infected by trematodes from *Metagonimus*, *Pygidiopsis, Microparyphyim* (Echinostomatidae) and *Nanophyetus* genera. 17 of 40 snails had stilet cercariae of tree different types. One snail emitted both *Pygidiopsis* and stilet cercariae. Infected *Phoxinus percnurus* were caught in lake near to the Anuj River. Opisthorchiids metacercariae were detected in 100% fish, which were fed to rats and one duckling. One specimen of adult *Metagonimus suifunensis* (confirmed by genetic data) was obtained in the intestine of one rat. In the duckling, the liver and two blind pouches of intestine contained a large number of adult *Opisthorchis* flukes. Both blind pouches were 5 times larger than usual size. The species of this parasite has not been determined, because whole parasites could not be extracted. They had a long body that was located in the tissues of the host's organs in several projections at once, while the parasite density did not allow separating individuals. However, anterior parts of parasites were collected for genetic analysis.

Gassi Lake. 145 specimens of *Koreoleptoxis* snails were examined, from which 14 were infected by *Metagonimus* spp. and *E. milvi*. The rats were fed by naturally infected fish. Four adult *C. sinensis* were detected in the liver of one rat.

Sindinskoe Lake. 157 specimens of *Parafossarulus* snails were examined. *Metorchis* sp. cercariae were detected in 18 of them. Infected fish were fed to two rats. On day 28 after infection, they were contained adult *C. sinensis* in their livers.

Petropavlovskoe Lake. The snails were not collected. Infected fish were fed to one rat. No trematodes were obtained in its liver and intestine.

The Kiya River. 63 specimens of *Koreoleptoxis* snails were examined, from which only one was infected by *E. milvi* cercariae. Fish were not examined.

The Bikin River. 348 specimens of *Koreoleptoxis* snails were examined, from which 14 were infected by *Metagonimus* spp., *Pygidiopsis* sp., *Centrocestus* sp. and *E. milvi* cercariae. Metgonimus was found in half of infected snails. Four snails had stilet cercariae. *Rhodeus sericius* (all specimens were infected) were fed to rats and ducklings. One rat contained eight *C. sinensis* in its liver. In the intestines of two ducklings, we obtained adult *Metagonimus* flukes and adult trematodes from the Cyathocotylidae family. In the gallbladders of two another ducklings, adult *Metorchis* were obtained.

The Amur Channel. In two rivers flowing into the Amur channel (Bychikha and Polovinka), fish were caught and checked for the presence of metacercariae. No metacaraciae were found. 326 specimens of *Koreoleptoxis* snails were examined. Three of them were infected by cercariae of *E. milvi, Centrocestus* sp. and stilet trematoda.

The Chirka River and two rivers flowing to it (Odyr and Tsypa). *Phoxinus percnurus* were caught in the Odyr and Tsypa Rivers, all of them were infected

by metacercariae by *Metagonimus* spp. Intensity of infection was higher in the Odyr River. Fish from both localities were fed to rats and ducklings. Ducklings were not infected, while 355 and 43 adult *Metagonimus* were found in the intestines of rats from the Odyr and Tsypa Rivers, respectively. 230 specimens of *Koreoleptoxis* snails were examined, from which one, five and four samples were infected by *Plagioporus* sp., *Metagonimus* spp., and *E. milvi* cercariae, respectively, and 29 snails emitted stilet cercariae of various genera.

Thereby, *Koreoleptoxis* snails emitted cercariae of *Metagonimus* spp. in seven of 10 investigated localities, where snails have been collected in this field trip. For two other localities, the number of collected snails was small, and it can be reason that these species were not obtained. At the same time, the absence of *Metagonimus* spp. in the Amur Channel, despite a large number of investigated mollusks. This part of the Amur Channel is located near the Ussuri river, and we plan to explore this area more thoroughly, since the absence *Metagonimus* spp. in the first intermediate and second intermediate hosts can lead to a disruptive selection of parasites from this genus. It seems likely, since we have revealed some differences in haplotypes of *Metagonimus suifunensis* from Primorsky Krai and the Khabarovsky Krai (data were not published). Currently, it is not obvious what species of *Metagonimus* were circulated in the Khabarovsky Krai, the issue would be clarified after obtaining molecular data later in the process of project. Nevertheless, the presence of *Metagonimus suifunensis* has already been confirmed for one locality (the Anuj River).

It is interesting that the list of obtained genera of parasites coincides with earlier described species for *Juga* snails from the Primorsky Krai [1]. However, not all species were detected in the Khabarovsky Krai due to the smaller size of samples: we examined only 2288 *Koreoleptoxis* snails, while Dr. Besprozvannykh examined 14 255 snails for infection by trematodes [1]. Despite the relatively small size of the sample in the Gur River, the high level of infection prevalence (25%) in the snails was revealed. The most of snails were infected by *E. milvi*, which infected kitten in the experiment of Besprozvannykh [1]. That is, it can be potential parasite of humans. The Gur River has very clean water, and many fishermen and their families are fishing here, as in other rivers, wherever the infected snails were collected. It should be noted that of the *Echinochasmus* genus is identified only *E. milvi*, while *E. suifunensis* are also distributed in the Primorsky Krai. At the same time, cercariae of *Nanophyetus* were obtained in only three localities with low prevalence. It was surprised, because representatives of this genus included in the list of trematodes with highest epidemiological significance in the Russian Far East. No *Paragonimus* cercariae (the fourth of the most dangerous species of trematodes in this region) were detected in the investigated *Koreoleptoxis* snails.

The study of the first intermediate host of *C. sinensis* was more difficult. Bithyniidae snails have not been discovered in all water bodies despite the presence of various representatives of the Opisthorchiidae family. In addition, it is interesting to find the new *Opisthorchis* species, and the plans are to continue the study in the near future.

Conclusion

The data obtained can serve as the beginning of a comprehensive study, including confirmation of the taxonomic status of parasites and their hosts based on molecular data and the study of phylogeography and historical processes in populations both parasites and their hosts.

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REFERENCES:

1. Besprozvannykh VV (2000) Fauna, biology, and ecology of parthenites and cercariae of trematodes in snails of genus *Juga* (Pachychilidae) from rivers in Primorsky Region. Vladivostok, Dalnauka, 121 p. (in Russian).
2. Bouvard V, Baan R, Straif K, et al. (2009) A review of human carcinogens – part B: biological agents. Lancet Oncol 10: 321–322. https://doi.org/10.1016/S1470-2045(09)70096-8.
3. Köhler F (2016) Rampant taxonomic incongruence in a mitochondrial phylogeny of *Semisulcospira* freshwater snails from Japan (Cerithioidea: Semisulcospiridae). J Molluscan Stud 82: 268–281. https://doi.org/10.1093/mollus/eyv057.
4. Shumenko PG, Tatonova YV, Besprozvannykh VV (2017) *Metagonimus suifunensis* sp. n. (Trematoda, Heterophyidae) from the Russian southern Far East: morphology, life cycle, and molecular data. Parasitol Int 66: 982–991. https://doi.org/10.1016/j.parint.2016.11.002.
5. Strong EE, Köhler F (2009) Morphological and molecular analysis of 'Melania jacqueti' Dautzenberg and Fischer, 1906: from anonymous orphan to critical basal offshoot of the Semisulcospiridae (Gastropoda: Cerithioidea). Zool Scr 38: 483–502. https://doi.org/10.1111/j.1463-6409.2008.00385.x
6. Tatonova YV, Shumenko PG, Besprozvannykh VV (2017) Description of *Metagonimus pusillus* sp. nov. (Trematoda: Heterophyidae): phylogenetic relationships within the genus. J Helminthol. https://doi.org/10.1017/S0022149X17001146.