Philophthalmus Lucipetus (Trematoda, Philophthalmidae) Cercariae in Molluscs Melanoides Kainarensis

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ABSTRACT

Cercariae of Philophthalmus lucipetus (Rudolphi, 1819) were detected in molluscs Melanoides kainarensis Starobogatov et Iz- zatullayev, 1980. Of the 4,629 examined individuals of M. kainarensis from Boshkhovuz, a warm body of water in the Karnabchul steppe (Nurabad District, Samarkand Province), 407 individuals (9.01%) were infected with cercariae and parthenitae of flukes from the genus Philophthalmus Looss, 1899, whose species was identified as Ph. lucipetus.

Key Words: Melanoides kainarensis, Philophthalmus lucipetus, Parthenitae, Cercariae, Molluscs.

INTRODUCTION

The geographical range of Philophthalmus lucipetus 1 is quite wide. Populations of this trematode were recorded in many countries in Europe, Asia, Africa, and Americas in various ecological groups of birds. Mature trematodes parasitise the conjunctival sac and cause a grave disease in agricultural and game birds. In CIS countries this trematode species was recorded in Anser anser in Ukraine ². Populations of Ph. lucipetus were also recorded in many other countries in geese, ducks, chickens, peacocks, turkeys, ostriches and rheas 3-10. Molluscs Melanoides tuberculatus, Fagotia acicularis, Amphimeleania holandri, Melanopsis praemorsa, Pleurocerca acuta and Tarebia granifera were identified as intermediate hosts of this trematode 11-14.

Mature forms of Ph. lucipetus has not yet been recorded in the wild birds of Uzbekistan. However, the recent record of cercariae in M. Kainarensis and identification of this species as a new intermediate host of this trematode in Uzbekistan, as well as the registration of an outbreak of the infection within a limited area, strongly demanded some comprehensive research.

The goal of this work was to establish the rate of infection with Ph. lucipetus cercariae in populations of M. kainarensis in Uzbekistan and reconstruct the life cycle of this trematode in an experiment.

MATERIALS AND METHODS

This work was based on the results of faunistic and experimental research carried out in 2010-2019 to study the fauna and morphology and biology of cercariae developing in freshwater mollusks. The mollusks were collected from a warm spring (Boshkhovuz in the Karnabchul steppe, Nurabad District, Samarkand province) following a commonly accepted method 15. The stationary survey was carried out between June and August in 2010-2018 and in January 2019. The total number of examined mollusk individuals from the spring was 4,629 (fig. 1). The species were identified using the following works 16-17. The collected mollusks were delivered to a laboratory, where each individual was placed in a separate glass with 50 ml of tap water at a temperature of 20-25 °С. Emerging cercariae were surveyed with a naked eye or using a МБС–10 binocular microscope.

The morphology of the cercariae that left the mollusks’ bodies and stayed in the water was studied using vital staining –0.05 % neutral red and 0.05 % Nile blue 18-20. Carmine acetoc was used in whole mount staining. The digestive gland, heart, and aorta were extracted from naturally infected mollusks M. kainarensis to study parthenogenetic forms. The collected mother and daughter rediae were examined through a microscope. The trematode larvae were photographed and sketched. Statistical data were processed using software Bio-stat 2007 and Microsoft Office Excel 2003.
RESULTS

Populations of M. Kainarenisis live in warm springs and streams and ponds resulting from these springs at a depth of 15-20 cm on stony or limy bottoms and show a density of 130-160 individuals per 1 m²(fig. 1).

Table 1: Natural rate of infection with Philophthalmus lucipetus cercariae in molluscs Melanoides kainarensis

| Time of the year | No. of examined individuals | Rate of infection (%) |
|------------------|-----------------------------|-----------------------|
| 2017 June        | 410                         | 21 (5.2)              |
| July             | 597                         | 63 (10.5)             |
| August           | 748                         | 82 (11.0)             |
| 2018 June        | 1,104                       | 63 (5.7)              |
| July             | 781                         | 71 (9.1)              |
| August           | 896                         | 100 (11.2)            |
| 2019 January     | 93                          | 7 (10.4)              |
| Total            | 4,629                       | 407 (9.01)            |

The prevalence shown by mollusks in the wild of Uzbekistan varied depending on the season and ranged between 5.2% and 11.2%.

Given below are the basic morphometric parameters of Ph. Lucipetus cercariae extracted from mollusk M. kainarensis and encysted adolescariae in the external environment.

Cercaria. The studied cercariae (20 individuals) had relatively large dimensions and in most cases showed a body length equal to the length of the tail. The body is oval and elongated, 0.53-0.65 mm long and 0.11-0.14 mm wide. The maximum width is recorded just in front of the ventral sucker. The tail is 0.46-0.52 mm long and 0.03-0.06 mm wide. The tip of the tail looks cut and has a sucker-like projection provided with glandular cells with ducts open at the tail tip. The subterminal oral sucker has a rounded shape and is 0.04–0.06 mm long and 0.04–0.05 mm wide. The ventral sucker is 0.06–0.07 mm long and 0.08 mm wide. The oral fissure is subterminal.

The prepharynx is well-developed, the pharynx is oval and is 0.03–0.04 mm long and 0.01–0.02 mm wide; the oesophagus is relatively long –0.10–0.12 mm – and bifurcates in front of the ventral sucker; the intestinal branches are long, reach the rear tip of the body and have blind ends. The glandular cells are numerous and have ducts opening in the front part of the body. The excretory system is typical of all flatworms and consists of a large number of flame cells, a system of ducts, and a urinary bladder. The excretory system is expressed by formula 2[(3+3+3)+(2+2+2)]=30. Sexual rudiments are
formed by cellular masses lying lengthwise in the space between the bifurcation of the aesophagus and the front part of the urinary bladder (fig. 2).

The evolution of cercariae into adolescariae takes relatively little time. At 20–25°C cercariae that have just left the body of a mollusk, their intermediate host, get encysted in 1.5–2 minutes. In 1–3 hours this process extends to 2.5–3 minutes (based on the observation of 40 cercariae, 20 individuals from each age group of larvae). The encystation is usually preceded by the attachment of the cercaria with its tail to a substrate and the tail’s detachment from the body. This phenomenon is characteristic only of this group of trematodes. The adolescariae usually are pear-shaped, 0.48–0.52 mm long, and 0.20–0.24 mm wide.

Identical figures were shown by Ph. Lucipetus cercariae extracted from other mollusk species, mainly M. tuberculatus 23-28. The larvae did not vary greatly in size depending on the mollusk host (Table 2).

Table 2: Comparative parameters of Philophthalmus lucipetus cercariae, mm.

| Body parts           | Pleurocerca acuta (acc.to West, 1961; USA) | Melanoides tuberculatus (Pinto and Melo 2010; Brazil) | Melanoides kainarensis (our data; Uzbekistan) |
|----------------------|--------------------------------------------|-------------------------------------------------------|-----------------------------------------------|
|                      | Limit                                      | Limit±m                                               | Limit±m                                       |
| Body: length         | 0.54–0.67                                  | 0.42–0.58                                             | 0.53–0.65                                    |
| width                | 0.12–0.13                                  | 0.11–0.14                                             | 0.12±0.01                                    |
| Oral sucker: length  | 0.06                                       | 0.05–0.06                                             | 0.04–0.06                                    |
| width                | 0.04–0.05                                  | 0.04–0.06                                             | 0.04–0.05                                    |
| Ventral sucker: length | 0.06–0.07                              | 0.06–0.07                                             | 0.06–0.07                                    |
| width                | –                                          | 0.06–0.08                                             | 0.07–0.08                                    |
| Pharynx: length      | 0.03–0.04                                  | –                                                     | 0.03–0.04                                    |
| width                | 0.01–0.02                                  | –                                                     | 0.01–0.02                                    |
| Oesophagus: length   | 0.10–0.11                                  | –                                                     | 0.10–0.12                                    |
| Head glands          | 20 pairs                                   | Numerous                                              | 20 pairs                                      |
| Excretory system     | 2[(3+3)+(2+2+2)]=30                        | –                                                     | 2[(3+3)+(2+2+2)]=30                          |
| Tail: length         | 0.93–1.14                                  | 0.30–0.48                                             | 0.46–0.52                                    |
| width                | 0.04–0.05                                  | 0.03–0.06                                             | 0.03–0.06                                    |

**DISCUSSION**

Various authors described about 36 species from the genus Philophthalmus 6-9. The validity of the number of species arouses doubts, since they are described on the basis of a limited amount of material, without taking into account the parasites’ changeability depending on a number of factors. The structure of the genus, species diversity, and biology are currently being studied and revised most actively 1-5. The research 29 showed identity between Ph. gralli and Ph. lucipetus. These authors presented Ph. gralli as a synonym for Ph. lucipetus, which quite corresponds with the priority principles. We agree with this viewpoint.

When studying mollusks M. kainarensis on 15 June 2013, we detected rediae and cercariae from the genus Philophthalmus, which were identified as the species Ph. lucipetus. No birds infected in a natural way were recorded in Uzbekistan. The parthenogenetic forms and cercariae found in mollusks M. kainarensis in an isolated body of water in Uzbekistan, probably, were brought by aquatic and semi-aquatic birds coming from other regions as part of their seasonal migration. The existence of certain biological pre-conditions led to the emergence of a new focus of infection in this territory. Mollusk M. kainarensis became a new intermediate host of this trematode.

As was already mentioned, a number of mollusc species, such as Pleurocerca acuta, Tarebia granifera, Fagotia acicularis, Amphimelatus holandri, Melanoides tuberculatus, Melanopsis praemorsa, and Melanoides kainarensis were identified as intermediate hosts of trematode Philophthalmus lucipetus, which ensures stability of the parthenit-mollusc system and facilitates the spread of the infection. The next stage in the life cycle of the cercariae is its penetration into the external environment. Ph. lucipetus larvae usually emerge at twilight or night, while some of them come out in the morning hours.
After floating for a while, cercariae usually attach themselves with the tip of their tail to the substrates of the water body. In our experiments, the larvae fixed themselves to the walls of the glasses or Petri dishes so tight that they stopped moving forward. When their tail tip is ‘immobilised,’ the cercariae place their body vertically and start making active pendular or circular movements to facilitate the detachment of the body from the tail. Soon after the body gets encysted and turns into an adolecascaria. The adolecascaria form several groups on the walls of the vessel (a glass or Petri dish), from 5 to 10 individuals in each group, gathering into separate microconglomerations.

A number of researchers noted the common morphological and biological characteristics of Philophthalmidae cercariae, which consist in the transformation of the tail due to its loss of the locomotor function into an anchoring organ11-15. The thing is that in free-living cercariae the oral and ventral suckers act as anchoring organs and contain secreting glandular cells whose ducts go as far as the surface of the suckers. The tail stem performs the locomotor function 16. This is a feature typical of cercariae of most trematodes. Unlike other trematode cercariae, the distal end of the tail in Philophthalmidae cercariae has a sucker-like organ provided with glandular cells secreting adhesive substance the cercariae use to stick to a substrate19-23.

The described morphological and biological characteristics shown by Philophthalmidae are quite functional. The above-mentioned transformations, probably, allow cercariae concentrate as densely as possible on a limited area on the surface of a substrate, accelerate the encystation of the parasite in the external environment and enhance the opportunity to parasitize definitive hosts.

By their morphological and biological features, the cercariae are, without exaggeration, unique. These features most brightly show themselves in this very group and are obviously of great importance for the study of trematodes’ phylogenesis.

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