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DISTRIBUTION OF TREMATODES *CRYPTOKOTYLE* (TREMATODA, HETEROPHYIDAE), IN FISH OF THE FAMILY GOBIIDAE IN THE ESTUARY WATERS AND THE BLACK SEA IN SOUTHERN UKRAINE

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Distribution of Trematodes *Cryptocotyle* (Trematoda, Heterophyidae) in Fish of the Family Gobiidae in Estuary Waters and the Black Sea in Southern Ukraine. Goncharov, S. L., Soroka, N. M., Pryima, O. B, Dubovyi, A. I. — The article describes occurrence and distribution of *Cryptocotyle* trematodes in fish in the waters of the Dnipro-Buh estuary and the Black Sea in Mykolaiv and Odesa Region. Study was conducted in 2015–2016. Two trematode species were found in natural waters of these regions*:* *Cryptocotyle cancavum* Crepli, 1825 and *Cryptocotyle jejuna* Nicoll, 1907. The latter species has not been previously registered in this region in southern Ukraine. Varying intensity of infection with *Cryptocotyle* metacercariae was observed in fish of *Gobiidae* family: *Mesogobius batrachocephalus* Pallas, 1814, *Neogobius melanostomum* Pallas, 1814, *N. fluviatialis* Pallas, 1814. The most affected species was *N. melanostomum,* with the prevalence of infection 59.2 %. Less infected were *N. fluviatialis* and *M. batrachocephalus,* with the prevalence of infection 30.4 % and 17 % respectively. The intensity of infection was the highest in *N. melanostomum* — 211 metacercariae per fish, followed by *N. fluviatialis* and *M. batrachocephalu* — 124 and 89 metacercariae respectively. Cryptocotyle was the most prevalent in the Dnipro-Buh estuary (cape Adzhigol, Mykolaiv Region) and much less prevalent in the waters of the Black Sea in Mykolaiv and Odesa Regions. Mean prevalence of infection was 31.4 %.
Key words: distribution, occurrence, *C. jejuna,C. cancavum,* Gobiids, intensity of infection, prevalence of infection, Black Sea, Dnipro-Buh estuary, Mykolaiv, Odesa.

Itroduction

Parasitic diseases area problem hindering the further development of fishery, the growth of the fish farms output, quality improvement, and the economic efficiency of the industry. It is vital to keep looking for new ways to achieve effective control over dangerous fish parasitic diseases. As part of this process, it is important to study parasites´ biology, their distribution, pathogenesis and course of disease in fish (Kurochkin, 1996).

It is known, that parasite has the most impact on a host when settling directly in host´s tissues. In such cases, the acutest negative effect on homoeostasisof the host organism is due to mechanical tissue damage, metabolism and immune system disruption, and often accompanied by high morbidity and mortality (Sudarikov, 2006). Metacercariae of Heterophyidae are a good example of this kind of parasitic impact. The genus *Cryptocotyle* consists of 8 species: *Cryptocotyle concavum,*Creplin, 1825; *Cryptocotyle lingua* Creplin, 1825; *Cryptocotyle jejuna,* Nicoll, 1907; *Cryptocotyle badamshini* Kurochkin, 1959; *Cryptocotyle cryptocoty-*

loides, Issaitschikow, 1923; *Cryptocotyle delamurei,* Jurachno, 1987; *Cryptocotyle quinqueangularis,* Skrjabin, 1923; *Cryptocotyle thapari,* McIntosh 1953 (Iskova et al., 1995; Gibson, 2002, 2005, 2008).

The life cycles of these trematodes include an intermediate host (gastropod) and a definitive host (piscivorous bird). Distribution of Cryptocotyle in fish, which can be an additional host, has not been studied in details in Ukraine. Many questions about the parasite biology, including pathogenesis, remain open: how does a parasite affect a host, what is the role of different piscivorous birds in disease spread, etc. There is also a possibility for a human to be a definitive host for this parasite (Sudarikov, 2006; Moshu, 2014.).

Cryptocotyle can live in the intestines of piscivorous birds, marine mammals, dogs and humans (Kurochkin, 1996; Sudarikov, 2006; Moshu, 2014).The intermediate stage of development, metacercariae, parasitizes the tissues of fish, mostly of Gobiidae family (Malek, 2004).

The first data on *Cryptocotyle* occurrence in fish of Gobiidae family in the Black Sea were reported by Isaychikov (1925). *Perccottus glenii* was found to be an intermediate host for *C. cancavum*. Ciurea (1924) found trematodes *C. jejuna* in *Larus argentatus cachinnans* and *Sterna hirundo* in Romania. Later, Rădulescu (1951) in the study of parasite fauna of gobiid fish in coastal region of Romania found metacercariae of *C. cancavum* in *N. melanostomus* and *Pomatoschistus marmoratus* Risso. Naidenova (1974) studied helminths fauna in greater details in the Black Sea region near Crimea. She found that *Gobius ophiocephalus* had the highest prevalence of infection (91.5 %), and *N. fluviatialis* had the highest intensity of infection (37–500 metacercaria).

Later, researchers conducted the study of parasites of gobiid fish in the bays of the Azov Sea and found that *C. concavum* and C. *lingua* had a significant impact on infectious disease burden in gobiid fish (Sarabeev, Domnich, 2001).

Occurrence of this parasite in marine and estuary waters of several countries including Russia, Germany, Bolivia, Britain, Bulgaria, France, Moldova, and Poland has been reported previously.(Gardner, Thew, 2006; Thieltges et al., 2006; Rolbiecki, 2006; Martinenko, 2016). In Ukraine, the first case was registered in domestic cat in Crimea (Stenko, 1977). Later, registered cases of *C. jejuna* infection in the Kerch Strait were found in *Larus cachinnans* Pallas, 1811 (Martinenko, 2012).

Intermediate host of *Cryptocotyle*is gastropod mollusc *Hydrobiaulvae* (= *Peringiaulvae*) (Pennant 1777), which inhabited waters of the Atlantic Ocean near the UK, France and Ireland (Field et al., 1999; Thieltges et al., 2006). In Ukraine, *Cryptocotyle* was registered in gastropod mollusc *Hydrobia acuta* Pennant, 1777 (Kudlai, 2011; Martinenko, Kornyychuk, 2013).

The aim of this work was to investigate waters of the Dnipro-Buh estuary and coast of the Black Sea, which geographically belong to Mykolayiv and Odessa Regions, to determine presence of *Cryptocotyle* in fish species and look for additional intermediate hosts.

Existing literature does not fully describe the biology of the parasite, pathogenesis of disease in fish, however, provides information that *Cryptocotyle* is widespread in numerous countries.

According to experts´ opinion, metacercariae of trematodes of Heterophyidae family can be a threat for human health when consuming a fish or fish products, which were not fully cooked (Kurochkin, Biserova, 1996; Sudarikov et al., 2006; Moshu, 2014). What is more, mature trematodes parasitize the digestive tract of cats, dogs and marine mammals (Gardner, Thew, 2006; Thieltges et al., 2006, 2009).

Methods

Fish was randomly chosen during a routine catches check. It was caught using fishing rods and bought from fishermen who caught it in the same area. Sampling of fish was conducted along the Black Sea coastline and in the area of the Dnipro-Buh estuary, within the administrative borders of Mykolaiv Region (Cape Adzhigol — 46°36´35.94″ N, 31°47´43.88″ E, Ochakiv — 46°36´26.77″ N, 31°32´19.90″ E, Rybakivka village, Berezan District — 46°37´18.10″ N, 31°23´56.25″ E) and part of the Black Sea in Odesa Region (Yuzhny — 46°36´43.71″ N, 31°05´23.01″ E, Odesa — 46°25´49.16″ N, 30°46´08.07″ E, Chornomorsk — 46°15´24.34″ N, 30°38´08.42″ E) (fig. 1).

During 2015–2016, 572 gobies were investigated in total. All fish that belonged to the Gobiidae family were undergone Ichthyopathological investigation. Clinical examination began with careful examination of a body surface. Oral cavity and gills were examined separately. Longitudinal dissection was performed on a ventral side of the body from anus to head. Each organ was extracted separately, including intestine with its content. Muscles were dissected in multiple areas and each section was carefully investigated. Brain, spinal cord and eye lens were also examined (Bikhovskaya-Pavlovskaya, 1985).

At autopsy, tissues were collected and examined using compressor MIS–7. Light microscopy was performed using the following equipment: Trinocular microscopes Micromed XS–4130 and binocular stereoscopic microscope Micromed XS–6320. Metacercariae were found on the body surface, fins and gills of the following species (Gobiidae family): *M. batrachocephalus, N. elanostomum, N. fluviatialis.*

After extraction of metacercaria from surrounding tissues, cysts were lysed in order to study larvae. Firstly, cysts were put in 0.5 % chymotrypsin solution (38–40 °C) and incubated for 7–10 minutes. This lysed surrounding tissues and allowed separation of the cyst. Next, cysts were transferred on the slide into thin layer of glycerol. Capsule was dissected used medical lancet. After cutting of a cyst wall, metacercaria left cyst by themselves without additional intervention (Soroka and Goncharov, 2015).

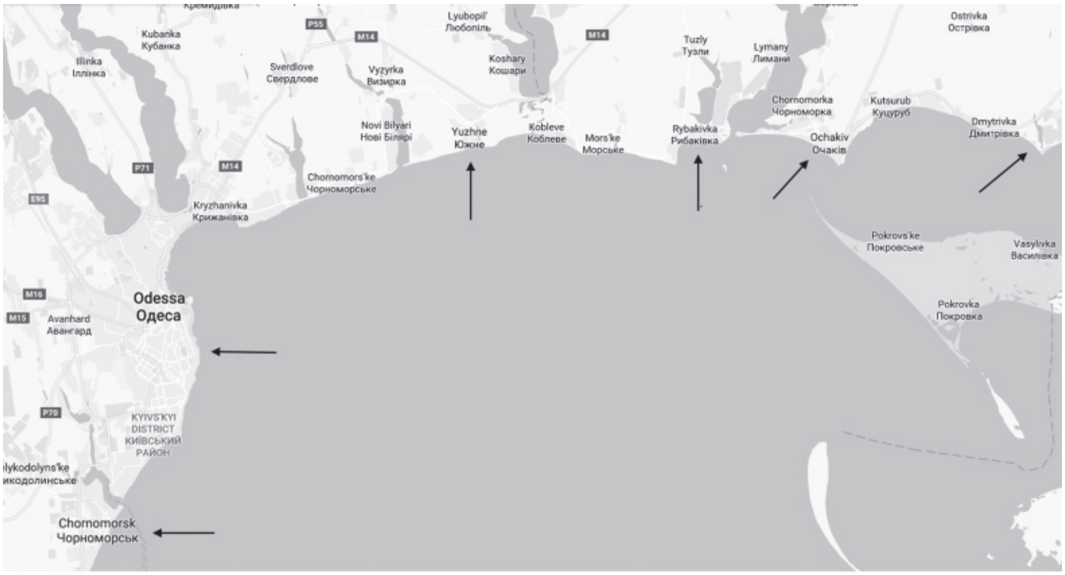

Fig. 1. Sites of material collection.

In order to study in details anatomical and morphological features of helminths, as well as for their identification, experimental animals (ducklings) were infected. Twenty 15-days-old ducklings of Beijing line were used in experiment. Weight range was 285–370 g. Fifteen ducklings were infected, whereas the control group consisted of 5 ducklings. Experimental group was fed with fish tissues which contained metacercaria of Heterophyidae and incubated for 25 days. After this period, all animals were euthanized; autopsy and parasitological examination were performed with special attention to the presence of mature trematodes in the intestine. The study was conducted in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986).

Obtained metacercaria and mature trematodes were rinsed in 0.9 % saline solution, stained with alum carmine, de-stained in acid alcohol solution, dehydrated in alcohol solutions of ascending concentration, cleared, and mounted in balm (Sudarikov, 2006).

Results and discussion

We conducted a full ichthyopathological investigation of obtained samples. During the external examination of the body surface and fins, clearly delineated black pigmented spots were found with no signs of inflammation in surrounding tissues (fig. 2).

We believe this is due to the fact that the life cycle of *Cryptocotyle* and cercariae migration has seasonal peaks, hence fish infection might have taken place some time ago and no signs of acute inflammation were present.

Behavioural changes and pathological changes in the internal organs of fish were not observed. Metacercariae of *C. jejuna* and *C. cancavum* were found in superficial tissues of the body, including fins and gills. However, on swabs microscopy from body surface and gills, no other parasites were found, and no metacercariae were found in hepatopancreas, gall bladder, eye lens, and tissues of the heart, kidneys, and brain.

Encysted metacercariae of *C. cancavum* had the oval-shaped body, 0.42 mm by 0.37 mm. The cuticle was thick, covered with small spines. Terminal oral sucker was round, 0.055 mm in diameter. Prepharynx was 0.011mm long; pharynx was oval, 0.038 mm long. They were followed by a divergence of intestine, trunks of which were directed to the posterior part of the body and terminated blindly posterior to testes. The genital sinus with rudimentary ventral round-shaped sucker was located in the posterior part of the body. Oval-shaped testes were symmetrically placed in the posterior part of the body. Ovary was 0.088 mm in length and situated in front of testes. Excretory organ had the opening at the posterior end of the body.

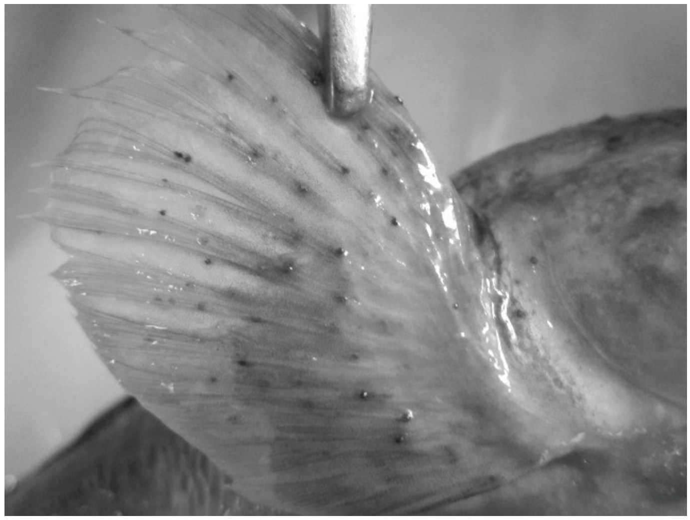

Fig. 2. Metacercariae of trematodes of Heterophyidae familyon the body surface and fins of *N. fluviatialis*.

Metacercariae of *C. jejuna* were first found in the waters of the natural reservoirs of studied region of southern Ukraine. Their cysts had an oval shape. The body of metacercariae was elongated, slightly pointed on anterior end and rounded on posterior end. The cuticle was covered with small spines. Oral sucker was located subterminally, 0.048 mm in size. Prepharynx was well defined, 0.013 mm, pharynx was small, ball-shaped. The esophagus had the same length as prepharynx. Intestinal trunks were well-visualized and blind-ended at the posterior end of the body, skirting the primordial testes. Genital sinus was located in the middle part of the body. Rudimentary ventral round-shaped sucker was located at the anterior edge of the sinus separated by the membrane. Primordial testes and ovaries were located in the posterior part of the body (Skryabin, 1952; Gibson, 2002, 2005, 2008). It is worth mentioning that encystation of metacercariae of both species wasrelatively simple, by loose cysts.

After experimental infection of ducklings with metacercariae of *C. jejuna,* the incubation period was 25 days. As early as on day 3 some ducklings experienced weakness, malaise and diarrhoea. Interestingly, in loose excrements, underdeveloped trematodes *C. jejuna* were revealed, which could potentially be due to their elimination by increased gut peristalsis in ducklings. Further, starting from day 5, diarrhoea did not lead to parasites´ elimination. This probably indicates that by that time parasiteshave securelyfixed themselves to themucosa of the gut. Mortality was not registered.

At autopsy of ducklings, acute catarrhal enteritis was diagnosed. The mucosa was inflamed, hyperemic and covered with abundant stringy mucus. Occasional petechial haemorrhages were observed. On the surface of intestinal mucosa, trematodes were seen with a naked eye. They were very noticeable because of their motility and prominent excretory bladder (fig. 3).

During further investigation and microscopy, mucus from the surface of the intestine revealed a large number of motile trematodes *C. jejuna* with clearly visible S-shaped excretory bladder, a hallmark of this species (fig. 4).

Selected trematodes were rinsed in saline and stained with alumcarmine by conventional method (Sudaricov, 2006).Mature trematodes *C. jejuna* had elongated body 0.94–1.47 mm long and 0.3–0.5 mm wide. Their cuticle was covered with small spines. Oralsucker was 0.085–0.072 mm in diameter, located subterminally. Genital sinus was located in the middle part of the body. Ventral sucker was rudimentary and covered with a membrane. Genital sucker had a look of the papilla and consisted of two parts. Next to the genital sucker, there was a genital opening. Prepharynx was relatively wide; pharynx was 0.062 mm, oval shaped, followed by a long esophagus. Bifurcation of the intestinal tube was located closer to the

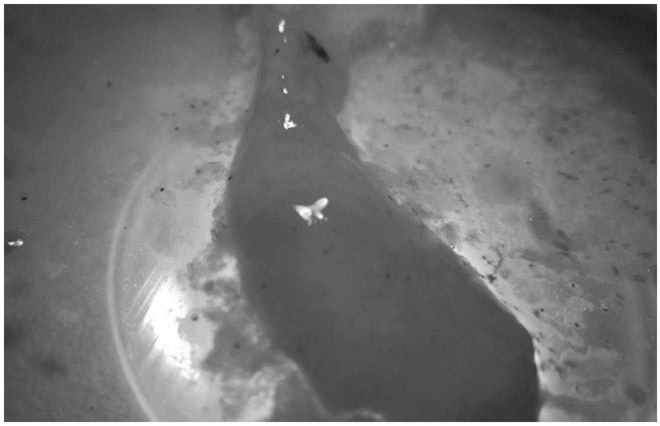

Fig. 3. Part of small intestines of duckling at autopsy. Visible trematodes *C. jejuna*in mucus and on mucosal surfaces.

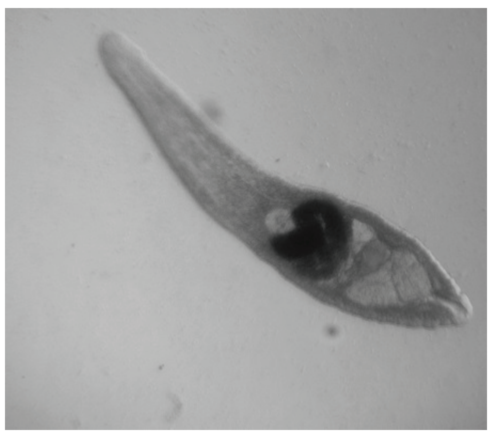

Fig. 4. *C. jejuna* in motionduring microscopy of mucus. x200 magnification.

oral sucker. Intestinal trunks went along sides of the body and almost reached the posterior end of the body. Testes were triangular, located in the posterior part of the body. The left testis was larger than the right one, 0.121 mm and 0.085 mm, respectively. Ventrogenital sac had a shape of elongated tube, located at the level of the ovary. Ovary was situated behind the right testis and had an irregular triangular shape, 0.68 by 0.101 mm. Vitellaria consisted of small follicles, which reached the posterior end of the body and were connected to each other. Uterus occupied a space between the left testis, the ovary and the genital sinus. Eggs were 0.03–0.034 mm in diameter. Excretory bladder with S-shaped trunk had two branches (n = 24) (fig. 5) (Skryabin, 1952; Gibson, 2002, 2005, 2008).

We conducted an analysis of gobies species that were affected by *Cryptocotyle* trematodes. The highest prevalence of infection was observed in *N. melanostomum,* 59.2 %, with an intensity of infection ranging from 11 to 211 metacercariae per fish. In *N. fluviatialis*,

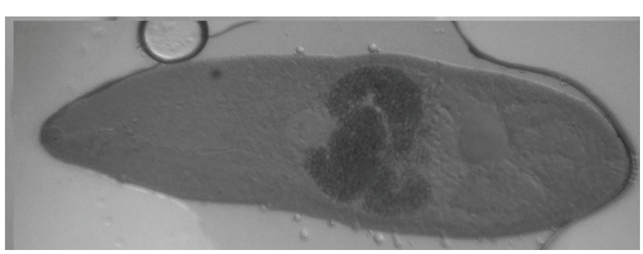

Fig. 5. *C. jejuna* stained with alumcarmine. x400 magnification.

the prevalence of infection was 30.4 % and the intensity of infection had a range from 9 to 124 metacercariae per fish. The lowest prevalence of infection was determined in *M. batrachocephalus.* In this fish mean prevalence of infectionwas17 %, and the intensity of infection had a range from 22 to 89 (table 1).

It should be noted that *Cryptocotyle* was the most prevalent in the region of Dnipro-Buh estuary (cape Adzhigol, Mykolaiv Region). Here the infection prevalence was higher compared to the Black Sea. This is a transitional area between the Dnipro and Buh rivers and the Black Sea. Conditions with varying salt concentration in these waters enable rich and diverse fish fauna and rich aquatic ecosystem. Alongside, this area is heavily involved in the routes of migratory birds because the Dnipro River and Dnipro-Buh estuary have a large number of floodplains, islands and wetlands that are now included in the "Black Sea State Reserve". This creates optimal conditions for nesting of piscivorous birds, the definitive hosts of *Cryptocotyle*, which play a key role in the spread of the parasite. In other areas of the Black Sea, indicators of gobies infection with *Cryptocotyle* varied slightly.

A significant contribution to the research of *Cryptocotyle* infection of gobiid fish in the Black Sea was made by Kvach (Kvach, 2004, 2006, 2014; Krasnovyd, Kvach, 2012). He thoroughly studied the occurrence of the parasite in the waters of the Dniester estuary and found that the most affected species was *Neogobius ratan*, with infection prevalence 87 %, but the intensity of infection was the highest in *N. fluviatialis* — 1500 metacercaria of *C. concavum*. Trematodes *C. lingua* were found only in *Neogobius syrman* Nordmann and *M. batrachocephalus* Pallas (only one of seven fish samples was infected), and these species had low intensity of infection. In the waters of Odesa region, *Neogobius eurycephalus* Kessler were mostly infected with *C. concavum* metacercaria, while *N. melanostomum* were mostly infected with *C. lingua*. *Cryptocotyle*s pp.were classified as the basis of helminthic fauna of gobiid fish in the Sukhoy estuary of the Black Sea. The most susceptible species were *N. fluviatialis* and *N. eurycephalus*. In Dnipro River, the prevalence of *C. concavum* infection in *N. fluviatialis* was 21 %.

Table 1. Species of gobies included in the studyand characteristics of their infection with metacercariae of *Cryptocotyle*

Species of fish	Number of examined fish	Prevalence of infection, %	Intensity of infection, range
Dnipro-Buh estuary (cape Adzhigol, Mykolaiv Region, 46°36′35.94″ N, 31°47′43.88″ E)			
Mesogobius batrachocephalus	18	38.8	32–89
Neogobius fluviatialis	74	55.4	13–121
Neogobius melanostomum	12	83.3	94–211
Black Sea (Ochakov, Mykolaiv Region, 46°36′26.77″ N, 31°32′19.90″ E)			
Neogobius fluviatialis	81	21.0	39–105
Mesogobius batrachocephalus	13	15.3	18–61
Black Sea (Rybakivka village, Berezan District, Mykolaiv Region, 46°37′18.10″ N, 31°23′56.25″ E)			
Neogobius fluviatialis	103	23.3	28–111
Neogobius melanostomum	24	62.5	108–179
Black Sea (Yuzhny, Odesa Region, 46°36′43.71″ N, 31°05′23.01″ E)			
Neogobius fluviatialis	62	38.0	12–96
Neogobius melanostomum	9	11.1	41
Black Sea (Odesa, 46°25′49.16″ N, 30°46′08.07″ E)			
Mesogobiu sbatrachocephalus	11	0	0
Neogobius fluviatialis	57	21.0	9–124
Neogobius melanostomum	4	25.0	48
Black Sea (Chornomorsk, Odesa Region, 46°15′24.34″ N, 30°38′08.42″ E)			
Mesogobius batrachocephalus	17	17.6	22–34
Neogobius fluviatialis	87	24.1	18–57

In the region of Dnipro-Buh estuary and the Black Sea in Mykolaiv and Odessa regions, the occurrence of the *Cryptocotyle* in fish was registered. During the ichthyopathological investigation, two species of trematode of Heterophyidae family were found: *C. cancavum* and *C. jejuna*. Metacercariae of *C. lingua* were not found, possible, due to their rare occurrence. Naidenova (1974) noted that metacercariae of *C. lingua* comprised only 0.8–1.2 % of total metacercariae in affected fish. Hydro-chemical parameters of the Dnipro–Buh estuary could also affect the occurrence and population size of intermediate host species and additional hosts.

The most prevalent fish infection was observed in the region of the Dnipro-Buh estuary (cape Adzhigol, Mykolaiv Region), while the least prevalent infection was in the Black Sea area, Mykolaiv and Odesa Regions. Mean prevalence of infection was 31.4 %. However, data on the distribution of *Cryptocotyle* in fish in other regions of Ukraine are limited or absent.

Cryptocotyle has epidemiological significance and therefore requires a great attention of specialists in human and veterinary medicine, focusing on the provision of safe fish products, study of disease distribution, pathogen biology and its impact on the fish.

Hence, fish is an intermediate host for *Cryptocotyle* and can be hazardous to mammals and humans. This fish requires careful investigation during veterinary expertise to mitigate the risk for public health. At the same time, legal documents and techniques, used in laboratory studies of the fish pathogen, do not classify *Cryptocotyle* as a threat to human health. Thus, the study of the pathogen, its intermediate, additional and definitive hosts remains important.

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