Zoodiversity, **56**(4):341-348, 2022
DOI 10.15407/zoo2022.04.341

Parasitology

UDC 598.115.31:595.132(282.247.32-197.4)

INFECTION OF DICE SNAKE, *NATRIX TESSELLATA* (REPTILIA, COLUBRIDAE), WITH *EUSTRONGYLIDES EXCISUS* (NEMATODA, DIOCTOPHYMATIDAE) IN THE MIDDLE AND LOWER DNIPRO RIVER BASIN

S. V. Yermolenko[1*], V. A. Gasso[1], A. M. Hahut[1], V. A. Spirina[2]

[1]*Research Institute of Biology, Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010 Ukraine*
[2]*Department of Microbiology and Immunology, Dalhousie University, 6299 South Street, Halifax, B3H 4R2, Nova Scotia, Canada*

**Corresponding author*
E-mail: yermolenko_sv@i.ua

S. V. Yermolenko (https://orcid.org/0000-0001-5496-0910)
V. A. Gasso (https://orcid.org/0000-0002-6094-9408)
A. M. Hahut (https://orcid.org/ 0000-0001-7942-6490)
V. A. Spirina (https://orcid.org/0000-0003-1336-8624)

Infection of Dice Snake, *Natrix tessellata* (Reptilia, Colubridae), with *Eustrongylides excisus* (Nematoda, Dioctophymatidae) in the Middle and Lower Dnipro River Basin. Yermolenko, S. V., Gasso, V. Y., Hahut, A. M., Spirina, V. A. — Dice snakes can be a paratenic host for a stage IV of *Eustrongylides excisus*, Jägerskiöld, 1909 larva due to specific diet of the snakes. The infection rate of *E. excisus* in *Natrix tessellata* (Laurenti, 1768) was studied in the basin of the middle and the lower Dnipro River in 2013–2017. We searched for nematodes in snakes from four sites: Prydniprovska Thermal Power Station, Majorova Balka, Zaporizhzhia; National Nature Park Velykyi Luh. The snakes from all studied areas had high prevalence of infection (more than 90 %). The lowest level of intensity (2.50 ± 0.60) and abundance (2.27 ± 0.58) were in ecosystems near Prydniprovska TPP. There was no significant difference between infection parameters in snakes from other groups. Capsules with larva localized in the gastrointestinal tract, liver, muscles, and lung. The most infected was the gastrointestinal tract statistical analysis showed significant differences with other organs. The lowest infection was found in the lung. Environmental pollution of the Dnipro River waters with pesticides and other pollutants accompanied by a climate change may influence on the *E. excisus* life cycle that needs additional studies.

Key words: eustrongylidosis, abundance, infection intensity, prevalence, Ukraine.

Introduction

The nematode *Eustrongylides excisus*, Jägerskiöld, 1909 is one of the most common roundworm parasites in the Palearctic fish. Typical definitive hosts are fish-eating birds of the orders Ciconiiformes, Anseriformes, Gaviiformes and Pelecanoformes. Caspian seal and sturgeons may also be occasional final hosts (Fagerholm, 1996; Kornyushin et al., 2004; Goncharov, 2018; Rusconi et al., 2022). Because of bird defecation and regurgitation, the nematode eggs get into the water; oligochaetes of the genera *Tubifex, Lumbricus,* and *Limnodrilus* ingest it and the larval form of the helminth begins to develop. The next intermediate host is fish that feed on benthos, such as Gobiidae and Cyprinidae. Some species of predatory fish, amphibians and reptiles can be paratenic hosts for *E. excisus* larvae at the third and fourth stages of development (Saglam & Arikan, 2006; Bjelić-Čabrilo et al., 2013). The nematode larvae of the third and fourth stages have also been detected in humans (Guardone et al., 2021; Honcharov et al., 2022).

E. excisus began to be observed in fish of the middle and lower parts of the Dnipro River in the first half of the 2000s. There are some reports of infection of round goby *Neogobius melanostomus* (Pallas, 1814), European perch *Perca fluviatilis* Linnaeus, 1758, pikeperch *Sander lucioperca* (Linnaeus, 1758) and wels catfish *Silurus glanis* Linnaeus, 1758 (Yesipova, 2013; Rubtsova, 2015). This may be related to changes in hydrological and climatic conditions, which led to an increase in breeding numbers of fish-eating birds, such as great cormorant *Phalacrocorax carbo* (Linnaeus, 1758) (Bulakhov et al., 2007), for which *E. excisus* is a common autogenous species (Bjelić-Čabrilo et al., 2013).

The dice snake *Natrix tessellata* (Laurenti, 1768) occupies a large areal that extends from Central and Southern Europe to Western China and Southwestern India (Gruschwitz et al., 1999; Liu et al., 2011; Šukalo et al., 2014; Jablonski & Kautman, 2017). It is one of the most common snake species in Ukraine (Kotenko et al., 2011; Gasso et al., 2020; Baranovski et al., 2021). Despite the fact that the species is thermophilic, there is a tendency for *N. tessellata* to move northwards in Ukraine. The northernmost finds have been recorded in Kyiv Region. This may be due to an increase in the number of food items and an increase in the mean annual temperature (Nekrasova et al., 2013). The diet of *N. tessellata* is dominated by fish of the families Gobiidae and Cyprinidae, the species composition of which may differ from region to region (Acipinar et al., 2006; Luiselli et al., 2007; Göçmen et al., 2011; Bakiev et al., 2011, Hutinec & Mebert, 2011; Weiperth, 2014). If an *E. excisus*-infected fish is ingested, the dice snake becomes a paratenic host for a stage IV of *E. excisus* larva (Sharpilo, 1976).

The aim of this study is to find out the degree of the dice snake infection in the ecosystems of the central and southern Dnipro River and determine the distribution of the nematode in the organs.

Fig. 1. Location of the studied: 1 — Prydniprovska Thermal Power Station; 2 — Majorova Balka, 3 — Zaporizhzhia City; 4 — National Nature Park "Velykyi Luh".

Material and methods

The research was conducted in 2013–2017. Sixty-five individuals of *N. tessellata* from four sites were studied. They were collected at the middle part of the Dnipro River, namely, the natural coastal ecosystem of Majorova Balka (48.262° N, 35.169° E), the sanitary protection zone of Prydniprovska Thermal Power Plant (Dnipro City) (48.400° N, 35.113° E), and the coastal ecosystem of the Zaporizhzhia City (47.886° N, 35.134° E). Natural coastal ecosystem of the National Nature Park "Velykyi Luh" (NPP "Velykyi Luh") (47.447° N, 35.133° E) is situated at the Kakhovske Reservoir, the lower part of the Dnipro River basin (fig. 1).

The captured snakes were brought alive to the laboratory for processing. The reptile abdominal cavity was dissected by a longitudinal ventral incision. Internal organs were removed from the body cavity and the skin was separated from the muscle tissue for visual inspection. The organs were carefully dissected in Petri dishes filled with 0.9 % aqueous sodium chloride solution (Düşen, 2012). Identified helminths were fixed in hot 70 % ethanol (de Vasconcelos Melo et. al., 2016). After fixation, the nematodes were examined with the use of the Carl Zeiss Amplival Microscope. Species identification of helminths was carried out according to Sharpilo (1976). Prevalence, infection intensity and abundance index were calculated according to standard methods (Bush et al., 1997). The study was carried out in accordance with the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986) and Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (Revision on August 8, 2021).

Data statistical analysis was performed by calculating the mean (x) and standard error (SE) values. Normality of distribution in the groups was carried out using the Shapiro-Wilk criterion. Nonparametric Kruskal-Wallis test was used for multiple comparisons of independent samples, and paired comparison was performed using Dunn's test in case of statistically significant differences. Statistically significant differences were considered at $p < 0.05$. Statistical analysis of the data obtained was performed using the Origin software, version 9.8 (Origin Lab Corp.).

Results

We found subcutaneous capsules located in the muscle tissue of the dorsal part of some dice snakes (fig. 2), which may be a manifestation of the snake infection by the nematode (Mihalca, 2011). The nematodes were also localised in connective tissue capsules on the surface of internal organs. Inflammation and mechanical damage to the organs have

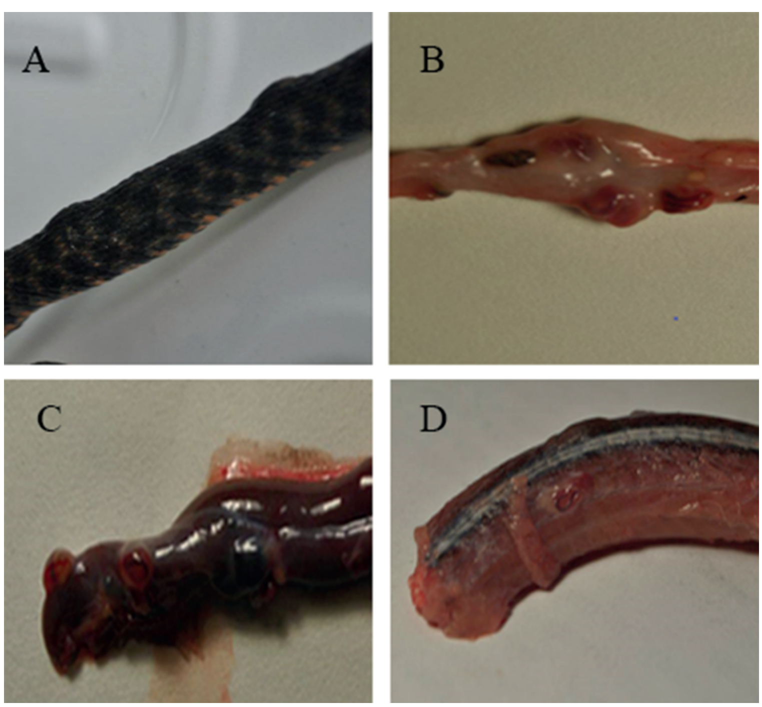

Fig. 2. Capsules with E. excisus larvae under the skin (A), on the gastrointestinal tract (B), on the liver (C), and on the musculature (D) of *N. tessellata*.

Table 1. *E. excisus* records for *N. tessellata* from sites of the middle and lower Dnipro River basin

Site	N	P, %	Intensity		Abundance
			MI ± SE	range	MA ± SE
Prydniprovska TPP	11	90.9	2.50 ± 0.60 [a]	1–7	2.27 ± 0.58[a]
Majorova Balka	26	84.6	11.35 ± 2.25 [b]	1–43	9.38 ± 1.96[b]
Zaporizhzhia	7	100	8.71 ± 2.39[b]	2–19	8.71 ± 2.39[b]
NNP "Velykyi Luh"	22	90.9	14.85 ± 2.49[b]	1–36	14.45 ± 2.33[b]

Note. P — prevalence; MI — mean intensity of infestation; MA — mean abundance; SE — standard error.

sometimes been observed near the capsules. Up to three *E. excisus* larvae were found in one capsule; some of nematodes were dead.

An inter-group comparison of infection intensity and abundance index of *E. excisus* larvae in dice snakes revealed some statistically significant differences. In all studied ecosystems, the prevalence of infection of the dice snakes was more than 80 %. In snakes from Zaporizhzhia, eustrongylidosis was detected in 100 % of investigated specimens. The lowest average intensity of infection was found in dice snakes from the population inhabited NNP Velykyi Luh. The average values of the infection intensity and helminth abundance index in *N. tessellata* from Prydniprovska TPP had the lowest values. A pairwise comparison of the infection indices of the dice snakes revealed significant differences between Prydniprovska TPP and all the other studied sites the snakes were collected from ($p < 0.05$) (table 1). Excluding the Prydniprovska TPP, no significant differences were found between the sites in terms of *E. excisus* invasion and abundance in the dice snakes.

A multiple comparison of the nematode abundance in the organs of snakes in all groups has been also revealed the significant differences. In the majority of the examined

Table 2. Distribution of nematodes *E. excisus* in organs of *N. tessellata* from sites of the middle and lower parts of the Dnipro River basin

Site	Index	Gastrointestinal tract	Liver	Muscles	Lung
Prydniprovska TPP (n = 11)	x ± SE	2.00 ± 0.63[a]	0.09 ± 0.09[bc]	0.18 ± 0.12[bc]	0[c]
	min-max	0–7	0–2	0–1	0
	P	81.8	9.09	18.2	0
Majorova Balka (n = 26)	x ± SE	5.19 ± 1.11[a]	1.34 ± 0.66[b]	2.77 ± 1,35[ab]	0.08 ± 0.05[c]
	min-max	0-19	0-16	0-35	0-1
	P	61.5	38.4	65.4	7.69
Zaporizhzhia (n = 7)	x ± SE	5.86 ± 1.39[a]	1.14 ± 0.51[bc]	1.71 ± 0.61[b]	0[c]
	min-max	1–12	0–3	0–4	0
	P	100	57.1	71.4	0
NNP "Velykyi Luh" (n = 22)	x ± SE	9.91 ± 1.60[a]	1.91 ± 0.46[b]	2.27 ± 0.36[b]	0.36 ± 0.36[c]
	min-max	0–24	0–8	0–7	0–8
	P	95.5	59.1	59.1	4.55

Note. P — prevalence, %; x — mean; SE — standard error; min-max — minimum and maximum number of parasites in organs.

dice snakes, more than half of the total number of nematodes were localised in the gastrointestinal tract. Less than 8 % of the snakes from Majorova Balka (0.08) and NPP Velykyi Luh (0.36) were found to have capsules on the lung, in the snakes of other groups no *E. excisus* larvae were detected on that organ. In the studied snakes from the Prydniprovska TPP, Zaporizhzhia and NPP Velykyi Luh the most nematodes were found on the gastrointestinal tract and according to the Dunn's test it differed from the abundance of the nematodes in other organs ($p < 0.05$). In snakes of Majorova Balka, no significant difference was found between the numbers of larvae in the muscular tissue (2.77) and in the gastrointestinal tract (5.19). Since in snakes from all studied ecosystems there are no significant differences between the nematode abundance in liver and muscles, it can be concluded that the distribution of nematodes in these organs is relatively even (table 2).

Discussion

In the studied ecosystems of the Dnipro River, *E. excisus* is a common helminth of the dice snakes, which should manifest a high infection of species that used as prey of the snake.

Goncharov (2017) notes that in predatory fish, the nematodes are largely predominant in muscular tissue. Apparently, the distribution of *E. excisus* in the dice snake organs and predatory fish has a different pattern in specific tissues.

A similar pattern of prevalence has also been reported for the dice snakes in Romania (85 %) (Mihalca et al., 2007). *E. excisus* has not been detected in helminth communities of the dice snakes caught in the Volga region (Kirilov, 2011), Armenia (Sargsyan et al., 2016) and some regions of Central and Western Asia (Shakarboev et al., 1999; Yossefi et al., 2014) with the exception of Bursa province, Turkey (Yildirimhan et al., 2007). Only one infested individual of *N. tessellata* was found in the southern part of the Volga delta in 1953 (Dubinina, 1953) (table 3).

Previous studies of the helminth fauna of the grass snake *Natrix natrix* (Linnaeus, 1758) from the ecosystems of the same Dnipro area have not detected *E. excisus* (Yermolenko et al., 2019) despite wide distribution and abundance of the snake species (Gasso, 2011). It is worth noting that cases of *N. natrix* infection with this nematode species were recorded in Bursa province, but the infection of the grass snakes was quite low (1 of 21 examined individuals) (Yildirimhan et al., 2007). The possibility of infection exists because bentophagous fish was observed in the diet of the grass snake (Hutinec & Mebert, 2011). In addition, the marsh frog *Pelophylax ridibundus* Pallas, 1771, which is known prey of *N. natrix*, is also possible host of *E. excisus* (Saglam & Arikan, 2006; Koyun et al., 2015).

Table 3. *E. excisus* records for *N. tessellata* from different regions

Region	Quantity, no. of snakes	Prevalence, %	Min–Max	Reference
Romania	20	85	3–22	Mihalca et al., 2007
Mazandaran province, Iran	9	0	0	Yossefi et al., 2014
Angren River, Uzbekistan	100	0	0	Shakarboev et al., 1999
Armenia	16	0	0	Sargsyan et al., 2016
Bursa Province, Turkey	24	46	1–6	Yildirimhan et al., 2007
Volga River Basin, Russia	58	0	0	Bakiev et al., 2011
Seaside part of the Volga River Delta, Russia	12	8.33	0–1	Dubinina, 1953
South Bulgaria	no data	9.1	2	Biserkov, 1996
Northeastern Bulgaria	–	17	1–10	Kirin, 2002

Presumably, unfavourable environmental conditions of the sanitary protection zone of Prydniprovska Power Plant may affect animal species included in the life cycle of the *E. excisus*. The surface waters in the area of the Prydniprovska TPP location have a high-level pollution of oil products, phosphates and nitrates (Kroik & Dorhanova, 2016). Environmental pollution of the Dnipro River waters with pesticides and other pollutants (Zarubin, 2013; Strilets, 2018; Kurchenko & Sharamok, 2020) accompanied by a climate change may influence on the *E. excisus* life cycle peculiarities and on a place of the dice snake in it that needs additional specific research.

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Received 22 June 2022
Accepted 3 August 2022