

Experimental infection of *Carassius auratus* (L., 1758) with the second stage larvae of the nematode Contracaecum rudolphii Hartwich, 1964

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ABSTRACT. Susceptibility of the goldfish to infection with the newly hatched second stage larvae of *Contracaecum rudolphii* was investigated under laboratory condition. A week after the larvae had been placed in the fish tank, the autopsied goldfish showed the nematodes in their intestine lumen. The majority of the larvae were dead. In week 2 and 3 of exposure, the larvae were being found exclusively in the intestinal wall; in week 4 and 5, the larvae, in addition to being present in the intestinal wall, were also found in the body cavity. No larvae were found in the fish autopsied in subsequent weeks.

Key words: *Contracaecum rudolphii*, goldfish, black cormorant, infection intensity, infection prevalence

Introduction

The definitive hosts of the nematode *Contracaecum rudolphii* are piscivorous birds, primarily black cormorants. The prevalence of *C. rudolphii* infection in cormorants in Poland and elsewhere is very high, almost 100% [1–5]. Due to their high mobility and migrations from one water body to another, those birds are very efficient carriers of the parasite and significantly contribute to its dispersal [6]. The nematode's eggs are excreted with the birds' faeces right into the water where the eggs develop embryos. The available literature contains few papers on *C. rudolphii* life cycle. The research, initiated by Thomas in 1937 [7, 8], showed fishes to be the sole intermediate hosts of the parasite (reported as *C. spiculigerum*). Subsequent experimental studies [9–11] involving the guppy (*Poecilia reticulatus*) and the mummichog (*Fundulus heteroclitus*) revealed the life cycle of *C. rudolphii* to be complex and to potentially involve two hosts. The first intermediate host may be freshwater or marine copepods, or benthic invertebrates (gammarids), while fishes are the second intermediate hosts or paratenic hosts. When small fishes are infected, crustaceans may be absent from the life cycle. While the guppies could be directly infected with newly hatched larvae, the infection prevalence and intensity proved higher when the nematode-bearing cyclopoids were the source of infection. The mummichog, larger than the guppies, were not amenable to direct infection with free-swimming nematode larvae, the infected cyclopoids being the only source of the parasites [9, 10]. In the mummichog, the larvae did not migrate to the body cavity, which was the case in the guppies, but – in most cases – became encapsulated in the intestine wall. On the other hand, Mosgovoy et al. [12] con-

sider copepods to be indispensable in the *C. rudolphii* life cycle, fishes being redundant. In their opinion, *Coenagrion* and *Agrion* dragonfly larvae are much better intermediate hosts than fishes. Szostakowska and Fagerholm [13] maintain that invertebrates may be skipped in the life cycle, fishes remaining the only hosts involved. As paratenic parasitism is rather common among anisakid nematodes, fishes are most probably only paratenic hosts for larval *C. rudolphii* [14]. Bartlett [10] maintains that fishes are intermediate hosts, a "sleeve" adhering to isolated larvae bearing evidence of moult during the larva's migration from the intestine wall to the body cavity. When in the definitive host, the larvae moult twice, grow, and reach sexual maturity.

According to Křie and Fagerholm [15], the *Contracaecum* nematodes show similar life cycles, the presence of crustaceans in the cycle being dependent on fish size. The above mentioned authors reported that the newly hatched *Contracaecum osculatum* larvae were capable of infecting small fishes only (*Pungitius pungitius*, *Zoarces viviparus*). In those fishes, scarce, non-encapsulated larvae were found mainly in the liver parenchyma. Larger fishes (*Pleuronectes platessa*, *Gadus morhua*) could be infected only via the already infected crustaceans or small fishes.

The high prevalence and intensity of infection of the black cormorants breeding in Poland suggests the common occurrence of the larvae in fishes. However, long-term studies on the fishes from water areas in the immediate vicinity of the cormorant colony failed to reveal the presence of the nematode [16, 17]. It was only in 2007 that larval *C. rudolphii* was recorded in the crucian carp (*Carassius carassius*) from Lake Selment Wielki and in the round goby (*Neogobius melanostomus*) from the Gulf of Gdańsk [13]. The prevalence of infection in both fish species was very low (2.4%). On the other hand, the nematode's larvae have been frequently found in fishes in water bodies of Chile [18, 19], Brazil [20], and in the Black Sea [21, 22], which may suggest the parasite's preference for warmer water for development, the cormorants being infected during wintering.

This work was aimed at investigating, under laboratory conditions, the susceptibility of goldfish to infection with the second stage larvae of the nematode *C. rudolphii,* and at analysing the larval migration routes in the fish body.

Materials and methods

Adult nematodes were collected from stomachs of cormorants shot near Ełk (the Province of Warmia and Mazury). The eggs, isolated from the terminal part of the adult female vulva, were suspended in the physiological salt solution (0.9% NaCl) and incubated at 23°C. The procedures used for egg development and hatching followed those described by Dziekońska-Rynko and Rokicki [11].

The goldfish (*Carassius auratus*) bred at the Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn were experimentally infected. Throughout the experiment, 20 goldfish individuals (22.02 g mean individual weight, 114.59 mm length) were kept in aerated flow-through 350 dcm3 tanks filled with 16°C1 temperature water. For 3 days, 5 ml of larval suspension (about 500 larvae per fish) were added to the tank.

Table 1. Prevalence and intensity of infection of the goldfish and location of *Contracaecum rudolphii* larvae

Week p.i.	Prevalence (%)	Intensity of infection	Location of larvae
1	100	75 (25 live) 56 (20 live)	Intestine lumen
2	50	5 (live)	Intestinal wall
3	100	7 (live) 3 (live)	Intestinal wall Intestinal wall
4	50	8 (live)	3 larvae in intestinal wall, 5 in body cavity
5	100	1 (live) 4 (live)	Intestinal wall Body cavity
Total number of larvae 73 (live)		73 (live)	

The fish were fed until 2 days before the infection and the onset of exposure; later on, the fish were offered the standard Nutra feed.

At one week intervals, 2 fish individuals were sacrificed and autopsied; all the organs were examined under the microscope for the presence of the nematode larvae. Those organs containing the larvae were digested, as described by Jackson et al. [23], in 1% pepsin (pH 2). Digestion, at 37°C, proceeded for 24 h. The larvae were measured and photographed under an Olympus microscope, using the Multiscan v.4.2 image analysis software.

Results

Data on the extent of infection and on the location of the larvae found in consecutive weeks post infection (p.i.) are summarised in Table 1.

A week after the larvae had been placed in the tank, they were found only in the fish intestine lumen, more than half of the larvae being dead. After week 2 and 3 p.i., the larvae were present in the intestine wall. When released from the digested intestine, all the larvae were motile and measured 264.25–288.45 m and 294.20–342.70 m after week 2 and 3, respectively. One of the two fish autopsied after week 4 showed the presence of 8 motile larvae: 3 in the intestine wall and the remaining 5 in the body cavity. The larvae measured 264.30–392.50 μm. After week 5, one of the autopsied fish revealed the presence of a larva actively penetrating the intestine wall, the other fish containing 4 motile larvae located in the body cavity. The larvae measured 274.98–458.45 μm. The fishes autopsied in the subsequent weeks (6–10) did not contain any larvae.

Discussion

The available literature contains few papers on the way the fish become infected with the nematode *C. rudolphii*. Laboratory studies on the guppies reported on by Huizinga [9], Bartlett [10], and the present authors [11] showed the fish to be equally vulnerable to the free-living second stage larvae and to the larvae derived from the experimentally infected intermediate hosts (cyclopoid copepods).

This study showed the second stage larvae of *C. rudolphii* to be capable of infecting the goldfish under laboratory conditions. However, despite a high number of larvae used in the experiment, both the prevalence and intensity of infection were very low. The maximum intensity of infection was 8 larvae per fish (week 4 p.i.). The weak infection could have resulted from inefficient penetration of the intestine wall by the second stage larvae. More than half of the larvae found in the fish intestine lumen a week after the onset of exposure were dead. The available literature lacks descriptions of intestine wall penetration by the larval *C. rudolphii*. Penetration enzymes (proteases, glycosidases, hyaluronidase, and aminopeptidases), found in the excretion-secretion (ES) products of numerous parasites [24–26] belong to the factors facilitating the parasite's entry to and settlement in the host's body. The enzymes secreted to the medium are regarded as serving multiple functions: they inhibit host's blood coagulation, defend the parasite from the host's immunoresponse, facilitate the parasite's migration within a tissue by decomposing the tissue barrier, facilitate larval hatching and moulting, and play an important part in larval feeding. In her study on larval *C. rudolphii* morphogenesis, Bartlett [10] reported the ES system in the second stage larvae, newly hatched from the egg, to be undetectable, a secretory pore being distinctly visible near the larval tooth in the larvae isolated from the experimentally infected crustaceans. The low-level infection parameters in the present experiment could have been caused by the larvae being unable to rapidly penetrate the fish intestine wall.

Another reason of such low level of infection may be sought in the larvae being susceptible to the host's digestive enzymes. Those enzymes, particularly proteases, are commonly known to form a strong barrier a parasite has to overcome before it may settle in the host's body. Those parasites dwelling in the intestine lumen are protected from digestive enzymes by, i.a., producing enzyme inhibitors [27, 28]. In their definitive hosts, the cormorants, the adult and larval *C. rudolphii* dwell in the stomach, hence they are most probably pepsin-resistant. As the goldfish lacks a separate stomach in the alimentary system, the nematode larvae were exposed to intestinal enzymes (trypsin, chymotrypsin, aminopeptidases) and could have been digested.

The fishes that were infected under natural conditions showed some differences in the location of and intensity of infection by the larvae, both the location and intensity depending on fish species and size. Torres and Cubillos [18] found encysted larvae of *C. rudolphii* only in the intestine wall of *Salmo trutta* from Rio Valdivia, while other fish species caught in that river (*Cauque mauleanum,*

Basilichthys australis, Galaxias maculatus and *Oncorhynchus mykiss*) contained the larvae in the mesentery [19] as well. Intensity of infection ranged from 8 to 1972 larvae per fish. In two fish species (*Neogobius melanostomus* and *Mesogobius batrachocephalus*) from the Black Sea, Kvach [21] found larval *C. rudolphii* in the mesentery, while Pronkina and Belofastowa [22] reported finding encysted *C. rudolphii* larvae in the gill ducts of the Black Sea's golden grey mullet (*Liza aurata*). Szostakowska and Fagerholm [13] found singular larvae in the mesentery of the round goby (*Neogobius melanostomus*) and in the intestine wall of two individuals of the crucian carp (*Carassius carassius*). In the heavily infected (500 larvae) crucian carp individual, the larvae were present in the intestine wall, in the mesentery, and in the vicinity of the liver.

The results of this experiment support Køie and Fagerholm [15] contention that the larval *Contracaecum* ability to penetrate the fish intestine wall depends on the proportion between the larval size and the intestine wall thickness. Small fishes may be infected by both the larvae and the already infected crustaceans [9–11]. Larger fishes, with a much thicker intestine wall, are much less vulnerable to infection. For the intestine wall to be penetrated, it is necessary that morphological structures (the larval tooth) and chemical effects exerted by penetration enzymes, as in other parasites, act in concert. Infection via the eggs of *C. rudolphii* or as a result of swallowing an egg-filled female by a fish, as contended by Szostakowska and Fagerholm [13], can be ruled out. The nematode's eggs have thin membranes and are very sensitive to temperature, moisture, and oxygen deficiency [11, 29]. The developing embryos are metabolically aerobic, anoxia producing irreversible developmental inhibition. Optimal conditions for the *C. rudolphii* egg development prevail only in the nearshore, well oxygenated water.

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