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Trichobilharzia regenti: The developmental differences in natural and abnormal hosts

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Abstract

Trichobilharzia regenti is a bird nasal parasite causing human cercarial dermatitis. Schistosomula are able to migrate via the bird nervous system and then, they mature and lay eggs in the nasal cavity. To some extent they can also migrate and develop in mammals. The present study has shown the developmental differences of *T. regenti* in the natural (ducks) and the abnormal (mice; inbred strains BALB/c, SCID) hosts. The study describes the following parameters of developing worms: length and width of the body, length and content of the intestine, development of the reproductive organs and characterization of surface and intestinal epithelium by lectin probes. The differences in length and width of schistosomula localized in the spinal cord of various hosts cannot be simply explained and may depend on yet unknown host factors. Moreover, there must be several physiological changes during the migration through the skin, the nervous tissue and the nasal cavity, enabling uptake and digestion of different host components. For example the intestine of schistosomula was mostly filled with light-brown pigmented granules until 6 days p.i. (probably of nervous tissue origin) while the older schistosomula and adult intestine was mostly full of dark-brown pigment (probably of blood origin). Reproductive organs were observed from day 9 p.i. in worms from ducks. Whereas ConA and PSA specifically bound to the surface and intestinal epithelium of schistosomula and adults, only the labelled UEA-I lectin could be used as a surface marker of cercaria–schistosomulum transformation. The results confirmed retarded development of parasites in abnormal hosts; the factor responsible for this phenomenon should be clarified in the future.

Keywords: *Trichobilharzia*; Schistosomes; Development; Cercarial dermatitis; Neuropathogen

1. Introduction

The cercariae of some species of Schistosomatidae and Diplostomatidae, e.g. *Trichobilharzia regenti*, *Diplostomum phoxini* and *Ornithodiplostomum ptychocheilus*, actively invade the vertebrate body epithelia and either migrate through the nervous system to the target organs/tissues [1,2] or they attack the nervous system as the final site of their location [3]. In the case of *T. regenti*, schistosomula enter the peripheral nerves of duck or mouse legs as early as 1 day p.i. and 1.5 day p.i., respectively. The peripheral nerves are used as route to the spinal cord. In the specific host (duck) schistosomula were found in the spinal cord from 2 to 15 days p.i. and in the brain from 12 to 18 days p.i. [1]. The larvae of *O. ptychocheilus* were observed in the cranial and spinal nerves of the fish intermediate host, *Pimephales promelas*, 2 to 8 h p.i. So, the peripheral nerves and the associated foramina seem to be the most probable gate to the central nervous system. The diplostomula were first observed in the neural canal, the spinal cord and the brain 1 h p.i., and the migration to the brain was completed 48 h p.i. [2]. The metacercariae of Diplostomatidae were unevenly distributed throughout the brain; the larvae of *D. phoxini* were aggregated in the cerebellum, medulla oblongata and optic lobes [3], and those of *O. ptychocheilus* were mostly localized in the optic lobes, cerebellum and brainstem [2].

Unfortunately, no detailed data on fluke development (growth and organogenesis) within the vertebrate nervous tissues are available at present; such data would be essential

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Table 1
Length and width (μm) of *T. regenti* schistosomula in the duck spinal cord (as a whole) and in the synsacral and thoracic spinal cords

Days p.i.	Spinal cord		Synsacral spinal cord		Thoracic spinal cord	
	Length ± S.D.	Width ± S.D.	Length ± S.D.	Width ± S.D.	Length ± S.D.	Width ± S.D.
3	278 ± 33	131 ± 16	278 ± 33	131 ± 16	ND[a]	ND[a]
6	578 ± 221	88 ± 21	599 ± 191	88 ± 19	480 ± 83	88 ± 11
9	1300 ± 545	72 ± 20	1302 ± 440	76 ± 16	1291 ± 366	55 ± 9
12	2086 ± 958	55 ± 18	2020 ± 701	55 ± 12	2871 ± 696	58 ± 15
15	1793 ± 1137	49 ± 20	2138 ± 905	51 ± 14	1201 ± 420	41 ± 18
18	Data were not evaluated[b]		Data were not evaluated[b]		Data were not evaluated[b]	

[a] The worms were only in the synsacral spinal cord.
[b] The worm body was fragmentary.

for understanding the pathogenic effect of these parasites. The present study aims to compare the development of *T. regenti* in specific and non-specific hosts with respect to the length and width of the body, length and content of the intestine, and development of the reproductive organs. The surface and intestinal developmental changes were also characterized by fluorescent lectin probes, mapping the appearance/disappearance of epithelial carbohydrate moieties.

2. Materials and methods

The following experimental hosts were used: ducklings of *Anas platyrhynchos* f. dom. (5- to 10-day-old), immunocompetent inbred mouse strain BALB/c (3-month-old) and immunodeficient inbred mouse strain SCID (1-month-old). The legs of ducklings and the tails and legs of SCID mice were immersed in water containing cercariae of *T. regenti* for 1 h. The tails and legs of BALB/c mice were exposed to cercariae for 1.5 h. In all experiments the animals were exposed to 1–5 thousands of cercariae. The development of schistosomula was observed and evaluated at 3, 6, 9, 12, 15 and 18 days post-infection (p.i.) in ducklings, 3, 6 and 9 days p.i. in BALB/c mice and 6 days p.i. in SCID mice.

Worms were isolated from the dissected nervous tissue (spinal cord and brain) on a sieve (mesh of 200 μm) immersed in Tris buffer (TBS; 20 mM Tris, 150 mM NaCl, pH 7.8) in a Petri dish. Schistosomula found at the bottom of the dish were washed in TBS. One sample of the isolated worms was observed under a light microscope equipped with Nomarski differential interference contrast, and a second sample was fixed in hot formaldehyde-based Baker's fixative [4]. The latter worms were used for measurement of their length and width, and length of the intestine. At least 20 worms were measured for each infection period and statistic evaluation was performed by non-parametric Mann–Whitney *U* test, non-parametric Tukey HDS test and parametric LSD test. A third sample of the schistosomula from the nervous tissue, cercariae and adults from the nasal cavity (18 days p.i.) were analyzed by lectin probes, enabling detection of surface and intestinal carbohydrates. Schistosomula, cercariae and adults were fixed in Bouin's fixative overnight, washed five times in 70% ethanol and embedded in JB4 resin (Polyscience, Inc.). Tissue blocks were cut to sections of 2 μm thickness. To avoid non-specific reactions, the material was blocked prior to lectin application with 2% bovine serum albumin (Sigma) in TBS for 1 h. A set of 10 commercial FITC-conjugated lectins (Vector Laboratories) was used. Lectins were applied at 100 μg/ml final concentration in TBS supplemented with 2 mM $CaCl_2$ and 2 mM $MnCl_2$. As a control, lectins were mixed with appropriate saccharides (200 mM concentrations excluding the UEA-I inhibitors, concentration of which was 100 mM) (see Vector Laboratories product information; http://www.vectorlabs.com) 30 min before application. All experiments were repeated three times.

3. Results

3.1. Growth

3.1.1. Natural host (ducks)

The length to width ratio of schistosomula body increased markedly with time (for worm dimensions, see Table 1); it was about 2:1 (3 days old worms), 18:1 (9 days p.i.) and 38:1 (12 days p.i.). The length differences (variability) between individual worms were significant from day 6 p.i.; e.g. 9 days p.i., the differences were usually 500 μm but in some cases up to 2000 μm. The location of worms influenced the length; the differences between worms from synsacral vs. thoracic spinal cords were significant 12 and 15 days p.i. In comparison with

Table 2
Length and width (μm) of *T. regenti* schistosomula in BALB/c and SCID mouse spinal cord

Days p.i.	Spinal cord	
	Length ± S.D.	Width ± S.D.
3 (BALB/c)	339 ± 87	83 ± 16
6 (BALB/c)	339 ± 62	83 ± 19
9 (BALB/c)	398 ± 129	88 ± 11
6 (SCID)	308 ± 57	137 ± 66