RESEARCH NOTE

TERMINAL PHASE OF BIRD SCHISTOSOMIASIS CAUSED BY *TRICHOILHARZIA REGENTI* (SCHISTOSOMATIDAE) IN DUCKS (*ANAS PLATYRHYNCHOS* F. *DOMESTICA*)

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Abstract. The terminal phase of the migration of *Trichobilharzia regenti* Horák, Kolářová et Dvořák, 1998 in the definitive host (*Anas platyrhynchos* f. *domestica*) was studied 12–27 days post infection (p.i.). Brain meninges were the last part of the nervous system where the worms were detected before their occurrence in the nasal cavity. In meninges, the parasites started to feed on red blood cells. Then the worms occurred in the nasal mucosa 14–25 days p.i. and the first immature eggs appeared 15 days p.i. The fully developed miracidia were recorded in the eggs from 17 days p.i. and freely in the nasal mucosa 19 days p.i. Infiltrates of lymphocytes, later also eosinophils and heterophils around the eggs and free miracidia, were observed from 15 and 19 days p.i., respectively. The haemorrhages occurring from 17 days p.i., and the granulomas with lymphocytes, eosinophils and heterophils forming around the eggs from 22 days p.i. were the most apparent pathological changes of nasal tissue.

Trichobilharzia regenti Horák, Kolářová et Dvořák, 1998 is a nasal schistosome of waterfowl (Horák et al. 1998). In the definitive host it migrates from the skin via nervous tissue, i.e. through the peripheral nerves and spinal cord to the brain (Horák et al. 1999, Hrádková and Horák 2002). Little is, however, known about the terminal phase of migration in the definitive host, where the worms leave the CNS and become localised in the nasal cavity. There were several aims of the present study: (1) determination of the place of passage of the worms from nervous tissue to the extraneural location, (2) description of the final location of the worms and distribution and development of eggs in terminal phase of migration, (3) evaluation of pathological changes in host tissues, and host cell reactions against the parasites being present in the nasal mucosa.

Trichobilharzia regenti is routinely maintained in our laboratory, using *Radix peregra* s. lat. as intermediate and *Anas platyrhynchos* f. *domestica* as definitive hosts. In order to characterise the terminal phase of the migration, 42 ducks were exposed to approx. 300–500 freshly shed cercariae. Ducks (2–5 specimens) were sacrificed on days 12, 13, 14, 15, 16, 17, 19, 21, 22, 24, 25 and 27 post infection (p.i.) and the nasal soft tissues and the nerves *n. olfactorius* and *n. opticus* were extracted. In addition, either bulbus olfactorius, hemispheres and meninges, or the brain as a whole were extracted from ducks sacrificed on days 12, 13, 14 and 15 p.i. The tissues were processed for classical histology (fixed with Bouin fixative, embedded in paraffin, sectioned at 6 µm and stained with haematoxylin-eosin).

Neither worms nor lesions were detected in bulbus olfactorius, *n. olfactorius* and *n. opticus*. Numerous worms were recorded 12 and 13 days p.i. in meninges of all ducks investigated. The worms were localised extravascularly and excep-

tionally also in the blood vessels (Fig. 1). Their intestinal content consisted of dark brown granules.

The first worms appeared in the nasal mucosa 14 days p.i. and persisted intact until 24 days p.i. Several worms with damaged surface were detected 25 days p.i. The worms were localised extravascularly, in the connective tissue between cartilage of the turbinate and the glandular epithelium of the nasal mucosa (Fig. 2). The intestine of adults contained dark brown granules.

Numerous eggs grouped in clusters of 5–12 specimens appeared 15 days p.i. (Fig. 3) and maximum number of eggs was recorded 22 days p.i. They were localised extravascularly in the connective tissue close to the cartilage, and due to growing abundance they became dispersed all over the nasal mucosa. The eggs recorded 15 and 16 days p.i. were immature. The first fully developed miracidia within the eggs appeared 17 days p.i. Since 19 days p.i. either eggs with fully developed miracidia, or miracidia already hatched and localised freely in the tissue were recorded (Fig. 4).

Focal haemorrhages up to 700 µm in diameter were dispersed all over the nasal mucosa from 17 to 27 days p.i. Infiltrates of lymphocytes were present around the accumulated eggs for the entire period of study. From 22 days p.i. granulomas containing lymphocytes, eosinophils and heterophils were formed around the eggs. Infiltrates of lymphocytes, eosinophils and heterophils without granuloma formation surrounded free miracidia. No cell reaction was recorded around adult worms.

According to the published data, schistosomula migrate through the spinal cord to the medulla oblongata and continued through the hemispheres to the bulbus olfactorius and nasal cavity (Hrádková and Horák 2002). Based on the migratory route described we supposed that the parasites migrate directly from the bulbus olfactorius to the nasal cavity and/or cranial nerves could be used in order to cross the distance between the CNS (brain) and the nasal cavity. Therefore, nerves *n. olfactorius* and *n. opticus* (both cranial nerves connecting the brain and the nasal cavity) were investigated. As we did not record any parasite or lesion, the hypothesis on migration through the cranial nerves was rejected. Subsequently, the brain (especially the bulbus olfactorius, hemispheres and meninges) was examined for the presence of parasites. Contrary to Hrádková and Horák (2002), meninges represented the only location where the worms were regularly detected on days 12–14 p.i., i.e. just before reaching the nasal cavity. Also Kolářová et al. (2001) recorded the worms in the duck brain mainly in the meninges; however, the exact intra- or extravascular location was not studied. Our results confirmed an extravascular location of most of the worms but several worms were seen also intravascularly. The intestine of worms in meninges contained dark brown granules, probably a product of haemoglobin digestion, as similarly reported in the intestine of mammalian and visceral bird schistosomes

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Figs. 1–4. Location of *Trichobilharzia regenti* in the terminal phase of infection in *Anas platyrhynchos* f. *domestica*. H&E. **Fig. 1.** Worm in the blood vessel of the brain meninges 12 days post infection (p.i., $\times 1,000$). **Fig. 2.** Adult worm 15 days p.i. ($\times 400$). **Fig. 3.** Immature eggs 15 days p.i. ($\times 400$). **Fig. 4.** Free miracidia 19 days p.i. ($\times 1,000$) located extravascularly in the nasal mucosa. BV – blood vessel; arrow – parasite.

(Blažová and Horák 2005). The granules differed from those in the intestinal content of *T. regenti* schistosomula migrating through the CNS and feeding on nervous tissue particles (Horák et al. 1999). This indicates another food source at the end of prepatent period when the worms localised in meninges probably started to take up and digest blood/red blood cells.

Because of the parasite location in the meninges, their absence in the cranial nerves and probable blood digestion we hypothesize that the parasites migrate to the nasal cavity from the meninges and, for this purpose, they use other tissues than the nerves, probably the blood vessels. This hypothesis, however, should further be verified.

Previous studies reported intra- and extravascular location of *T. regenti* adults in the nasal cavity (Horák et al. 1998, Kolářová et al. 2001), with migration of the worms from the blood vessels to the nasal tissue at the end of the patent period (Horák et al. 2002). The location of adult worms of several other *Trichobilharzia* species found in the nasal tissue was intravascular (Fain 1956). Contrary to these findings, the worms in the nasal cavity were localised extravascularly in our study. The above cited references and our results are in accordance concerning the extravascular location of the eggs.

Parasites present in the nasal cavity caused serious pathological changes of the soft nasal tissue. Granuloma formation

around accumulated eggs was the most apparent pathological reaction, and this is in agreement with the generally accepted view that the eggs trapped in tissues are the major cause of pathological changes in schistosomiasis (e.g. Modha et al. 1998). Recent descriptions of the influence of *T. regenti* on the duck host were focused mainly on histopathological changes in the CNS (Kolářová et al. 2001), resulting in neuromotor disorders (Horák et al. 1999). Besides CNS injuries, Kolářová et al. (2001) studied also the nasal tissue of two ducks 23 days p.i. The authors described similar type of reaction as reported here, with eosinophils and heterophils around the eggs and empty shells after the hatching of miracidia.

In conclusion, the brain meninges were recognized as a place where parasites start to feed on different food source (probably the red blood cells) and leave the CNS. The absence of worms in cranial nerves and the detection of some specimens within veins in the meninges indicate that local blood vessels might be used for transport of worms from the CNS to the nasal area. The worms reach the nasal cavity 14 days p.i. and start to lay eggs 15 days p.i. The haemorrhages occurring from 17 days p.i. and granulomas with lymphocytes, eosinophils and heterophils forming around the eggs from 22 days p.i. are the most important pathological changes of the nasal tissue.

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