Trichobilharzia regenti, a pathogen of the avian and mammalian central nervous systems

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SUMMARY

The development of nasal avian schistosomes of the genus *Trichobilharzia* in their final host is poorly known. Therefore, an experimental infection of ducklings (*Anas platyrhynchos f. dom.*) by *T. regenti* was performed. The infection resulted in leg paralysis and orientation/balance disorders of birds. The examination of the duck's spinal cord and brain confirmed the presence of developing parasites in pre-patent as well as patent periods. The absence of the worms in other tissues strongly supports our hypothesis that the parasite migrates through the central nervous system (CNS) to its final location in bird nasal mucosa. The injury level is probably dependent on number of parasites as well as yet unknown host factors. The affinity to the CNS seems to be high; also by exposure of experimental animals to low cercarial doses the growing worms in the CNS were found. In addition to the generally accepted view that bird schistosomes may cause cercarial dermatitis of mammals (including man), there is evidence of a partial development of *T. regenti* in mouse CNS; in certain cases leg paralysis was also recorded. Therefore, the pathogenesis spectrum caused by bird schistosomes in birds/mammals needs to be reconsidered.

Key words: *Trichobilharzia*, schistosomes, cercarial dermatitis, neuroinfection.

INTRODUCTION

Avian schistosomes of the genus *Trichobilharzia* are separated into 2 groups inhabiting either visceral or nasal areas of their host bodies (Blair & Islam, 1983). Using the vascular network, the larval and pre-adult stages of the visceral group members migrate within the host in order to reach their specific target organs/tissues. Once mature, the worms residing in blood vessels start to lay eggs. There is an exception in the case of *T. szidati*, adults of which leave the veins and are found in the intestinal wall tissue of the avian host (Neuhaus, 1952).

In the case of nasal schistosomes, development and migration of parasites within the host body is unknown and the worms and eggs were reported exclusively from the nasal area (for review see Blair & Islam, 1983). In our observation, laboratory infections of ducklings by *T. regenti*, the newly discovered member of nasal schistosomes (Horák, Kolářová & Dvořák, 1998), have led in some animals to leg paralysis and balance/orientation disorders. In order to determine whether the parasite is responsible for the symptoms, we examined the central nervous system (CNS) of the infected animals. As *Trichobilharzia* larvae are able to cause cercarial dermatitis of mammals (including man), infection experiments of laboratory mice with subsequent CNS examination were also performed.

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MATERIALS AND METHODS

The parasite *T. regenti* has been maintained in our laboratory since 1997. In this life-cycle, ducks (*Anas platyrhynchos f. dom.*) and freshwater lymnaeid snails (*Radix peregra*) served as final and intermediate hosts, respectively. In our experiments, legs of ducklings (5–10 days old) and tails and legs of adult mice (*Mus musculus*, strain BALB/c, approx. 8 weeks old) were immersed for 1 h in a beaker containing water with freshly emerged cercariae of *T. regenti*. Unless stated otherwise, the infection doses were 1500–2500 cercariae/animal; in certain cases the dose was 50 (2 mice killed on day 10 p.i.) or 500 (3 ducklings killed on days 6, 8 and 10 p.i.; 2 mice were killed on day 10 p.i.) cercariae/animal. Fourteen ducklings and 8 mice were killed on 3–23 days p.i. and on 10 days p.i., respectively; 4 separately infected mice (infection dose 1500–2500 cercariae/animal) were examined by day 23 p.i. Spinal cords and brains of the animals were examined as a squashed tissue under a light microscope ($\times 100$). As a tissue specificity control, examination of other body organs (heart, lungs, liver, kidney, mesenterial

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Table 1. Occurrence of *Trichobilharzia regenti* in the central nervous system of ducklings

Tissue/day p.i.	3*	6*	7*	8*	10*	11*	13†	14*	15*	16*	21†	23*
Synsacral s.c.‡		■	■	■	■		■	■	■	■		■
Thoracic s.c.	■	■	■	■	■	■	■	■		■		■
Cervical s.c.			■		■	■	■	■	■			■
Cerebellum						■	■	■				■
Hemispheres					■	■	■					
Ocular lobes + n.							■			■		
Nasal lobes						■						■
Nasal mucosa							■	■	■	■	■	■

■, Worms detected.
* One duckling was examined.
† Two ducklings were examined on the same day p.i.
‡ s.c., Spinal cord; n, nerves.

veins) was performed in some animals. The location and developmental stage of the parasites in the CNS were determined; the number of parasites was not assessed. Selected samples of the infected tissue were fixed in Bouin's fixative at 4 °C overnight, washed in 70 % ethanol, dehydrated and embedded in paraffin blocks. Histological sections (7 μm) were routinely stained in haematoxylin/eosin, haematoxylin/PAS or Gomori trichrome.

RESULTS

Duck model

In 6 out of 9 ducklings, a leg paralysis (complete or partial, depending on yet unknown factors) was observed by day 11 p.i.; 5 animals were sacrificed earlier and, therefore, paralysis could not develop. This ratio of affected/unaffected animals is higher than in routine parasite maintenance, where paralysis appears in about 30–40 %. The paralysis of afflicted animals continued until the patent period, i.e. day 20 p.i. and later. In the case of 2 ducklings, the paralysis disappeared just before the patent period. The infected animals exhibited orientation/balance disorders; in certain cases, petechiae in the nasal mucous tissue were seen and, rarely, massive nasal haemorrhage occurred.

Examination of the CNS (Table 1, Fig. 1 A, B, D) revealed schistosomes in 12 out of 14 animals exposed to cercariae; in 2 ducklings killed on day 21 p.i., the first had worms in the nasal mucosa only and in the second the infection did not establish. The migrating worms appeared in the thoracic spinal cord by day 3 p.i. By day 6–7 p.i., the young flukes occurred also in synsacral and cervical spinal cords. In the course of a heavy infection, some worms in the synsacral spinal cord were destroyed and black-pigmented; the nerve mass around the destroyed parasites was partly changed to a gelatinous matrix. The brain, i.e. cerebellum, hemispheres, ocular lobes/nerves and nasal lobes, started to be invaded by the parasites between days 10 and 13 p.i. The adults appeared in the nasal region by day 13 p.i. and the first immature eggs were detectable by day 14 p.i. Eggs were never observed in the CNS. Other body organs (see Materials and Methods section) had no developing parasites, except for lungs where schistosomula were found on 2–3 days p.i.

Mouse model

By day 10 p.i., in 2 out of 8 infected mice (2/8) a paralysis of the hind legs developed; the paralysed mice were exposed to 1500–2500 cercariae/animal. One infected mouse (infection dose 500 cercariae/animal) became paralysed as early as day 4 p.i. and the symptoms ceased by day 7 p.i. The separately infected 4 mice were sacrificed by day 23 p.i.; they were without apparent symptoms.

All mice examined by day 10 p.i. hosted developing worms in the CNS (Fig. 1 C) (the lumbar spinal cord (3/8), thoracic spinal cord (7/8), cervical spinal cord (7/8), medulla oblongata (1/8) and cerebellum (3/8)). The hemispheres, nasal lobes and nasal mucosa were parasite free. Also other organs (see Materials and Methods section) did not contain parasites. Although worms that were delayed in their development (a schistosomulum comparable to the parasites from ducklings after 3–6 days p.i.) were usually found, in 2 cases young adults (worms growing comparably to those in ducklings) were detected in the thoracic spinal cord. A high affinity of worms to the nervous system was confirmed by exposing mice to 50 cercariae only; also in this case, the schistosomula were found in all 3 parts of the spinal cord. In 4 mice infected for 23 days, no living parasites were found in the CNS.

Feeding of parasites

During the migratory phase coincident with CNS impairment (in both ducklings and mice), a high

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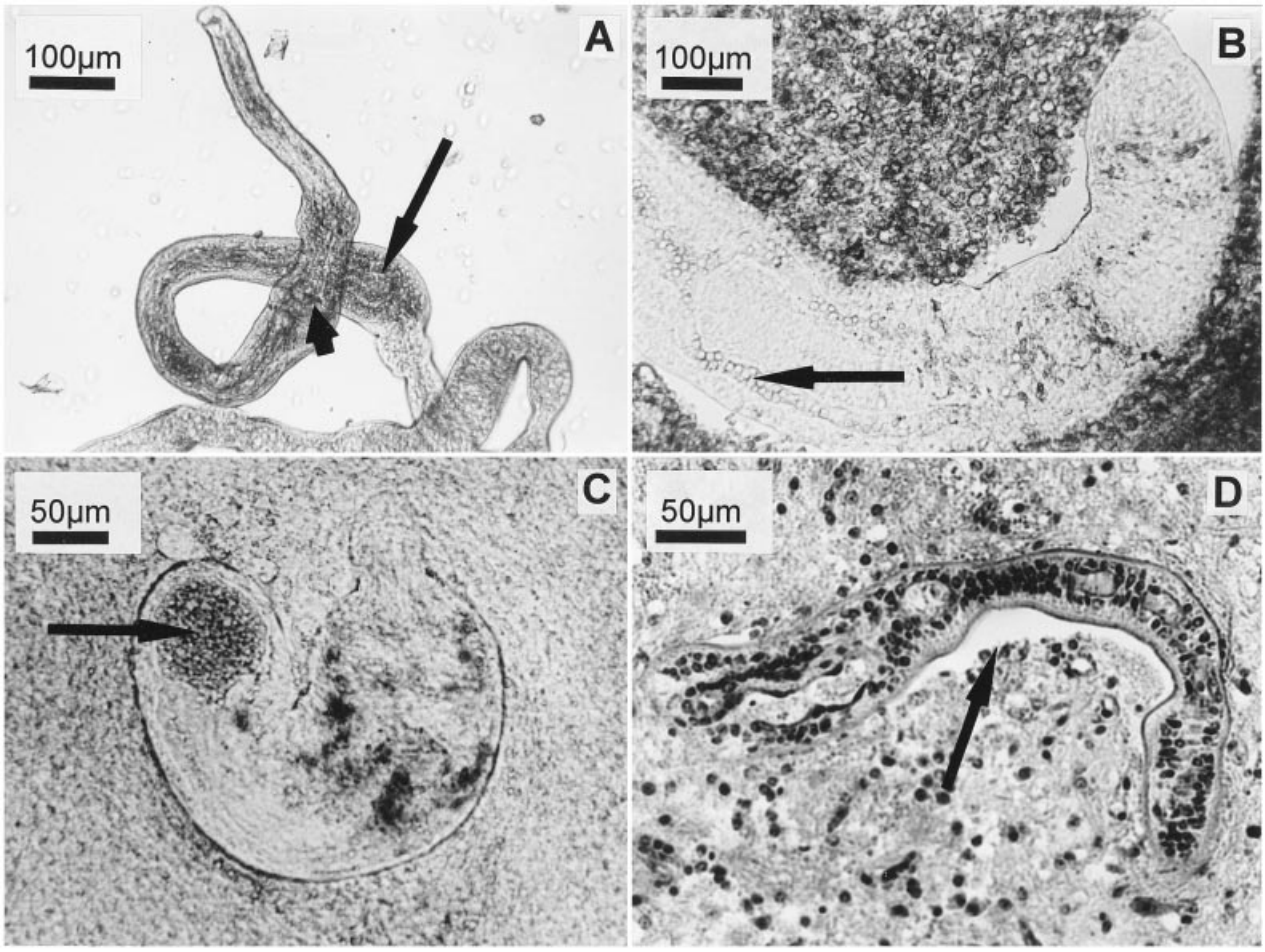

Fig. 1. Development of *Trichobilharzia regenti* in the central nervous system. (A) Adult male from synsacral spinal cord of a duckling (day 14 p.i.); several organs of the anterior body end – oral sucker, acetabulum (short arrow) and canalis gynaecophorus (long arrow) – are visible. (B) The intestine of growing worms (thoracic spinal cord of a duckling; day 7 p.i.) is filled by unidentified cells and granules (long arrow) of the surrounding nerve tissue. (C) The intestinal content (long arrow) is visible also in worms developing in mouse cerebellum (day 10 p.i.). (D) Histological examination confirmed that the worms (synsacral spinal cord of a duckling; day 14 p.i.) are located extravasally in the nerve tissue – there is no evidence for vessel walls around parasites (long arrow).

number of undetermined cells/granules of the surrounding host tissue was found in the parasite intestine; the worms apparently did not feed on host erythrocytes in this stage. When located in the nasal area of ducklings, the gut was filled by a dark blood-derived pigment (haematin) showing feeding of worms on red blood cells.

Histology

A study of sections of the infected spinal cord and cerebellum of both experimental animals (duck, mouse) confirmed that the developing worms migrated and developed directly in the nervous tissue; they were not residing within blood vessels. An inflammatory reaction with lymphocyte infiltration and undetermined degenerative changes was sometimes present around parasites or their tracks caused by migration in the nerve tissue.

DISCUSSION

Location in the nasal area of a host represents an unusual event in schistosomes. In mammals, only 1 species (*Schistosoma nasale*) occupies this site (see Rollinson & Southgate (1987) for review). Migration of the parasite to this location is, however, poorly described. The same is true for avian schistosomes. Nasal schistosomes of the genus *Trichobilharzia* have not been characterized during their migratory phase in the final host; only patent infections with adult worms in the nasal area have been reported (Blair & Ottesen, 1979; Blair & Islam, 1983; Palmer & Ossent, 1984; Islam, 1986*a*; Horák *et al*. 1998). In 1 case where the infected host died in the pre-patent period, the immature males of *T. arcuata* were found in the lungs and heart (Islam, 1986*b*).

In our experiments, it has been confirmed that young flukes of *T. regenti* are able to migrate and

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grow extravasally in the spinal cord and brain of ducklings and cause certain CNS dysfunctions. The parasite seems also to be pathogenic to mammals. The worms were found in the spinal cord and brain (mainly cerebellum) of mice infected by *T. regenti* cercariae and were absent from hemispheres, nasal lobes and nasal mucosa. The parasites were also found in the lumbar spinal cord of 1 rat (results not presented in this study). Certain parasites were inhibited in their development or even killed by the mouse host; nevertheless, many parasites were able to feed on nerve tissue and several individuals grew into young adults.

The relation of infection dose and pathology is confusing. Whereas a low dose of cercariae (50–500) resulted in subclinical infection of ducklings, high doses were followed by a broad spectrum of clinical symptoms, from leg paralysis, balance disorders and heavy rhinorhagia to an asymptomatic state. It is not known why a heavy infection can proceed without symptoms in some animals. Nevertheless, an effective killing of parasites by an immune attack is highly improbable: an asymptomatic infection resulted also in large numbers of adults and eggs in the nasal mucosa.

In our view, nasal schistosomes are highly specialized to the CNS. The worms appeared in the spinal cord and brain of animals (ducklings, mice) independently of the infection dose, i.e. even exposure to a low number of parasites (50 cercariae) resulted in the infection of CNS. Moreover, the worms were not present in other body organs. It should be pointed out that there is no general pattern in *Trichobilharzia* distribution in duckling CNS during late infection; whereas some parasites reached the nasal region and started to produce progeny, other worms remained within the CNS, mainly the spinal cord. The occurrence of many living/killed worms in the synsacral spinal cord raises a question concerning the orientation ability of worms within the CNS and/or the factors influencing their migration towards the nasal mucosa.

It is not yet clear whether all parasites reaching the nasal mucosa of a duckling migrate exclusively through the CNS or if some individuals can find the target tissue *via* the blood system without entering the CNS. In *T. arcuata*, immature males were collected from the lungs and heart by day 12 p.i. (Islam, 1986*b*). In *T. regenti* the schistosomula can be found in the lungs of ducklings 2–3 days p.i.; by day 3 p.i. they already invade the thoracic spinal cord. As the worms are not detected in the nasal region until they appear in the brain, it seems that the CNS represents the main route by which they reach the final location in the nasal mucosa. Frequency of the parasite occurrence in the CNS, advanced development, and active feeding of worms on CNS-associated cells also support our hypothesis that migration through the CNS represents an obligatory part of the life-cycle. However, the precise site and time of parasite entry into the CNS is not known; we can hypothesize that the blood supply of the spinal cord (e.g. *arteria spinalis*) might represent this gate. Nevertheless, this point needs to be further studied.

Three important points may be presented in conclusion. (a) The development of nasal bird schistosomes occurs in the CNS of the infected host; spinal cord and brain represent probable routes of parasite migration to the nasal mucosa. (b) In the CNS, the parasites develop outside of the blood vessels, directly in the nerve tissue, and feed on nerve-associated cells. The worms do not, therefore, parasitize solely the blood system of vertebrates. (c) The role of avian schistosomes as pathogens should be reconsidered. In birds, nasal schistosomes may cause serious clinical symptoms in the form of paralysis, balance disorders, rhinorhagia, etc. In mammals (including man), these parasites are usually known as agents of cercarial dermatitis. Nevertheless, although they are unable to reach adulthood in mammals, they possess the potential to invade the CNS of mice and, in certain cases, to induce CNS injury leading to paralysis. It should, however, be noted that the final clinical status is not simply derived from the infection dose, i.e. number of parasites which penetrated the skin and reached the CNS. The resulting pathology is certainly based on a particular host–parasite interaction which is probably influenced also by the size of the host and its immune reactions towards the parasite.

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