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PENETRATION OF *TRICHOBILHARZIA* CERCARIAE INTO MAMMALS: DANGEROUS OR NEGLIGIBLE EVENT?

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Summary:

Bird schistosomes and cases of human cercarial dermatitis occur worldwide, but the number of cases is not monitored. Experiments with two schistosomes, namely *Trichobilharzia szidati* and *T. regenti*, show that they possess potent tools to penetration bird and mammalian skin, as well as exhibit species-specific migration patterns within vertebrate bodies. Therefore, the infections may affect different organs/tissues *e.g.* lungs or spinal cord. In this minireview, the adaptations and pathogenic effects of bird schistosomes in experimental mammals are discussed, and some ideas/hypotheses on risks to humans from exposure to bird schistosome cercariae are expressed.

KEY WORDS : cercarial dermatitis, *Trichobilharzia*, *Schistosoma*, *Lymnaea*, *Radix*, cathepsin.

There are numerous reviews on bird schistosomes (mainly the genus *Trichobilharzia*) and cercarial dermatitis and, therefore, one may ask, why recall this topic? Of course, in comparison with human schistosomes infecting about 200 million people in tropical and subtropical countries and causing death of 11,000 people/year (Molyneux, 2006), the impact of bird schistosomes on human (or animal) health is substantially lower. Bird schistosomes and cases of human cercarial dermatitis occur worldwide (even in cold subarctic areas like Iceland); the disease can be reported from regions with fresh-, salt- or brackish water bodies, including geothermally warmed lakes (Skírnisson & Kolářová, 2005). The number of cases is not monitored probably due to self-curing character of the disease; only local epidemics are regularly reported (see e.g. Kolářová, 2007 for review). In line with this, only a few research laboratories are focused on bird schistosomes/cercarial dermatitis.

In total, two experimental models of bird schistosomes were established: *Trichobilharzia szidati* (= *T. ocellata*, see below for explanation) and *T. regenti*. It is of interest that the above mentioned model parasites employ different strategies in vertebrate hosts – they belong to visceral and nasal inhabiting schistosomes, respectively. In order to reach the target organ/tissue, the visceral schistosomes migrate via blood system, whereas *T. regenti* (the only bird nasal schistosome for which behavior within final hosts in known) migrates via peripheral nerves and central nervous system (see Horák *et al.*, 2002 for review). In this minireview we present the current knowledge on mechanisms of *Trichobilharzia* entry into vertebrate hosts. As well, the adaptations and pathogenic effects of bird schistosomes in experimental mammals are discussed, and some ideas/hypotheses on risks to humans from exposure to bird schistosome cercariae are expressed.

HOST FINDING AND PENETRATION

In order to find a proper vertebrate host and attach to its skin surface, *Trichobilharzia* cercariae respond to external or skin-associated stimuli (*e.g.* shadow, warmth, ceramides, cholesterol). After finding a suitable entry site the larvae (cercariae) penetrate the skin. Among other stimuli, unsaturated fatty acids also trigger the process of penetration (see Haas, 2003 for review). Nevertheless, these penetration stimuli are not strictly host-specific, because cercariae of bird schistosomes readily penetrate also the skin of non-permissive hosts-mammals (humans). This phenomenon can probably be explained by a high content of penetration signals (fatty acids) in the mammalian skin (Haas & van de Roemer, 1998).

Skin disruption is facilitated by products of cercarial penetration glands. There are at least two groups of compounds being synthesized within the penetration glands: lectins and histolytic enzymes. Lectin-like proteins specific for some non-charged saccharides (laminarin and lactulose) and glycosaminoglycans were found in *T. szidati* and *T. regenti* cercariae (Horák *et al.*, 1997; Mikeš *et al.*, 2005). Up to now, there is no direct evidence for a role of lectins in skin penetra-

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tion; specificity of these proteins indicates they might be involved in recognition of glycosaminoglycans in the host extracellular matrix.

A more thorough characterization of cercarial penetration tools was performed with histolytic enzymes of *T. szidati* and *T. regenti*. In their cercarial homogenates, serine and cysteine peptidase activities were detected. In *T. szidati* (in the original paper called *T. ocellata*, see below for explanation), the presence of a trypsin-like serine peptidase similar in some detail to *Schistosoma mansoni* elastase was reported by Bahgat *et al.* (2001) and Bahgat & Ruppel (2002). However, the activity of *S. mansoni* elastase is chymotrypsin-like (Salter *et al.*, 2000). In subsequent experiments the presence of an elastase orthologue in both *T. szidati* and *T. regenti* was not confirmed (Mikeš *et al.*, 2005; Dolečková *et al.*, 2007; Kašný*et al.*, 2007). Recent data indicate that the homogenates of cercariae can be contaminated by trypsin-like peptidases of snail origin (Salter *et al.*, 2000; Mikeš *et al.*, 2005; Dolečková *et al.*, 2007) and, therefore, the role of serine peptidases in general and cercarial elastase in particular as penetration tools of *Trichobilharzia* cercariae remains questionable.

On the other hand, there is strong evidence for the presence of cysteine peptidases in cercarial secretions of *T. szidati* and *T. regenti* (Mikeš *et al.*, 2005). This class of enzymes was studied mainly in *T. regenti*, in which cathepsin B-like enzymes were identified in sporocysts with developing cercariae (Dolečková *et al.*, 2007), mature cercariae penetrating vertebrate hosts (Kašný *et al.*, 2007) and schistosomula migrating through the host nervous tissue (Dvořák *et al.*, 2005). Whereas cercarial cathepsins were capable of degrading native keratin and collagens (types II, IV) indicating their role in skin penetration (Kašný *et al.*, 2007), the cathepsins from the intestine of schistosomula cleaved myelin basic protein (major protein component of the nervous tissue) demonstrating their function as digestive enzymes (Dvořák *et al.*, 2005).

The penetration process is accompanied by physiological and morphological changes of free-living cercariae; they become schistosomula and fully adapted to endoparasitism. As a consequence of these changes, the transformed worms need to avoid any further contact with the outer environment. Therefore, in the case of *T. szidati*, they follow chemical gradients of L-arginine and D-glucose to find deeper tissues and blood vessels (Grabe & Haas, 2004). During the process of penetration, cercariae (like in human schistosomes) shed their glycocalyx and build a new surface double membrane of the tegument. All this happens not only in the bird host, but also in the non-permissive experimental mammals (mice) (Horák *et al.*, 1998a). As well as, these surface changes can be observed in *in vitro* transformed and cultivated worms (unpublished results).

PATHOGENIC EFFECT ON MAMMALS

Mammalian (human) infections caused by *Trichobilharzia* and other bird schistosomes are usually linked with cercarial dermatitis; the parasites do not mature in mammals. In fact, cercarial dermatitis occurs in the already sensitized people, *i.e.* in people that had repeated contacts with schistosomes and can mount an efficient allergic-type hypersensitivity response (see Horák & Kolářová, 2005 for review). Such response comprises both the cellular (*e.g.* neutrophils, eosinophils, macrophages, $CD4^+$ lymphocytes and mast cells) and the humoral (specific antibodies, cytokines) immune reactions polarized towards Th2 (Kolářová *et al.*, 1994; Kouřilová *et al.*, 2004).

However, the course of infection after the initial contact of mammals (humans) with these worms remains rather mysterious. Experimental data show that the well pronounced cercarial dermatitis associated with immune protection is absent and the antigen-stimulated lymphocytes produce a mixed Th1/Th2 cytokine response (Kouřilová *et al.*, 2004). This suggests that the worms may escape from the skin and migrate through the mammalian body (e.g. Horák *et al.*, 1998a; Horák *et al.*, 1999; Horák & Kolářová, 2000; Blažová & Horák, 2005; Chanová *et al.*, 2007); to a certain degree, this can also happen after repeated contacts with the agent. Similarly to infections in birds, the worms can be found predominantly in the mammalian blood system, the lungs (visceral schistosomes, e.g. *T. szidati*), or in the nervous tissue (*T. regenti*). As a consequence of these larval migrations, some organs (lungs, spinal cord) can be impaired and associated disorders (including e.g. leg paralysis in mice infected by *T. regenti*) may develop (Horák *et al.*, 1999; Chanová *et al.*, 2007). Surprisingly, no immune cell-mediated damage to *T. szidati* worms in the lungs of experimental mice was detected. The intestine of some lung schistosomula was filled with host blood (Horák & Kolářová, 2000). Body growth of worms in the non-permissive host (mice) was reduced in comparison to that in infected ducks (Blažová & Horák, 2005). Therefore, we deduce the failure of bird schistosomes in mammals (at least in the case of non-sensitized animals/humans) might be caused by a lack of nutritional or signal factors in the non-permissive host body (Horák & Kolářová, 2000; Chanová *et al.*, 2007).

From the medical viewpoint, the fate/survival of *Trichobilharzia* worms after penetration into humans remains to be clarified. It was discussed by French authors that humans repeatedly exposed to cercariae of bird schistosomes sometimes present alterations in their general health status, accompanied by pulmonary disorders (Bayssade-Dufour *et al.*, 2001). Nevertheless, there is no direct evidence that the worms occur

either in the human lungs or even in the peripheral nerves/spinal cord (for infections by *T. regenti*), inducing possible neurologic disorders. As detection of specific antibodies in serum confirms only a previous contact with bird schistosomes (*e.g.* Kolářová *et al.*, 1994), validation of hypotheses on migration and pathogenic effect of these worms in humans requires implementation of new species-specific diagnostic tools (*e.g.* detection of parasite DNA in samples other than blood).

MEDICAL/VETERINARY IMPORTANCE OF SPECIES IDENTIFICATION

As mentioned above, particular bird schistosomes (see visceral *vs* nasal) can impair the host in various ways due to their affinity to different host organs/tissues. Therefore, the identification of schistosome species in the field and estimation of risks associated with infections by these worms seem to be essential from epidemiological and clinical viewpoints. Unfortunately, the taxonomic situation in bird schistosomes in general and the genus *Trichobilharzia* in particular is rather problematic. *Trichobilharzia* represents the largest genus within the family Schistosomatidae, covering over 40 species of bird schistosomes. In Europe, these worms have been recognized for about 150 years; the larval stage called *Cercaria ocellata* was described from *Lymnaea stagnalis* collected near Berlin in Germany in 1855, already (La Valette, 1855). Unfortunately, the original drawing of these cercariae does not correspond with our current knowledge of *Trichobilharzia* cercarial morphology, and subsequent descriptions of adults of *T. ocellata* (e.g. Brumpt, 1931) are poor (see Rudolfová *et al.*, 2005 for details). Contrary to this, many older findings of cercariae and/or adults from various localities and different snail/bird hosts were determined as *T. ocellata* (*C. ocellata*), ignoring possible species richness of the genus *Trichobilharzia* (see Horák *et al.*, 2002 for review, Rudolfová *et al.*, 2005). During the last two decades, the situation in Europe has changed substantially due to the discovery of three distinct species: *T. franki* (Müller & Kimmig, 1994), *T. regenti* (Horák *et al.*, 1998b) and *T. salmanticensis* (Simon-Martin & Simon-Vicente, 1999). Recently, molecular techniques (sequencing of nuclear and/or mitochondrial genes) revealed that the spectrum of European bird schistosomes is even broader and contains yet unknown species (Rudolfová *et al.*, 2005). It was thought that particular *Trichobilharzia* species are strictly host-specific in terms of their intramolluscan development (see Horák *et al.*, 2002 for review). Accepting this view, snail species found at a particular locality could predetermine parasite occurrence and associated infection risks. However, in light of recent molecular analyses, the concept of strict intermediate host specificity was weakened: a) One species of *Trichobilharzia* may use closely related snail species for its development. This was shown in compatibility tests (experimental infections) where *T. franki* developed not only in *Radix auricularia*, but to a certain degree also in *R. ovata*; similarly *T. szidati* used both *L. stagnalis* and *Stagnicola palustris* as intermediate hosts (Kock, 2001). b) One snail species may be used by more *Trichobilharzia* isolates/species. For example, there is evidence for an undescribed *Trichobilharzia* isolate/species developing in *Lymnaea stagnalis*, the usual intermediate host of *T. szidati* (Rudolfová *et al.*, 2005). c) Last but not least, morphological observations and molecular analyses of lymnaeid snails (namely the genus *Radix*) document confusion in systematics, *i.e.*, commonly used morphological characters for snail identification must be employed with caution. It is now clear that taxa and identification keys to these snails need major revision (Bargues *et al.*, 2001; Pfenninger *et al.*, 2006).

CONCLUSION

At least three hot topics can be seen in the research of cercarial dermatitis and *Trichobilharzia* interactions with vertebrates (humans): a) Future preventive measures can be focused not only on management of snail-free (infection-free) recreational water bodies (e.g. human intervention in the area of Lake of Annecy, France), but also on direct protection of the skin of people in contact with the infection agent (e.g. potential use of inhibitors blocking parasite penetration, or the already published high protection provided by cream formulation with niclosamide; Wulff *et al.*, 2007). b) The spectrum of bird schistosomes and their species-specific behavior/adaptations need to be studied further. Of course, such experimental work should also include the identification of snails as vectors of infection agents. c) Experimental infections of small mammals with cercariae of *T. szidati* or *T. regenti* demonstrate transitory worm migration within mammalian bodies. Therefore, in the case of human infections, it should be clarified in the future if there is any health risk other than cercarial dermatitis. In order to check this possibility, new diagnostic tools (e.g. based on DNA detection) need to be developed and introduced.

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