RECOVERY OF AVIAN SCHISTOSOME CERCARIAE FROM WATER USING PENETRATION STIMULANT MATRIX WITH AN UNSATURATED FATTY ACID

THADDEUS K. GRACZYK AND CLIVE J. SHIFF
Department of Molecular Microbiology and Immunology and Department of Environmental Health Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

Abstract. Avian schistosome cercariae that emerge from aquatic snails can penetrate human skin causing cercarial dermatitis resulting in serious skin disease in sensitized and immunocompromised people. A trap developed for Schistosoma mansoni cercariae was tested for recovery of avian schistosome cercariae. A matrix with an unsaturated fatty acid, linoleic acid stimulates attachment and penetration of Trichobilharzia spp. cercariae, and the immobilized larvae can be subsequently visualized. The number of trapped cercariae exceeded by 3 to 7 times the number of larvae expected on the surface of the trap, based on their random distribution in the water. Recognition, attachment, and penetration of Trichobilharzia spp. cercariae led to injection of more secretory products into the stimulant matrix than by Schistosoma mansoni cercariae. This method can assist in the identification of waters infected with avian schistosome cercariae so that human exposure to these parasitic larvae can be minimized.

INTRODUCTION

The three anthropophilic species of schistosome cercariae, Schistosoma mansoni, Schistosoma haematobium, and Schistosoma japonicum, produce a local skin reaction, dermatitis schistosomica. The avian schistosome cercariae also produce a local skin reaction known as freshwater cercarial dermatitis, i.e., “swimmer’s itch”, or marine cercarial dermatitis, e.g., “sea-bather’s eruption” or “clam-digger’s disease”. Freshwater or marine cercarial dermatitis is manifested by macular eruptions, diffuse erythema, persistant itching, and purpuric lesions.

The similarity of skin lipid composition between aquatic birds and humans may contribute to the erroneous penetration of avian schistosomes. Schistosoma mansoni cercariae are sensitive to stimuli from unsaturated fatty acids present in lipids on human skin surface. A doubly-unsaturated fatty acid, linoleic acid incorporated in a solid matrix, stimulated S. mansoni cercariae to adhere and penetrate the surface. Linoleic acid-treated matrix successfully performed like a trap in irrigation canals in Egypt and in ponds in Zimbabwe in recovering waterborne S. mansoni larvae.

Free sterols, cholesterol, fatty acids, and triglycerides stimulated penetration of Austrobilharzia terrigalensis and Austrobilharzia variglansis cercariae (agents that cause marine dermatitis) in vitro. Surface skin lipids of aquatic birds and humans have similar composition, and host-finding and penetration responses of avian schistosome cercariae, and if so, to determine if the stimulant matrix can trap these waterborne larvae. We selected Trichobilharzia spp. larvae because this genus is a notorious agent incriminated in severe and painful freshwater dermatitis.

MATERIALS AND METHODS

Trichobilharzia spp. cercariae originated from naturally infected Physa acuta snails collected from ponds located in a migratory wildfowl refuge. Infected snails were maintained in the laboratory. All experiments were carried out with freshly shed cercariae and dechlorinated tap water that had stood for several hr at room temperature to permit disappearance of air bubbles. The cercariae population consisted of a mixture of larvae from 3–4 snails. The trap consisted of a glass microscope slide, 22 × 75 mm, to which stimulant matrix in the form of clear nail varnish (Pavlon Ltd., Nyack, New York, NY) containing 1.78 × 10⁻² mM of linoleic acid (Aldrich Chemical Co., New York, NY) was applied as described previously. Control slides contained only the clear nail varnish base.

Fifty Trichobilharzia spp. cercariae were added to each of 5 glass petri dishes (5.3 cm in diameter) filled with 25 ml of prepared tap water. The slide with stimulant matrix was left in each petri dish for 30 min and then gently removed. Five control slides were similarly treated. In another experiment carried out in quadruplicate, five hundred Trichobilharzia spp. cercariae were introduced into a clear plastic dish, 22 × 14 × 4 cm, filled with 1.0 L of water. The slide with stimulant matrix was placed at the bottom center of the dish for 30 min and then gently removed with forceps. Control slides received identical treatment. Several independent trials were performed utilizing stimulant matrix exposed in a petri dish to various numbers of Trichobilharzia spp. cercariae to insure optimal staining and visualization of the trapped larvae.

Both dish types were located on a horizontal dissecting microscope (with the light off) so that the matrix was in the center of the examination area to allow observation of cercarial behavior patterns. The period of intermittent swimming versus the length of the resting phase was measured in the plastic dish with the aid of a stop watch to obtain 30 measurements from 3 trials (n = 90).

Linoleic acid-treated and untreated slides removed from the dishes were subjected to the same treatment. A drop of hematoxylin was added to the matrix surface, a 22 × 22 mm coverslip (4.8 cm²) was applied, and the area under the coverslip was examined microscopically and the cercariae were
Controls. In the container with the control slides, *Trichobilharzia* spp. cercariae preferentially remained near the water surface where they tended to be motionless. Eventually they showed an intermittent swimming movement ($\bar{x} = 7.3 \pm 1.4$ sec) directed mainly horizontally. The swimming movement alternated with a passive sinking ($\bar{x} = 2.5 \pm 0.8$ sec). Despite their movements most of the larvae were located in the upper layer of the water.

Stimulant matrix. Cercarial behavior in the container with linoleic acid-stimulant matrix differed from that of the controls. The larvae showed fast intermittent swimming ($\bar{x} = 11.1 \pm 2.4$ sec) directed predominantly vertically. The passive sinking was less frequent, of shorter duration ($\bar{x} = 1.5 \pm 0.9$ sec), and predominantly occurred near the bottom of the container. The durations of the swimming and sinking phases of cercariae stimulated by linoleic-acid matrix differed significantly from the unstimulated larvae (two sample $t$-test; $t = 1.55, P < 0.02$).

In both types of container, *Trichobilharzia* spp. cercariae attempted to penetrate the linoleic-acid stimulant matrix within the first 15 min of exposure. After 30 min, the untrapped cercariae were lying dead at the bottom. Remarkably, these larvae were swollen, tailless, and their ventral sucker protruded. Very few dead cercariae of normal appearance were found in the containers with control slides. *Trichobilharzia* spp. cercariae vigorously attached, probed, and attempted to penetrate the linoleic-acid stimulant matrix without creeping (Figures 1–4). A small fraction (less than 20%) of cercariae probed the matrix, and attempted to penetrate after a short time of creeping on the matrix surface (not exceeding 5 sec). The cercariae that probed the matrix left round gaps in the stimulant matrix (Figure 3); no gaps were present in the matrix of control slides. During probing a small portion of the penetration gland content was injected with a strong force into the stimulant matrix (Figure 2). Penetration was initiated by strong acetabular attachment to the stimulant matrix and through this junction the contents of the penetration glands were vigorously pumped into the matrix with a strong force (Figure 4). Injection of the penetration gland content was accompanied by contraction and elongation of the cercarial body and protrusion of the ventral sucker stained with hematoxylin.

FIGURES 1–4. Attachment and penetration responses of *Trichobilharzia* spp. cercariae induced by linoleic-acid stimulant matrix; larvae stained with hematoxylin.

**FIGURE 1.** Cercaria trapped by the stimulant matrix; detachment of the tail (open arrow), protrusion of the ventral sucker (arrow head), and extended area of proteolytic enzymes injected from the penetration glands into the stimulant matrix (closed arrow). Bar = 150 μm.

**FIGURE 2.** Cercaria in the act of probing the stimulant matrix; acetabular attachment with the matrix (open arrow), a small portion of proteolytic enzymes injected from the penetration glands into the matrix (arrow head), and eyespots (closed arrow). Bar = 40 μm.

**FIGURE 3.** A round gap in the stimulant matrix digested by proteolytic enzymes of a probing cercaria. Bar = 40 μm.

**FIGURE 4.** Cercarial body captured by the stimulant matrix; anterior and penetration organ (open arrow), protrusion of the ventral sucker (closed triangle), extended area of proteolytic enzymes injected from the penetration glands into the stimulant matrix (large closed arrow), and eyespots (small closed arrow). Bar = 55 μm.
sucker which enhanced horizontal attachment and movement by acting as a support and anchor. Approximately 10–15 sec after initiation of penetration, the tail was shed and the cercarial body movement slowed down and became motionless. Unlike in a few cercariae retrieved from the control slides, cercariae trapped by the linoleic-acid stimulant matrix shed their tails, their ventral suckers protruded, and the contents of the penetration gland secreted into the matrix formed extensive dark spots near the larvae (Figures 1, 4). Unstained larvae were less visible but still recognizable. Slides with trapped cercariae that were stained after approximately 3 weeks, open air-dried, and stored yielded satisfactory visualization and enumeration of the larvae.

**DISCUSSION**

Despite the fact that cercarial dermatitis is reported across the United States and around the world, the diagnosis is always presumptive as it is based on self-reported symptoms and exposure to water.\(^1\)\(^2\) This parasitic infection is now considered an emerging disease in Europe due to the increased public and economic impact of recreational and occupational outbreaks.\(^3\)\(^4\) Progressively more evidence has shown that bird-specific schistosomes can cause serious problems, i.e., neuropathology and neurologic and neuromotor disorders.\(^5\)\(^6\)

Recognition and penetration of linoleic-acid stimulant matrix by avian schistosome cercariae, *Trichobilharzia* spp., described in the present study was more intense than such responses showed by *S. mansoni* cercariae under similar conditions.\(^6\) The linoleic-acid stimulant matrix performed well as a trap for *S. mansoni* cercariae in natural waters in Egypt and Zimbabwe with retrieval rates from 30% to 100%.\(^6\) This stimulant matrix can be also used for trapping waterborne larvae of avian schistosomes. Identification of waters infected with bird schistosome larvae will certainly help to minimize human exposure. The method reported here is inexpensive, the stimulant matrix preparation is straightforward, identification of trapped cercariae is easy, and water-exposed traps can be stored dry prior to staining and examined at a convenient time.\(^6\) The matrix surface is sufficiently elastic so that larvae are firmly immobilized by the anchorage of their surface spines which prevents them from being washed off when the trap is recovered from water.

Behavioral patterns of avian and human, i.e., *S. mansoni*, schistosome cercariae are similar after these larvae are exposed to human skin.\(^3\) *Schistosoma mansoni* cercariae showed chemokinetic responses to linoleic acid when the source of the stimulus was distant.\(^7\) It is believed that similar to *S. mansoni*, stimulation of *Trichobilharzia* spp. cercariae was activated by micelles of linoleic acid released from the matrix and suspended in the water, and these micelles induced the irreversible penetration responses of the larvae. Suspension of hydrophobic fractions of chicken skin surface lipids in the water can also explain a large increase in the number of *Austrobilharzia terrigalensis* cercariae when the source of the stimulus was distant.\(^7\)

The role of the penetration-gland content injected into human skin is not well understood in the pathogenesis of cercarial dermatitis.\(^1\)\(^2\) Our stimulant matrix technique allows for direct quantitative comparison of the amount of the penetration-gland content injected by stimulated cercariae of *S. mansoni*\(^6\) and *Trichobilharzia* spp. During penetration *Trichobilharzia* spp. secreted quantitatively far more material than *S. mansoni* under the same conditions.\(^6\) Thus, invasion of human skin by avian schistosomes is associated with a heavier load penetration-gland secretion that is far more intense than during invasion by *S. mansoni*, an important factor which has not yet received attention in the pathogenesis and severity of cercarial dermatitis particularly in previously sensitized people.

Acknowledgments: We thank F. Barnes for her technical assistance.

Financial support: The study was supported in part by a grant (H630–951–2002) from the AKC Fund of New York, New York, and The Center For A Livable Future, Johns Hopkins University, Baltimore, Maryland.

Authors’ addresses: Thaddeus K. Graczyk, Johns Hopkins, University, School of Hygiene and Public Health, Department of Molecular Microbiology and Immunology, 615 North Wolfe Street, Baltimore, MD 21205; Tel: (410) 955-0105. Clive J. Shiff, Johns Hopkins University, School of Hygiene and Public Health, Department of Molecular Microbiology and Immunology, 615 North Wolfe Street, Baltimore, MD 21205; Tel: (410) 955-1263, Fax: (410) 955-0105. Reprint requests: Thaddeus K. Graczyk, Johns Hopkins University, School of Hygiene and Public Health, Department of Molecular Microbiology and Immunology, 615 North Wolfe Street, Baltimore, MD 21205.

**REFERENCES**