THE PENETRATION OF MAMMALIAN SKIN BY CERCARiae OF
TRICHOBILHARZIA SP. (TREMATODA: SCHISTOSOMATIDAE) FROM
SOUTH AFRICA

C. C. APPLETON and KRISTEN BROCK, Research Institute for Diseases in a Tropical Environment, South African
Medical Research Council, P.O. Box 17120, Congella 4013, South Africa

ABSTRACT
APPLETON, C. C. & BROCK, KRISTEN, 1986. The penetration of mammalian skin by cercariae of
Trichobilharzia sp. (Trematoda: Schistosomatidae) from South Africa. Onderstepoort Journal of Veterinary
Research, 53, 209-211 (1986)
The ability of the cercariae of Trichobilharzia sp., the commonest avian schistosome in South Africa, to
cause dermatitis in man is confirmed, and their progress through and resultant histopathology in rodent skin are
documented. These cercariae reach the subcutaneous tissue within one hour after exposure, and some migrate to
the lungs. Between 46 and 97 h later, those stranded in the skin have died, while those in the epidermis are
extruded by 97 h. There were haematological changes in the peripheral blood and a rapid rise and fall in the
mitotic rate of the basal epithelium of the epidermis.

INTRODUCTION
The most widespread avian schistosome in South Africa is an undescribed species of Trichobilharzia
(Appleton, 1982; 1986). It is a common parasite of the spurwing goose, Plectropterus gambensis, and its inter­
mediate host is the freshwater snail, Lymnaea natalensis. Anecdotal reports suggest that the cercariae of this
verbatim host is the freshwater snail, Lymnaea natalensis. Anecdotal reports suggest that the cercariae of this
blood-fluke are a cause of dermatitis in man (Appleton, 1984). This paper presents experimental evidence con­
firming this and describes the histological responses of mouse skin to penetration by these cercariae.

MATERIALS AND METHODS
Twenty unsensitized BALB/c mice were anaesthetized with a 1:4 ml dilution of pentobarbitone sodium (Sagatal)
at a dosage of 0.1 ml/mouse. Each mouse was then exposed for one hour to 2 to 7 cercarai (mean 4.4) freshly
emerged cercariae of Trichobilharzia sp., using the ring method of Smithers & Terry (1965). The diameter of the
rings used was 11.6 mm and the area of skin exposed was therefore 105.7 mm². Biopsies of the exposed skin of 2 mice
were then taken after each of the following intervals: 1, 3, 20, 28, 46, 73, 97, 120 and 216 h. The skin was then serial-sectioned at 5 mm and stained in haematoxylin and eosin. A control series of sections was
made of skin from an unexposed animal. Smears of blood of exposed mice to be recorded parallel to the
series of skin biopsies. The lungs of 3 animals were examined for evidence of cercarial migration by teasing the
them apart in 0.9 % saline (Bacha, Roush & Icardi, 1982).
A mitotic index, M/(I+M) X 100, where M = total mitotic and I = total interphase cells (Hsu, 1973), was
for the basal epithelium across the width of sectioned lesions in the epidermis. Between 34 and 883
(mean 333) cells were counted for each lesion, with 5-10 replicates per time interval.
Unless otherwise stated all measurements are given ± standard deviation.

RESULTS
Laboratory confirmation of the ability of the cercariae of Trichobilharzia sp. to cause dermatitis in man was
demonstrated by exposing the forearms of 3 volunteers to these larvae. In 2 out of 3 cases this resulted in the
development of itchy, erythematosus papules similar to those of avian schistosome dermatitis, as described by
Lethbridge (1979) and Shimuzu, Matsuoka & Ando (1981). A short-lived, prickling sensation was felt 5–10 min after the cercarai were pipetted onto the skin. These "pinpricks" itched for a further 30–60 min.
Small, hard papules with a diameter of approximately 4.5 mm, surrounded by an area of erythema, appeared
5–30 h later and itched for the subsequent 2–3 days. These papules persisted for 8–9 days before fading gradu­
ally.

Penetration of mouse skin
From the number of tails recovered from the rings after the exposures it was estimated that 85.2 % of cercarai
attempted to penetrate. Of these, approximately 41 % were found adhering by means of suckers to the outer
surface of the epidermis, while the remaining 59 % had entered the skin (Table 1). The mean depth of penetra­
tion, viz., outside of corneous layer to the centre of the
inflamed area, was 0.57 ± 0.09 mm (n=9), with a maximum recorded depth of 0.72 mm. It is noteworthy that, in
sections taken after 97 h, there was no evidence of cercarai in the epidermis, though they could still be found in the dermis. Except in 2 instances where they had not completely penetrated, all cercarai seen in the
sections were in cross-section. This indicates that they were lying at right angles to the presumed axis of
penetration.

While in most cases cercarai could be seen to have penetrated through the corneous layer of the epidermis,
there was evidence that some cercarai used hair follicles as an entry route, causing a folliculitis. Up to and includ­
ing 46 h, morphological features of the cercarai, such as the acetabulum and the pigment granules of the eye­
spots, could be recognized in the sections. At 73 h and later, only the pigment granules could be recognized,
and these were usually scattered and no longer in aggregations as shown by Van der Roemer & Haas (1984) for
fresh cercarai. Cercarai were therefore considered to have died between 46 and 73 h post-exposure. This com­
pares favourably with the LT50 of 60 h for cercarai in water (Fig. 1). Only one schistosomulum, measuring
529 µm, was recovered after 97 h from lung tissue from a mouse exposed to 300–350 cercarai. Some of these
schistosomula do therefore migrate from the dermis to the lungs.

Inflammatory response

Cercariae in the epidermis elicited very little inflammation. At 1 h post-exposure, a few lymphocytes and neutrophils were seen around the points of entry, and at 3 h, superficial ulcers had developed at these sites where the epidermis had been disrupted. A sparse population of neutrophils was visible around the cercariae themselves.

By 20 h the inflammation, consisting mostly of neutrophils, had become diffuse, and occasional macrophages and lymphocytes were present around the cercariae. Inflammatory crusts of packed neutrophils in keratin had formed at the penetration sites, and an unusually marked rate of mitotic division could be seen in the basal epithelium of the epidermis in these regions. At 28 h the cell population around the invading cercaria showed a chronic inflammatory response consisting mainly of lymphocytes and histiocytes. In contrast to earlier sections, very few neutrophils were present. By 46 h the response has changed and was again dominated by neutrophils. One cercaria was found lodged in a layer of connective tissue between 2 layers of muscle and had clearly passed through the outer of these layers.

At 73 h the inflammation had become reduced to localized patches of infiltration around the remains of the cercariae and measured 131.6 ± 15.5 x 99.3 ± 16.2 μm (n=17). These were mixed cell populations, consisting of lymphocytes, histiocytes and neutrophils. This situation persisted through the 120 and 216 h sections. By 120 h, however, these focal responses had assumed a granulomatous character with the appearance of several multinucleate giant cells in each granuloma and an increase in size to 193.9 ± 38.0 x 144.1 ± 42.2 μm (n=8). At 216 h, areas of dark pink coloration could be seen within the cytoplasm of these giant cells and are believed to be the remnants of the cercariae. No eosinophils were seen in any of the sections.

The mitotic index rose rapidly after exposure from a normal level of 0.4 % to 9.4 % after 21 h (Fig. 2). A slightly higher rate was probably reached a few hours earlier, at approximately 16 h post-exposure. This high level dropped just as rapidly to 2.0 % at 46 h and continued falling gradually to reach 1.7 % by 216 h.

Differential counts

Counts over a period of 194 h post-exposure (Fig. 3) provided evidence of 3 haematological changes of successively decreasing magnitude in the peripheral blood, viz., between 4-23 h, 46-65 h and 73-200 h. These corresponded respectively to (1) the initial neutrophil-dominated response, as recorded in the 3 and 22 h sections, the change to (2) a lymphocyte-dominated response and (3) to the death of the cercariae, coupled with the reduction of the responses to focal ones. After 100 h post-exposure, the lymphocyte and neutrophil levels showed only slight changes from normal, and these corresponded to the formation of granulomata around the remains of the stranded cercariae. Eosinophils were rare in these smears.

DISCUSSION

Like many species of avian schistosome, the cercariae of *Trichobilharzia* sp. from South Africa do cause dermatitis in man. These cercariae are able to penetrate mammalian skin rapidly and reach the vascularized dermal layers as well as the subcutaneous tissue within 1 hour of exposure. In previous studies (Brackett, 1940; Macfarlane, 1949), the cercariae of several North American and New Zealand species of *Trichobilharzia* were found not to have penetrated deeper than the Malpighian layer of the epidermis, though in both cases human skin was involved.
Yang, Tchou, T’ang, Ho & Luo, (1965) noted that Trichobilharzia spp. in China penetrated to the subcutaneous tissue of white mice within 1.5 h, while in human skin they failed to go beyond the papillary layer of the dermis. In Japan, Shimuzu et al. (1981) failed to locate cercariae of Giganobilharzia in sections of exposed human skin, but noted that all evidence of cellular infiltration was confined to the epidermis and papillary dermis. In the present study, as described in this paper are comparable with those following exposure to other species of Trichobilharzia (Brackett, 1940; Macfarlane, 1949; Shimuzu et al., 1981; Yang et al., 1965). The intensity and speed of the reactions would probably be accelerated in sensitized subjects.

Estimates of the longevity of these cercariae in unsensitized skin vary from 21 h (Yang et al., 1965), 29 h (Brackett, 1940), 31 h (Shimuzu et al., 1981) to <42 h (Macfarlane, 1949). The criteria used by these workers to indicate death are a general breakdown of normal cercarial structure and, more particularly, nuclear degeneration and cellular pyknosis. In the present study, as was noted earlier, identifiable cercarial structures were seen up to 46 h and most cercariae were therefore considered to have died between 46 and 73 h post-exposure.

Another feature of the present series of sections was the disappearance of cercariae from the epidermis of skin 97 h or more after exposure. This is probably due to their having been extruded as a result of an accelerated rate of skin growth in the affected areas (Macfarlane, 1949) and exemplified by the high level of mitotic activity recorded here up to approximately 28 h post-exposure (Fig. 2).

Lung migration by cercariae of avian schistosomes has been demonstrated for species of Trichobilharzia and Austrobilharzia in laboratory rodents and monkeys (Bacha et al., 1982; Olivier, 1953; Yang et al., 1965). The finding of only a single Trichobilharzia schistosomulum in the lungs in the present case shows that at least a small proportion of cercariae do enter the bloodstream and reach the lungs. The important question that we, in common with other workers who have investigated this consequence of schistosome dermatitis, still have to answer is whether or not this migration also takes place in man. The fact that Olivier (1953) demonstrated its occurrence in monkeys suggests that it might also occur in man, but the observations of Brackett (1940) and Macfarlane (1949) that congeneric cercariae failed to reach the dermis of human skin, or, as Yang et al. (1965) reported, penetrated only its outer layer, tend to militate against this.

ACKNOWLEDGEMENTS

We are indebted to Dr V. Chrystal, University of Natal Medical School, for advice on the interpretation of the histopathology and to Mr A. Saikoolal, Medical Research Council, for help with the exposure and bleeding of the rodents. This paper is published by permission of the South African Medical Research Council.

REFERENCES


SMITHERS, R. S. & TERRY, R. J., 1965. The infection of laboratory mice with cercariae of Schistosoma mansoni and the recovery of the adult worms. Parasitology, 55, 697–700.
