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THE STRUCTURE OF THE SHELL AND POLAR PLUGS OF THE EGG OF THE WHIPWORM, *TRICHURIS TRICHIURA* (NEMATODA: TRICHURIDAE) FROM THE SAMANGO MONKEY (*CERCOPITHECUS ALBOGULARIS*)

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ABSTRACT

APPLETON, C. C. & WHITE, BELINDA J., 1989. The structure of the shell and polar plugs of the egg of the whipworm, *Trichuris trichiura* (Nematoda: Trichuridae) from the Samango monkey (*Cercopithecus albogularis*). *Onderstepoort Journal of Veterinary Research*, 56, 219–221 (1989)

The structure of the shell of the egg of *Trichuris trichiura* was examined using light microscopy as well as scanning and transmission electron microscopy. The results confirmed its three-layered structure and provided evidence that the cores of the polar plugs, which could be seen to be extensions of the shell's middle layer, could be lost *en bloc*, either mechanically or chemically, and in this way probably provided an exit for the first stage larva at hatching.

INTRODUCTION

Eggs of the nematodes belonging to the whipworm family Trichuridae are characterized by being thick-walled, tanned, devoid of sculpture and having bipolar "plugs", "protuberances" or "opercula" which give the eggs a barrel shape. This thick eggshell undoubtedly plays an important role in promoting the survival, development and infectivity of the enclosed embryo (Wharton, 1980). The nature and purpose of the polar "plugs" are less clear and although accounts of their structure vary, they are presumably involved in the hatching process.

The structure of the shell and polar "plugs" has been described for 3 species of *Trichuris*, viz. *T. vulpis* from the dog (Inatomi, 1960), *T. suis* from the domestic pig (Wharton & Jenkins, 1978) and *T. trichiura* from man (Xianquin, Songshan, Wenquin, Boxia, Wensu & Li, 1986). The methodology employed by the above authors has differed; Inatomi (1960) used light microscopy, Wharton & Jenkins (1978) transmission electron microscopy (TEM) and several histochemical techniques while Xianquin *et al.* (1986) used scanning electron microscopy (SEM). In the present study, the eggs of *T. trichiura* were also examined but from a different host, the Samango monkey (*Cercopithecus albogularis*), using all 3 forms of microscopy.

Whipworms live in the caeca of their hosts and have a direct life cycle in which the eggs, containing undifferentiated embryos, are voided in the faeces. Over the subsequent period of approximately 3 weeks, depending on the prevailing temperature and moisture levels in the soil, the embryo develops to a 1st stage larva within the egg. It hatches only after having been ingested by another host.

MATERIALS AND METHODS

Samango monkeys (*Cercopithecus albogularis*) harbouring *T. trichiura* infections were collected at Karkloof, Natal (30° 24′ S; 30° 17′ E) in November 1987.

Light microscopy

Trichuris trichiura eggs were recovered from fresh *C. albogularis* scats using the formol-ether sedimentation technique of Allen & Ridley (1970). They were then measured to the nearest 0,1 µm with a micrometer eyepiece and photographed using a Leitz Laborlux 12 photomicroscope. Measurements were expressed as means ± standard error.

Scanning electron microscopy

Eggs were collected as described above and preserved in 4 % formalin until they were processed as follows for SEM: after collecting on 5 µm Millipore filter paper, they were rinsed in distilled water, placed in 2 % osmium tetroxide for 2 h, rinsed again, dehydrated through a graded alcohol series and critical point dried (cpd) in a Hitachi HCP-2 critical point drier. Others were air-dried.

With one exception, female worms dissected from *C. albogularis* were fixed in 4 % formalin and thereafter fractured under liquid nitrogen using a razor blade also cooled in liquid nitrogen. Pieces containing uterus were then dehydrated and critical point dried. A single specimen was fixed in 3 % glutaraldehyde buffered in 0,05 M phosphate buffer and critical point dried as described above, except that this specimen was rinsed in 0,05 M phosphate buffer and post-fixed in 2 % osmium tetroxide in the same buffer. Examples of these uterus-containing pieces were then mounted on brass stubs before being coated with gold palladium using a Polaron E500 sputter coating unit and viewed on a Hitachi S-570 SEM.

Transmission electron microscopy

Pieces of 4 % formalin-fixed female worm containing uterus were rinsed in distilled water, post-fixed for 2 h in 2 % osmium tetroxide, rinsed and dehydrated through a graded alcohol series before being embedded in Spurr's resin. Ultrathin gold sections were cut, collected on copper grids, stained with uranyl acetate, followed by lead citrate and

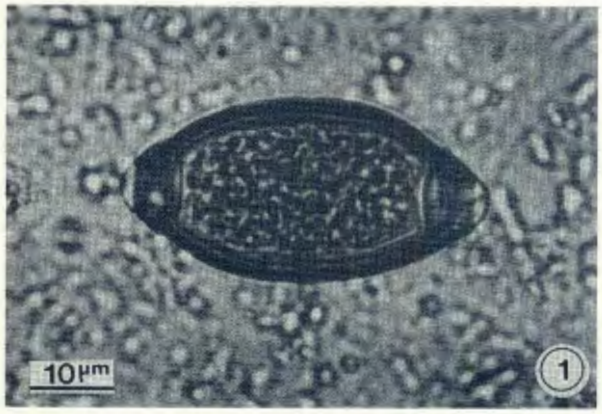

FIG. 1 Light-microscope photograph of the egg of *T. trichiura* from a faecal sample

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FIG. 2 TEM transverse section through the egg-shell of an intra-uterine egg, showing the vitelline layer (V), the chitinous layer (C), the lipid layer (L) and the embryo (E). The lamellae of the chitinous layer are clearly visible

FIG. 3 SEM micrograph of an intra-uterine egg, showing the collapsed polar plug

FIG. 4 TEM section of the polar plug of an intra-uterine egg, showing the expanded lamellae of the chitinous layer (arrow) and the core area (ca)

FIG. 5 SEM micrograph showing the empty core area of the plug (arrow) of an intra-uterine egg. The lamellae are also visible

viewed at 80 kV using a Jeol 100CX TEM. Measurements of egg-shell layers were made to either the nearest 0,1 nm or µm and expressed as means ± standard error.

RESULTS

The dimensions of *in utero T. trichiura* eggs were 62,4 ± 0,3 × 38,4 ± 0,4 µm (n=30) while those recovered from scats were slightly smaller, measuring 60,1 ± 0,4 × 26,9 ± 0,2 µm (n=30). Light microscopy showed the egg-shell to be multi-layered (Fig. 1) but the number of these constituent layers was not clear. TEM sections however, showed a shell comprising 3 layers (Fig. 2). The innermost layer varied considerably in thickness from 180 nm to 2,1 µm (mean 834,7 ± 157,1 nm; n=15). The thicker middle layer, which in the central part of the egg measured 1,7 to 3,8 µm (mean 2,3 ± 0,2 µm; n=15), consisted of 10 bands of electron-dense material alternating with less dense ones. The outermost layer, which could be seen to cover the entire egg, was the thinnest (30,4 to 144,0 nm; mean 61,4 ± 6,6 nm; n=20). Layering was indistinct when viewed using SEM.

Under the light microscope the outer surface of the polar plugs was seen to be convex in both fresh and preserved eggs (Fig. 1), but had collapsed during preparation for SEM (Fig. 3) and was recessed

into the collars of the egg-shell. TEM sections revealed the cores of the plugs to be extensions of the shell's middle layer (Fig. 4). The concentric arcs visible on one side of the plug can be seen to be continuations of the bands which comprise this layer but clearly do not traverse the core itself. The electron density of the plug cores was not uniform, being less dense in the peripheral areas. Using SEM on a fractured specimen, the contents of these cores could not be seen at all (Fig. 5).

While the outer surface of the *in utero* eggs had a wrinkled appearance, that of the voided egg was smooth and devoid of any decoration. No pores could be seen.

Discussion

The shell of the trichurid egg has been variously reported to comprise 1, 2 and 3 layers by Inatomi (1960), Xianquin *et al.* (1986) and Wharton & Jenkins (1978) respectively. The three-layered structure observed in the present material (Fig. 2) therefore confirms the findings of the latter authors. The innermost layer conforms to their lipid layer, the middle one to their chitinous layer and its bands to their "lamellae". The outermost layer equates with their vitelline layer.

The structure of the polar plugs, like that of the shell itself, has been the subject of debate. While the collapsed nature of the outer surface might have been an artefact of the preparation techniques, the fact that it had not happened in fresh or preserved eggs suggests that it could well occur in the natural environment after the eggs have been voided from the host. The variation of the electron density of the plug's core so clearly seen in Fig. 4, supports the contention of Wharton & Jenkins (1978) that its composition may differ from that of the rest of the shell and may perhaps be more susceptible to enzymatic action during the hatching process in the host's caecum. Failure to see any substance to the cores of fractured specimens under the SEM indicates that these contents were lost, either mechanically or chemically, during preparation. This loss led Xianquin *et al.* (1986) to conclude, mistakenly, that the "plug" was an empty cavity. The lack of sculpture is not surprising because trichurid egg-shells lack a 4th layer, the uterine layer, which forms the proturberances and spines that decorate eggs such as those of *Ascaris* spp.

Conclusions

Using *T. trichiura* from *C. albogularis,* this study has confirmed the 3-layered structure of the trichurid egg-shell and polar "plug" as proposed by Wharton & Jenkins (1978). It has also provided visual demonstration that, while the cores of the "plugs" are indeed continuations of the chitinous layer of the shell, they differ in composition from that in the adjacent shell wall. The absence of this core material in air-dried and critical point dried eggs suggests that it is a "weak-spot" which can be lost during the hatching process and that the term "plug" is an appropriate one.

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References

Allen, A. V. H. & Ridley, D. S., 1970. Further observations on the formal-ether concentration technique for faecal parasites. *Journal of Clinical Pathology,* 23, 545–546.

Inatomi, S., 1960. Submicroscopic structure of the egg shell of helminth. II. A study on *Trichuris vulpis. Acta Medicinae Okoyama,* 14, 257–260.

Wharton, D. A. & Jenkins, T., 1978. Structure and chemistry of the egg-shell of a nematode (*Trichuris suis*). *Tissue & Cell,* 10, 427–440.

Wharton, D., 1980. Nematode egg-shells. *Parasitology,* 81, 447–463.

Xianquin, M., Songshan, W., Wenquin, Z., Boxia, W., Wensu, H. & Li, W., 1986. The operculum-plug area and membranous structure of the eggs of *Trichuris trichiura. Scanning Electron Microscopy,* 111, 1015–1018.