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SCANNING ELECTRON MICROSCOPY OF THE EGG AND THE SECOND STAGE LARVA OF *TOXOCARA VITULORUM*

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دراسة سطحية بالميكروسكوب الإليكتروني للبيوض والطور اليرقي الثاني لطفيل توكبوكارا فيتللورم

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الملخص

تم الوصف السطحي بالميكروسكوب الإليكتروني للبيوض والطور اليرقي الثاني لطفيل توكسوكارا فيتللورم. والبيوض بيضاوية الشكل سطحها مزخرف بالحواف المتداخلة تحصر بينها منخفضات ضحلة. واليرقات تتميز بأحجامها الصغيرة والمدببة الأطراف وفتحة الفم مستديرة أو مثلثة الشكل وطرفية الموقع وتحاط بست (٦) شفاه غير واضحة المعالم. تمتد الحواف الكيتينية على جانبي الطرف الأمامي لليرقات. ويوجد اثنان من الثقوب الحسية هلى جانبي فتحة الفحم والفتحة الأخراجية كبيرة ومستديرة وتقع على السطح البطني للجسم ويبين السطح الخارجي لليرقات عوارض خطية غير واضحة. أما فتحة الشرح فهي مثلثية الشكل وتقع قبل نهاية الجسم على السطح البطني.

Key words: Surface topography – Egg, Larva, *Toxocara vitulorum*

ABSTRACT

The surface topography of the egg and 2nd stage larvae of the nematode *Toxocara vitulorum* are described by scanning electron microscopy. The eggs are oval and their surfaces are characterized by the presence of distinct ornamentations in the form of interconnecting ridges and shallow invaginations. The larvae have small cylindrical bodies with rather pointed extremities. The mouth opening is terminal and rounded or triangular in shape and is surrounded by six large ill-defined lips. Two prominent lateral alae are found slightly posterior to the cephalic end of the larva. Two large amphidial pores are found on the lateral sides of the mouth opening. The excretory pore is a large rounded opening on the ventral surface of the body. The cuticular surface of the larva is ornamented with ill-defined transverse striations. The anal opening is triangular and located subterminally on the ventral surface of the posterior extremity of the body.

INTRODUCTION

Toxocara vitulorum is an ascaridid nematode which is common in calves and is possibly a major cause of human toxocariasis in tropical and subtropical countries (1). The hatching larvae migrate into the soft tissue of hosts other than bovines to cause ocular and visceral larva migrans (2).

Identification of nematode eggs and their larvae is important in the taxonomy and control of these parasites. The pitted outer layers characteristic of the eggs of ascaridids, including *Toxocara* species (3) have been studied by Scanning Electron Microscopy (SEM) (4,5,6). However, little has been reported for SEM of *T. vitulorum* eggs (7,8). Also, no description is available of the 2nd stage larvae except by light microscopy (9). The present work aims at studying the surface topography of *T. vitulorum* eggs and its 2nd stage larvae by SEM.

MATERIAL METHODS

The embryonated eggs and the mechanically hatched 2nd stage larvae of *T. vitulorum* were washed in phosphate buffer and dehydrated in a series of ethanol, then transferred to mixtures of amylacetate and alcohol (2:1, 1:1 & 1:2) before being dried in CO_2 critical point drier and coated with gold/ palladium and examined in Jeol SEM.

RESULTS

The eggs of *T. vitulorum* are rounded to oval in shape each measuring 68-95 µm in diameter. The surface of the egg is ornamented with prominent ridges. These ridges interdigitate leaving in between, small circular or polygonal depressions (Figures 1 & 2). The 2nd stage larvae were seen emerging from the mechanically broken shell (Fig. 3). No definite operculum was seen on the egg. The larva is small cylindrical with a rather narrow anterior extremity. It is ensheathed in a loose cuticle that forms several wrinkles, particularly at the cervical region (Figs. 4-6). The mouth opening is roughly circular to triangular. It lies on the top of the cephalic plate, and is surrounded with six massive and ill-defined lips. In some larvae, the cuticular incisions separating these lips are not deep, making some of them appear as being fused together (Figs. 7, 8 & 9). Two large amphidial pores are found on the cuticular surface, on both lateral sides of the mouth opening (Fig. 8). The body cuticle of the larva appears transversally striated. These striations are not as regular as those reported in the adult worm (Figs. 10 & 11).

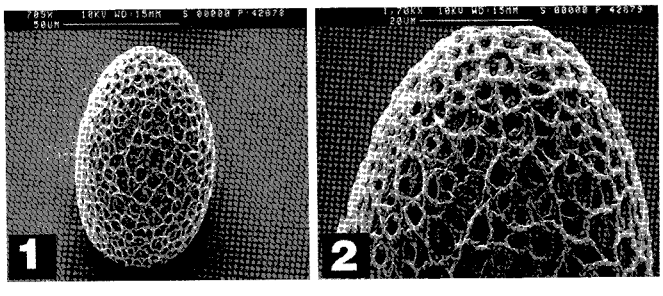


Fig. 1: The egg surface, showing characteristic ridges and depressions.

Fig. 2: Egg surface enlarged, showing interconnecting ridges and depressions.

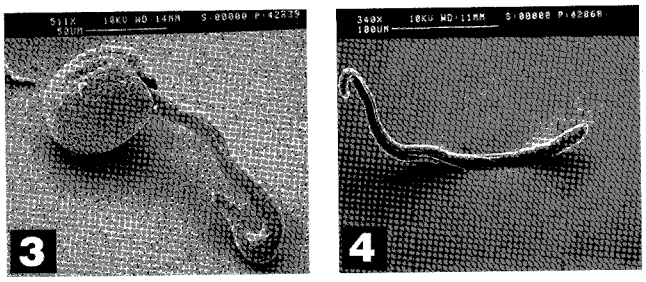


Fig. 3: Larva emerging from the egg. Note absence of operculum.

Fig. 4: The whole larva, showing a rather pointed anterior end.

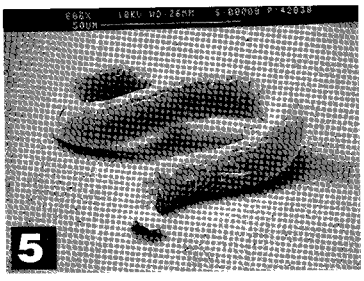


Fig. 5: Whole larva enlarged.

Two prominent lateral alae are commencing a short distance posterior to the celphalic extremity and extend on both lateral sides of the larva (Figs. 6 & 7).

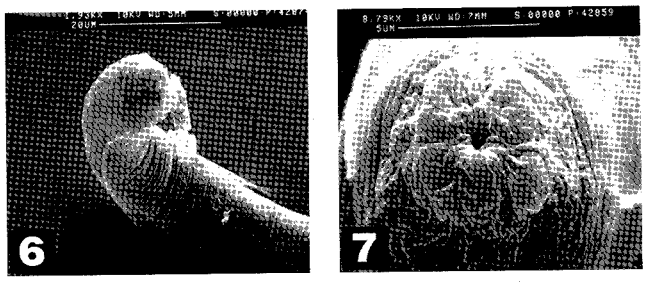


Fig. 6: Anterior extremity, showing lateral alae and excretory pore.

Fig. 7: En face view, showing mouth opening, lips and lateral alae.

The excretory pore (2.2 µm diameter) is distinctly wide, forming a simple circular opening on the ventral surface, at the anterior half of the body (Figs. 6, 10 & 11). The anal opening is smaller (1-2 µm) located on the ventral surface of the most posterior narrow knob (Fig. 12).

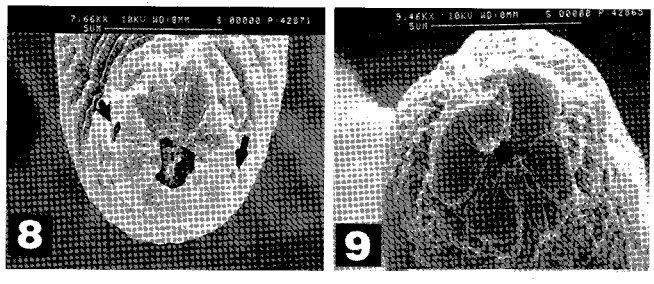
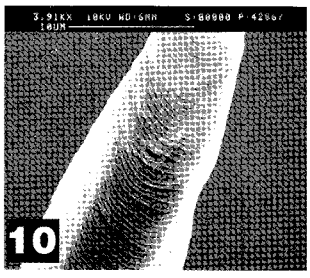


Fig. 8: En face view, showing amphidial pore (arrow).

Fig. 9: En face view, showing mouth opening and lips.



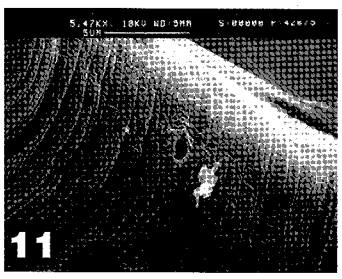


Fig. 10: Anterior extremity, showing body transverse striations and excretory pore.

Fig. 11: Enlarged cuticular surface, showing poor cuticular transverse striations and excretory pore.

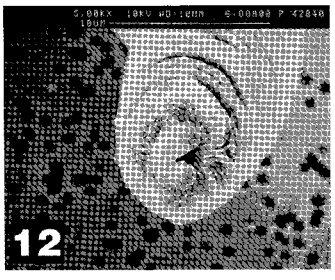


Fig. 12: Posterior extremity of the larva, showing anal opening and the surrounding cuticular thickening (knob).

DISCUSSION

The distinct ridges on the surface of *T. vitulorum* eggs, as revealed in the present study, are similar to those occurring on the egg shells of the ascaridid nematodes *Ascaris lumbricoides, A. suum, T. canis* and *T. mystax* (Figs. 4, 10). However, Mohamed *et al.* (8), using both SEM and TEM reported the presence of specific morphological differences between the egg shells of *T. canis* and *T. vitulorum*; this was basically in the nature and thickness of the surface ridges and in the relative thickness of the shell layers.

The presence of six lips in the larva, while only three lips are known to occur in the adults, suggests that each two lips in the larva will fuse together in the adult worm and the number of these lips will be reduced to only three (developmental modification). A similar view was also held by Chitwood (Fig. 11)), who reported that the arrangement of the cephalic papaillae and the number of lips in the primitive nematodes are reduced in the advanced ones (phylogenetic modification).

Previous contributions to the morphology of larval stages of *T. vitulorum* were based almost on observations with the light microscope (Fig. 3). The present study appears to be the first report describing the detailed morphology of the infective larva of *T. vitulorum* by SEM.

Lichtenfels *et al.* (Fig. 12) using SEM established the morphological characteristics of the infective larva of the nematode *Haemonchus contortus* and reported on the usefulness of SEM in obtaining information on the ultrastructure of those medically important parasites, including provision of new characters for studies in systematics and for species identification and improved capabilities for separating developmental stages.

New information on the second stage larva of *T. vitulorum* provided herein include description of the mouth opening, lips, amphidial pores, lateral alae, excretory pore and anal opening.

The value of the present study will be apparent after comparative information for larvae of related species become available. This study may be helpful in histopathological diagnosis of Toxocariasis in man.

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