

SCANNING ELECTRON MICROSCOPY OF INFECTIVE LARVAE OF *HAEMONCHUS CONTORTUS* OF DOMESTIC RUMINANTS*

N. Jeya Thilakan¹ and V. Sathianesan

Department of Parasitology
College of Veterinary and Animal Science
Kerala Agricultural University
Mannuthy, Trichur, Kerala

ABSTRACT

The external features of infective larvae of Haemonchus contortus, the large stomach worm of ruminants were described and illustrated in detail from scanning electron microscope.

Key words : *Haemonchus contortus*, infective larvae, SEM

Haemonchus species are bloodsuckers and are important parasites of domestic ruminants.

ACKNOWLEDGEMENT

We thank Dean, for the facilities provided and Dr.T.Sree Kumaran, SEM Laboratory, Department of pathology, College of Veterinary and Animal Sciences, KAU, for the scanning electron micrographs and ICAR for financial assistance by way of Junior Research Fellowship to the first author.

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Haemonchus contortus (Rudolphi, 1803) is the prime species occurring in the mucosa and contents of the abomasum of sheep goats, cattle and numerous other ruminants in many parts of the world being especially important in areas with a hot, moist climate. In India, it is considered to be the most important intestinal parasite of sheep and goats (Sood, 1982). The infective larvae is of special interest because it is the form that can persist on pasture and is the larva that is used routinely in identification of the species. In India previous contributions to the morphology of infective larval stages of *Haemonchus contortus* were almost all been based on observations with the light microscope (Sathianesan and Peter, 1977, Tripathi, 1968 and Chauhan *et al* 1973). SEM study of infective larvae of nematodes is more accurate than the routine methods for identification since the ultra structural differences are also seen (Lichtenfels *et al.*, 1990). Fine structure characteristics, as seen with SEM of infective third stage larvae of *H.contortus* has not been adequately described and there fore are presented here in.

* Part of M.V.Sc. thesis of the first author submitted to the Kerala Agricultural University

1. Assistant Professor, Department of Veterinary Parasitology, Madras Veterinary College, Chennai-600 007.

MATERIALS AND METHODS

Infective larvae preparation for SEM was done as per Nakamura *et al.*, (1984) and Lichtenfels *et al.*, (1990). Freshly collected live larvae from Coproculture were cleared of all the adhering dirt and debris by repeated washings with distilled water. They were then killed by immersing in boiling water for 10 seconds, quickly cooled by addition of ice and pelleted by centrifugation at 3000 rpm for 5-10 minutes. The supernatant was discarded slowly and the larval pellet was separated out. The larvae were then fixed in 20 volumes of 4% glutaraldehyde at room temperature for 24 hr. After fixing they were rinsed thrice each time for 10-15 min with 0.1 M phosphate buffer solution to remove the fixative, followed by washing with distilled water for 30 min to remove the buffer salts and other electrolytes present in the fixative. Dehydration of fixed specimens was carried out in ascending grades of ethanol and finally with several washes in 100% ethanol, the duration being 10-15 min. They were then critical point dried, mounted in metal stubs, gold coated and examined in a Hitachi model 530 scanning electron microscope at an accelerating voltage of 15 k.v. scanning electron micrographs were also taken by using Ilford 35 mm black and white film. The measurements of larvae were taken under light microscope using micrometer. Camera lucidia drawings of infective larvae were also done.

Results and Discussion

The length of the infective larvae ranged from 675-780 μ m and 19.5-24 μ m wide (Fig. 1a). The larval sheath was not loose, but was projecting beyond the head end (Fig. 2a). The length of the Oesophagus is 127-157 μ m (Fig. 1b). The oesophagus was not well demarcated (Fig. 2a). A central dark longitudinal line was seen through out the middle of the body (Fig. 2b). The tail end of the larva was pointed (Fig. 2c). The length of the tail was 55-66.5 μ m (Fig. 1c). The tail sheath end was also pointed (Fig. 2c). The length of the tail sheath was 63.5-77 μ m long. The intestinal cells were clear in

light microscope and also the kink in tail sheath was prominent under light microscope (Fig. 1b & c).

Previous contributions to the morphology of infective larvae of *Haemonchus contortus* have almost all been based on observations with the light microscope. Emphasis has been on certain characteristics of the infective larvae, especially the shape and size and its major organs such as the oesophagus, intestinal cells, tail nerve ring, excretory pore, anus rectum etc. for distinguishing the species from other soil borne nematode larvae that infective domestic ruminants. These light microscope morphological studies were of Tripathi (1968), Chuhan *et al.*, (1973) and Sathianesan and Peter (1977).

Previous work on the fine structure of infective larvae of nematodes are scanty. Only a few authors studied the infective larvae of nematodes, like *Strongyloides planiceps* (Arizona *et al.*, 1976), *Angiostrongylus cantonensis* (Ho lian yin *et al.*, 1984) *Nippostrongylus braziliensis* (Nembo *et al.*, 1993), *Anisakis simplex A* (Larizza and vovals 1995) and *Strongyloides papillosus* (Jeya Thilkan and Sathianesan 1999). Lichtenfels *et al.*, (1990) described the external morphology of infective larvae of *Haemonchus contortus* under SEM. According to them the larval stages had an inner circle of 6 labial papillae, an outer circle of 6 labial papillae and 4 somatic papillae, and lateral amphidial pits. But the above structures were not identified in the present study. The ultimate value of the basic observations described herein will be determined after similar information for other related species becomes available. Some possible uses for this sort of information include provision of new characters for studies in systematics and for species identification. Thus comparative studies of morphology of larvae under SEM may be of great scientific interest.

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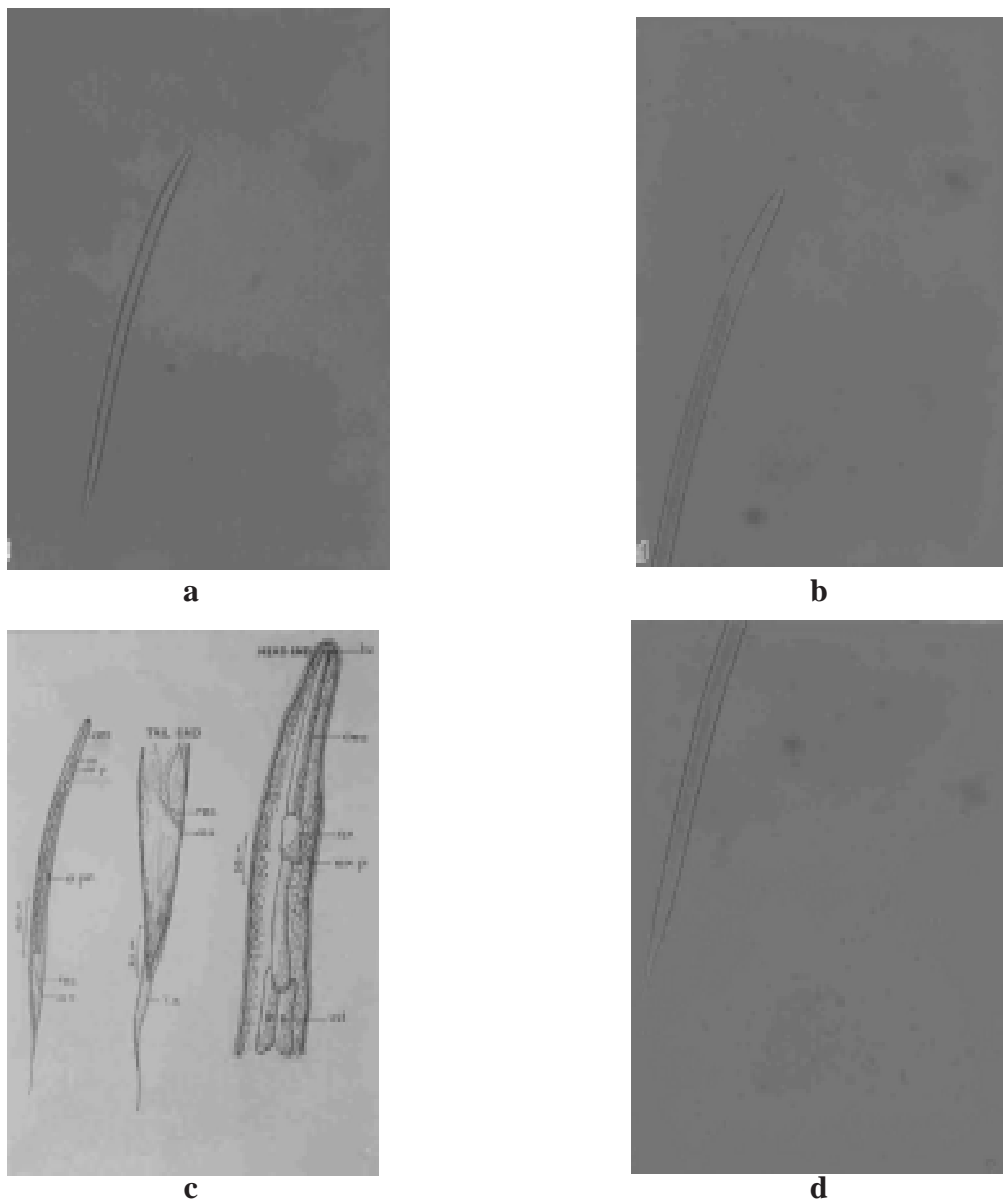


Fig. 1

Showing light photomicrographs of infective larva *Haemonchus contortus*

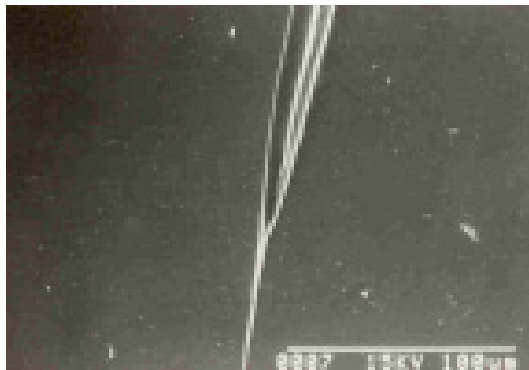
a. entire larva, b. Head and showing the clear view of oesophagus and packed intestinal cells (x250), c. Tail and showing the kink in tail sheath (250), d. camera Lucida drawings showing the various parts of larvae (bc-buccal capsule, oesoesophagus, nr-nerve ring, ex.p-excretory pore, g. pr-genital primordium, intestinal cells, rec-rectum, an-anus, t.s-tail sheath)



a



b



c

Fig. 2

Showing scanning electron micrographs of infective larva of *Haemonchus contortus*

- a. head and of the larva showing sheath projecting beyond the head and ill-demarcated oesophagus,
b. The middle portion of larva showing central longitudinal line, c. Tail end showing the pointed tail and tail sheath.