Dept. of Zoology, Faculty of Science, Assiut University.

LIGHT AND SCANNING ELECTRON MICROSCOPICAL OBSERVATIONS ON ADULT HAEMONCHUS PLACEI (NEMATODA: TRICHOSTRONGYLOIDEA) IN CAMELES AT ASSIUT, EGYPT

(With 18 Figures)

By
NAWAL A. M. MAZEN
(Received at 30/3/2005)

ملاحظات بالميكوسكوب الضوئي والألكتروني الماسح لديدان الهيمونيكس بلاسى في الجمال-أسيوط-مصر.

نوال عبد السميع محمد مازن

الدراسة أظهرت وجود تجويف فمى صغير به لسان ظهرى و 7 حلمات شفية داخلية و 7 حلمات شفية داخلية و 7 حلمات شسفية خارجية و ٤ حلمات جسدية و ٧ ثقب أمقيديا جانبية. الحلمات الرأسية دائمة وتشبه الشوكة. الثقب الاخراجي موجود. كيس التسافد الذكرى له فصوص جانبية مدعمة باشبعة رفيعة. الشوكة السفادية للذكر مزودة بفتحة قريبة من نهايته. الفتحة التناسلية للأنثى مغطاة بزائدة لسانية والفتحة الشرجية لها حافة ظهرية وحافة عضلية بطنية.

SUMMARY

The tegumental ultrastructure of adult *Haemonchus placei* was studied by scanning electron microscopy (SEM). Adult worms were recovered from fourth stomach or abomasum of cameles. The study revealed the presence of a small buccal cavity with a dorsal lancet, 6 internal labial papillae, 6 external labial papillae, 4 somatic papillae and two lateral amphidal pits. The cervical papillae were prominent and spine-like, measured (370µm). The excretory pore was present and measured (315 µm) in diameter. The male bursa bursa had elongated lobes supported by long, slender rayes. The small dorsal lobe was asymmetrical with Y-shaped dorsal rays. The spicules are long, each provided with a small barb and pore near its extremity. The vulva of the female by a linguiform process or flap, and the anal pore at the posterior end of the body had a simple dorsal rim and a muscular ventral rim.

Key words: Nematode, Trichostrongylidae, Haemonchus placei, morphology, scanning electron microscopy.

INTRODUCTION

Scanning electron microscopy (SEM) was used to examine the surface ultrastructure of worms *Haemonchus placei* (Place, 1893) which is a blood sucking abdominal nematode responsible for extensive losses in sheep and cattle, ruminant especially in tropical areas (Urquhart *et al*, 1991). Also, revision of the genus *Haemonchus* was carried out by Gibbons in (1979). In another study, Gibbons (1986) illustrated some scanning electron micrographs of *Haemonchus placei*. The pattern of cuticular ridg of *Haemonchus placei* was studied by Lichtenfels *et al*. (1986).

The aim of the present study is to provide additional information on morphology of the adult male and female of *Haemonchus placei* by using light and scanning electron microscopy.

MATERIALS and METHODES

Fifteen sexually mature adults (5 males and 10 female) of *Haemonchus placei* were collected from fourth stomach (abomasum) of naturally infected camels in Assiut, Egypt. The worms were and cleaned with 2% sodium cacodylate buffer (pH 7.2).

Diagnosis of species was confirmed on the basis of light microscope examination and using the keys of Lichtenfels, *et al.* (1988). For scanning electron microscope, the nematodes were transferred in fresh 4 % glutiraldehyde for 2hs, postifexed in 1 % osmium tetraoxide in cacodylate (pH 7.2) for 1h.at 4C and dehdrated through a series of ethanols, they were dried in a liquid Co2 critical point apparatus, ladd, 2800. The specimens were placed on precleaned aluminum stabs, coated with gold in an ics and examined by the scanning electron microscope at an accelerating voltage of 15 kv.

RESULTS

The present study reveald that the camel (Camelus dromedarius) is a new host for the adult worms of (Haemonchus placei). According to the key of Lichtenfels, et al. (1988), the anterior ends in both sexes had similar cuticlar surface including cuticular transversal and ridges or synlophe (Fig.3) cuticular ridges which were absent at the beginning of

the worm (neck) (Fig.1). The synlophe of *Haemonchus placei* has 34 ridges in the posterior half of the oesophagus function is locomotion or the attachment of the host (Durette – Desset, 1969). On the ventral side of the anterior end, the excretory pore is situated and measured 270 – 360 (315 μ m) in diameter (Fig.2). The cervical papillae are spine – like 340 – 400 (370 μ m) and situated on the lateral sides of the anterior end (Fig.4). A small mouth is surrounded by 6 internal labial papillae, 6 external labial papillae, 4 somatic papillae, and small amphidal apertures are also present, (Fig. 5a&b).

The bursa of the male had elongate lobes supported by long rays, while the dorsal lobe is asymmetrical and supported a Y- shaped dorsal ray (Figs 6a&b). On each lateral side, at the beginning of the copulatory bursa, one single prebursal papillae can be observed (Fig.6a). The spicules of the male measured 400-480 ($440~\mu m$)in length, the surface of spicules are smooth. The dorsal tips of spicules each had a single barb at unequal distances, the barb on the left spicule is 40-55 ($47.5~\mu m$) distant from the tip, and the right spicule 25-35 (30um) in length (Fig.13). There is a single small pore at the end of spicule (Fig.13). Dorsal lobe (DL) and -Y- shaped dorsal ray with one pair of papillae on each extremity (double arrows) (Fig.12).

The vulva is located at the beginning of the posterior third of the female body 75-80 (77.5um) from the anterior extremity, it is covered by an anterior flap which takes the shape of a protruding knob (Fig.14). The surface of the vulval flap is covered by cuticle with transversal striae.

Scanning electron observations showed longitudinal cuticular ridges present only in the first and second third of the body. The anal pore is situated on the ventral side of the posterior end of the body (Fig.18). The tail of female length 360-700 (530 μ m) in diameter (Fig.16). SEM observations showed one botton like phasmid right / left 115-200 (157.5 μ m) distance from distal tip of tail (Fig.8 μ).

DISCUSSION

Haemonchus placei (Place, 1893); Ranson, 1911, is primarly a parasite of domestic cattle (Bas taurus) but it has been found also in domestic sheep, white tail deer (Odocoileus virginianus), and pronghorn antelope (Antilocapra americana) (Lichtenfels et al. 1994). Haemonchus placei is not universally accepted as a separate species (Gibbon, 1979). Lichtenfels et al. (1986) described differences between Haemonchus

placei and Haemonchus contortus in the percentage of the body covered by the synlophe, distinctive characteristic of the spicules, female reproductive tract, and female tail which allowed Haemonchus similis to be separated from Haemonchus placei and Haemonchus contortus.

The present study recognized *Haemonchus placei* in a new host the camel (*Camelus dromedaricus*), according to key to species of *Haemonchus* in North America by Lichtenfels et al. (1988). The means of the distances from spicule barb to distal tip can also be used to separate *Haemonchus placei* from *Haemonchus contortus* males. Females of *Haemonchus similis* can be distinguished from those of *Haemonchus placei* by their significantly shorter tail length.

The main differences between the three species of *Haemonchus* according to Lichtenfels *et al.* (1994) depend on the shape of vulval flap and cervical papillae, tip of spicules and number of longitudinal ridges. Both *Haemonchus similis* and *Haemonchus placei* bear 17 dorsal and 17 ventral longitudinal cuticular ridges in region of the posterior half of the oesophagus, but in *Haemonchus contortus* there are only 15 ridges dorsally and 15 ventrally, this number was constant in both males and females. In the present work, the morphological characteres agreed with that described to *Haemonchus placei*. Gibbons (1979) showed that the relative position of the spicule barbs are quite variable among the species of the genus *Haemonchus*.

The caudal bursa, characteristic of the order: Strongylata, consists of two latero-ventral lobes a dorsal lobe which may or may not be fused with the latero-ventral lobes (Durette – Desset, 1985). The caudal papillae are generally situated at the extremines of the bursal rays as also described by Chitwood and Chitwood (1950). Caudal sensory structures are situated at the end of long peduncles (bursal rays) which extend into the bursa.

In the present work the vulva was located at the beginning of the posterior third of the body of the female at 77.5 µm distance from the extriemety. Chitwood (1957) grouped the females of *Haemonchus* depending on the presence or absence or shape of vulvar flaps into three basic types linguiform; knobbed and smooth without flaps.

Variation in vulvar region were also noticed in specimens from different hosts (Roberts, 1954). The present observation showed that the vulva was covered with a flap a protruding knob and supported by two lateral knobs. Daskalov (1972) considered that the vulvar flaps have no taxonomic importance and are age related. Privious work on immature forms of *Haemonchus* Lichtenfels et al. (1990) showed that phasmidal

<u> Assiut Vet. Med. J. Vol. 51 No. 105 April 2005</u>

ducts opening on the body wall halfway between the anus and the distal ends of the lateral alae slightly ventral to the alae. On the other hand the phasmids in this work was observed near to the posterior extremity of adult which agreed with Naem and Seifl, (1998). Recent transmission electron micrograph studies by Wang and Chen (1985) and a special staining method for nematode secretion by Premachandran et al. (1988) have demonstrated that phasmids have a secretory function. However, phasmids are also believed to be chemoreceptors and the secretory function may be limited to the production of substances that serve as a filter for chemoreception (Carta and Baldwin, 1990).

The present study indicates the presence of differences in the cuticular ridges of adults *Haemonchus placei* in different parts of their bodies. The nematode cuticle has important basic functions both as a structural framework and acting to protect the organisms from dehydration, abration and predation in free living species and by parasitic nematodes (Naem and Seifi, 1998).

REFERENCES

- Carta, L.K.; and Baldwin, J.G. (1990): Ultrastructure of phasmid development in Meloidodera floridensis and M. charis (Heteroderinae). Journal of Nematology, 22: 362 385.
- Carta, L.K.; and Baldwin, J.G. (1990): Ultrastructure of phasmid development in *Meloidodera floridensis* and *M. charis* (Heteroderinae). Journal of Nematology, 22: 362 385.
- Chitwood, B.G. and Chitwood, M.B. (1950): An introduction to nematology. Monumental Printig co. Baltimore, Maryland, 372 P.
- Chiwood, M.B. (1957): Intraspecific variation in parasitic nematodes Syst. Zool. 6: 19 23.
- Durette- Desset, M.C. (1969): Les system daretes cuticulaires chez les Nematodes Heligmosomes parasites de Murides australiens. Ann de Parasitol. et Comp. 44: 733-747.
- Durette-Desset, M.C. (1985): Trichostrongyloid Nematodes and their vertebrate hosts: Reconstrction of the phylogeny of aparasitic group. Advan Parasitol., 24: 239-306.
- Daskalov, P.B. (1972): Haemonchus contortus : factors determining the polymorphism of linguiform females. Exp. Parasitol.32: 364-368.

- Gibbons, L.M. (1979): Revision of the genus Haemonchus Cobb, 1898: (Nematoda:Trichostrongyloidea). Sys.Parasitol. 1: 3-24.
- Gibbons, L.M. (1986): SEM guide to the nematode parasitology. United Kingdom 199 P.
- Lichtenfels, J.R.; Pillit, P.A. and Lejambre, L.F. (1986): Cuticular ridge pattern of Haemonchus placei (Nematoda:Trichostrongyloidea). Proc. Helm. Soc. Wash. 53: 94-101.
- Lichtenfels, J.R.; Pillit, P.A. and Lejambre, L.F. (1988): Spicule lengths of the ruminant stomach hybrids. Proc. Helm. Soc. Wash. 55: 97-100.
- Lichtenfels, J.R., Gamble, H.R. and Purcell, J.P. (1990): Scanning electron microscopy of the sheated infective larva and parasitic third stage of *Haemonchus contortus* (Nematoda: Trichostrongyloidea). J. Parasitol. 76: 248-253.
- Lichtenfels, J.R.; Pillit, P.A. and Hoberg, E.P. (1994): New morphological characteres for identifying individual specimens of *Haemonchus spp.* (Nematoda: Trichostrongyloidea) and a key to species in numinant of North America. J. Parasitol. 80: 107-119.
- Naem, S. and Seifi, H. (1998): Scanning electron microscopical observations on adult Haemonchus controtus (Nematoda). J. Fac. of Vet. Univ. of Tehran, Vol. 53. No. 3 & 92-99.

LIST OF FIGURES SCANNING OF MICROGRAPHS

- Fig. 1a: The anterior end of neck without transverse cuticular ridges.
- Fig. 1b: The anterior part with longitudinal & transversal ridges and cervical papillae.
- Fig. 2: Excretory pore (EP).
- Fig. 3: Middle part of the body cuticle, transversal striae.
- Fig. 4: Magnification of lateral side of anterior cervical papillae (CP).
- Fig. 5a: Lateral view of mouth, dorsal lancet (DL), internal labial papillae (ILP)., external labial papillae, somatic papillae (SP).
- Fig. 5b: Magnification of buccal cavity.
- Fig. 6a: Copulatory bursa of male, and prebursal papillae.
- Fig. 6b: Opened copulatory bursa and spicule.
- Fig. 7: Posterior end of female showing the anus.
- Fig. 8: Posterior end of female showing the phasmides.
- Fig. 9: Magnification of egg Haemonchus placei.

LIGHT MICROGRAPHS

- Fig. 10: Anterior end showing cervical papillae. X91.8.
- Fig. 11: Anterior end, showing labial papillae. X165.
- Fig. 12: Copulatory bursa showing prebursal papillae X13.2.
- Fig. 13: Spicules tip, left spicule (LS) with pore, Right spicule(RS) with pore X41.25.
- Fig. 14: Vulval flap X33.
- Fig. 15: Vulva opening X33.
- Fig. 16: Tail of female showing position of vulval flap X10.89.
- Fig. 17: Eggs of Haemonchus placei X33.
- Fig. 18: Posterior end of female showing anal opening X16.5.







