

## Studies on Strongyloidiasis of the Peacock

### III. Scanning Electron Microscopy of *Strongyloides pavonis* Sakamoto et Yamashita, 1970

Tsukasa SAKAMOTO, Isaburo KONO and Nobuhiro YASUDA

(Laboratory of Veterinary Pathology)

Received for Publication September 10, 1980

#### Introduction

The light microscopical structures of *Strongyloides* have been studied for the taxonomical necessity by many investigators. However, the ultrastructure of *Strongyloides* has been observed by few workers. Particularly, there is no report dealing with the electron microscopy on the structures of avian *Strongyloides*. The authors<sup>4,5)</sup> reported on the light microscopical features of *Strongyloides pavonis*. The present paper extends the previous studies and reports the results of observations on the superficial structures of the infective larvae, free-living male and female adults and parasitic female adults of *Strongyloides pavonis*.

#### Materials and Methods

The feces from the chicken artificially infected with *Strongyloides pavonis* Sakamoto et Yamashita, 1970 were cultured on the clay plate in the Petri dish containing water at 28°C. The adults were collected from the surface of feces on the plate more than 48 hours after incubation. The infective larvae having migrated into water were collected from the water around the clay plate 4 or 5 days after incubation.

The infective larvae were given orally or subcutaneously to chicken. The parasitic females were obtained from the caeca of the chicken 7 to 14 days after infection. Namely, the caeca were removed, slit longitudinally, and washed in saline solution to remove their contents. The mucous membrane was scratched from the caeca, and placed in saline solution at 37°C for a half hour to remove parasitic females. The adult females then were concentrated by gravity from the combined washing and fixed in cold buffer solution at pH 7.4.

The cleaned larval and adult worms were fixed in 1% cold glutaraldehyde solution with phosphate buffer at pH 7.4 for 2 hours. After being rinsed away with phosphate buffer solution, the worms were followed by post-fixing in 1% osmium tetroxide solution with phosphate buffer at pH 7.4 for 2 hours, and washed three times in the phosphate-buffered solution. The fixed specimens were dehydrated in ethanol series by the routine method, followed by the three changes of pure isoamylacetate, and dried by the critical point method using liquid CO<sub>2</sub>. The dried worms were placed on block covered with double-sided adhesive tape or silver-paste, sputter-coated with gold,

---

This work was presented at the 82nd Meeting of the Japanese Society of Veterinary Science, on 9th-11th October, 1976, in Morioka, Japan.

and observed at accelerating voltage of 5 to 30 kV with scanning electron microscope.

## Results

### 1. Filariform infective larvae

The mouth is simple pore. The dorsal and ventral boundary-cuticles of the oral opening are slightly elevated. The oral opening is flanked by distinct, lip-like, bilateral elevations. A pair of papilliform amphids bounded distinctly are situated lateral to the oral opening. The size of amphids is 0.7 to 0.9  $\mu\text{m}$  in transversal length and 0.25  $\mu\text{m}$  in width. The transversal striations are recognized at intervals of 0.8 to 1.2  $\mu\text{m}$ . Two lateral alae run from about 20  $\mu\text{m}$  posterior to the anterior end of the worm toward the posterior end. The width between the two lateral alae is 0.6 to 0.8  $\mu\text{m}$ . A slit-like line is seen between the alae. The width of external fold of lateral alae is about 0.7  $\mu\text{m}$ . Accordingly, it is at the level of middle part of the worm that the total width of both the lateral alae is the widest, measuring about 2.3  $\mu\text{m}$ . The width of lateral alae is narrower as getting nearer to the posterior end. Two alae are united into one at the part of posterior end. Lateral alae reaching to the posterior end remain as cuticular folds. Therefore, the posterior end shows an asteroid shape having six side-folds. Anus appears to be a pouch-like depression, 3.2  $\mu\text{m}$  in width. Phasmids are situated on the lateral cord about 2.3  $\mu\text{m}$  posterior to the anal opening. The anal pore is 0.05  $\mu\text{m}$  in diameter, and surrounded by cuticular elevation of 0.33  $\mu\text{m}$  in diameter.

### 2. Adult parasitic female

Mouth is funnel-shaped and situated on the tip of cephalic dome. Oral opening is Y-shaped slit, surrounded by circumoral elevations consisting of three pairs of lobes composed of dorsal, lateral and subdorsal lobes. Four tiny papilliform projections are situated dorsal and ventral and lateral to oral opening. A pair of papilliform amphids are situated lateral to the lateral lobes of circumoral elevation. The amphid is 0.8~1.2 $\times$ 0.25  $\mu\text{m}$  in size and bounded distinctly. The transversal striations were recognized over entire cuticular surface excepting the anterior and posterior ends. The lateral cord is V-shaped groove, running from the anterior part toward the posterior end, but is not accompanied with lateral alae. A spherical papilla (cervical papilla) was recognized on the lateral surface at the level of excretory pore situated on the ventral side of cervical part. Vulva opens on the hemispherical elevation of cuticle situated on the ventral side of middle part of the body. The vulvar opening is a transversal slit. A pair of papillae are situated on the lateral side at the vulvar level. Anus appears to be a round, pouch-like depression which is 0.37  $\mu\text{m}$  in diameter. There are a pair of phasmids on the lateral surface of the tail. The phasmids are round depressions, approximate 0.086  $\mu\text{m}$  in diameter, in which spherical protuberance measuring 0.036  $\mu\text{m}$  in diameter is contained. The posterior end is smooth and hemispherical, 0.37  $\mu\text{m}$  in diameter.

### 3. Adult rhabditiform male

Mouth is situated on the tip of the anterior end, and funnel-shaped. Oral opening is a vertical slit and flanked by a pair of labiate, bilateral elevations. The buccal capsule is noted obviously. The gentle, round bulge is recognized lateral to the labiate elevation. Transversal striations are recognized at intervals of 0.6~0.9  $\mu\text{m}$  on the entire cuticular surface. Eight pairs of papillae consisting of 3 pairs of preanal papillae, 2 pairs of adanal ones, and 2 or 3 pairs of postanal ones, are arranged on the surface of the posterior end of male. The papillae are hemispherical, measuring

2 to 3  $\mu\text{m}$  in diameter, and have a tiny protuberance about 1  $\mu\text{m}$  in diameter in the center of each papilla. Tail is curved to the inside and tapers off to a point.

#### 4. Adult rhabditiform female

The structures of anterior part and striations of cuticle are similar to those of males. Vulva is situated on the ventral surface of middle part of the body. Vulvar opening is transversal slit and surrounded by a cuticular elevation. Anus is pouch-like and opens to the posterior direction. The groove runs from the anus to the posterior end. The tail tapers off to a point.

#### Discussion

The light microscopical structures of many species of genus *Strongyloides* were described by a number of workers. Some uncertainties, however, still exist concerning some of the structures, especially papillae on the circumoral and caudal papillae. Nichols<sup>3)</sup> observed light-microscopically the tissue-stage larva of *Strongyloides stercoraris*. He stated that the recognition of the presence, and ascertainment of type or lack of lateral alae in the transverse section of mid-intestinal level were helpful for the differentiation of tissue-stage larva. Inatomi *et al.*<sup>2)</sup> measured the depth and width of lateral alae of filariform larvae of *S. stercoraris*, *S. fülleborni* and *S. ratti* at the esophagus and mid gut levels, and stated that the pattern of ala at the mid gut level is different among the species of infective larvae. The lateral ala of *S. pavonis* revealed different patterns among the respective levels as shown in the schematic patterns of Figure 1. Inatomi *et al.*<sup>2)</sup> also described that the average widths between transverse striations of *S. stercoraris*, *S. fülleborni* and *S. ratti* are 0.91, 0.86 and 1.12  $\mu\text{m}$ , respectively. The width between transverse striations of *S. pavonis* was 0.8~1.2  $\mu\text{m}$ . Arizono *et al.*<sup>1)</sup> studied the surface structures of parasitic female, infective larva, and free-living adult male and female of *S. planiceps* by scanning electron microscopy. The findings

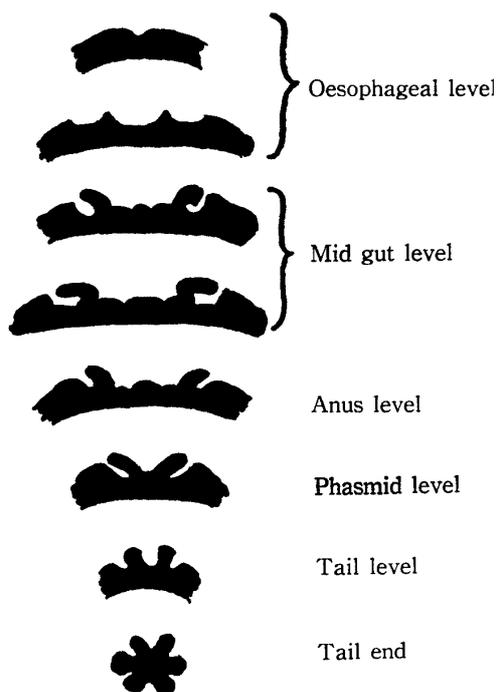


Fig. 1. Schematic pattern of lateral alae of *Strongyloides pavonis*.

of superficial structures of *S. pavonis* observed in the present experiment appear to be essentially analogous to those of *S. planiceps*.

### Summary

The surface structure of infective larva, parasitic adult female, and free-living adult male and female were observed using scanning electron microscope.

The infective larva has oral opening surrounded by circumoral elevation indistinctly divided into three lobes. Obvious double alae are observed on the lateral cord. The sectional pattern of lateral ala is different among the respective levels of the body.

The parasitic adult female has an oral opening surrounded by circumoral elevation which was indistinctly divided into three pairs of lobes. V-shaped groove (lateral cord) without lateral ala runs longitudinally on the lateral surface of the entire body. Vulva opens on the hemispherical elevation situated on the ventral side in the middle part of the body. Each pair of amphids, cervical papillae, vulvar papillae and phasmids are situated on the lateral surface at the levels of cephalic part, excretory pore, vulva and caudal part, respectively.

The free-living adults have a mouth which has a vertical slit-like opening and is flanked by a pair of liberate, bilateral elevations situated on a gentle bulge.

The free-living adult male has eight pairs of caudal papillae consisting of 3 pairs of preanals, 2 pairs of adanal and 2 or 3 pairs of postanals on the caudal surface.

The vulva of free-living adult female is a transversal slit-like opening surrounded by a cuticular elevation, and situated on the ventral surface of the middle part of the body. Its anus opens to the posterior direction.

The distance between the transversal striations of cuticular surface of filariform infective larva, of parasitic adult female and of free-living male and female are 0.8 to 1.2, 1.0 to 1.2 and 1 to 1.2  $\mu\text{m}$ , respectively.

### References

- 1) Arizono, N., Matsuo, K. and Yoshida, Y.: Scanning electron microscopy of *Strongyloides planiceps* Rogers, 1943. *Jap. J. Parasit.*, **25**, 468–474 (1976) [Japanese with English abstract]
- 2) Inatomi, S., Sakamoto, D. and Itano, K.: Studies on the submicroscopic structure of body surface of larval nematodes. *Jap. J. Parasit.*, **12**, 16–39 (1963) [Japanese with English abstract]
- 3) Nichols, R. L.: The etiology of visceral larva migrans II. Comparative larval morphology of *Ascaris lumbricoides*, *Necator americanus*, *Strongyloides stercoralis* and *Ancylostoma caninum*. *J. Parasit.*, **42**, 349–399 (1956)
- 4) Sakamoto, T.: Avian *Strongyloides* and strongyloidiasis. *Shiyu (J. Grad. Ass. Dep. vet. Med., Kagoshima Univ.)*, No. 10, 14–24 (1968) [in Japanese]
- 5) Sakamoto, T. and Yamashita, J.: Studies on strongyloidiasis of the peacock II. *Strongyloides pavonis* n. sp. (Nematod: Strongyloididae) from the green peafowl *Pavo muticus* Linnaeus. *Jap. J. vet. Res.*, **18**, 163–171 (1970)

**Explanation of figures**

All figures are scanning electron photomicrographs of *Strongyloides pavonis*.

- Fig. 2. Anterior end of infective larva.  $\times 3,000$   
Fig. 3. Anterior end of infective larva.  $\times 10,000$   
Figs. 4 and 5. Lateral alae and transverse striations on the surface of infective larva.  $\times 3,000$   
Fig. 6. Posterior end of infective larva.  $\times 12,000$   
Fig. 7. Anterior end of parasitic adult female.  $\times 3,600$   
Figs. 8 and 9. Anus of infective larva.  $\times 12,000$   
Figs. 10 and 11. Phasmid on the lateral surface of tail of infective larva.  $\times 12,000$   
Fig. 12. Anterior end of free-living adult male.  $\times 3,300$   
Fig. 13. Spiculum and caudal papillae on the surface of posterior end of free-living adult male.  $\times 1,200$   
Fig. 14. Posterior end of parasitic adult female.  $\times 2,400$   
Fig. 15. Posterior end of parasitic adult female.  $\times 1,200$   
Fig. 16. Anus opening of parasitic adult female.  $\times 6,000$   
Fig. 17. Tail end of parasitic adult female. An arrow shows a phasmid.  $\times 3,600$

