

## CONTRIBUTIONS ON THE PASSALURUS AMBIGUUS LIFE CYCLE AND SCANNING ELECTRON MICROSCOPY STUDIES

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### SUMMARY

The life cycle of *Passalurus ambiguus* was studied experimentally. The 4th and 5th stage larvae were studied morphologically. The prepatent period was determined and the seats of egg deposition were demonstrated.

Histopathological studies were carried out from the infected region of the gastro-intestinal tract and perianal region of experimentally infested rabbit. The anterior end of the obtained adult worms was studied by scanning electron microscope.

### INTRODUCTION

Rabbit parasites were studied by many authors in Egypt [ Abdel-Rhman 1988 Hegazi 1988 & Ibrahim 1990] but only sporadic literature reports have dealt with the biology and life cycle of the nematode *Passalurus ambiguus*

This work aimed to clarify some aspects of the life cycle and the developmental stages of *P.ambiguus* and to describe cephalic morphology of the worm by scanning electron microscopy.

### MATERIAL AND METHODS

Infective *P.ambiguus* eggs were obtained by scraping of the perianal region, from the surface of the faecal pellets; and the rectum of the naturally infected rabbits , eggs were prepared for experimental infection according to the method of Fetisov (1967) . Infective eggs were counted according to Gorden & Whitlock (1939) using a Mc-master slide.

Fifteen 6-week-old male rabbits, free from intestinal worms were used . Each was infected with 1 ml saline containing about 500 eggs [3rd stage larvae were active in about 80% of these eggs ] by stomach tube.

Two rabbits were sacrificed at 10, 15, 20, 30, 40, and 50 days post infection. Scraping and examination of intestinal contents for larval development were done at each time period. The other three rabbits were retained to determine the prepatent period and the site of egg deposition.

For histopathological studies, specimens from small intestine, caecum , appendix rectum and perianal region were fixed in formalin, embedded in paraffin, sectioned, stained with H & E and examined according to Harris (1898).

For scanning microscopy, the anterior end of the adult worm was fixed for 2-4 hr, in aqueous 4%

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glutaraldehyde and 1% osmium tetroxide in a ratio of 3:1. The materials were washed in distilled water, dehydrated through ethanol and amyl acetate dried using carbon dioxide, fixed to stubs with colloidal carbon and coated with gold palladium in a sputtering device. Specimens were examined and photographed using a scanning microscope (SEM) model JSM-T20. JEOL (Japan) Accelerating voltage : 19 K.V. Magnification: 2500,X.

## RESULTS

The eggs obtained from perianal scrapings and the surface of the faecal pellets contained mostly 3rd larval stage in different shapes oriented along the long axis of the egg, wide at one pole narrow or tapering at the other pole (Plate 1).

Experimental infection of non infected rabbits revealed that the infective eggs passed through the stomach to the intestine and released the infective larvae there, which developed deeply in the crypts and mucosa of the intestine for a short time (15 days). They were released in the lumen of the small intestine as 4th stage larvae (20-30 days). Larvae migrate to the caecum and appendix to develop and moult into 5th stage larvae within the mucosa and crypts (40 days). The juvenile 5th stage larvae developed to adult worms in the lumen of the rectum and perianal region (50 days).

The 4th stage larvae obtained from the small intestine at day 20 were small sized (0.43-0.68 mm length), spindle shaped, blunt anteriorly. The oesophageal bulb was clear and the nerve ring was posterior to it. The intestinal caeca were not clearly developed (Plate 2 Fig.1).

The 5th stage larva recovered from the appendix at 40 days was larger in size (0.72-0.87mm length), blunt anteriorly, narrow posteriorly, with

a clear bulbed oesophagus. The intestinal tract was more clearly visible and developed than the 4th stage larvae (Plate 2 Fig.2).

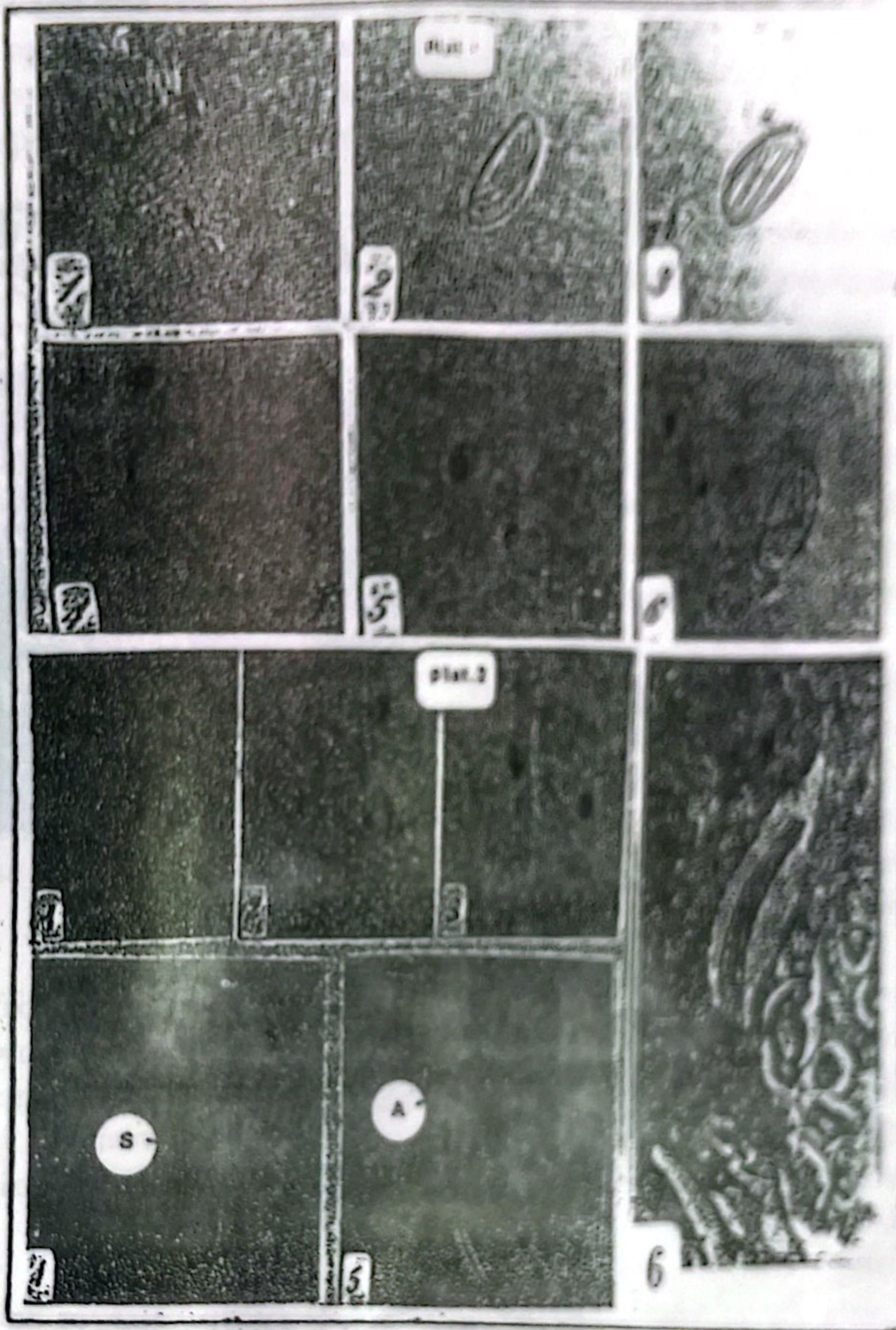
The immature worms obtained at 50 days were larger in size (1.25-1.93mm length) than 5th immature larva, blunt anteriorly and narrow and pointed posteriorly. The prebulbar oesophagus appeared at this stage and the larvae increased in the width. The intestine was fully developed at this stage (Plate 2 Fig.3). At higher magnification (X 40) the posterior end could be sexually differentiated by the appearance of early spicule in immature males and by the long narrow tail posterior to the anus in immature females (Plate 2 Fig.4 and 5). For the 3 rabbits retained to determine the prepatent period, eggs were recovered on the surface of the faecal pellets at 58, 64 and 67 days post infestation for rabbits, 1, 2 and 3 respectively.

The oviposition of *P. ambiguus* eggs occurs mostly in the rectum and on the perianal region. Eggs leave the female through a tube, ovipositor or finger like uterine tube (Plate 3-1) projected from the valvular region, about 1.59 mm length. The adult female contracted to the side opposite to the vulvar opening and as the uterine tube proceed, eggs were deposited in the mucosa of the rectum the perianal region and over faecal pellets. Eggs were present in patches in the crypts and mucosal layer of the rectum (Plate 3-2).

Histopathological examination revealed desquamated epithelial cells with eosinophilic exudate and some leukocytes in the intestinal lumen. Leukocytic infiltration consisting mainly of lymphocytes and eosinophils were observed in lamina propria along with congested capillaries. Erosion of the small intestinal mucosa was the predominant lesion. Longitudinal sections of the developing larvae were found in the crypts and mucosa of appendix (Plate 2-6).

Plate 1: F  
Plate 2: J





**Plate 1:** Eggs collected from perianal scraping and surface of the faecal pellets (X40) (1-6).

**Plate 2:** Different developmental stages of *P. ambiguus*:

- 1- 4th stage larva (X40).
- 2- 5th stage larva (X40).
- 3- Juvenile adult worm (X40).
- 4- Immature male posterior end (X40).
- 5- Immature female posterior end (X40).
- 6- 5th larval stages in the appendix mucosa.





**Plate 3-1: *P. ambiguus*: ovipositor**

**3-2: *P. ambiguus* egg patches in intestinal mucosa.**

**3-3: *P. ambiguus* anterior end (dorso-lateral view) by scanning electron microscopy (X 2500).**

**P. = papilla, T. = teeth.**



By scanning electron microscopy of the anterior end of the adult worm, the dorsolateral view revealed four well developed papillae: two medium (one dorsal and one ventral) and two lateral at the mouth opening. At the base of the mouth three teeth were present; located at the rim of the oesophageal opening two dorsal and one ventral (Fig.4).

## DISCUSSION

Egg deposition occurred through the egg tube or ovipositor as reported by Fetisov (1967). Most eggs were deposited on the surface of faecal pellets and in the crypts and mucosa of the rectum and on the perianal region. This observation differs from the opinion of Boecher (1953), who reported that the release of eggs at the perianal region occurred after the death and disintegration of female worms. However the egg patches found in the mucosa contained 2-20 eggs suggesting that these eggs were produced via the uterine tube.

The development from infective larvae to adult male and female worms and their morphology was nearly in agreement with the observation of Boecker (1953) and Fetisov (1967). However they did not mention or discuss the immature stages.

Histological sections revealed deep and rapid parasitic invasion of the small intestine; larvae were clearly also visible in longitudinal section of appendix at 40 days.

Scanning electron microscopy of the anterior end of the adult worms at the dorsolateral view showed clearly 3 teeth (two dorsal and one ventral) for the first time.

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