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ULTRASTRUCTURE OF THE INTESTINAL NEMATODE PARASITE: *ENTEROBIUS VERMICULARIS* FROM EGYPTIAN SCHOOL-CHILDREN

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Abstract

Enterobius vermicularis is nematode the commonest human parasites particularly among children aged school based on behavioral and ecological factors that facilitate spreading. To ensure the phylogenetic relation to Nematoda ultrastructure of *Enterobius vermicularis* worms and male spermatogenesis criteria must be studied

This study was performed on school children from both rural and urban areas to identify the *Enterobius vermicularis* worm in fecal samples. The adults were examined macroscopically and microscopically as well as by scanning electron microscopy (SEM), for strain identification and spermatogenic criteria were examined by transmission electron microscopy (TEM).

The SEM showed the criteria of different parts of the adult female and TEM clarified the males' spermatogenic criteria that showed many ribosomes, rough endoplasmic reticulum (RER), mitochondria, and golgi bodies in spermatocyte's cytoplasm. A prominent nucleolus and a perforated nuclear membrane are features of a large nucleus.

Key words: Egypt, SEM, *Enterobius vermicularis*, Parasite, TEM, Spermatocyte

Introduction

Enterobius vermicularis, or the pinworm, is one of the commonest nematode infections of worldwide distribution; with an original of *E. vermicularis* was named *Oxyuris vermicularis*. Humans are the only natural host for this infection. Transmission occurs in people who is living in crowded environments and usually occurs within families, as small, thin, and white worms (Rawla and Sharma, 2022). The infective eggs can survive for 2-3 weeks on fingers, under fingernails, and on surfaces such as linens, clothing or toilet seats (Song *et al,* 2003). Although pinworm infection can affect all people, it most commonly occurs among children, institutionalized persons, and household members of persons with pinworm infection, as treatable with over-the-counter or prescription medication, but reinfection occurs (CDC, 2020).

Males and females contract infections at a rate of about 2:1. However, those between the ages of 5 and 14 showed a female majority of infection. Children in school age and those from tropical regions are more at risk. Ingestion of pinworm eggs via fecal-oral pathway causes infection most frequently (Wang *et al*, 2016) higher among children with human leukocyte antigen genotype conferring the high risk for type 1diabetes or asthma (Gale, 2002).

Some patients mainly children with and without symptoms usually connected with enterobiasis, e.g. anal pruritus with infection and infection is typically benign (Bøås *et al,* 2012). Itching in the perianal region is one of the signs of pinworm infection most frequently. Because of the scratching, there may be perianal erythema. Some patients reported watery diarrhea. Female genitourinary infections were documented (Wendt *et al*, 2019). Also, appendicitis due to enterobiasis was reported (Zaghloul *et al,* 2015).

Adult female is 8 to 13mm x 0.5mm thick, with a sharply pointed posterior end. Adult male, which is much smaller and has a curled posterior end, is 2 to 5mm x 0.2mm thick. Translucent eggs have a surface that sticks to things. Thick shell was flattened on one side (D shaped) measures 50 to 60mm x 20 to 30mm. Except for large clumps of millions of eggs that are just barely visible, the eggs are invisible to the naked eye due to their small size and lack of color. A growing embryo or a pinworm larva may both be present in one egg. The larvae reach a length of 140-150 mm (Gutiérrez, 2000).

For better diagnostic results, the examination is typically performed in the early morning. The diagnosis is ruled out if the examination is negative for five mornings in a row. Since that *E. vermicularis* are typically only sporadically discharged in the stool, a stool examination is not helpful in the identification of the condition. To rule out other causes, it was recommended to analyze stool sample (Meade and Watson, 2014).

This study aimed to present the ultrastructure of *Enterobius vermicularis* (Egyptian strain) worm and its male spermatogenesis criteria by a scanning (SEM) and transmission (TEM) electron microscopy.

Materials and Method

This study conducted on school children from both rural and urban areas. The samples were collected after approval of Local Ethics Committee of Zagazig University, Faculty of Science. Written informed consent was obtained from authorities of Egyptian ministry of Education and from students' parents under supervision of responsible consultant doctor in each school.

Conducted children were subjected to clinical examination from September 2017 to September 2018. Fresh stool samples were collected from each child in a clean, covered and labeled plastic container. All the specimens were immediately transferred to Zoology Department Laboratories, Faculty of Science, Zagazig University.

Fecal samples were collected in clean plastic covered boxes, mothers were asked to handle sample immediately after defecation. Samples were examined macroscopically for color, odor, consistency, mucous, blood or adult worms. Samples were processed using Formalin ethyl-acetate concentration technique. After concentration a drop of sediment was placed onto a microscope slide and covered with a cover-slip, and microscopically examined at 40X.

Adults were collected directly from feces fixed in 5-10% formalin and transferred into 70% ethanol, dehydrated through an ascending ethanol grades, washed in absolute ethanol for two changes and then dried in hexamethyl disilizane solution. Samples were mounted onto aluminum stubs, sputter coated with 4.5nm of gold/palladium, and examined with Scanning Electron Microscope at 3 kV with the SEI detector (Saleh *et al,* 2022). Anterior and posterior abdominal regions were removed to have clear reproductive organs (two testes in males and uterus with mature spermatozoa in females). Examination was done in Transmission Electron Microscope, in TEM Unit, Faculty of Science, Alexandria University.

Results

Mature worms are white, tiny, and have transversely striated cuticles. There is a cephalic vesicle, and the anterior end has three lips that surround a triangular-shaped mouth. Lips have an apical ridge that is larger towards the lateral edges and narrower in the middle. Laterally, the dorsal lip has two cephalic papillae. Each subventral lip has one cephalic papilla and one amphidial pore laterally on the ventral and dorsal sides of the lip, respectively.

Three flattened blade-like teeth are present in the anterior pharynx; the anterior most projections of each tooth have an outer surface that is flattened or nearly flattened, an inner luminal surface that is concave caudally, and nearly uniform denticles with smooth edges on the apical margin of the teeth. There are lateral alae in both sexes. The tail has a long, conical shape with a point at the end. In longitudinal sections of testis *E. vermicularis*, sperm cells can be seen at many stages, including immature spermatozoa and spermatogonia. The distal portion of the testis is where spermatogonia are found. These are tiny, undifferentiated polygonal cells, and the majority of the cell is taken up by the nucleus.

Spermatogonia get bigger and more active as they transform into spermatocytes. A highly developed synthetic apparatus, including numerous ribosomes, rough endoplasmic reticulum (RER), mitochondria, and Golgi bodies, may be found in the cytoplasm of the spermatocyte. A robust nucleolus and a perforated nuclear membrane are features of the big nucleus. Little clumps of dense fibrous material, produced by the synthetic machinery, are dispersed throughout the cell's cytoplasm. Large spherical filamentous patches that enclose bundles of thick fibers surround the nucleus. The spermatids of other nematode species have been shown to have FBs, which are quite similar to the patches. Fusion of the filamentous material patches results in the formation of FBs.

Along the edges of the fibrous bodies (FBs) and in the cytoplasm that divides the nearby FBs, mitochondria are found. The early spermatids' plasma membrane develops filopodia. A main cell body (MCB) plus a residual body make up late spermatids. The core nucleus of the MCB has abundant chromatin but no nuclear envelope. The cytoplasm is home to ribosomes, Golgi bodies, RER cisternae, mitochondria, and FBs, but their boundaries are less clearly defined than at the previous stage. The MCB's plasma membrane sprouts a lot of filopodia.

Details were given in figures (1, 2, 3, 4, 5, &6).

Discussion

The rates of intestinal nematode infections are high in children. These parasites are largely contagious, and poverty is a factor in their spread. As a result, locations with poor hygiene have a higher incidence of them (WHO, 2019). With more than 200 million people globally carrying the parasite, *Enterobius vermicularis* is the most common helminthes that affects humans. The parasite favors youngsters aged 7 to 11 and infects 30% of them; the female-to-male infectivity ratio is 3:1. Geographically, temperate climates are where the parasite is more frequently found. Infectivity is heightened by elements like overcrowding and poor sanitation (Smolyakov *et al,* 2003).

In the present study, SEM examination of the *E. vermicularis* (Egyptian stain) worms clarified that the worms are minute to small white with cuticle transversely striated. The anterior end carried three lips surrounding a triangular shaped mouth. Lips rimmed with an apical ridge that is narrow medially and wider at lateral margins. Dorsal lip has 2 cephalic papillae laterally. Each subventral lips has 1 cephalic papilla laterally on ventral side of lip and 1 amphidial pore laterally on dorsal side of lip. Anterior pharynx possesses 3 flattened bladelike teeth, with anterior most projection of each tooth flattened or nearly flattened on outer surface, concave caudally, convex to triangular shaped on inner luminal surface with nearly uniform denticles with smooth edges on apical margin of teeth. Lateral alae present in both sexes. This agreed with Hugot and Tourte-Schaefer (1984), they showed that male *E. vermiculris* have a total body length (3.0-3.85mm), a wider range of excretory pore lengths (607-725), and a wider range of spicule lengths (68-141). Female *E. vermicularis* have a big esophageal bulb (220 long by 220 broad), a long tail (1.83 mm), and are distinguished by having a single crested lateral ala along the length of the body (10.13-12.0mm long & 600-650 wide).

By TEM examination, the spermatozoa of *E. vermicularis* share the main features with male gametes of other nematodes. They are bipolar cells without acrosomes and axonemes, but contain a nucleus with a condensed chromatin, which is not surrounded with a nuclear envelope.

Most nematodes have spermatids and immature spermatozoa that are homologous to FBs, which are structures that develop during spermatogenesis in other worm species. When the sperm are activated in the female uterus, they are retained in immature spermatozoa and give birth to the cytoskeleton of the pseudopodium. The FBs, which are formed in the cytoplasm without the assistance of spermatocytes and contain the proteins required to construct the pseudopodium (Yushin, 2003).

Although the presence or absence of FBs in the cytoplasm cannot be considered as a defining trait of the entire nematode order, yet it was used to identify specific species, genera, and possibly other taxa within the order (Yushin, 2002). Lack of FBs in nematodes didn't mean that sperm cells being unable to make cytoskeleton proteins. Spermatocytes and spermatids are biosynthetically active cells with a well-developed synthetic apparatus in some species, such as *Pandoripharynx unidentatus*, originally known as *Panduripharyn pacifica* (Dashchenko, 1989).

Mature spermatozoa of these species have a typical pseudopodium. Consequently, the chromadorids *Neochromadora poecilosoma, Paracytholaimus pugettensis,* and *Halichoanolaimus possjetiensis*, as well as many other nematodes, development of cytoskeleton in mature spermatozoa of *P. unidentatus* didn't involve a morphologically visible FB intermediate stage (Yushin, 2004).

A number of nematodes belonging to phylogenetically distant taxa (oxiurids, strongilids, trichuroids, mermithids, and dioctophymids) were also shown to lack FBs in the sperm cells development (Poinar and Hess-Poinar, 1993).

Spermatozoa of several nematodes shared characteristics such as the lack of a flagellum, a unique arrangement of centrioles, and an amoeboid shape with amoeboid motility due to cytoskeleton components (Jamieson and Tudge, 1999. Besides, they lacked an enveloped nuclear and poor nuclear chromatin condensation (Justine, 2002). Nematode spermatozoa include several mitochondria, special membrane-based parts derived from Golgi complex (Alberti, 2005). The spermatozoa's large size is caused by their substantial cytoplasm, which is packed full of numerous components (Balsamo *et al,* 2007).

Conclusion

Egyptian *Enterobius vermicularis* was the main strain detected in all samples. This was clarified by SEM of adults and TEM of spermatozoa specific fertilization. Studies on sperms and variety in development mechanisms to highlight their phylogenetic relation are ongoing and will be publish in due time elsewhere.

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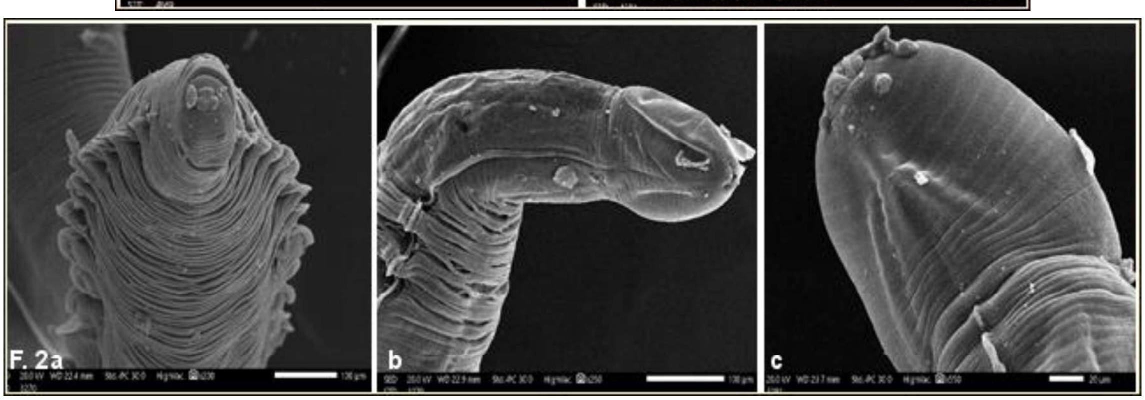
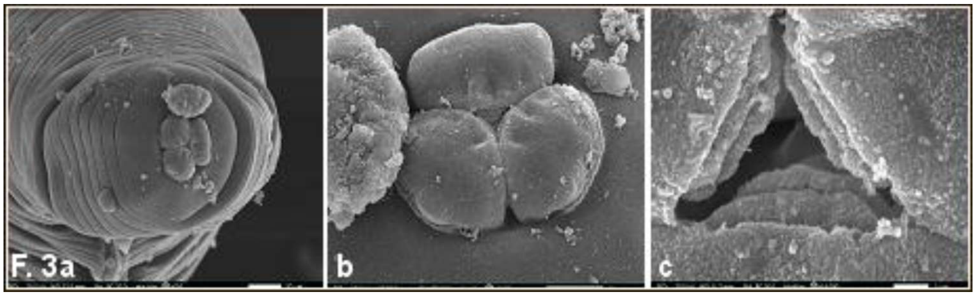
Explanation of figures

Fig. 1: SEM images of female *Enterobius vermicularis* whole worm.
Fig. 2: SEM images with ventral (a), lateral (b) and apical (c) views of cephalic end of *E. vermicularis*.
Fig. 3: SEM images of cephalic end (a) and pharyngeal teeth (b), apical view (c) of *E. vermicularis*.
Fig. 4: SEM images of single crested alae of cephalic oesophageal mid-corpus region (a) and mid-body region (b) of *E. vermicularis*.
Fig. 5: SEM images of posterior end of female *E. vermicularis.*
Fig. 6: SEM images showed vulva (v) in tail of female *E. vermicularis*.
Fig. 7: TEM of spermatocyte a- Spermatogonia, b- Developing sperms, c- Sperms different shapes, d- Patches of filamentous material

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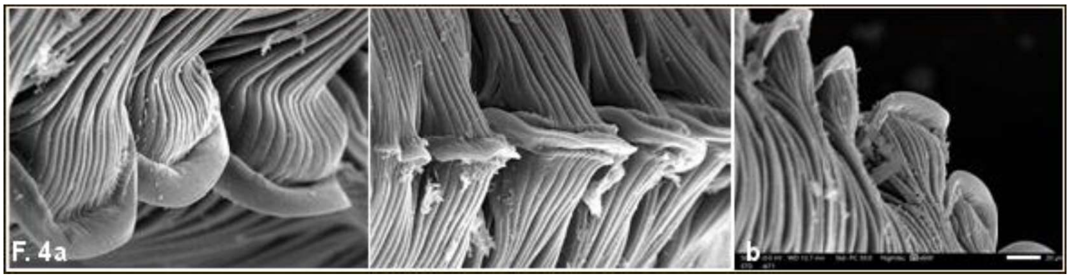
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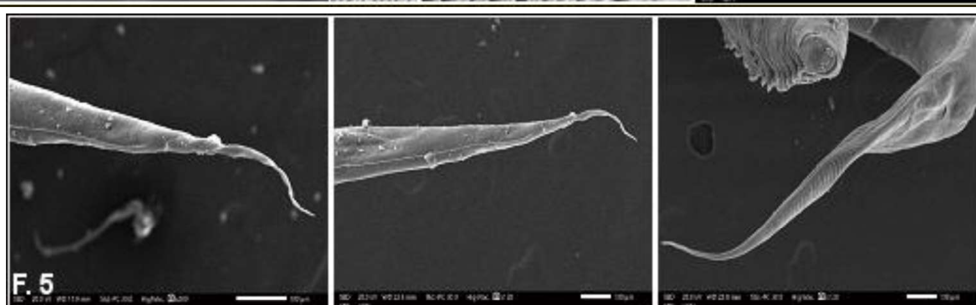
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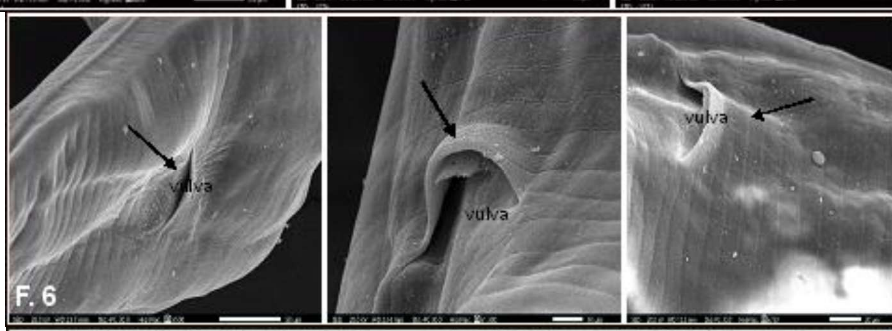
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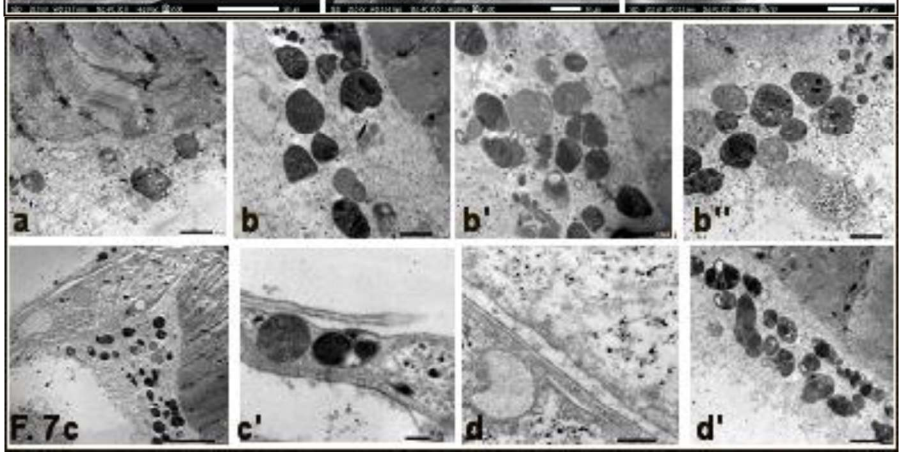
F. 6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