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Advanced Approach in Differentiation Study in *Hymenolepis nana* and *H. Diminuta* by Scanning Electron Microscopy

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Abstract: Scanning electron microscopy (SEM) succeeds in revealing novel microtopographical features of the host-parasite relationship, as well as proving invaluable in helminthes taxonomy. *Hymenolepis nana* and *H. diminuta* are commonly maintained in laboratory rodents and used in many experimental model systems of tapeworms infections. In the present work, the length of worm of *Hymenolepis* nana and *H. diminuta* were approximately up to 1-3 cm × 1mm and 6 cm × 3.5 mm, respectively. The SEM revealed that the scolex of *H. nana* and *diminuta* appears roughly rectangular and is provided with an armed suker and rostellum. The rostellum is provided with hymenolepid hook in *H. nana and an unhooked rostellum in H. diminuta*. These suckers occupy the corners of the rectangular scolex. Fine structural of the tegumental surface of both *H. nana* and *H. diminuta* is densely covered with microtriches which are of the same shape and were never seen to be branched. SEM of intestinal mucosa of infected rat with *H. nana* revealed alteration in villous architecture. The purpose of this study is to use SEM technique as advanced approach for differentiating between *H. nana* and *H. diminuta*.

Key words: Scanning electron microscopy % SEM % Rat % *H. nana* % *H. diminuta*

INTRODUCTION

Hymenolepis nana and *H. diminuta* are the most common cestodes in humans, domestic and wild rats, mice and human [1]. The body of tapeworms is differentiated into an expanded anterior end, scolex, bearing hold fast organs, un-segmented neck and a strobila which is usually very elongated and ribbon-like, ranging from few millimeters to 10-20 m or more, divided into 4-4000 segments or proglottides [2-4].

The cestode tegument is, however, unique in bearing microvilli (microtriches) apparently serving to increase the area of absorptive surface. Each mature segment has a complete set of male and female genitalia. Immature newly formed segments are nearer to the neck, mature segments are more posterior and those at the end are gravid segments [1, 5].

Scanning electron microscopy succeeded in revealing novel microtopographical features of the host-parasite relationship, as well as proving invaluable in helminthes taxonomy and in assessing the efficacy of test substance in drug screens [6-8].

Few literature are found concerning the surface of *Hymenolepis* by SEM. Fine structural studies of the tegumental surface of cestodes have demonstrated that the surface cytoplasm is extended as microtriches, consisting of cylindrical cytoplasmic bases capped by dense structures termed "shafts" [9].

SEM studies of cestode surface have shown that it provides as a suitable technique for obtaining information regarding the three dimentional relationship of surface structures. Berger and Mettrick [10] reported polymorphism in the microtriches from *H. diminuta*, *H. microstoma* and *H. nana* by SEM. They suggested that the microtriches played a role in locomotion of these organisms within the host's gut.

Several workers have examined the pathological effects of larval cestodes in man and mammals [11, 12]. Martin and Holland [13] examined by SEM the intestinal morphology of rats given 1, 10, 100 cystecercoids of *H. diminuta*, they found that the presence of this tapeworm cause extensive villous atrophy and fusion. Also, they observed colonization of the upper region of ileum by long filamentous bacteria in rats infected with *H. diminuta*.

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The purpose of this study was to use the SEM technique as a comparative diagnostic tool for morphological differentiation between some surface topography features of *H. nana* and *H. diminuta*. As well as the pathological effects in intestinal morphology of rats infected *H. nana*.

MATERIALS AND METHODS

Parasites Samples Collection: *H. nana* eggs were obtained from infected humans in National Hepatology and Tropical Medicine Research Institute. Approximately 2000 *H. nana* eggs were inoculated into 5-week-old male white mice [14]. Adult worms were dissected from the small intestine approximately 14 days post-inoculation. While hymenolepis species worms were obtained from naturally infected *Rattus norvegicus* from Abu Rawash area, Giza, Egypt. Rats were killed by cervical dislocation and entire small intestine was removed from the gut and rinsed in physiological saline.

Scanning Electron Microscopy (SEM): The worms and pieces of the intestinal tissue (which opened longitudinally) were fixed at 4°C for 24 hr in 5% glutaraldehyde in 0.1 M tris-hydroxy methyl aminomethane meleat solution pH 7.4 [15]. The samples were rinsed for an hour in a fresh phosphate buffer salin (pH.7.4) and dehydrated in an ascending series of ethanol. The specimens were placed in liquid CO_2 then mounted on metal stubs coated with gold and examined with a Joel SEM (Joel Corp., Mikaka, Japan) operated at 15 Kv. This work was carried out in central laboratory of national research center, Cairo, Egypt.

RESULTS

SEM of *H. Nana*: The length worm is approximately 1-3 cm × 1mm. The scolex appears roughly rectangular and is provided with a comparatively large hooked rostellum. The rostellum lies at the center of the scolex and is provided with pointed and posteriorly directed hooks. The neck and then is followed by gradually immature and mature proglottides. Genital atria are unilateral; each has a rounded opening and is surrounded with ordinary microtriches. The entire tegumental surface is densely covered with microtriches of uniform size and density. The microtriches were overlapped that it was difficult to observe their bases. Gravid segments are more posterior (Fig1 a-e).

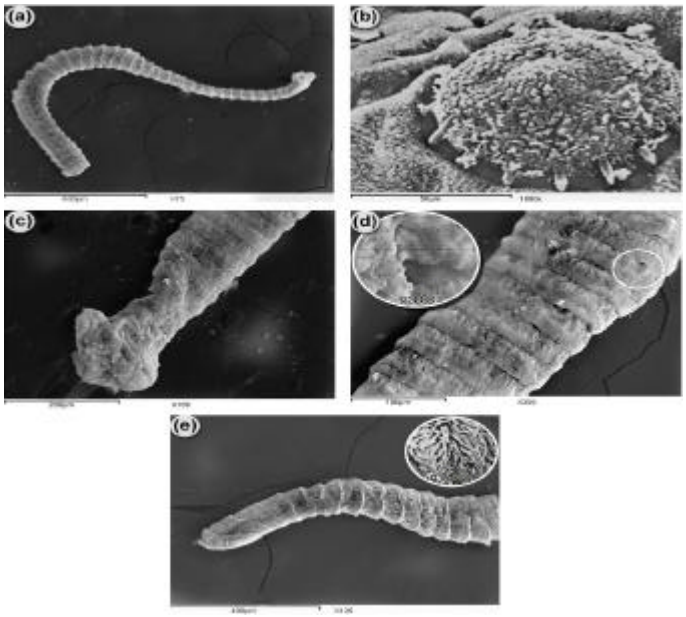

Fig. 1: Scanning electron micrographs of *H. nana*:
- a) Adult *H. nana* with scolex, neck and entire strobila (x 75).
- b) The rostellum with a ring of hymenolipid hooks and muscular border covered with distinct microtriches (x1000).
- c) Anterior part of *H. nana* (x180).
- d) Segments with unilateral genital pore (x 300, x 2000).
- e) Segment covered with distinct microtriches (x120, x 15000).

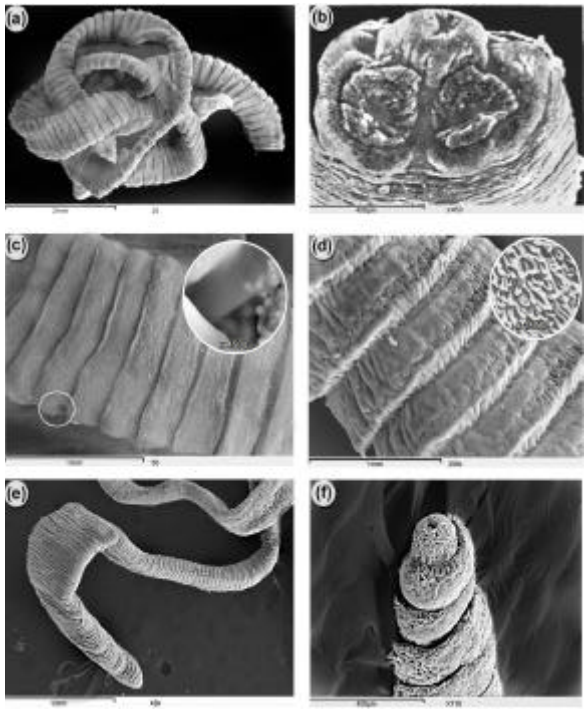

Fig. (2): Scanning electron micrographs of *H. diminuta*:

a) Adult *H. diminuta* (x20).
b) Scolex provided with an armed sucker and rostellum, the muscular borderof sucker were covered with distinct microtriches. (x450).
c, d) The genital pore was covered with microtiches (x4500, x 15000).
e) Integument of gravid segments (x40).
f) Excretory pore (x110).

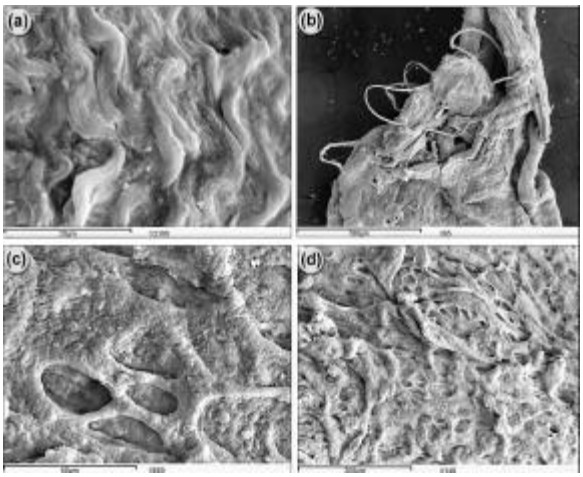

Fig. 3: Scanning electron micrographs of the intestine of mice infected with *H. nana*:

a) Anterior region of the illum of mice show that the villi are reduced in height and fused to give irregularly shaped ridges of tissue (x 2300).
b) Region of the ileum of mice with numerous long filamentous bacteria on the surface of mucosa associated with the presence of worms (x65).
c, d) Damaged and fused epithelial cells which may the scolex of *H. nana* becomes attached to the wall of intestine (x 800, x240).

SEM of *H. diminuta*: The length of worm is approximately up to 6 cm × 3.5 mm. The scolex of *H. diminuta* is provided with an armed sucker and rostellum as observed by SEM is presented in. The suckers located bilaterally on the dorsal and ventral surface. The suckers occupy large portion of the available surface area, thus suggesting their important functional role. The rostellum lies at the center of the scolex and has no hooks. Genital atria are unilateral and, similar to *H. nana* and surrounded with ordinary microtriches. The scolex and proglottides are all covered with posteriorly directed microtriches of uniform shape (Figs. 2a-d).

SEM of Intestine Removed from Infected Mice with *H. nana*: The intestine had showed some degree of villous atrophy and fusion. The villi were reduced in height and fused to form long ridges of tissue which gave a rippled, wave-like appearance. Long filamentous structures identified as bacteria were observed in large numbers, the bacteria appeared to be attached to the entrocytes by hold fast or attachment segments. However, small depressions were occasionally observed in the mucosa of infected animals and it is tentatively suggested that these represent the attachment site of *Hymenolepis*. A shallow groove was also seen in association with triangular-shaped protuberances. Villi in the center of the groove were further atrophied (Fig.3 a- d).

DISCUSSION

Scanning electron microscopy (SEM) succeeds in revealing novel micro topographical features of the host-parasite relationship, as well as proving invaluable in helminthes taxonomy. *H. nana* and *diminuta* are cosmopolitan in their distribution, but the former is more common in warmer climates, while the latter is a common parasite of rats and mice and only occasionally infects man [16-18].In the present study, the length of full strobilia of *H. nana* and diminuta were approximately up to 1-3 cm × 1mm and 6 cm × 3.5 mm, respectively. The SEM revealed that the scolex of *H. nana* and *diminuta* appears rectangular and is provided with a comparatively large rostellum and suckers. The rostellum lies at the center of the scolex and is provided with pointed and posteriorly directed hooks in *H. nana* and has no hooks in *diminuta*. This is in agreement with the view held by Rothman [19] and Ubelaker *et al*., [20]. In the present work, the fine structural of the tegumental surface of both *H. nana* and *H. diminuta* is densely covered with microtriches. This is in agreement with Ashour [9] who studied the surface ultra-structure of two cestodes, *H. nana* and *H. diminuta*, by SEM. He found that the scolex, suckers, rostellum and strobilia are all covered with dense populations of microtriches which are all of the same size and shape except on the scolex and suckers which are slightly more slender than those on the strobilia. Presently study the microtriches appeared of uniform shape and length along the strobilar surface and were never seen to be branched.Moreover, Abouzakham *et al*. [21] studied the cytological structure of tegument of *H. nana* by SEM. They found that the surface of *H. nana* is covered with dense populations of microtriches occur on scolex proper, suckers and strobilia; with an average density of 20/Fm^2. On the other hand, Jha and Smyth [22] reported polymorphic microtriches on the rostellum of *Echinococcus granulosus*. In the present study neither *H. nana* nor in *H. diminuta* this polymorphism was observed. Andersen [23], using SEM compared the surface structure of adults and larvae of three species of *Diphyllobothrium* and reported the presence of regional difference in microtiches appearance in the larva, but not in the adult cestodes. Also, polymorphism was observed in *Diphyllobothrium.*

In the present study the intestinal mucosa showed alterations in villous architecture, formation of shallow grooves and along filamentous bacteria also were observed. This runs in full agreement with Martin and Holland [13] who examined the intestinal morphology of rats given cysticercoids of *H. diminuta* by SEM. They found that the presence of this tapeworm cause extensive villous atrophy and fusion. The most extreme changes in mucosal architecture were observed adjacent to the mature proglottides of the worm. Bacterial over growth of the small intestine has occasionally been reported [24] but the factors influencing this change in habitat are not fully understood. Tannouk and Savage [25] suggested that dietary conditions and stress may affect bacterial colonization of the intestinal tract. Mettrick [26] and Smyth [27] showed that *H. diminuta* has a high rate of carbohydrate fermentation, which leads to the release of several acids, including lactic acid and it is possible that the presence of these substances may alter the physiochemical conditions along the intestine and thus facilitate colonization of the more anterior regions of the gut by these microorganisms. Gross alterations of intestinal mucosa ha observed in association with many nematode parasites. *Nippostrongylus brasiliensis* in rats [28,29], *Trichinella spiralis* in guinea pigs [30] and *Ascaris lumbricoides* in man [31] all cause varying degrees of villous atrophy and fusion. Flattening of the villous surface has also been observed in mice harboring *H. microstoma* [32].

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