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Original Paper

Histopathological study on rodent small intestine infected with an adult tapeworm, *Hymenolepis diminuta* recorded at Sohag, Egypt

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Abstract
Rodents play a significant role in public health, Chiefly due to their role as carriers or reservoirs of microbes and parasites of zoonotic importance. The main objective of the present study was to make a histopathological study on the small intestine of rodents infected with an adult tapeworm, *Hymenolepis diminuta* collected from two sites at Sohag, Egypt. The study revealed that The infection percentages of the present rodent with the adult tapeworm *Hymenolepis diminuta* were (12% females) and (16% males) and (12% females) and (8% males) in first and second sites, respectively. Histopathologically, the lumina of the present rodent intestine contained tapeworm. Presence of the tapeworm in lumina of the infected rodent intestine lead to excessive mucin secretion in luminal debris. Some intestinal villi appeared blunt and reduced in height. The intestinal muscularis layers were thickened. Moreover, inflammatory cells infiltration in the connective tissue core of the villi and crypts were observed. Erosion and adhesion of the tip of villi were observed in the intestine. The proliferating activity of the enterocytes was evidently increased and mitotic figures were observed not only in the intestinal crypts but also in the epithelium covering the middle third of the villi. Crypts and villi hyperplasia of intestine were observed.

Key words: Rodents, intestine, histopathology.

Introduction

Endo-parasites of rodents play an important role in the zoonotic cycles of many diseases. Several studies on endo-parasites of commensal and forest rodents have been carried out in Malaysia (Leong *et al.*, 1979; Ambu *et al*., 1996). Studies on the endo-parasites of rats have been carried out in several countries such as Malaysia (Leong *et al*., 1979; Chooi and Sani, 1985; Ambu *et al*., 1996; SyedArnez *et al.*, 2006; Paramasvaran *et al*., 2009).

Mezaros *et al.*, (1983) presented the first data on the helminth species of the house mouse and other rodents. Different authors have reported a wide range of rat helminthic parasites all over the world, (Nama and Parihar, 1976). in India, (Seong *et al.*, 1995). in Korea, (Yen *et al.*, 1996). in China, (Namue and Wongsawd, 1997). in Thailand, (Davoust *et al.*, 1997) in France, (Abd el-Wahed *et al.*, 1999). in Egypt, (Ceruti *et al.*, 2001). in Italy, (Stojcevic *et al*., 2004). in Croatia, (Al-Zihiry, 2006) in Iraq and Kataranovski *et al.* (2010) in Serbia.

Hymenolepis diminuta is the most common of the rat tapeworms all over the world (Ahmed *et al.*, 2015). Rats and other rodents are the definitive host, while arthropods such as fleas, Lepidoptera and coleopteran act as intermediate host (De Carneri, 2004). The occurrence of genus *Hymenolepis* in wild rats was reported by (Simmous and Walkey, 1971; Gardner and Schmidt, 1988). (Joseph, 1974) reported the presence of *Hymenolepis diminuta* in gray squirrel from Indiana. Also, (Seong *et al.*, 1995) and Iannacone and Alvariño (2002) studied intestinal parasites of rats. (Fagir and Rayab, 2009) described two species of cestodes, *Hymenpleis nana* and *H. diminuta* from Nile rats in Sudan.

Histopathologically, the tapeworm *Hymenolepis diminuta* is a chronic parasite living in the small intestine of rats, mice and humans (Dwinell *et al.*, 1998; Kosik-Bogacka and Kolsaa, 2012). This tapeworm produces some pathological changes in the entire small intestine of the host such as disappearance of intestinal villus, inflammatory swelling, and small bowel erosion and reduction of height (Fal and Czaplicka, 1991). In rodents, *H. diminuta* infection results in an increase in crypt depth as well as number of goblet cells in the villus of intestinal epithelium (Mckay *et al* 1990; Webb *et al*., 2007). Also, infected rats may also have mucosal mastocytosis and smooth muscle hypertrophy (Dwinell *et al.*, 1998). On the other hand, the presence of tapeworm *Hymenolepis diminuta* in rat small intestine caused reduction in intestinal permability and absorption of electrolytes

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and fluid (Podesta and Mettrick, 1974) and electro-physiological parameters (Palmer and Castro, 1986; Kosik-Bogacka *et al*., 2010).

The present study aims to describe the collected adult tapeworm from the caught rodents and study histopathological changes in the infected small intestine of rodents.

Materials and methods:

The present work was carried out in two trapping area, (Shandwell farm and surrounding houses, Sohag, Egypt). The study area was chosen for the present work because it is highly infested with rodents also the selected area occupied with different fruit trees (palm and mango), summer and winter field crops and vegetables

All rodents (Genus: *Rattus*) were trapped alive using specially made wire traps, each of which measures (24 x 11 x 9 cm) in both sites. Traps were baited with meat and carrot and sampling was carried out randomly.

50 rodents (25 individuals from each site) were caught alive from two localities in north east of Sohag city during the period of collection from May 2010 till April 2011. The collected rodents were dissected in the laboratory, and the small intestine of rodents were removed from the digestive tract, put in separate labeled Petri-dishes containing saline solution, and then examined separately under stero-binocular microscope searching for adult tapeworms.

The adult tapeworms were picked up by delicate forceps and put in another clean Petri-dish containing the saline solution. They were relaxed in cold water during 24 hours, fixed in hot alcohol-formalin- acetic acid, stained with carmine stain, dehydrated in graded series of ethyl alcohol, cleared in toluene and mounted on slide with Canada balsam. After preparation of adult tapeworms for study, they were examined and measured with an ocular micrometer of Olympus microscope (mm) and photographed.

For histopathological studies, small pieces of rodent small intestine were fixed in Carnoy's fluid dehydrated in graded series of alcohols, embedded in paraffin wax, sectioned at 5-7µm and stained with haematoxylin and eosin. The stained sections were examined under a research microscope and then photographed.

Identification of the present parasites was carried out according to the keys of Joyeux and Kobozieff (1928), Yamaguti, (1959), Leong *et al*. (1979), Miyazaki (1991) and Ambu *et al*. (1996) for adult tapeworm. The present host was identified by Harrison& Quah (1962), Medway (1983) and Payne *et al*. (1985).

Results:

50 rodents (25 individuals from each site) were collected and dissected in the laboratory. Table (1) shows the number of infected organs of rodent females and males with adult tapeworm, *Hymenolepis diminuta* in the two sites. In the first sites, 3 individuals of rodent females (12%) and 4 males (16%) were infected with the adult. While, in the second sites, 3 individuals of rodent females (12%) and 2 males (8%) were infected with the adult tapeworm.

Adult tapeworm	Infected organ	First site		Second site	
		Female	Male	Female	Male
	Intestine	3	4	3	2
% of infection		12	16	12	8

Table 1: Number of infected organs of rodents with adult tapeworm, *Hymenolepis diminuta* in the two sites of collections.

During gross examinations, small intestine of rodents showed adult tapeworms which was described as follows:

1- Scolex and suckers:

Macroscopic and microscopic examinations of the present rodent small intestine showed presence of adult tapeworms. The number of tapeworms ranged from 1 to 9 per host. The fresh worm is white in colour, filiform and cylindrical in shape. The body of tapeworm is differentiated into an expanded anterior end, scolex bearing hold organs, un-segmented neck and strobila which is very elongated. The scolex is slightly wider than the neck with unarmed rostellum . The four suckers are unarmed simple and cup shaped (Pl. 1A, B, C).

2- Segments:

Immature, mature and gravid proglottids are broader than long. It is important to note that all hymenolepidids have a single set of reproductive organs per segments, and genital pores are unilateral (Pl. 1D).

3- Male reproductive organs:

The male reproductive organs involve three testes (one poral and two aporal) and cirrus sac. Testes are spherical in shape and located in the median field. It is noted that single poral testes

separated by ovary from the two aporal ones. Cirrus is smooth and surrounded with an oval cirrus sac which is short and not reaching middle of segment and open into genital atrium just dorsal to the vagina (Pl. 1E, F).

4- Female reproductive organs:

The female reproductive organs involve multilobed ovary lying in the middle of segments with oval vitteline glands, an oval seminal receptacle and uterus. Uterus is a large transverse sac divided into an irregular network, and filled with spherical eggs. Eggs in all examined specimens are immature. They have two shells and without hooks. The space between the shells is filled with a loose cellular mass (Pl. 1G-J).

The embryos are spherical and vary in diameter. Table (2) summarized the measurements (mm) of adult tapeworm.

Parameters	Minimum		Maximum		mean	
Characters	Length	Width	Length	Width	Length	Width
Scolex	0.19		0.24		0.23	-----
Suckers	0.08	0.05	0.09	0.06	0.09	o.o6
Immature segments	0.12	0.7	0.20	1.4	0.3	1.15
Mature segments	0.19	1.74	0.48	1.79	0.23	1.76
Testes	0.13	0.13	0.19	0.15	0.16	0.14
Seminal receptacles	0.19	0.09	0.26	0.26	0.22	0.13
Gravid segments	0.29	2.05	0.67	2.1	0.48	2.01
Egg	0.04	0.03	0.09	0.06	0.07	0.04

Table 2: Measurements (mm) of adult tapeworm *Hymenolepis diminuta*.

The intestine of rodents is a long coiled tube and its wall consists of serosa, muscularis, submucosa and mucosa. These layers are arranged serially one below the other.

Histopathologically, the lumina of the present rodent intestine contained tapeworm. Excess mucin secretion were observed in luminal debris. Some intestinal villi appeared blunt and reduced in height. The intestinal muscularis layers were thickened. Moreover, inflammatory cells, infiltration in the connective tissue core of the villi and crypts were observed. Erosion and adhesion of the tip villi were observed in the intestine. The proliferating activity of the enterocytes was evidently increased and mitotic figures were observed not only in the intestinal crypts but also in the epithelium covering the middle third of the villi. Crypts and villi hyperplasia of intestine were observed (Pl. 2A-J).

Disscusion:

Hymenolepis diminuta is a cosmopolitan parasite, which has been commonly reported from the intestine of rodent hosts worldwide. The present adult tapeworm were observed in the lumen of intestine of rodents. This observation recorded by some authors such as Balachandra and Ranade (1978), Jawdat and Mahmoud (1980), Dwinell *et al.* (1998a, b), Webb *et al.* (2007), Singla *et al.* (2008) and Kosik-Bogacka and Kolasa (2012).

The number of infected organs of present rodent females and males with adult tapeworm *Hymenolepis diminuta* in the two sites reported as follows: in the first sites, 3 individuals of rodent females (12%) and 4 males (16%) were infected with adult cestode. While, in the second sites, 3 individuals of rodent females (12%) and 2 males (8%) were infected with adult *H. diminuta*. The incidence value for *H. diminuta i*n different types of rodents all over the world was reported 37% and 67% in Nile delta and Upper Egypt (Fahmy *et al.*, 1969) respectively, 41% in Suez canal (Abdel Aal. and Abou Eisha 1997), 13% in Khuzestan (Sadjjad and Massoud, 1999), 25% in Iraq (Al-Zihiry, 2006) and 50% in India (Singla *et al.*, 2008).

The tapeworm *H. diminuta* was reported in most studies done all over the world. It was recorded in Iraqi (Mahmoud, 1974; Al-Barwari *et al*., 1987; Al-Zihiry, 2002), in Thailand (Chitchang *et al*., 1978), Kuala lumpur (Leong *et al*., 1979), USA(Levi *et al*., 1987), Jamaica (Cohen, 1989), Nigeria (Miafiana *et al.*, 1997), Iran(Sadjjadi and Massoud, 1999), the Philippines (Claveria *et al.*, 2005) and in Egypt (Mohammad and Hegazi, 2007). On the other hand, hundred cases of children have been reported in humans infections with *H. diminuta* (Levi *et al.*, 1987; Panpiglione *et al*., 1987 and Cohen, 1989;).

The present tapeworm is medium in size, scolex with four suckers, rostellar hooks are absent. There are multistrobila its length is less than width 3 male reproductive organs involve three spherical testes, one poral and two aporal. Cirrus sac is oval. While the female reproductive organs involve multilobed ovary lying in the middle of the segments with oval vitteline glands, seminal

receptacle oval, uterus filled with eggs. According to Hussein (1986); Macko and Hanzelova (1997); Casanova *et al.*, (2001) and Al-zihiry (2006), the morphological characters of the present tapeworm corresponding to the adult tapeworm, *H. diminuta* .

Histopathologically, the lumina of the present rodent intestine contained tapeworm, *Hymenolepis diminuta* . Excessive mucin secretion were observed in luminal debris. Some intestinal villi appeared blunt and reduced in height. The intestinal muscularis layers were thickened. Moreover, inflammatory cells, infiltration in the connective tissue core of the villi and crypts were observed. Erosion and adhesion of the tip of villi were observed in the intestine. The proliferating activity of the enterocytes was evidently increased and mitotic figures were observed not only in the intestinal crypts but also in the epithelium covering the middle third of the villi. Crypts and villi hyperplasia of intestine were observed. Similar findings were reported by some authors Podesta and Mettrick (1974), Fal and Czaplicka (1991) and Kosik-Bogacka and Kolasa (2012). In rodents, *H. diminuta* infection results in increase in crypt depth and number of goblet cells in the villus of intestinal epithelium (Mckay *et al.* 1990; Dwinell *et al* 1998; Webb *et al.* 2007).

Abbreviations:

Ad = Adult of tapeworm
AT = Aporal Test of adult
CA = Cirrus of Adult
CR = Crypts of intestine
EA = Eggs of Adult
FF = Fine Fragmentation
GP = Genital Pores of adult
HA = Head of Adult
HI = Hepatic Inflammatory reaction
HP = Hyperplasia of intestine
ICI = Inflammatory cells inflitration
IO = Intestine Organ of rodent
IS = Inner Shell of eggs
MI = Mitoses of Intestine epithelium
NA = Neck of Adult
NW = Net Work reticulation of intestine epithelium
OA = Ovary of Adult
OS = Outer Shell of eggs
PT = Poral Testes of adult
RA = Rostellum of Adult
SA = Scolex of Adult
SI = Stroma of Intestine
SR = Seminal Receptical of adult
SUA = Sukers of Adult
SW = Sinusoid Widening
UA = Uterus of Adult
VA = Vagina of Adult
VG = Vitelline Gland of adult
VI = Villia of Intestine

List of Photos:

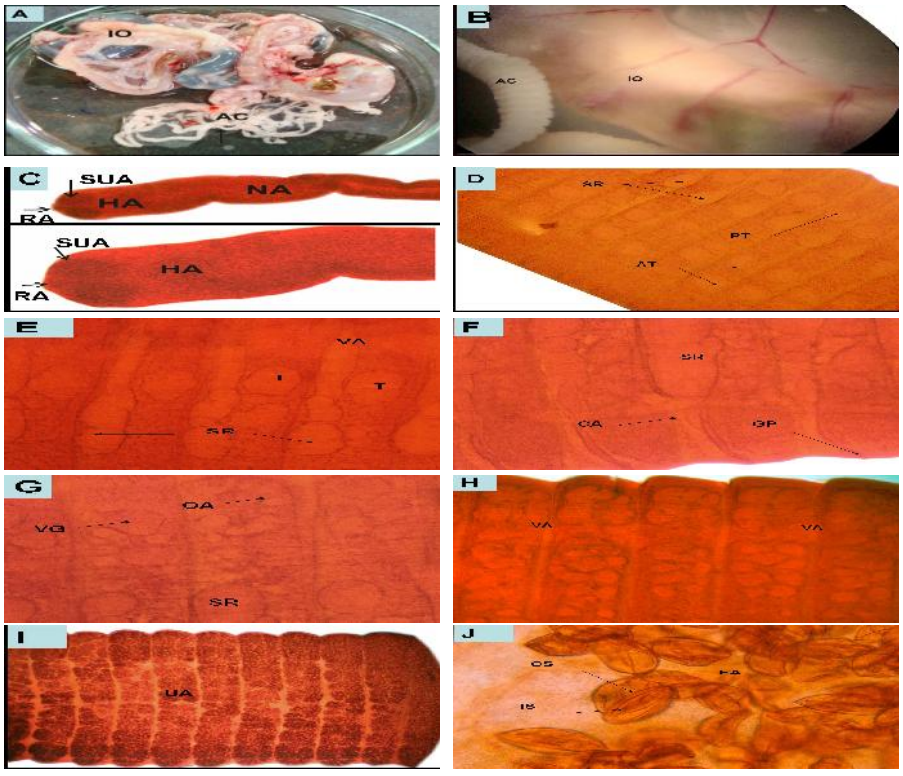

Plate 1: Photographs and Photomicrographs of dissected rodent small intestine infected with adult tapeworm, *Hymenolepis diminuta* showing.

A- Adult worm inside the intestine
B- Congested intestine of rodent
C- head, neck and suckers
D- Poral and aporal testes.
E- testes, seminal receptacles and vagina of matue segments.
F- cirrus and genital pores.
G- ovary and vitelline gland.
H- Pregravid segments with developing reticulate uteri
I- gravid segments with developing eggs in uteri
J- outer and inner shells of eggs

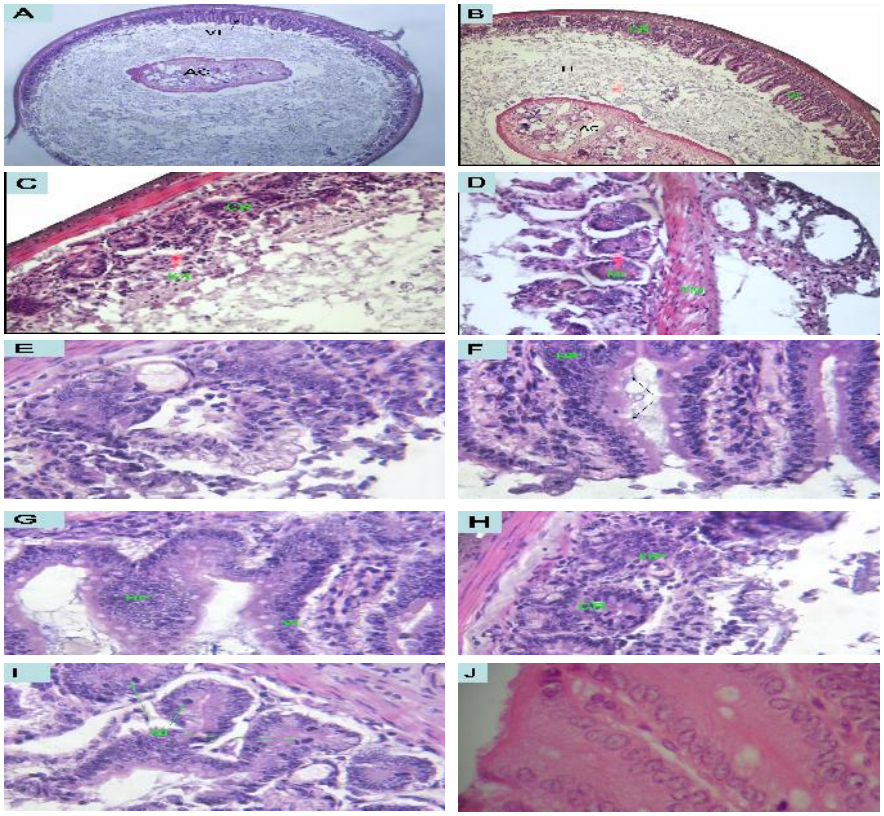

Plate 2: Photomicrographs of transverse histological sections through rodent small intestine infected with adult tapeworm, *Hymenolepis diminuta* showing.

A- worm inside the intestine lumen.
B- structure of intestine
C- Inflammatory cells infiltration
D- Thickened of muscular layer
E- erosion and adhesion of villi and crypts
F- brush border of villi disappeared
G- hyperplasia of villi
H- hyperplasia of crypts
I- Mitotic figures of crypts
J- Fusion and liquefactions of villi

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الملخص العربى

دراسة نسيجية مرضية على الأمعاء الدقيقة للقوارض المصابة بالطور البالغ لدودة الفأر الشريطية سجلت في سوهاج، مصر

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ملخص

تلعب القوارض دورا هاما في مجال الصحة العامة، بسبب أن معظم هذه القوارض تنقل أو تخزن الميكروبات والطفيليات التى تصيب الحيوان بالأمراض المختلفة. تم في هذا البحث دراسة موقعين في محافظة سوهاج، مصر لعمل دراسة نسيجية على الأمعاء الدقيقة للقوارض المصابة بالطور البالغ لدودة الفأر الشريطية. وقد أوضحت الدراسة بأن نسبة إصابة القوارض محل الدراسة بدودة الفأر الشريطية هى كالآتى: (١٢٪ للإناث) و (١٦٪ للذكور) فى الموقع الأول و (١٢٪ للإناث) و (٨٪ للذكور) في الموقع الثاني. تبين من خلال الدراسة المرضية النسيجية للأمعاء الدقيقة للقوارض المصابة بدودة الفأر الشريطية وجود إفراز مفرط للمخاط فى تجويف الأمعاء الدقيقة. كما ظهر انخفاض حاد فى ارتفاع بعض الخملات والكهوف، وكذلك زيادة فى سمك الطبقات العضلية المعوية. وعلاوة على ذلك، لوحظ وجود التهابات فى النسيج الضام للخملات والكهوف المعوية، وتآكل والتصاق وتضخم فى بعض الزوائد في الأمعاء و تزايد فى نشاط الانقسام الميتوزى لبعض الخلايا المعوية المختلفة.