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HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDIES ON CESTODE PARASITES INFECTING DOVES (*STREPTOPELIA SENEGALENSIS*) FROM EGYPT

By

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Abstract

Birds live in open environment carry a great risk of parasitic infections. The most important of these parasites are pathogenic cestodes. In February 2016, a total of 10 laughing dove (*Streptopelia senegalensis*) were examined grossly and microscopically. This study estimated ultrastructural features and histopathological impacts of 27 cestodes parasites isolated from the intestine of laughing dove (*S. senegalensis*) collected from Damietta Governorate. Data on morphology, hosts and infection intensity of the parasites were provided. The intestine was opened longitudinally examined looking for helminthes which examined by using both light and electron microscopy for identification.

Fine morphological criteria of cestodes in the present study are shown by using both light and electron microscopy. Microtriches, the surface features among cestodes comprised two basic types that actually play in the lives of cestodes and an integral role in the formation of certain "hard" structure in cestodes**.** At present some surface features "microtriches" are illustrated through scanning electron microscopy. Tissue samples were also taken for histopathological examination.

Keywords: Cestode parasites, Ultrastructure, Doves, *Streptopelia senegalensis*

Introduction

The laughing dove (*Streptopelia senegalensis*) is a small pigeon that is a resident breeder in Sub-Saharan Africa, the Middle East to the Indian Subcontinent as well as in Egypt (Madkour and Mohamed, 2019) This small long-tailed dove is found in dry scrub and semi-desert habitats where pairs can often be seen feeding on the ground (Bird Life International, 2015). Helminthes are major parasites of poultry animal. Several species of cestodes may live in the intestinal tract of chicken Zubeda *et al.* (2014). *Trypanosoma hannae* Pittaluga, 1905 was reported in laughing dove, *S. senegalensis* (Bennett *et al*, 1994), Ayadi *et al.* (2017) in Tunisian oases reported 10% of doves were West Nile virus (WNV) seropositive and 4% were Usutu virus (USUV) seropositive and recommended to study the dove ecto-parasites. Also, Ayadi *et al*. (2018) studied avian haemosporidian parasites in the laughing dove *Spilopelia senegalensis* and detected 2 new *Haemoproteus lineages*, related to other *Haemoproteus* transmitted by their biting midges. Lukášová *et al.* (2018) in South Africa by PCR in brains detected *Toxoplasma gondii* and *Neospora caninum* in red eyed dove (*Streptopelia semitorquata*), and laughing dove (S. *senegalensis*). Besides, Morsy *et al.* (1999) in Nile Delta Egypt reported zoonotic mite index as 4.74 on house sparrow (*Passer d. niloticus*), served as host for 25 mite species and 7.22 on laughing dove (*Streptopelia s. aegyptiaca*) served host for 28 mites species.

Cestoda (helminthes) were studied (Justine, 2003). *Raillietina* is a genus of tapeworms of family Davaineidae, order Cyclophyllidea are zoonotic parasites as well as in birds (Jawad, 2012). Structures ornamenting the surfaces of cestodes were first observed in the 19th century (Chervy, 2009). Several monographs focusing on specific cestode orders provided SEM data for the microtriches of a substantial number of species and /or genera. In his monograph on the trypanorhynchs, Palm (2008) presented one or more SEM image of microtriches on scolices of over 50 species with wide-array of trypanorhynch families; he also gave TEM images to interpretation of some of

the more interesting microthrix forms seen in certain members of this order. The microtriches structure, most specifically their possession of an electron-dense cap, makes them unique among the cellular surface elaborations seen in animals (Chervy, 2009). With respect to their pathological effects, severe lesions on the intestinal walls and diarrhea could arise that ostensibly resulted in illness (Unwin *et al,* 2012). Under heavy infection, *R. echinobothrida* caused conspicuous intestinal nodules in chicken, with characteristic hyperplastic enteritis associated with the formation of granuloma or nodular tapeworm disease (Jawad, 2012). Beetles and houseflies inhabiting poultry houses act as intermediate host for some cestodes (Baba and Oyeka 2005).

The present study aimed to study incidence of the cestodes infection in the laughing dove (*Streptopelia senegalensis*) by morphologically and histopathologically by using light microscopy and SEM in Damietta Governorate, Egypt.

Material and Methods

Collection of birds and cestodes: A total of 10 birds of laughing dove were collected by capturing by bird hunting nets during February 2016. The birds were immediately dissected and their intestine were removed and completely examined for parasites (Keymer, 2000). Nine *Raillietina echinobothridia,* eleven *Cotugnia digonophora* and seven *Amoebotaenia cuneata* were gathered from intestine of *Streptopelia senegalensis*. Cestodes were identified by standard keys (Wardle and Mcleod, 1952; Yamaguti, 1959; Sawada, 1965). Flukes were fixed in 10% formalin, flattened with minimal cover slip pressure, and stained with aceto-carmine stain. Specimens were then dehydrated in ethanol series, cleared in xylene and mounted in Canada balsam (Garcia and Ash, 1979). Pictures were taken with Digital Camera on Research Microscopy (Micros MCX 100). Histopathological study: Specimens of the intestines were fixed in 10% formaldehyde and washed under running tap water over night to remove excess fixative. They were dehydrated in ascending series of ethanol, in xylene-alcohol and then cleared in two changes of xylene, 30 minutes each. They were transferred into a mixture of xylene and melted paraffin wax for an hour and then two changes of pure paraffin wax, 30 minutes for each. Specimens were embedded in pure paraffin wax. Serial sections were cut at a thickness of 5-6 µ using rotary microtome. Sections were stained in haematoxylin and eosin (Drury and Wallington, 1967). Finally, stained sections were cleared in xylene, mounted in Canada balsam and examined by light microscopy.

SEM examination: Seven specimens of cestodes were gently removed from their hosts, cleaned with a brush in physiological saline solution, fixed for 24 hours at 4°C in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 and dehydrated with ethanol and critical point dried. They were mounted on metal stubs and coated with a gold film by sputtering, before examined with JEOL- JSM 5200 LV Field Emission SEM equipped.

Results

Seven specimens of 10 samples of laughing dove were infected with three species of Cestodes: *Raillietina echinobothrida*, *Cotugnia digonophora* and *Amoebotaenia cuneata*. The worms were medium in length with scolex, numerous immature and mature proglottids.

Morphological study*: R. echinobothrida* small sized cestode, edges of proglottid serrate, scolex large and subglobular (Figs. 1, 5 & 6h). The morphology and histology of scolex, mature and gravid proglottides of *C. digonopora* were given (Figs. 2, 5 &6h). The worms were medium in size, flattened, with many segments, consisted of scolex, imamture, mature and gravid segments. Scolex was large and quadrangular in shape (Figs. 1&5). Worms of *A. cuneata* were flattened. Scolex was small in size, globular in shape, slightly longer than broad and broad in the middle. Neck was long, wide longer

than bro-ad. Mature proglottids were medium in size, rectangular in shape, broader than long with irregular concave or convex lateral margins (Figs. 1, 5 & 6h).

Tegument description: Several monographs focused on specific cestode orders provided SEM data for microtriches. Filitriches, and especially spinitriches, come in a wide variety of forms. It was observed among the body region, particularly among scolex (Fig. 6a) and among proglottides (Figs. 6f & g) within individual species (Figs. 6b, c, d, e, g). Spinitriches possess relatively short prongs that were not more than half of the spinithrix length. These spinitriches were broadest basally, taper to a point, with straight sides rather than concave or convex (Fig. 6g).

Histopathological examination: Light microscopy of non-infected intestine hematoxylin and eosin stained sections showed that the intestine was composed of three main layers; mucosa, submucosa and muscularis (Fig. 4a, b). The intestinal mucosal epithelium composed of a single layer of columnar and mucous cells. The muscularis composed of an outer longitudinal and an inner circular muscle layer then intestinal lymph follicle (Fig. 4b). At the light microscope level, *Raillietina echinobothrida*, *Cotugnia digonophora* and *Amo botaenia cuneata* caused the major histopathological impacts included degeneration, necrosis, hyperplasia, dwarfism of villi and chronic inflammation at the attachment site to alimentary canal tissue. Light microscopy examinations of stained intestine sections showed that the attachment of the flukes exerted a compression against the intestine tissue at the attachment site causing necrosis, hyperplasia, degeneration and dwarfism of villi (Figs. 4c & d). At attachment site, cells were destroyed with complete superficial tissue erosion (Figs. 4e & f). Histopathology revealed enlarged columnar epithelial cells and villi associated with internal cestode infection (Fig. 4g). Intestine section also showed that the flukes filled the intestinal lumen resulting in intestinal obstruction (Fig. 4h).

Scanning electron microscopy examination showed the surface of non-infected intestine (Fig. 7a) was histologically normal. The surface ultrastructure of the infected intestine of laughing dove showed at the attachment site (Figs. 7b & c) regressive phenomena prevailed: superficial tissue erosion and destruction. Details were given in figures (1, 2, 3, 4, 5, 6 &7).

Discussion

The present study showed that the laughing doves were infected by three species of Cestodes: *Raillietina echinobothrida*, *Cotugnia digonophora* and *Amoebotaenia cuneata*, which were more common in warm seasons. Jassim (2016) reported that *R. echinobothiridia* from collared dove *Streptopelia decaocto.* Al-Rammahi *et al.* (2013) detected *R. echinobothiridia* in domestic and wild columbides birds. Both Nayyef (2012) and Omer *et al*. (2015) recorded *R. echinobothiridia* infected wild pigeons. Parsani *et al*. (2014) reported *R. echinobothiridia* infected *Columba livia.* The genus *Cotugnia* included one species *C. digonopora* from *Gallus gallus dometicus* (Diamare, 1893). The present *C. digonopora* agreed with Parsani *et al*. (2014) and Zubeda *et al* (2014) who reported *C. digonopora* from pigeon *C. livia.* Silva *et al* (2016) described *A. cuneata* from chickens, *Gallus domesticus*.

In the present study, SEM showed the scale-like spines (microtriches) covered the rostella base of some Davaineinae. Their arrangement and position on rostellar spines agreed with some other cestodes (Chervy, 2009). In the present study, intestine showed dwarfism of villi, hyperplasia, degeneration and tissues' necrosis as well as enlargement in columnar epithelial cells and their villi. These pathological data agreed with Samad *et al.* (1986) who reported that *R. echinobothrida* affected blood values and intestinal tissues of domestic fowls. Also, Nayyef (2012) reported similar intestinal pathological lesions in pigeons infected with

Raillietina spp., as infiltration of mononuclear cells and increase in the goblet cells number.

Conclusion

Cestodes parasites infected the doves. This was due to food availability and diversity of hosts. Light and SEM microscopies showed their characteristic morphology and pathological impacts in doves' intestine.

Recommendation

The morbidity rate of the\parasites especially *Raillietina echinobothridia* on pigeons must be considered. Zoonotic role of laughi- ng dove and migratory or resident birds must be in mind of zoology, parasitology and veterinary medicine authorities

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List of abbreviations

l	lumen,	lf	intestinal lymph folli-	hy	hyperplasia
v	villi	cf	cestode flukes	deg	degeneration
mu	muscularis	n	necrosis		

Explanation of figures

Figs. 1, 2 & 3: cestode flukes, F.1:*Raillietina echinobothridia*, a scolex, b mature segments, c gravid segments, F.2: *Cotugnia digonophora,* a scolex, b mature segments, c gravid segments and F.3: *Amoebotaenia cuneata,* a scolex, and b mature segments.

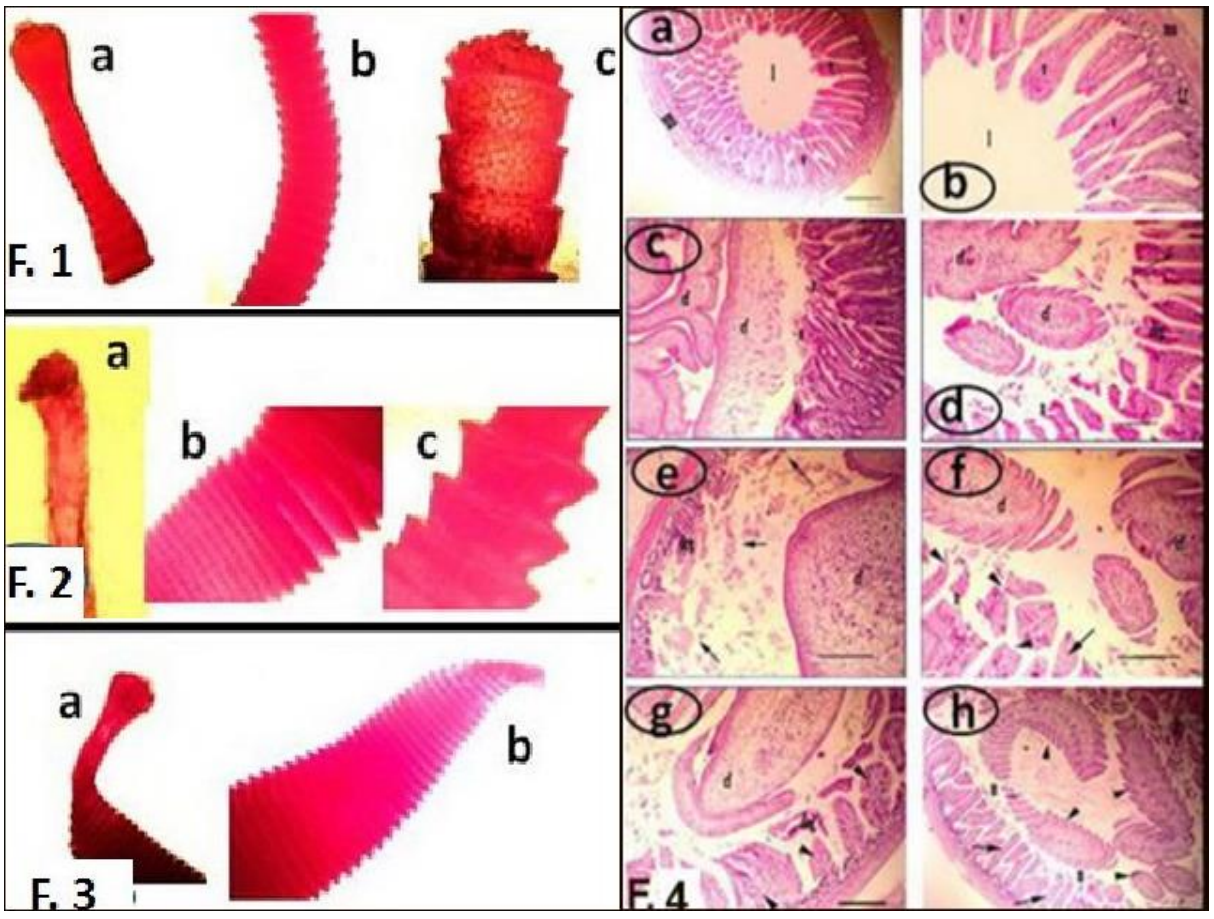
Fig. 4: Hematoxylin and Eosin staining of dove small intestine sections. a, b intestine of non-infected dove showing normal histology. l, lumen; v, villi covered with epithelium in mucosa; mu, muscularis; lf, intestinal lymph follicle. Scale bar: 100µm. c, Intestine section showed cf, cestode flukes attached with intestinal wall causing n, necrosis; hy, hyperplasia. Scale bar: 100µm. d, Intestine section showing dwarfism of villi (arrow); n, necrosis; deg, degeneration. Scale bar: 100µm. e, showed complete superficial tissue erosion of villi (arrows); deg, degeneration. Scale bar: 200µm. f, showed destruction and erosion of villi (arrowheads); n, necrosis; dwarfism of villi (arrow). Scale bar: 250 µm. g, Intestine section showed enlarged columnar epithelial cells and villi (arrowheads); deg, degeneration. Scale bar: 250µm. h, Photomicrograph showed intestinal obstruction due to cestode flukes (arrowheads); n, necrosis; dwarfism of villi (arrows). Scale bar: 200µm.

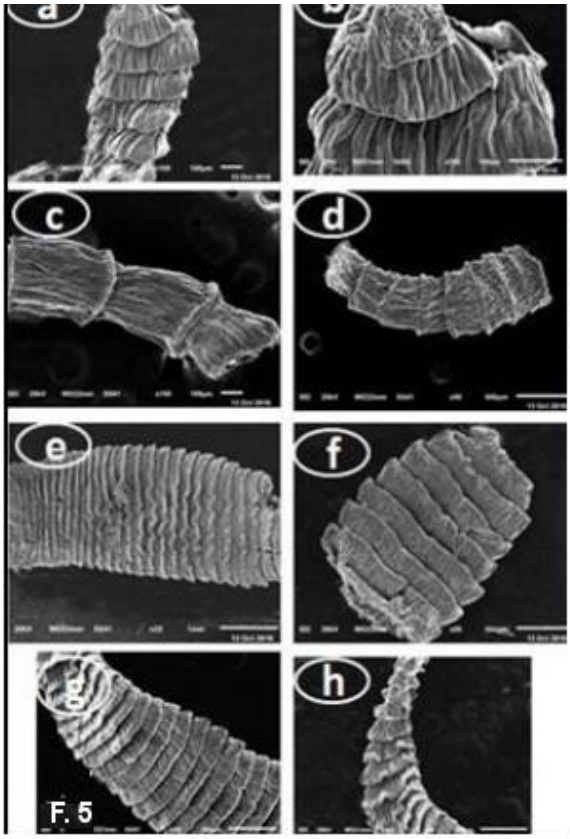
Fig. 5: Scanning electron micrographs of the cestode flukes body. a, b, c, d *Raillietina echinobothridia* a and b scolex c, gravid segments and d, mature segments. e, f, *Cotugnia digonophora* e, mature segments f, gravid segments. g and h mature segments of *Amoebotaenia cuneata*.

Fig. 6: Scanning electron micrographs (a – g) illustrating some shapes of filitriches & spinitriches. a, b, c, d, e *Raillietina echinobothridia* a, surface of apical region of scolex b, Strobila, gladiate spinitriches c, d, hastate spinitriches and acicular filitriches. e, Proximal bothridial surface; cyrillionate spinitriches and papilliform filitriches. f, g, *Cotugnia digonophora* f, mature segments g, Strobilar scutes; inset detail shows scutes composed of capilliform filitriches. h, cestode flukes separated from dove intestine and i, cestode flukes protrude from small intestine.

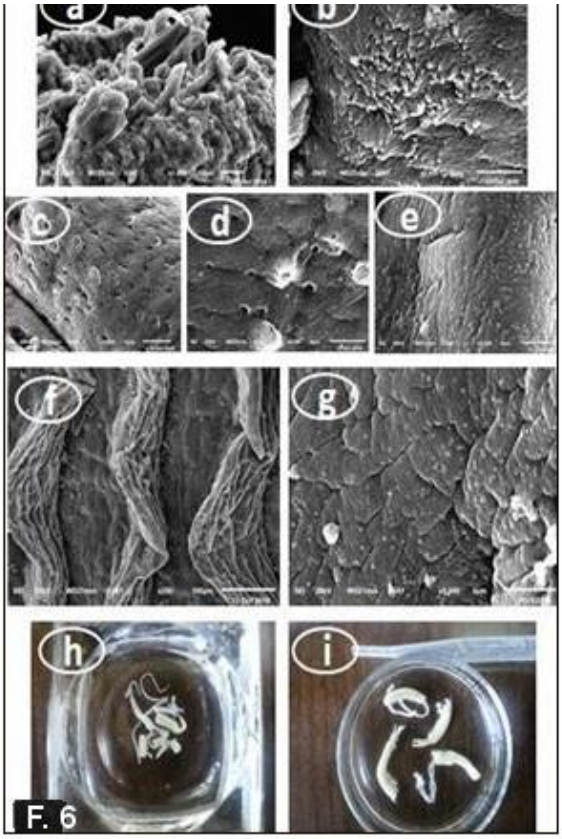
Fig. 7: SEM of dove small intestine. a, surface of non-infected intestine. b, c, SEM revealed erosion and destruction of infected intestine tissue (arrowhead)

Note, cestode dropped from intestine during specimen processing.

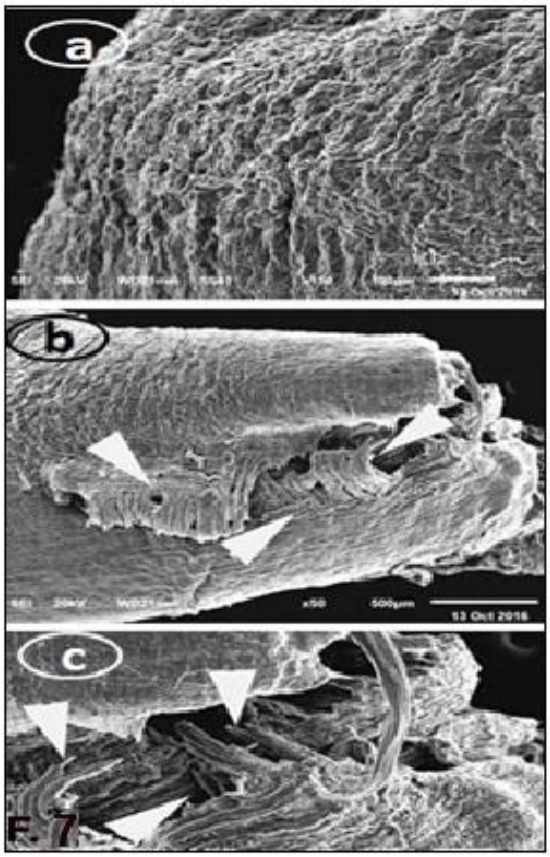




F. 5



F. 6



F. 7