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Surface ultrastructure of *Cardiocephalus longicollis* (Digenea: Strigeidae) from Herring gull, *Larus argentatus* and its associated pathological lesions

A. A. ABDEL-AAL, *M. F. M. SOLIMAN, *I. M. SHALABY

Department of Parasitology, Fac. Vet. Med.; *Department of Zoology, Fac. Sc., Suez Canal University, Ismailia, Egypt,
E-mail: *maha_soliman@hotmail.com*

Summary

Surface ultrastructure of strigeid trematode, *Cardiocephalus longicollis* (Rudolphi, 1819) from herring gulls, *Larus argentatus* (Pontoppidan, 1763) was investigated with highlighting on its pathogenic effect on intestine of gulls. Scanning electron microscopy showed that the digitiform processes of the holdfast organ, which appeared solid at light level, were pedunculated and formed hollow structure-like suckers and surrounded with longitudinal ridges and ornamented with fine tubercles. Outer surface of the processes was provided with minute needle-like spines. These modifications might explain the tight attachment and penetration of the flukes to the intestinal mucosa of infested gulls. Surface ultrastructure of hind body was smooth with exception of some interrupted longitudinal ridges, minute pores and elongated slits. Hind body was terminated posteriorly with an adhesive disc of the copulatory bursa, which was provided with minute papillae of sensory function around the genital opening. Intestinal histopathology showed that the aggressive invasion of *C. longicollis* to the intestine resulted in formation of numerous fibrotic nodules on the surface of the intestine accompanied with hemorrhagic spots and a severe destruction to the intestinal mucosa which might impair feeding, digestion and absorption processes.

Key words: *Cardiocephalus longicollis*; light microscopy; ultrastructure; histopathology

Introduction

Herring gull, *Larus argentatus* (Pontoppidan, 1763) has been considered a particularly good sentinel species for monitoring the overall health of an ecosystem because it is a top predator that consumes primarily fish and thus bioaccumulates persistent, lipophilic, organic pollutants (McCleary, 2001). The digenetic trematode, *Cardiocephalus longicollis* (Rudolphi 1819) had been recorded in Egypt since Szidat (1928) recovered it from *Larus* spp. Sudarikov (1959) recorded the genus *Cardiocephaloides* which was very similar to genus *Cardiocephalus*. Niewiadomska (2002) reviewed the family Strigeidae and concluded that the two previous genera are synonyms. Few studies had been done on the occurrence and description of this trematode (Jennings & Soulsby (1957) from herring gulls, *L. argentatus* in Britain; El-Sokkary (1992) from the gulls, *L. fuscus* in Egypt and Roca *et al.* (1999) from Audouin's gulls, *L. audouinii* in Spain). The life cycle of *C. longicollis* was completed between *L. argentatus* and *L. ridibundus* as final hosts and several marine fishes of family Sparidae and Scombresocidae which contained metacercariae of tetracotyle type (Prevot & Bartoli, 1981; Bartoli & Prevot, 1986; Lewis *et al.*, 2002). In spite of the unique structure of the forebody of *C. longicollis*, there is no available information on the surface ultrastructure. Surface ultrastructures of helminth parasites, as revealed by scanning electron microscopy, are helpful for not only taxonomic studies but also for immunological and drug efficacy studies. (Chai *et al.*, 2002). No information about the pathogenic effect of *C. longicollis* on the gulls is known while there were few studies on other trematodes (Appleton, 1984; Appleton & Randall, 1986). Thus, the present study was performed to observe the surface ultrastructure of this trematode and to study the histopathological changes in the intestine of the infested gulls.

Materials and Methods

A total of 30 herring gulls, *L. argentatus*, were collected from the shores of El-Manzalah Lake near Port-Said, Egypt. It had been noticed that there was an unexplained high mortality among *Larus* sp. population during the sampling time. Each bird was necropsied and its alimentary tract was removed, divided into several segments, incised, scrapped and shacked in physiological saline. The sedi-

ments were examined macro-and microscopically for helminth parasites. About 100 specimens of the trematode (*C. longicollis*) were collected, washed in saline and fixed in AFA (Alcohol, Formalin, Acetic Acid equal volumes) and processed for light microscopy according to Kuntz and Chandler (1956). Some specimens were fixed in 2.5 % glutaraldehyde in phosphate buffered saline for 4 hrs, then post fixed in 1 % osmium tetraoxide for 1 hr. The fixed specimens were dehydrated, air-dried, mounted on metal stub and coated with a thin layer of gold (Bozzola & Russel, 1992). The coated specimens were observed with high voltage scanning electron microscopy (Jeol JSM 35C at 30KV). Specimens of intestine were immediately removed out from gulls, fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thickness and stained with H&E for histopathological examination (Bancroft *et al.*, 1990).

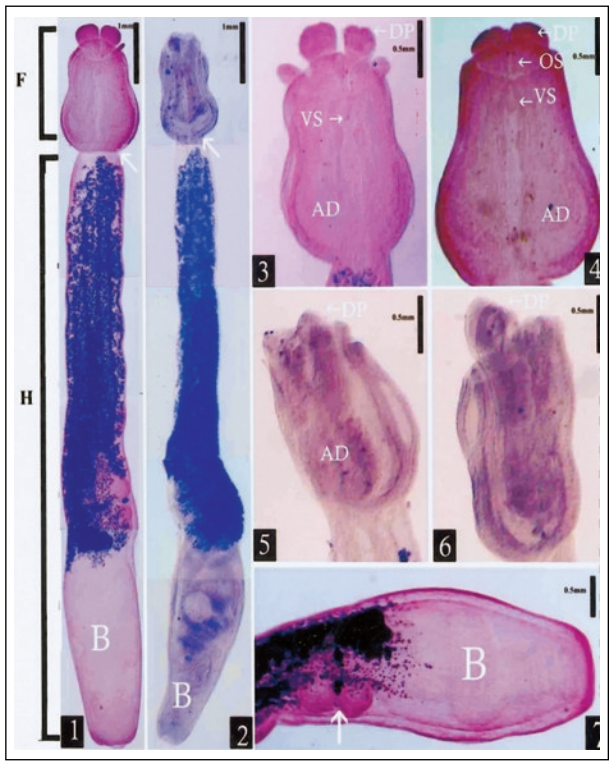
Results

Light microscopic observations

More than 100 specimens of *C. longicollis* were collected from the small intestine of infested gull. The fresh worms were pink in color. The mature worms, which contain eggs, varied from 8.2 – 15 x 0.8 – 1.2mm in maximum length and width with an average of 13.7 x 0.98 mm. Body was distinct bipartite. Forebody was small, cordiform and pear-shaped (Figs. 3, 4). The neck was attenuated and hind body was cylindrical, long and 5 – 8 times longer than the forebody (Figs. 1, 2). Ventral sucker was feeble (Fig. 3). Holdfast organ was well developed with four protrusible digitiform processes (Figs. 3, 5). Gonads situated in the posterior half of the hind body anterior to the copulatory bursa (Fig. 7). Vitellarium confined to hind body. Copulatory bursa was evaginable and voluminous (Figs. 1, 2). Genital cone at the base of the bursa sometimes protruded posteriorly (Fig. 1). Eggs were numerous large-sized, oval-shaped and measured 120 – 122 x 75 – 80 µm.

Scanning electron microscopic observations

Tegument of the forebody was provided with longitudinal ridges and corrugations, which spread on the outer surface. The outer surface of the digitiform processes was provided with sharp cuticular projections in the form of needle-like spines (Fig. 8). The digitiform processes were pedunculated to form hollow structure-like suckers, which surrounded with longitudinal ridges and ornamented with fine tubercles. The longitudinal ridges were curved internally at the rim of the opening of the cavity (Figs. 9, 10). Each of the two digitiform processes originated from one root (Fig. 9). In some cases, the opening of the hollow organ was narrow. Consequently, the wall became thick and the longitudinal ridges clumped with each other (Fig. 9). In other conditions, opening of the hollow organ was wide and the wall became thin and the longitudinal ridges spaced from each other (Fig. 11). The tegument of the hind body was interrupted by longitudinal ridges, minute pores and elongated slits (Fig. 12). The posterior extremity of the hind



Figs. 1, 2. Stained and unstained specimens of *Cardiocephalus longicollis* showing forebody (F), attenuated neck (φ) and hind body (H) with copulatory bursa (B); Fig. 3. Forebody with 4 digitiform processes (DP), feeble ventral sucker (VS) and adhesive organ (AD) Fig. 4. Forebody with 2 processes overlapping the other ones (DP), oral sucker (OS), ventral sucker (VS) and adhesive organ (AD); Fig. 5. Forebody (lateral view) with partial protruded digitiform processes, which connected to adhesive organ (AD); Fig. 6. Forebody (lateral view) with one protruded and other retracted processes (DP); Fig. 7. Posterior portion of hind body with two testes (η) and copulatory bursa (B)

body contoured with ovoid or circular adhesive disc of the copulatory bursa. There was an irregular genital opening nearly at the middle of this disc and large number of minute papillae which were regularly distributed on the complete outer surface of the adhesive disc (Fig. 13).

Histopathological findings

Gross morphology of intestine of *Larus* sp. infested with *C. longicollis* showed a presence of many nodules like structure on the outer surface of the alimentary canal and a pronounced hemorrhage. Histopathologically, sections of intestine revealed the presence of severe damage to the intestinal tissue due to both invasion of parasite and elicitation of an intense host reaction. Numerous fibrotic nodules were clearly visible on the serosal surface of the intestine that was localized at the interface between external muscular layer and intestinal serosa (Fig. 14). In addition, general atrophy for the villi had been noticed (Fig. 14). Section of nodules showed that cellular infiltration and fibrosis were the main component of these nodules (Fig. 15). Sections at the aggregation site of *C. longicollis*, showed the aggressive invasion of parasite to the intestinal tissues that

accompanied by cellular infiltration in muscular and fibrous layers of the intestine, reduction of intestinal brush border microvillus length and density, which resulted in a destruction or complete lacking of contact mucosal epithelia and presence of many hemorrhagic spots that reflect occurrence of hemorrhage at the site of parasite attachment (Figs. 16 – 19). No evidence of any inflammatory response was noted around the worms in the lumen.

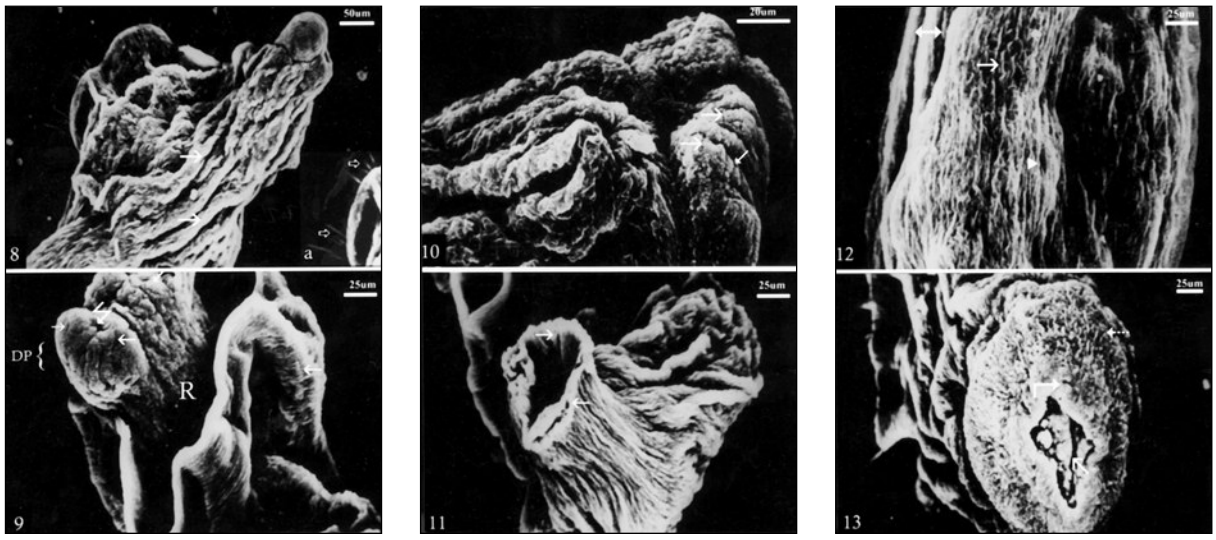


Fig. 8. Foreboby (lateral view) showing digitiform processes, which covered with needle-like spines, (α), longitudinal ridges and corrugations of the tegument (γ); Fig. 9. Digitiform processes pedunculated anterior end (DP) forming a hollow structure-like sucker (7) which surrounded with longitudinal ridges (γ) and ornamented with fine tubercles (λ), each two processes originated from one root (R). Notice the thinking of the wall of the hollow organ (ϕ); Fig. 10. Over view of digitiform processes with longitudinal ridges; it is curved internally (γ) and subdivided transversely with tubercles (λ); Fig. 11. High magnification of hollow structure-like sucker of digitiform process with dilated opening (γ) and thin wall (ϕ); Fig. 12. Tegument of hind body with interrupted longitudinal ridges (γ), elongated slits (ι) and pores (υ); Fig. 13. Posterior extremity of hind body showing the adhesive disc (;) of copulatory bursa with irregular genital opening (φ) and many papillae (3)

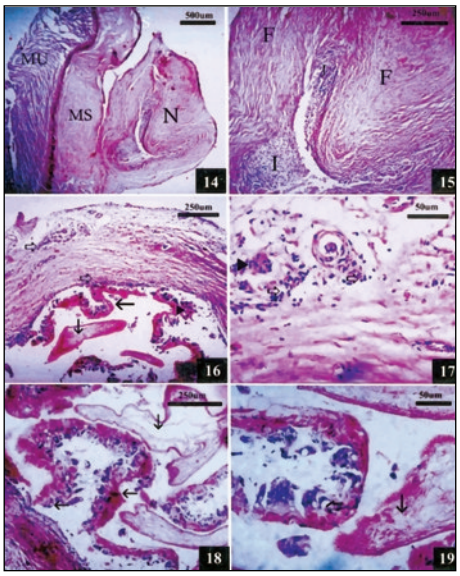


Fig. 14. Sections of intestine of herring gull infested with *C. longicollis*, showing formation of nodules (N) on the serosal surface of the intestine; MU: mucosa; MS: musculature. General atrophy for the villi was noticed; Fig. 15. Section of nodules showing its fibrotic component (F) and cellular infiltration (I); Figs. 16 – 19. Sections at the aggregation site of *C. longicollis*, showing the aggressive invasion of parasite (ι) to the intestinal tissues, cellular infiltration (α), hemorrhagic spots (υ) and destruction or complete lacking of contact mucosal epithelia (ϕ)

Discussion

The strigeid trematode which detected from small intestine of herring gulls (*L. argentatus*) during this study was identified as *C. longicollis* depending upon the morphological characteristics in which the fluke was demarcated into small, cordiform forebody, attenuated neck and long cylindrical hind body with evaginable voluminous copulatory bursa. These findings were similar to those of Yamaguti (1958) and Niewiadomska (2002). Our specimens were very similar to those of El-Sokkary (1992) who added that the species found in the gull (*L. fuscus*) in Egypt was very close to *C. longicollis* rather than other species of the genus. In addition, Niewiadomska (2002) stated that the genus *Cardiocephalus* had a well-developed holdfast organ. However, our study added that the holdfast organ was terminated anteriorly with four digitiform processes. At least one of them was protruded and functioned.

The surface ultrastructure of the forebody of *C. longicollis* showed that the digitiform processes which appeared solid at light level, was pedunculated and formed a hollow organ like-sucker, surrounded with longitudinal ridges and ornamented with fine tubercles. In our opinion, this structure helped this trematode to attach tightly to the intestinal mucosa of the infested gull. Also, the outer surface of these processes was provided with minute sharp needle-like spines, which help in penetration of the intestinal mucosa.

This structure functional relationship was in line to those of Valentin *et al.* (1998) and Chai *et al.* (2002) for the trematode *Philophthalmus lucipetus* and heterophyid trematode (*Pygidiopsis summa*), respectively. Also, the surface ultrastructure of the adhesive disc of copulatory bursa was provided with minute papillae, which spread regularly all over the surface around the genital pore. The function of these papillae is probably helping in cross-fertilization between two partners. However, this point needs further investigation.

The impact of parasitism on wild birds can be quantified in terms of the enhanced risk of population extinction. In this regard, the pathogenic effect of *C. longicollis* on herring gulls has not been investigated. Significant severe pathological changes attributable to the presence of parasites in intestine were observed in this work. In a study on a related parasite, *C. physalis*, Randall and Bray (1983) found that the parasite were responsible for several mortalities in chicks of Jackss penguin. Accordingly, the cause of death among herring gulls population noticed during sampling time was likely due to the aggressive destruction of mucosa, internal hemorrhage and formation of nodules. Often, tissue damage to the intestine of birds results in interrupted feeding, disruption of digestive processes or nutrient absorption, dehydration, anaemia and increased susceptibility to other disease agents (Friend & Franson, 1999).

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