Pathology Induced by *Sphaerirostris picae* (Acanthocephala, Centrorhynchidae) in the Hooded Crow *Corvus corone cornix* (Aves: Corvidae) from North Delta of Egypt

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Abstract: The present study describes the pathological manifestations of the acanthocephalan, *Sphaerirostris picae* (Rudolphi, 1819) Golvan, 1960 in the small intestine of the hooded crow "*Corvus corone cornix*" collected from the northern parts of Nile Delta in Egypt. Histological and histochemical alternations of infected ileum were illustrated using heamatoxylin and eosin stain, alcian blue method for mucin and Malaty's modified simultaneous coupling azodye method for acid and alkaline phosphatases. Examinations of 25 birds showed that the infected ileum only harbored the acanthocephalan worm, no other helminthes were observed. The proboscis of the acanthocephalan pierced the mucosal epithelium, its lamina propria and reached the external muscularis causing compression and erosion of the villar epithelium, shortening and abrasion of the intestinal villi and destruction of the glands (crypts) apposing the everted worm proboscis. Noticeable cellular infiltration, hemorrhage and marked destruction, thickening and vacuolation of stromal connective tissue surrounded the acanthocephalan preasoma, as well as in the submucosa were detected. A marked increase in the number of goblet cells in both crypts and villi was observed. The intestinal epithelium exhibited a detectable increase in acid phosphatase activity in both villi and crypts while alkaline phosphatase showed moderate decrease in the villi and detectable decline in the crypts epithelium.
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Key words: *Sphaerostris picae,* Acanthocephalae, *Corvus corone cornix*, Histopathology, Phosphatases, Egypt.

1. INTRODUCTION

The pathological changes in the parasitized tissues brought Abot by acanthocephalans form an important factor in determining the ability of these parasites to adapt at their environment. The body of acanthocephalans consists of a trunk or metasoma, freely lying in the intestinal lumen and the praesoma (proboscis and neck) that has the ability to penetrate into the intestinal wall of the host. **Wanstall *et al.* (1986)** revealed that the common consequence of acanthocephalan infection in vertebrate host is damage to the mucosal epithelium of the gut by the main body (metasoma). In addition, the attachment organ (praesoma) can damage the gut at the attachment site.

Palmer and Meerveld (2001) referred to the inflammation of the host induced by intestinal parasites, resulting in altered gastrointestinal function, namely enhanced secretion and propulsive motility of the gut. **Sanil *et al.*, 2010** reported that the acanthocephalan histopathology in the intestine as revealed by loss/degeneration of the intestinal villi, formation of granular tissues and capsule are associated with host immune responses. Depending on their attachment mechanism, they are able to seriously disrupt the integrity of the mucosal gut layer, inducing lesion of wide degree from shallow erosions to deep ulcerations with hemorrhage and perforation of the gut wall. Invasion/migration of the acanthocephalans into uncommon locations has also been reported (**Nickol, 2006**).

The hooded crow is a widely distributed bird and a common resident inhibiting cultivated land and open and wooded terrain in the Nile Delta and Valley in Egypt (**Tharwat, 1997**). Although its food habits include wide range of intermediate and paratenic hosts (insects, mollusks, worms, small vertebrates and carrion), its entire intestine has been reported as microhabitat of only one acanthocephalan species; *Sphaerirostris picae* with no infection of any other helminth species (Radwan, personal communication). To the best of our knowledge, no information is available due to the pathology induced by *S. picea* infection in the hooded crow.

The present study was undertaken aiming to determine the histopathological changes in the intestine of the hooded crow *Corvus corone cornix* by the invasive action of the well armed proboscis of the big - sized acanthocephalan; *S. picae*. The mucopolyscharrides and phosphatases have been localized in the intestinal tissues.

2. MATERIALS AND METHODS

Sampling

Twenty five hooded crows were collected from the agricultural areas around Kafr El Sheikh Governorate. Birds were transformed alive to the laboratory, anesthetized and dissected. The intestines were incised longitudinally and examined in 0.7% physiological saline for the parasite infections. Some acanthocephalan specimens attached to the wall of the

intestine were dissected out with the aid of fine needles and forceps, and then fixed in cold 70% ethanol.

Whole mount preparation

For preparing whole mount of collected acanthocephalans, specimens were stained in Mayer carmine and cleared in Terpiniol according to **Amin, 1998**. Acanthocephalan specimens were identified according to **Dimitrova *et al.*, 1997 and 2000**.

Histopathological examination

Pieces of 1 cm in diameter of infected ileum were fixed in Bouin's solution, dehydrated in an ethanolic series, cleared in xylene and embedded in paraffin wax. 5 µm paraffin sections were stained with haematoxylin and eosin (HE) and mounted in Canada balsam. Slides were examined with Olympus CX31 equipped triocular light microscope and representative photomicrographs were taken with E-330 DC 7.4V digital camera. For comparison uninfected host tissue was also sectioned and examined.

Histochemical examination

Alcian blue method for demonstrations of acid mucoplysaccharides was done (**Steedman, 1950 and Lison, 1954**).

Suitable parts of both infected and uninfected ileum were used for the preparation of cryostat sections of 8µm thickness. The frozen sections were treated according to Malaty's modified simultaneous coupling azodye method (1971) for detection of the activity of acid and alkaline phosphatases. Red granules of the Azo dye deposits indicate sites of acid phosphatase and bluish granules indicate sites of alkaline phosphatase activity.

3. RESULTS

All the examined birds appeared healthy without any external clinical manifestation concerning the weight, activity and feather appearance.

S. picae is a large-sized acnthocephalan (Figure 1a) with well armed proboscis (Figure 1b). It was restricted to the ileum; no infection was detected in the duodenum, jejunum or cecae. Heavily infected parts of the ileum appeared swollen with the anterior ends of some worms deeply embedded. Along all the number of examined infected intestine (25), no worms were found outside the intestine. At the site of parasite attachment, the surface of the ileum appeared thickened (Figure 1 c).

Histopathological observations

Sections of the ileum of uninfected hooded crow showed the normal features of bird ileum consisting of an outer serosa, muscularis externa with outer longitudinal muscle fiber and thick inner circular muscle fiber. The intestinal glands (crypts) are closely packed in the demarcated lamina propera. The villi are tall and regular arranged with columnar epithelial lining included mucus secreting goblet cells and the lumen is narrow (Figure 2a). Strips of smooth muscle fibers forming the muscularis mucosa extend in-between the intestinal glands (crypts) and villi. A core of lamina propria with diffuse lymphatic tissue and small blood vessels extend inside the intestinal villi (Figure 2b, c).

When the penetration of S. *picae* proboscis through the wall of the ileum is shallow, it reaches the mucosal and submucosal layers only, and this is accompanied by blunting, shortening and destruction of the intestinal villi (Figure3a), and compression and erosion of their columnar epithelium apposed the everted worm proboscis, with noticeable increase in the number of goblet cells which open in the inter-villous space (Figure 3e). A marked destruction of intestinal gland (crypts) and an increase in the number of goblet cells were detected (Figure 3c, d).

Marked cellular infiltration was seen in the stromal connective tissue surrounding the everted proboscis in both submucosa and muscularis (Figure 3a). The serosa and muscularis presented destruction, thickening and vacuolation of the stromal connective tissues (Figure 3b, f). Dilation and congestion of blood vessels in stromal connective tissue between the muscle bundles have been detected (Figure 3f).

The deep penetration of the worm preasoma to reach muscularis layer was accompanied by full thickness of mucosa and submucosa with cellular infiltration and hemorrhage surrounding the penetration site which enclosed poorly differentiated lymphocytes (Figure 4a).

The tissues around the proboscis were hemorrhagic and granulated (Figure4b). The epithelial cells of the intestinal glands near the site of proboscis attachment, exhibited crypts hyperplasia and hyperchromatic nuclei and mitotic activity (Figure 4 b&c). There was noticeable fibrosis and dense fibrous stroma around the worm proboscis (Figure 4d). In the submucosal layer, dilation and congestion of blood vessels and stromal lymphocytic infiltration was clearly observed (Figure 4f). The presence of aggregation of lymphocytes and large number of eosinophil granulocytes and fibroblasts in the inflammatory sites, suggest inflammatory responses (Figure 4e).

Histochemical study

I- Acid mucopolysaccharides

A moderate alcian blue stain revealed the presence of acid mucopolysaccharides in the mucus secreting goblet cells in the villi and crypts of uninfected sections (Figure 5a). Due to the erosion of the mucosal epithelium facing worm attachment sites, negative reaction of mucopolysaccharides was detected in these areas. However, villi and crypts near but not facing the attachment sites showed strong alcian blue reaction as a result of the increase in number of goblet cells (Figure 5b).

II- Enzymes

Acid phosphatase

Acid phosphatase activity in the control ileum was intensed in the epithelial cells lining the villi and crypts (Figures 5c, e). In infected ileum, the intestinal epithelium exhibited a detectable increase in acid phosphatase activity in both villi and crypts (Figures.5d, f). Strong reaction was noticed at the worm attachment sites (Figure 5d).

Alkaline phosphatase

Alkaline phosphatase was detected in the intensely strained border of the intestinal epithelium of the villi and crypts (Figures 5g, i). In infected sections there was moderate decrease in the enzyme activity in the villi and detectable decline in the crypts cells (Figures 5 h, j). No reaction was detected around the worm attachment sites (Figure 5h).

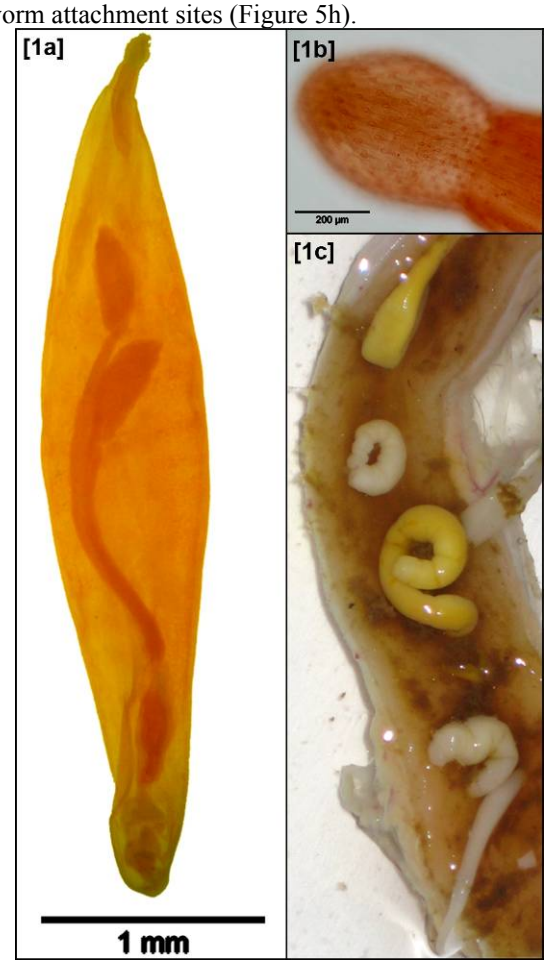


Figure 1: Whole mount of adult male *S. picae* (Mayer's carmine stain).

Figure 1b: Enlarged heavily armed proboscis of *S. picae* (Mayer's carmine stain).

Figure 1c: Infected intestine of the hooded crow opened to show thickening at the site of *S. picae* attachment.

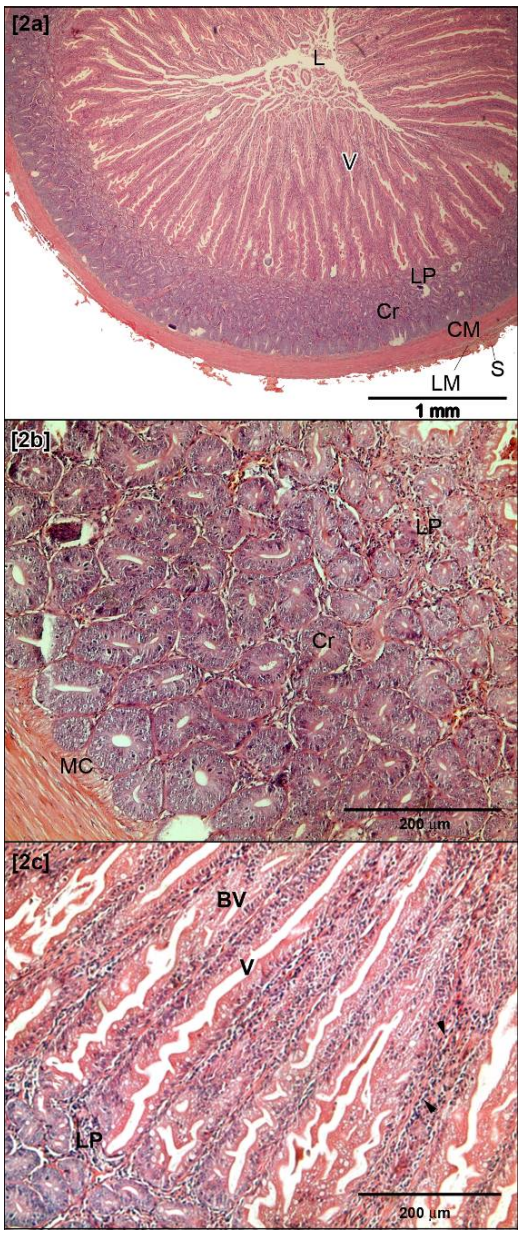


Figure 2. Light micrographs of histological sections through the ileum wall of hooded crow (heamatoxylin and eosin preparation)

Figure 2 a: Transverse section of uninfected ileum to show normal features; outer serosa (S), muscularis externa with outer longitudinal muscle fiber (LM) and thick inner circular muscle fiber (CM), cross sections of intestinal gland (crypts) (Cr) closely packed in demarcated lamina propera (LP), tall regularly arranged villi (V) and narrow lumen (L).

Figures 2 b, c: Magnified parts of the previous section to show strips of smooth muscle fibers; muscularis mucosa (MC) extended in-between the intestinal glands, tall regular intestinal villi (V) exhibiting a columnar lining epithelium with strait border and goblet cell (GC),and core of lamina propria with diffused lymphatic tissue (arrow head) and small blood vessels (BV).

Figures (3-6): **Light micrographs of histological sections through the ileum wall of hooded crow naturally infected with *S. picae***

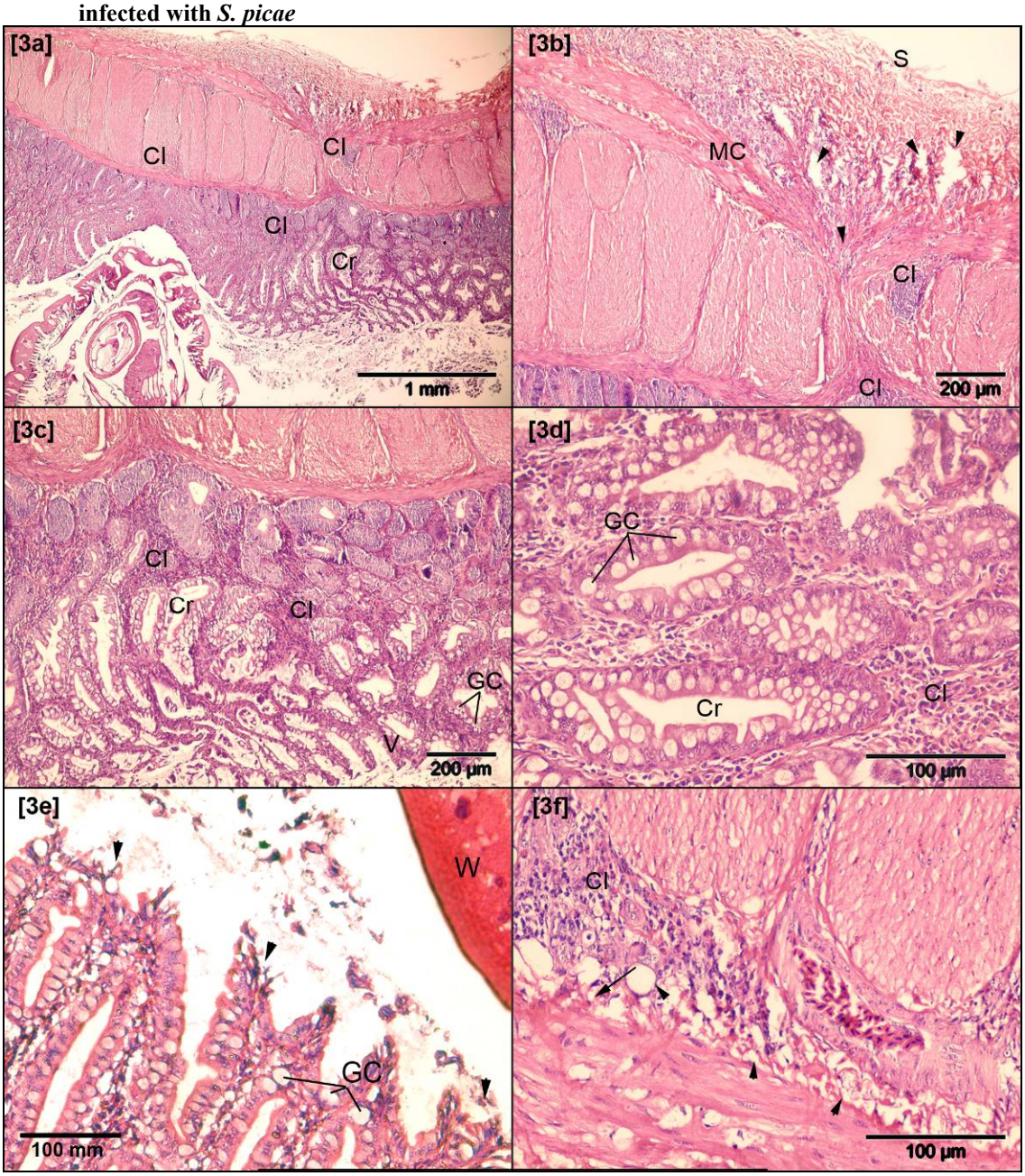


Figure 3a: Longitudinal section exhibit invasion of the worm to the ileum wall causing noticeable destruction of its layers by *S. picae* preasoma. Note the blunting, shortening, and destruction of the villi, compression and erosion of epithelial cells (arrowheads), marked cellular infiltration (CI) and heamorrage (H) in the stromal connective tissue.

Figure 3b: Magnified parts of figure 4a showing the muscularis mucosa (MM) and serosa (S) layers with destruction, thickening and vacuolation of stromal connective tissue (arrowheads), and marked cellular infeltiration (CI).

Figure 3c: Magnified parts figure 4a showing the mucosal layers with disorganization and destruction of both villi (V) and crypts (Cr), notable cellular infeltiration (CI).

Figure 3d: High magnification of intestinal glands (crypts) (Cr) showing the increase in number of goblet cells (GC) and remarkable cellular infiltration (CI) between the crypts.

Figure 3e: High magnification of intestinal villi showing destruction of villi with erosion of villous epithelium (arrowheads) facing the integument of the worm (W) and increase of the goblet cells (GC) that open into the intervillous spaces.

Figure 3f: Magnified parts figure 4a showing dilation and congestion of blood vessels (arrow) and vacuolization and destruction in stromal connective tissue between muscle bundles (arrowheads).

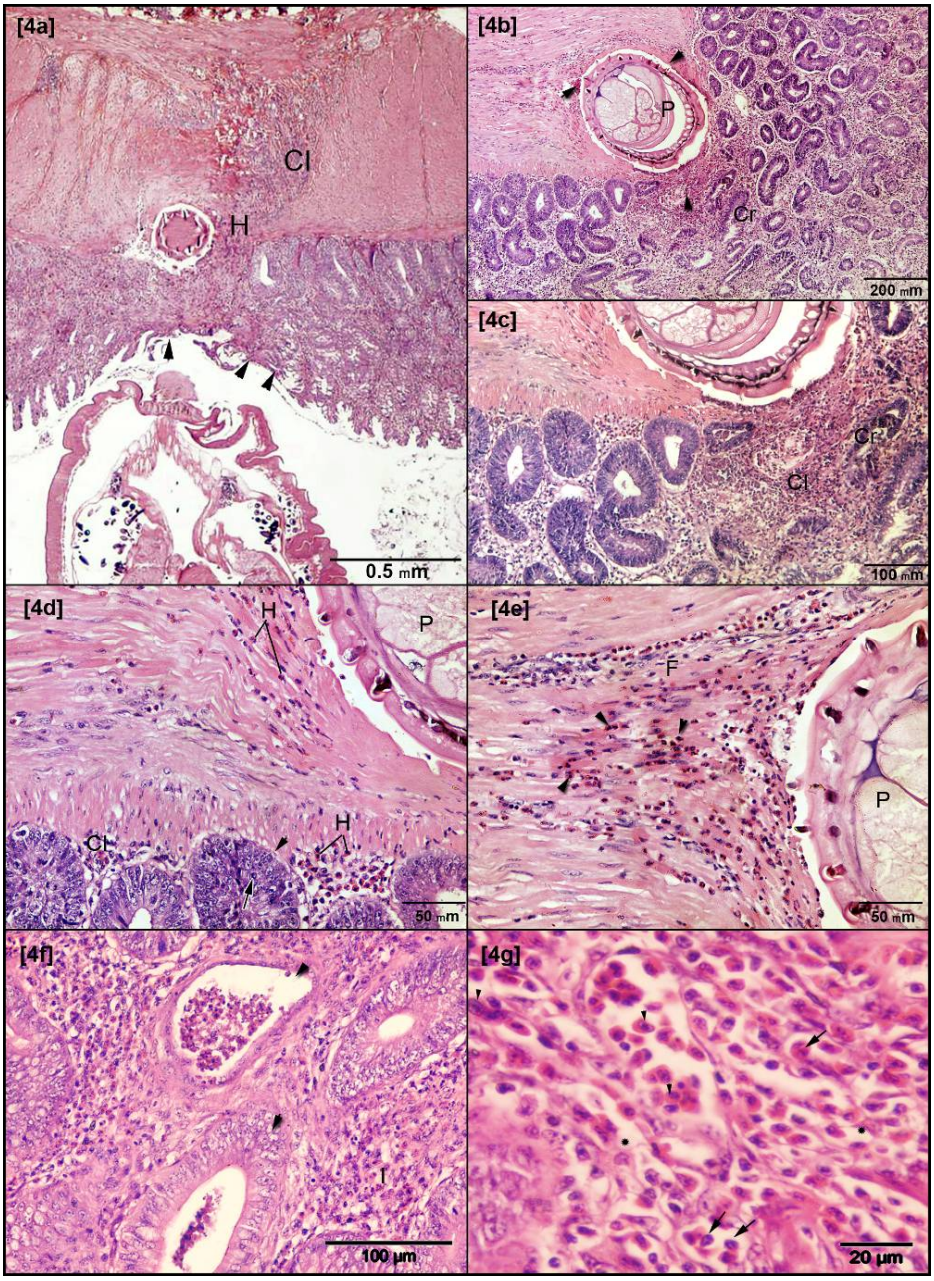


Figure 4a: Longitudinal section shows that deep penetration of the worm to reach the muscular layers is accompanied by destruction and blunting of the villi and crypts (arrowheads) and full thickening of the mucosa and submucosa with cellular infiltration (CI) and hemorrhage (H) surrounding the penetration site which encloses poorly differentiated lymphocytes.

Figure 4b: Magnified parts of figure 4a shows the destructed intestinal glands (crypts) (Cr), villus (V) atrophy and crypts hyperplasia(arrows). Note that the thickness of mucosa and submucosa surrounding the worm is replaced by poorly differentiated lymphocytes (arrowheads)

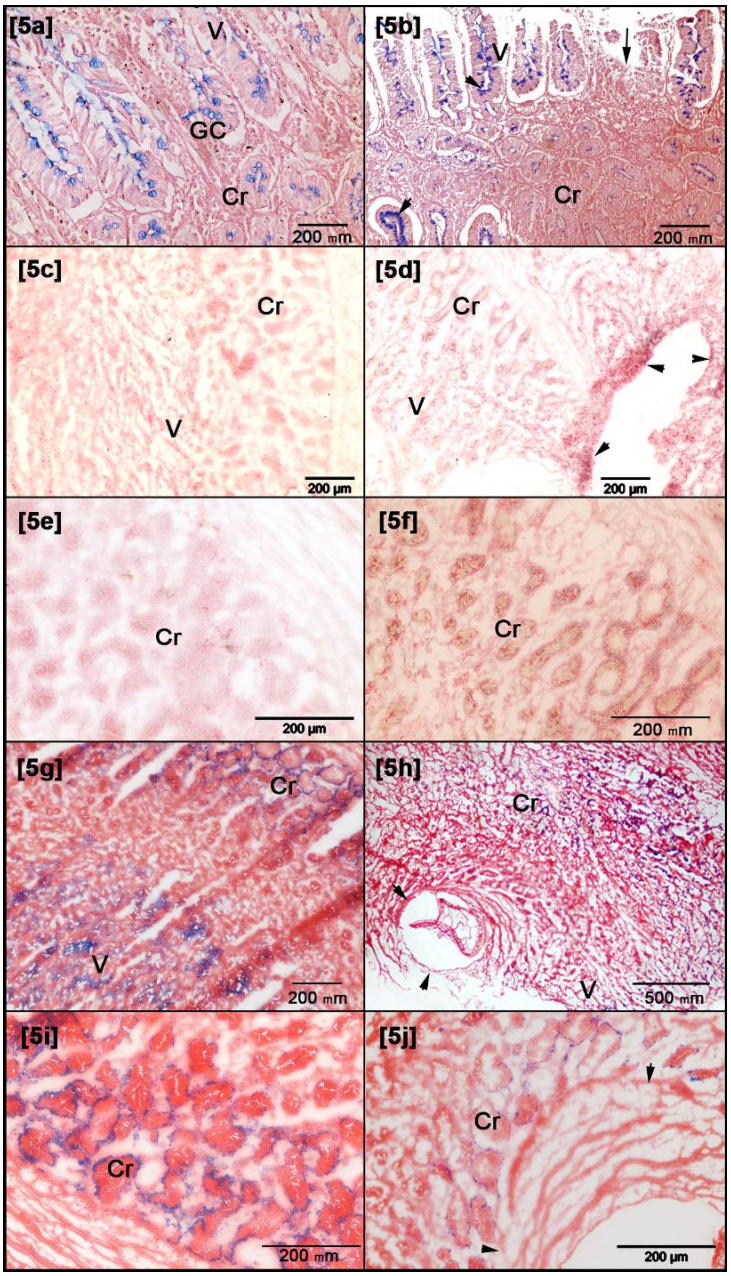
Figure 4b, c: Magnified parts of figure 4a shows high stromal cellular infiltration (arrowheads) around the proboscis (P) and destruction of crypts (Cr) towards the invasive proboscis.

Figure 4d: High magnified part of figure 4a exhibits heamorragic reaction (H) in muscle bundles and at the base of crypts surrounding the proboscis (P). Note that the epithelial cells of intestinal glands exhibit crypts hyperplasia (arrows) and hyperchromatic nuclei and mitotic activity (arrowheads).

Figure 4e: High magnified part of figure 4a exhibits noticeable fibrosis (F) around the worm proboscis (P), active fibroblasts (arrowheads) and dense fibrous stroma (arrows).

Figure 4f: Another magnified part of intestinal mucosa to show crypts hyperplasia (arrows), dilated and congested blood vessels (arrows) and stromal lymphocytic infiltration (I).

Figure 4g: Magnification of cellular infiltrated area surrounding the proboscis, aggregation of lymphocytes (arrowheads), eosinophil granulocytes (arrows) and fibroblasts (star) at the site of inflammation.



Figures 5a, b: Histochemical localization of acid mucopolysaccharides

Figure 5a: Intestinal mucosa of uninfected crow exhibits normal distribution and activity of mucus secreting goblet cells (GC) in both crypts (Cr) and villi (V).

Figure 5b: Intestinal mucosa of infected crow show decrease in number of mucus secreting goblet cells (GC) in the site of worm attachment in both destructive atrophid crypts and villi (arrows), while those nearby the worm site show high active goblet cells (arrowheads)

Figures 5c-f: **Histochemical localization of acid phosphatase:**

Figures 5c and 5 e: Acid phosphatase reaction in uninfected intestine presenting moderate activity intensed in the epithelial cells lining the villi (V) and crypts (Cr).

Figures 5d and 5 f: Acid phosphatase reaction in infected intestine presenting detectable increase in activity mainly in crypts cells (Cr), and epithelial lining of the villi (V). Strong reaction is noticed in the proboscis attachment sites (arrowheads).

Figures 5g-j: Histochemical localization of alkaline phosphatase

Figures 5g and 5i: Alkaline phosphatase reaction in uninfected intestine presenting strong activity in the strained border of the epithelium of the villi (V) and crypts cells (Cr).

Figures 5h and 5j: Alkaline phosphatase reaction in infected intestine presenting moderate decrease in enzyme activity in the villi (V) and detectable decline in the crypt cells (Cr). Negative reaction is noticed in the proboscis attachment sites (arrowheads).

4. DISCUSSION:

Persson (1974) and Galaktionov and Bustnes (1996) proposed that the pathogenic effects of helminths on birds might be manifested as a reduction in their populations. **Sala and Martorelli (2007)** referred to many underlying processes, including, food resource shortage, environmental contaminants, inclement weather, infectious diseases, sibling competition, and internal parasitism which may interact together and lead to render a host more susceptible to the effects of parasitism.

Acanthocephalans have been reported in recurrent mortality events in many birds such as the common eider (**Camphuysen *et al.*, 2002**), mute swan (**Sanford, 1978**), Olrog's Gulls (**Sala and Martorelli., 2007**) and ducklings (**Hollme´n *et al.* ,1999**). **Taraschewski, 2000** and **Sanil *et al.*, 2010** observed that the histopathological changes due to acanthocephalan infections depend on various factors such as species of parasite and host, nature of the infected tissues and host-parasite interactions. The nature and thickness of the various host tissue layers, length of the neck and proboscis, presence or absence of a proboscis bulb and the nature of spination in the acanthocephals also affect the pathological outcome.

Although the present observations revealed that *S. pica* is injurious to the hooded crow's ileum tissue and create histological alternations, bird with unusually moderate parasite load (2-5 worm/cm^2) appeared healthy without any clinical manifestations. In agreement with **Taraschewski (2000)**, the probosces of penetrated worms appeared more or less evaginated. The author reported that the proboscis of the adult would not completely evert during the penetration of host tissues.

The penetration of the intestinal wall seems to be both mechanic and chemical. The advancement of the large-sized *S. picae* with well armed proboscis through the wall of intestine may be due to partial evagination and invagination of the preasoma in combination with contractions of the metasoma. The degree of penetration depends on the probosces orientation relative to host intestinal wall. If the proboscis is directed transversally to the mucosal layer, shallow penetration will happen, and the pathological effect will be limited in the mucosal and submucosal layers. This action may induce blunting, destruction and fusion of the intestinal villi and glands (crypts), compression and erosion of their columnar epithelium apposed the penetrated worm, as well as an increase in the number of goblet cells in both villi and crypt epithelium. In this case the inflammatory infiltration will poorly develop especially in muscular and stromal connective tissue layers. On the other hand if the proboscis is directed obliquely, the penetration will be deeper reaching the muscular layer where the host inflammatory reaction will be more pronounced and associated with hemorrhage and extensive cellular infiltration including aggregation of lymphocytes, presence of numerous eosinophilic granulocytes and the appearance of fibroblasts. This inflammatory response may be either induced directly by *S. picae*, or indirectly by bacteria and other gut pathogens introduced the damaged intestinal wall. This reaction did not appear to damage the worm, where the inflammatory cells were not seen within the integument of *S. picae*. This observation is corroborated by the findings of **Krasnoshchekov and Lisitsyna (2009)** on the cystacanth of *S. picae* in the tissue of its paratenic host; *Lacerta agilis* (**Linnaeus, 1758**) .

According to **Schelhaas (1980)** and **Cortan *et al.*(1999)**, these tissue changes may represent building up of cell mediated immunity to the causative agent, and in the initial stage, the neutrophils and macrophages aggregate at the site of infections. The authors also suggested that macrophage engulf the necrotic tissue and dead cells, and such development of cell mediated immunity, lead to the generation of specifically sensitized lymphocytes. **Nickol, 2006** explained that the parasite which induces fibrosis in the intestinal wall along with the associated biochemical reactions will induce loss of gut motility. Furthermore, **Sanil *et al.* (2010)** referred to reduction of the absorptive area available for the digestive and absorptive functions of the animal due to damage of the intestinal folds.

The present investigation revealed that dilation and congestion of blood vessels and stromal lymphocytic infiltration often emerge at considerable distance from the worm praesoma. This agrees with the finding of **Thurston *et al.* (1998)**, who explained such change as a sign associated the inflammatory reactions and infiltration in this layer. In addition, the pronounced thickening of the ileum surface around the site of attachment could be due to the detectable hyperplasia in the crypts. This finding agrees with the study of **Sanil *et al.* (2010)** on the intestinal wall of red snapper infected with *Tenuiproboscis* sp.

The present findings are corroborated by the report of **Amin *et al.* (2010)** on the same species infecting the intestine of *Pica pica* Linnaeus, 1758 (Magpie). The author referred to the possibility of the acanthocephalan to migrate through the musculature into the abdominal cavity causing more destruction to the penetrated layers.

Krasnoshchekov and Lisitsyna (2009) suggested that such mechanical injuries would be purulent but does not produce inflammation associated with eosinophils and neutrophils formation. On the other hand, there are evidences in literatures on the modifying effect of the parasite, in particular, on production of substances inducing eosinophilic taxis

and neutrophilic migration (**Linghtowlers, Rickard, 1988 and Taraschewski, 2000**).

In the present study, the erosion of the host intestinal epithelium towards the worm proboscis may be also a result of proteinases secretion through the pores of channels in the acanthocephalan tegument. Some acanthocephalans (*Pomphorhynchus laevis*, **Müller, 1776**) have been reported to secrete trypsin-like proteinases that were necessary for the complete and quick perforation of the fishes' intestinal wall (**Polzer and Taraschewski, 1994**).

Helminth infections are typically associated with considerable goblet cells hyperplasia (**Artis *et al.*, 2004**) which is related to intestinal protection and worm expulsion (**Amin, 1998**). As expected, a detectable increase of goblet cells in the intestinal epithelium was recorded near the attachment sites. In support of the role of goblet cell derived mucus in worm expulsion, *in vitro* experiments have demonstrated that increased intestinal mucus viscosity at sites surrounding *Nippostrongylus brasiliensis* inhibits worm movement (**Ishikawa *et al.*, 1994**). Moreover, isolation of the goblet cell secreted protein RELMβ/FIZZ2 and its incubation it in vitro with parasitic nematodes, resulted in impaired chemotactic function in the worm (**Anthony *et al.*, 2007**).

To the best of our knowledge, the present study is the first to detect the enzymatic histochemical changes in the bird's parasitized intestine with adult acanthocephalan. The results showed an increase in the level of acid phosphatase in the ileum of infected birds which may reflect a muscular damage and cellular infiltration at the penetration sites. Acid phosphatase is a lysosomal enzyme that play a vital role in the physiology of the intestine . The increase of the activity of this enzyme may be related to autolysis of any foreign substances and microbial agents. Similar observations have been reported in the intestine of birds and rodents with a trematode infection (**Bassiouni *et al.*, 1985 and Abo-Shafey, 1992**)

Regarding to the role of alkaline phosphatase in transport of glucose 6- phosphate from the intestinal lumen, the moderate decrease in the level of this enzyme in the infected ileum, especially in the villi, may be explained by the destruction of the intestinal epithelium and altering of its absorption power. Similar observation has been detected by **Boulus *et al.* (1981), Hamdy and Saleh (1983) and Abo-Shafey *et al.* (1992)** on the small intestine of mice had a trematode infection. Otherwise **Matta (1980)** reported that the alkaline phosphatas activity of *Ascaridia galli* infected intestine was found to increase around the parasite and at the sites of the damaged tissues.

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3/2/3012