

HISTOPATHOLOGICAL STUDIES ON DONKEYS INFESTED WITH LARVAE OF GASTEROPHILUS SPECIES

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INTRODUCTION

The larval stages of *Gasterophilus* flies are obligatory parasite of the alimentary tract of equines. The infestation of donkeys with these larvae is a common parasitic problem in Egypt (Botros, 1942; and Hilali et al., 1987). The 1st instar larvae occurred in the oral cavity of horses causing stomatitis and destruction of the oral epithelia (Zumpt, 1965; Cogley et al., 1982; and Cogley, 1989). The 2nd and 3rd - instar larvae were attached in the pharynx, stomach and duodenum of horses causing inflammation, formation (Zumpt, 1965; Lapage, 1968; Waddel, 1972; Shefstad; 1978 and Soulsby, 1982

The available literature revealed that there is no previous histopathological studies carried out on donkeys infested with *Gasterophilus* spp. There are few references on the pathogenesis of *G. intestinalis* in horses (Faulkner and Kingsote, 1936; Shefstad, 1978 and Cogley, 1989).

The aim of the present investigation was to contribute to our knowledge on the locations of the different instar larvae of *Gasterophilus* spp. in donkeys. Besides, a comprehensive histopathological study was carried out for the infested parts of alimentary tract (tongue, pharynx, stomach and duodenum) due to

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migration and location of the various developmental instar to know the most alarming species to the host.

MATERIALS AND METHODS

The present study was carried out on 56, 35, 116 and 91 infested tongues, pharynxes, stomachs and duodena respectively. They were dissected from 120 donkeys, slaughter at the slaughter-house of the National circus at Giza governorate during the period from January 1987 up to the end of December 1987.

The infested organs were examined macroscopically to demonstrated the normal location and habits for each larval instar of *Gasterophilus* species. Identification of the larvae was carried out according to Zumpt, 1965.

For histopathological examination, autopsies were performed immediately after slaughtering of each donkey. The tissues were taken from the infested parts of tongue, pharynx, stomach and duodenum, then fixed in 10% neutral formaline. After proper fixation, the tissues were routinely dehydrated, cleared and embedded in paraffin wax. Sectioning of the tissues were done at 4 micron, then they were stained with Haematoxyline and Eosin (H & E) and mounted in Canada balsam on glass slides (Carleton et al., 1967).

RESULTS

I- Locations of the different instar larvae:

The tongues were infested only with first-instar larvae of *G. intestinalis* and *G. pecorum* (Fig. 1). The larvae were located in the dorsal surface in the area between the free portion of the tongue until 3 cm before vallate papillae. The 2nd - instar larvae of *G. pecorum* occurred in tunnels at the root of the tongue and continued in their tunnels in the pharynx

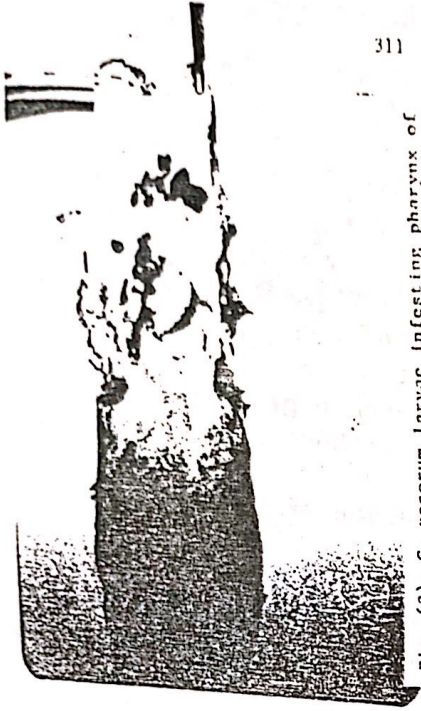


Fig. (2): G. pecorum larvae infesting pharynx of donkey.

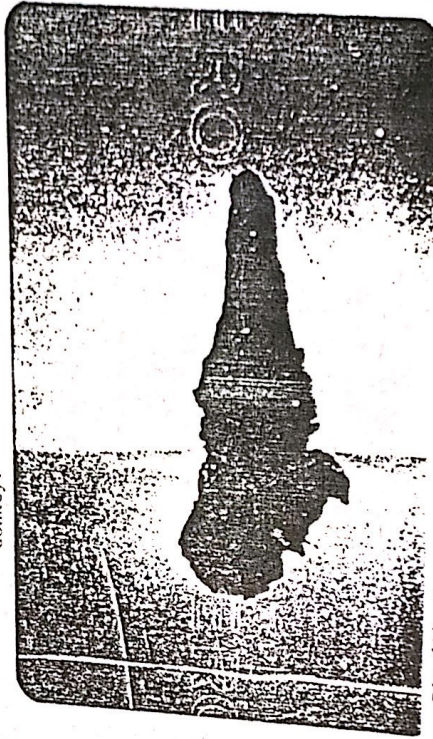


Fig. (4): G. nasalis larvae infesting duodenum of donkey.



Fig. (1): G. pecorum first-instar larvae infesting the dorsal mucosa of the tongue.



Fig. (3): Gasterophilus larvae (G. intestinalis and G. pecorum) infesting stomach of donkey.

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(Fig. 2). The pharynxes harboured the 2nd and 3rd - instar larvae of *G. pecorum*.

The 2nd and 3rd - instar larvae of *G. intestinalis* together with the 3rd - instar larvae of *G. pecorum* were attached near the boundary of both glandular and non-glandular epithelia of the stomach (Fig.3). The larvae attached to gastric mucosa of non-glandular epithelia formed clusters in the most of infested stomachs, but only few larvae were attached to glandular epithelia in heavy infested stomachs without forming clusters.

The 2nd and 3rd - instar larvae of *G. nasalis* were located in the duodenum (Fig. 4). The larvae were mainly attached near the pylorus opening and the first part of the duodenum. In heavily infested duodenum, the larvae may obstruct the duodenal passage causing dilatation of the duodenum.

II- Histopathological observations:

Tongue

Histopathological examination of the tongue revealed a well demarkated longitudinal line of reaction in the submucosa, which represents the track of migration of the 1st-instar larvae. The line of reaction was represented by a rather diffuse aggregation of eosinophils arranged in a longitudinal manner parallel to the surface of the tongue (Fig. 5), with reactive fibroplasia appeared in and around the line of reaction. Other locations in the submucosa, showed focal areas of eosinophilic cell aggregation, and fibrous C.T. condensation taking a longitudinal arrangement parallel to the surface through which there were a heavy zone of eosinophils.

Pharynx

The infested pharynx showed a line of reaction in the submucosa resembling that described in the tongue, but it was more apparent and severe. The diffuse



Fig. (5): Tongue showing a longitudinal line of eosinophilic aggregation in the submucosa. Stain H & E (X 125).

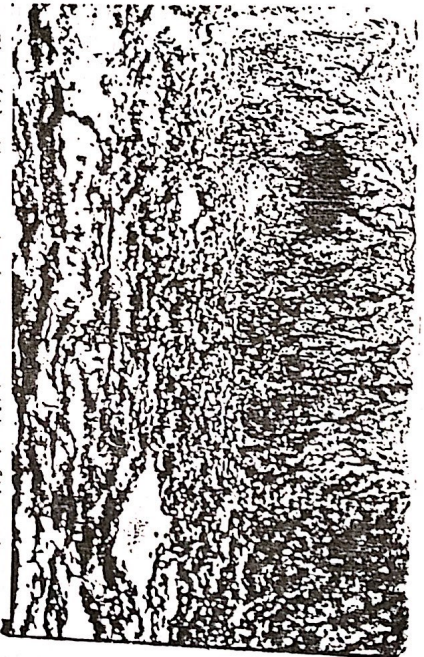


Fig. (6): Pharynx showing eosinophilic infiltration in the submucosa and extending between the folds of the mucosa. Stain H&E (X 250).



Fig. (7): Stomach showing an ulcer extended up to the middle part of the lamina propria. Stain H&E (X 250).

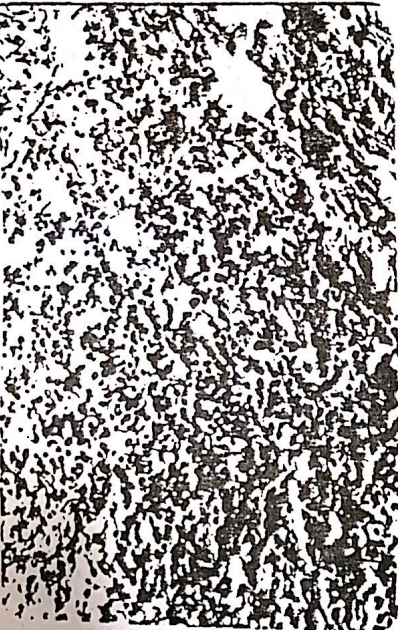


Fig. (8): Duodenum showing heavy leukocytic infiltration, eosinophils, macrophages and lymphocytes in the submucosa, near the ulcerated surfaces. Stain H&E (X 250).

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eosinophilic cell infiltration in the submucosa was extended between the folds of the mucosa and around the basal layer of the epithelium (Fig. 6).

Stomach

Microscopic examination of the stomach revealed desquamation and destruction of some areas of the stratified squamous epithelium leaving a large irregular bare surface. In some locations, the destruction was more severe and extended deeper to involve the lamina propria constituting an ulcer with raised borders and more depressed base. The borders of the ulcer consisted of necrosed darkly stained cells which appeared as fragmented or diffuse deeply stained eosinophilic areas. The base consisted of necrosed cells and tissue debris extending to the middle part of the lamina propria (Fig. 7). In the areas adjacent to the ulcer, there was necrosis of the epithelial lining with increased fibroblasts and collagen fibers deposition around the necrotic masses. At the lamina propria, there were an increased number of blood capillaries together with inflammatory cell infiltration mainly eosinophils, macrophages and lymphocytes. Areas showing early desquamation exhibited marked activity of leucocytic infiltration specially eosinophils with proliferation of blood capillaries in the underlying lamina propria. Some areas showed desquamation of keratin and the remaining layers of the stratified squamous epithelium showed hydropic degeneration.

Duodenum

The duodenum showed desquamation and destruction of the mucosa forming an ulcer with necrotic epithelial cells and tissue debris. At the lamina propria, around the submucosal glands and near or around the aggregated surface, there were heavy leucocytic aggregation mainly eosinophils with some macrophages and lymphocytes (Fig. 8). Some areas showed diffuse

fibrous tissue proliferation in the most basal part under the ulcer and others showed apparent atrophy of the submucosal glands, decrease in the number of these glands and showed widening of the interstitial tissues.

DISCUSSION

The locations and migratory behaviour of the different *Gasterophilus* spp. in the alimentary tract of donkeys were demonstrated in this study. The 1st-instar larvae of *G. intestinalis* and *G. pecorum* were located in the tongue mucosa, the 2nd-instars of *G. pecorum* in the pharynx, the 3rd-instars of *G. pecorum* in both pharynx and stomach. The 2nd and 3rd-instar larvae of *G. intestinalis* were located in the stomach, while those of *G. nasalis* were in duodenum. Similar results were reported in horses by Zumpt (1965), and Cogley et al. (1982), and Lancaster and Meisch (1985). Therefore, it could be concluded that the locations of *Gasterophilus* spp. infesting donkeys were similar to those infesting horses.

Migrating 1st-instar larvae within the tongue of horses have been reported to cause a little damage (Edwards, 1982) or generally no appreciable reaction (Soulsby, 1982). Our histopathological findings demonstrated that there was a significant damage to the tongue of donkeys which was represented by a well demarkated longitudinal line of reaction in the submucosa with diffuse eosinophilic cells aggregations and reactive fibroplasia. These results were in accordance with those recently reported by Cogley (1989) who added that the lesion contained microabscesses.

The lesions observed in the pharynx were similar to those described in the tongue, but they were more severe. The severity of the lesions may be either due to the more sensitivity of the pharyngeal tissues than that of the tongue or the more developed mouth

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hooks of the 2nd-instar larvae of *G. pecorum* than that of the 1st-instar larvae of *G. intestinalis*. It is also possible that the 2nd -instar larvae stay a longer time in the pharynx than the 1st-instars and hence induces a heavier reaction. Our histopathological findings agreed with that reported in horses by chereshnev (1954), Zumpt (1965) and Lancaster & Meisch (1985) who stated that *G. pecorum* larvae attached to the pharynx caused a severe inflammatory process around the larvae. Wejda (1961) reported that the *G. pecorum* larvae infesting horses caused a great difficulty in the swallowing. This difficulty could be attributed to the C.T. proliferation in the pharynx which was reported in our study.

The pathological effects of *G. intestinalis* and *G. pecorum* larvae in the stomach were mainly represented by ulcer formation. Similar results were also reported in horses by Starkoff (1942), Waddell (1972) and Shefstad (1978). they also added that abscess formation, peritonitis and squamous cell tumour were observed. In this study, the inflammatory cell infiltration of the submucosa of the stomach were eosinophils, lymphocytes and macrophages. Shefstad (1978) observed the same cells while examining the lesions in horse stomach using Scanning Electron Microscop. Also, our findings demonstrated hydropic degeneration in the stratified squamous epithelium. This result agreed with Faulkner and Kingscote (1936) who found the 2nd and 3rd instar larvae of *G. intestinalis* produced a marked oedema in the submucosa of horse stomach.

The histopathological changes in the duodenum demonstrated in this study were similar to those described in the stomach with addition of a heavy leucocytic aggregation and involvement of the submucosal glands of the duodenum. This finding was in agreement with Smith et al. (1972). The difference in the pathological reaction observed may be due to the fact that the duodenum is glandular tube and that the larvae were attached mainly to the non-glandular portion of the stomach.

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From the above mentioned discussion, it could be concluded that the *G. pecorum* larvae could be considered as the most alarming species for donkeys due to their effects on pharynx, which is the most essential organ for both the digestive and respiratory system.

SUMMARY

The histopathological alterations of infested parts with different larval stages of *Gasterophilus* spp. were studied in details. The 1st-instar larvae of *G. intestinalis* and *G. pecorum* were located in the tongue. The 2nd-instars larvae of *G. pecorum* were found only in the pharynx, while those of the 3rd instars were observed in both pharynx and stomach. The 2nd and 3rd instar larvae of *G. intestinalis* and *G. nasalis* were found in the stomach and duodenum respectively. It has been concluded that *Gasterophilus* spp. larvae causes eosinophilic reaction with reactive fibroplasia and necrosis in the tongue, pharynx, stomach and duodenum. Ulcer formation in the mucosa of stomach and duodenum were observed.

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