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Histopathological changes in the liver and stomach of *Didelphis virginiana* (Didelphimorphia: Didelphidae) during natural infection with *Gnathostoma turgidum* (Nematoda: Gnathostomidae)

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Abstract

Gnathostoma turgidum is a nematode parasite that exploits the stomach of Virginian opossums, *Didelphis virginiana,* in Latin America. The opossum is the definitive host of *G. turgidum* in the wild. Intrahepatic growth and maturation of the parasite, subsequent migration to the stomach and spontaneous expulsion are common. However, the histopathological lesions caused by *G. turgidum* are poorly described. A better understanding of the life cycle of this parasite and the pathological changes in natural host–parasite interactions could help to clarify the progression of human infections caused by *Gnathostoma binucleatum*. The aim of this work was to study morphological changes in the liver and stomach of *D. virginiana* during natural infection and adult worm expulsion. Three opossums naturally infected with *G. turgidum* were captured from an endemic area of gnathostomosis. Three uninfected opossums captured from a non-endemic area were used as controls. The opossums were sacrificed at different stages of infection (March, May and December), and a histopathological study of their livers and stomachs was conducted. Injuries in livers were observed by histopathology – areas of necrosis and collagen septa were identified. Parasites caused nodules with necrosis on the periphery of lesions, and collagen fibres were also observed in stomachs. Collagen septa may be caused by antigenic remains of the parasite. Further immunological studies are necessary to verify that stimulation is caused by these factors.

Introduction

The nematode *Gnathostoma turgidum* has been reported in eight Mexican states, including Sinaloa (Pérez-Álvarez *et al*., 2008; Díaz-Camacho *et al*., 2009), which has been described as an endemic area of human gnathostomosis with more than 10,000 estimated human cases. *Gnathostoma binucleatum*, a related species, has been reported to be the causative agent of the human disease (Almeyda-Ártigas *et al*., 2000). Both *Gnathostoma* species have been reported in the endemic area, suggesting that they co-inhabit the same location. The prevalence of *G. turgidum,* an annual parasite of *Didelphis virginiana,* is highest during May and June (Nawa *et al*., 2009). It has been reported that advanced third-stage larvae (A3L) localize to the liver of infected animals from February to April, and subsequently adult worms are located in the stomach from April to July (Díaz-Camacho *et al*., 2010). The beginning and end of synchronous *G. turgidum* egg expulsion in the faeces of infected *D. virginiana,* as well as the expulsion of adult worms from May to September by spontaneous curing, have been reported (Torres-Montoya *et al*., 2014). However, the histopathological changes of *D. virginiana* caused by infection with *G. turgidum* are unknown. Although *G. binucleatum* is the reported causative agent of human gnathostomosis, the histopathology of lesions has been poorly studied, because it is difficult to obtain samples of the parasite from infected humans, due to its migratory behaviour. Experimental infection with *G. binucleatum* in dogs has been reported; however, stomach histopathology has not been studied adequately

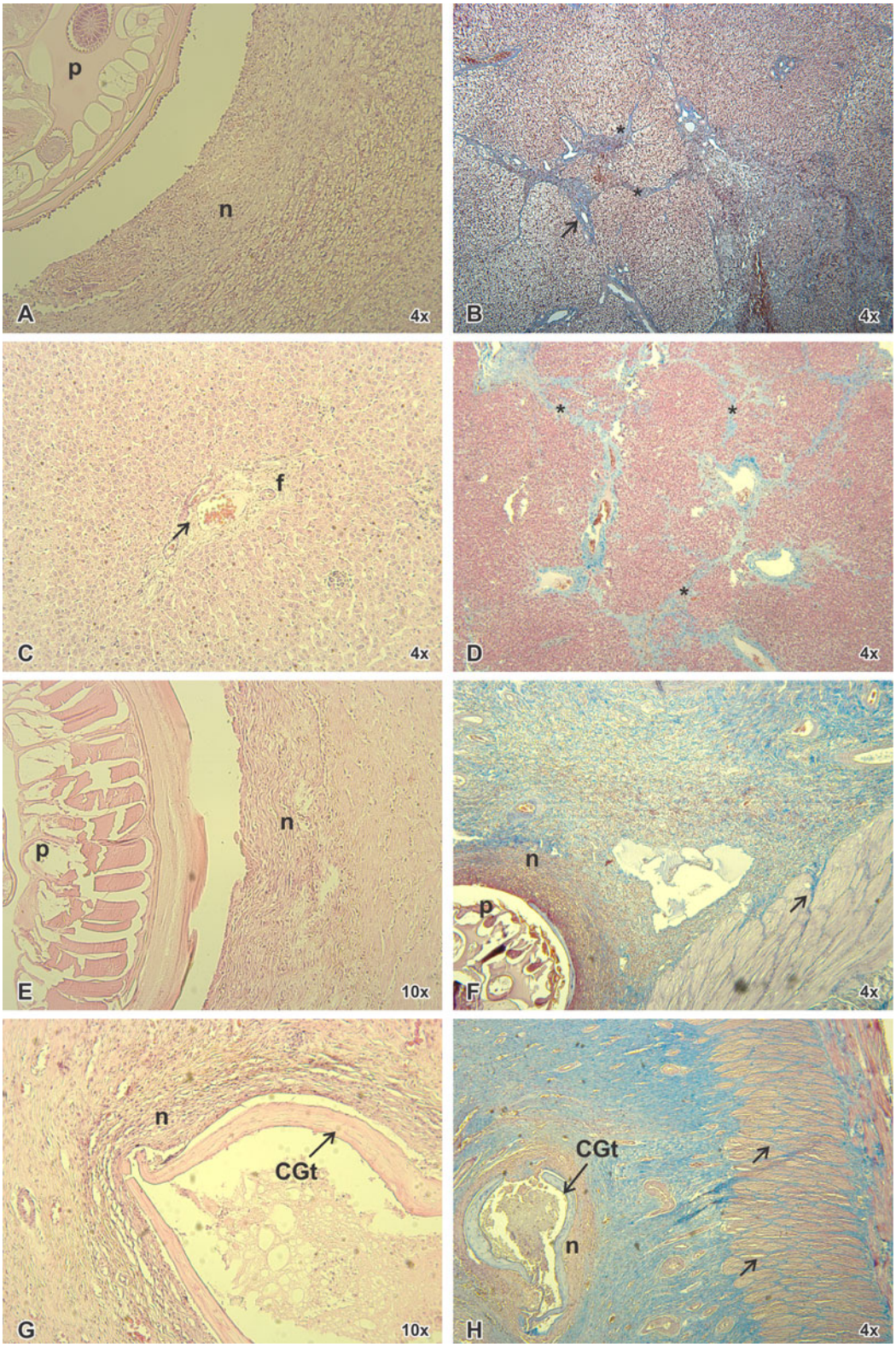

Fig. 1. Liver histopathology of *D. virginiana* infected with *G. turgidum*. (A) Liver damage of T1, sacrificed in March, suggests necrosis (n) around the cuticular surface of the parasite (p) (stain HE). (B) Liver of T1 with formation of collagen septa (*) between the periportal structures (arrow) (stain Masson). (C) Representative image of livers of T2 and T3, similar damage in both, with periportal fibrosis (f) in portal vein (arrow) (stain HE). (D) Representative image of livers from opossums T2 and T3, where thickening in the collagen septa (*) is shown (stain Masson). (E) Stomach histopathology of T2, necrosis (n) in the periphery of the lesion caused by the parasite (p) (stain HE). (F) Section of T2 stomach with a parasite (p), necrosis (n) in the periphery of the nodular lesion and infiltration of collagen fibres (arrow) in the muscle layer (stain Masson). (G) Stomach of T3, necrosis (n) in the periphery of the nodular lesion and apparent remains of cuticle from an adult *G. turgidum* worm (CGt) (stain HE). (H) Stomach of T3, apparent cuticular surface of parasite (CGt) in the nodular lesion with necrosis (n) over the periphery, and apparent thickening of collagen fibres in the muscular layer (arrows).

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(Álvarez-Guerrero *et al*., 2012). In addition, it is not clear whether *G. turgidum* is also involved in human gnathostomosis. As such, further studies are needed to achieve a better understanding of the life cycle of the parasite and to describe the pathological changes associated with natural host–parasite interactions. Such studies could further clarify the progression of human infections caused by *G. binucleatum*. In this report, we studied the histopathology of the liver and stomach of wild *D. virginiana* infected with *G. turgidum*.

Materials and methods

Three opossums suspected to be infected with *G. turgidum* were captured in an endemic area of Sinaloa (Torres-Montoya *et al*., 2014). All of them were positive for *Gnathostoma*, based on stool examination. Three opossums captured from a non-endemic area of *G. turgidum* in Navolato, Sinaloa (24°45′55″N, 107°42′7″W) were used as a negative control. Experimental and control specimens were registered as (T1–T3) and (TC1–TC3), respectively. Opossums were transported to the lab and kept under natural conditions of humidity, temperature and light in 50-cm^2 cages, without access to external food. They were fed with commercial dog food and had access to water *ad libitum*. Animals were sacrificed in March (T1), May (T2) and December (T3) to follow the process of infection and spontaneous curing. Opossums were sedated with 10 mg/kg of intramuscularly administered tiletamine-zolazepam (Zoletil50, Virac, Guadalajara, Jalisco, México). After applying an intra-cardiac dosage of 50 ml potassium chloride (KCl) to sacrifice the opossums, necropsies were performed to collect liver and stomach samples. Samples were fixed with 10% formalin–phosphate-buffered saline (PBS), processed in paraffin wax, and stained with haematoxylin–eosin (HE) and Masson's trichrome (Marcos *et al*., 2007). Histopathology was performed using an optical microscope (PrimoStar, Zeiss, Oberkochen, Germany).

Results and discussion

Histopathology of the liver collected in March (T1) showed fibrous connective tissue, hepatocyte necrosis and haemorrhage, with polymorphonuclear cells surrounding the parasitic structure in the liver parenchyma (fig. 1A). Periportal fibrosis and formation of collagen septa between the portal structures were observed (fig. 1B). In animals T2 and T3, inflammatory infiltration of macrophages and periportal hyperplasia were observed, along with some evident haemorrhagic areas (fig. 1C). Histopathology showed a thickening of the collagen fibres and the presence of inflammatory cells inside the fibrotic areas (fig. 1D). Stomach histopathology in T1 showed normal histology. *Anchylostoma* spp. and *Turgida turgida* adults were also observed; however, nodule lesions in stomachs caused by these nematodes have not been described previously. Stomach histopathology of T2 showed an adult worm between the lamina propria and muscular tissue surrounded by apparent necrosis (fig. 1E), along with collagen fibres in the muscular layer (fig. 1F). In T3, a nodule in the stomach caused by the parasite was observed, as well as necrosis in the periphery of the lesion, inflammatory infiltrate and infiltration of the muscular layer with possible thickening of the collagen fibres (fig. 1G, H).

In the current study, the establishment of collagen septa in the liver and stomach of opossums infected with *G. turgidum* was observed. Even though the fibrogenic process in the stomach has not yet been described for this type of infection, it has been shown that in bovine liver infections with *Fasciola hepatica*, fibrosis is observed in at least two-thirds of the liver parenchyma (Raadsma *et al*., 2007). Moreover, it seems that the degree of fibrosis is correlated with the parasite load in other helminth infections, suggesting that establishment, and even partial reversibility, of fibrosis is directly proportional to the number of parasites and degree of damage (Marcos *et al*., 2007). In the present study, the beginning of collagen septa formation was observed, and interconnection of the septa in liver tissue increased with infection time. Collagen septa were observed after 7 months, when the parasite had left the liver or had been expelled by the opossum, as has been described previously in other animal models (Friedman. 2008). However, more studies are needed to verify that *G. turgidum* causes fibrosis, and to determine if the state of fibrosis depends on parasite load.

Stomach histopathology revealed similar pathology as that caused by other nematodes, such as *Spirocerca lupi* in dogs (Dvir *et al*., 2010), suggesting that remains of *G. turgidum* may cause a chronic inflammatory response, and fibrosis proceeds even after the parasite has been expelled. Further immunological studies are necessary to verify that this stimulation is caused by antigenic remains of the parasite, and to determine how these factors contribute to the chronic inflammatory process.

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Conflict of interest. None.

Ethical standards. Animals used in this article were captured with permission from the Mexican Wildlife Animal authorities (SEMARNAT, 02197/12) and were maintained following guidelines from the Bioethical Animal Committee of the Universidad Autónoma de Sinaloa.

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