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Metazoan and protozoan pathology of wild opossums (*Didelphis virginiana*) in Mexico[1]

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ABSTRACT.- García-Valle J.L., Ramírez J.A.R., García-Prieto L., Ramírez-Hernández C., Ramírez-Romero R., Macedo-Barragán R.J., López-Mayagoitia A., Martínez-Burnes J. & García-Márquez L.J. 2023. **Metazoan and protozoan pathology of wild opossums (*Didelphis virginiana*) in Mexico.** *Pesquisa Veterinária Brasileira 43:e07282, 2023*. Facultad de Medicina Veterinaria y Zootecnia, Universidad de Colima, Av. Universidad 333, Col. de las Víboras, 28040 Colima, México. E-mail: ljgm_cmv@hotmail.com

The aim was to describe the incidence and lesions caused by metazoan and protozoan parasites in *Didelphis virginiana* opossums in Mexico. From 2019 to 2021, twenty *D. virginiana* were collected from the state of Colima, Mexico. *Post mortem*, parasitological, and histopathological studies were performed to identify and describe their lesions and findings. All opossums(20/20) presented at least one type of parasite of the twelve found; the most abundant were nematodes 58.33% (7/12) *Didelphostrongylus hayesi*, *Turgida turgida*, *Cruzia* sp., *Viannaia* sp., *Trichuris didelphis*, Trichostrongylidae and *Gnathostoma* sp. Protozoa 25% (3/12), including *Sarcocystis* sp., *Besnoitia* sp., and *Sarcocystis* sp. A single specimen of *Mathevotaenia* sp., 8.3% (1/12), and *Paragonimus mexicanus* 8.3%, were identified (1/12). The most affected systems were the digestive, respiratory, and musculoskeletal. The most abundant parasites were *Turgida turgida* (20/20) 100%, *Cruzia* sp., (16/20) 80%, and *Viannaia* sp. (6/20) 30%, followed by *Sarcocystis* sp. cysts, (6/20) 30%. The associated parasitic lesions were: *D. hayesi* produce eosinophilic granulomatous bronchopneumonia, *P. mexicanus* eosinophilic focal granulomatous pneumonia, and *Besnoitia* sp., focal eosinophilic lymphoplasmacytic interstitial pneumonia. *Gnathostoma* and *T. turgida* caused severe eosinophilic granulomatous focal gastritis. *Viannaia* sp., *Sarcocystis* sp., *Mathevotaenia* sp., and Trichostrongylidae; caused diffuse eosinophilic mucosal enteritis. *Cruzia* sp., and *T. didelphis*, induced diffuse eosinophilic mucosal typhlitis. *Sarcocystis* sp. cysts were detected in the cytoplasm of muscle fibers without histological changes.

INDEX TERMS: *Didelphis virginiana*, wild opossums, protozoa, metazoa, parasites, pathology, Mexico.

RESUMO.- [**Patologia de metazoários e protozoários em gambás selvagens (*Didelphis Virginiana*) no México.**] O objetivo deste artigo foi descrever a frequência e as lesões causadas por parasitas metazoários e protozoários em gambás (*Didelphis virginiana*) no México. De 2019 a 2021, vinte Gambás (*D. virginiana*) foram coletados no estado de Colima, México. Estudos *post mortem*, parasitológicos e histopatológicos foram realizados para identificar e descrever suas lesões. Todos os gambás (20/20) apresentaram pelo menos um tipo de parasita dos doze encontrados, os mais abundantes foram

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os nematóides 58,33% (7/12) *Didelphostrongylus hayesi*, *Turgida turgida*, *Cruzia* sp., *Viannaia* sp., *Trichuris didelphis*, Trichostrongylidae e *Gnathostoma* sp. Protozoários 25% (3/12) *Sarcocystis* sp., *Besnoitia* sp. e *Sarcocystis* sp. Só uma única espécie de *Mathevotaenia* sp., 8,3% (1/12) e *Paragonimus mexicanus* 8,3% (1/12). Os sistemas mais acometidos foram o digestivo, respiratório e musculoesquelético. Os parasitas mais abundantes foram *T. turgida* (20/20) 100%, *Cruzia* sp., 80% (16/20), e *Viannaia* sp., 30% (6/20), seguidos pelos cistos de *Sarcocystis* sp., 30% (6/20). As lesões observadas causadas pelos vários tipos de parasitas foram: *D. hayesi* produziu broncopneumonia granulomatosa eosinofílica, *P. mexicanus* gerou pneumonia granulomatosa focal eosinofílica e *Besnoitia* sp. produziu pneumonia intersticial linfoplasmocítica eosinofílica focal. *Ganthostoma* e *T. turgida* geraram uma gastrite focal granulomatosa eosinofílica grave. *Viannaia* sp., *Sarcocystis* sp., *Mathevotaenia* sp. e Trichostrongylidae causaram enterite mucosa eosinofílica difusa. *Cruzia* sp. e *Trichuris didelphis* causaram tiflite mucosa eosinofílica difusa. Cistos de *Sarcocystis* spp. foram detectados no citoplasma das fibras musculares, sem alterações histológicas.

TERMOS DE INDEXAÇÃO: *Didelphis virginiana*, protozoários, metazoários, parasitas, patologia, México.

INTRODUCTION

Mexico ranks third in the world in the number of wild animal species, with 564, only behind Brazil with 648 and Indonesia with 670. The 564 species in Mexico are grouped into 200 genera, 46 families, and 13 orders, representing approximately 13% of the world's diversity. Eight marsupials species are distributed in Mexico, within the order Didelphimorphia, in seven genera (*Marmosa, Tlacuatzin, Caluromys, Chironectes, Didelphis, Metachirus,* and *Philander*). The genus *Didelphis* is one of the most widely distributed, with two species: *Didelphis virginiana* and *Didelphis marsupialis*. These species are considered generalists, opportunists, and successful, capable of inhabiting different environments, even with anthropogenic disturbances (Cruz-Salazar et al. 2014). In Mexico, there is a great diversity of ecosystems where marsupial species such as *D. marsupialis, D. virginiana,* and *Philander opossum* stand out, in which 16, 30, and 17 helminth taxa have been recorded, respectively (García-Prieto et al. 2012, Acosta-Virgen et al. 2015). The helminth species recorded in *D. virginiana* in Mexico are Trematoda: *Brachylaima* sp., *Brachylaima virginiana*, *Paragonimus mexicanus*, *Rhopalias coronatus*, *Rhopalias macracanthus*, and *Duboisiella proloba*. Cestoda: *Mathevotaenia* sp., *Mesocestoides* sp., Acanthocephala: *Oligacanthorhynchus tortuosa*, *Oncicola luehei*, *Pachysentis gethi*, *Porrorchis nickoli*. Nematoda: *Cruzia* sp., *Cruzia americana*, *Cruzia tentaculata*, *Didelphonema longispiculata*, *Didelphostrongylus hayesi*, *Gnathostoma turgidum*, *Gongylonema mexicanum*, *Turgida turgida*, Trichostrongylidae gen. sp., *Trichuris didelphis*, *Trichuris minuta*, *Viannaia didelphis*, *Viannaia* sp., and *Viannaia viannai*, (García-Prieto et al. 2012, Acosta-Virgen et al. 2015). Zoonotic protozoa reported in *D. virginiana* in Mexico include *Trypanosoma cruzi* and *Toxoplasma gondii,* with prevalences of 55% and 5.3%, respectively, in Mexico (Panti-May et al. 2021). *D. virginiana* is currently in the conservation status of least concern according to the red list of categories and criteria (Pérez-Hernández et al. 2016). This species inhabits Central America, from Costa Rica to Mexico and in the United States at the east of the Rocky Mountains, and north to southwestern Ontario, Canada (Pérez-Hernández et al. 2016).

It is considered essential to know the species of parasites present in *D. virginiana* in the different ecosystems and their possible role in the biological cycle and the zoonotic potential. This will help to understand the ecological dynamics in the host-parasite interaction to develop conservation and protection programs. Moreover, the description of the lesions that parasites produce would help us to understand the animals' health condition. Opossums and their interaction as synanthropic animals represent a risk to public and veterinary health, due to the role they play as spreaders of diseases (Bezerra-Santos et al. 2021). This research aimed to describe the incidence and lesions caused by metazoan and protozoan parasites in the *D. virginiana* opossums in Mexico.

MATERIALS AND METHODS

Animal Ethics. All procedures and protocols in animals were carried out after the approval of the Ethics Committee of the FMVZ of the "Universidad de Colima", No.3/2019.

Study local and contextualization. From June 2019 to May 2021, twenty *Didelphis virginiana* were collected from the main highway and road networks in the state of Colima (to the North 19°31', to the South 18°41' North latitude, to the East 103°29' and the West 104°41' of West longitude, with an average altitude of 570 meters above sea level. The average annual temperature in this area is around 25°C, with a maximum of 38°C and a minimum of 7°C. The average annual rainfall is 983 millimeters). Only those complete corpses in a good state of preservation, without autolytic changes, were collected. Location, identification, sex, total length, weight, age (young adult), and body condition were recorded for each animal. They were sent to the Pathology Laboratory of the "Facultad de Medicina Veterinaria y Zootecnia" (FMVZ) of the "Universidad de Colima" to perform the corresponding *post mortem* study.

***Post mortem* examination.** During the necropsy, the parasites were identified, and all the organs were examined in detail.

Parasitological examination. For the helminthological examination, the parasites were extracted from the tissues with fine brushes and placed in Petri dishes with 0.85% physiological saline solution. Nematodes were fixed in 70% ethanol and mounted in lactophenol; cestodes and trematodes were fixed in hot 4% formalin, preserved in 70% ethanol, stained with Meyer's paracarmine and mounted in Canada balsam (Lamothe-Argumedo 1997). For the identification of the helminths, the keys of Anderson et al. (2009), Gibson et al. (2002), and Khalil et al. (1994) and according to the structural morphological characteristics under light microscopy, the cysts are tentatively compatible with *Besnoitia* sp. and *Sarcocystis* sp. Voucher specimens of some parasites were deposited at the National Helminth Collection (CNHE 5998, 5999, 7076, 7547), housed in the "Instituto de Biología", "Universidad Nacional Autónoma de México" (UNAM), Mexico City.

Histological examination. Tissue sections of 1cm^2 from all organs were fixed in buffered formalin at 10% pH7.2, processed with routine histological techniques, cut at 5µm thickness, and stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) (Prophet et al. 1995). For the identification of metazoan and protozoan parasites in the tissues, it was based on Gardiner & Poynton 1999 and Gardiner et al. 1998.

RESULTS

All the animals collected were preserved well and showed good body condition. The captured *Didelphis virginiana* were adult animals, 70% (14/20) male, and 30% (6/20) female; the average total length was 78cm, with a range of 65cm to 90cm. The opossums' average weight was 1.6kg, with a range of 1.0kg to 2.5kg.

One hundred percent (20/20) of the *D. virginiana* were infected with a parasite, and the most abundant was nematodes 58.33% (7/12) followed by protozoa 25% (3/12) a single cestode parasite 8.3% (1/12), and trematode 8.3% (1/12), as shown in Table 1. There have been frequent cases of parasitic co-infections of up to three parasites sharing a single host, such as *Turgida turgida*, *Cruzia* sp., and *Didelphostrongylus hayesi*, or *T. turgida*, *Cruzia* sp., and *Sarcocystis* sp.

The lesions caused by the nematode, trematode, and protozoan parasites in the respiratory system were very varied. In the case of *D. hayesi*, the lungs were pale and elastic, with parasites in the bronchi and lung parenchyma. Microscopically, there were abundant eosinophils in the interstitium and the bronchial mucosa with focal granulomas associated with adult parasites and eggs. This microscopic change was typical of eosinophilic granulomatous bronchopneumonia with bronchiectasis, bronchiolitis obliterans, and pulmonary fibrosis. In *Paragonimus mexicanus* the lungs were hyperemic and showed nodules (1x2cm) in the cranio-dorsal region with two intralesional parasites per nodule. Microscopically, these nodules showed abundant eosinophils and fibroblasts in the interstitium associated with the presence of the adult parasite and its eggs. These changes were interpreted as focal eosinophilic granulomatous pneumonia with pulmonary fibrosis and intralesional parasites. In *Besnoitia* sp., the lungs were pale pink with focal nodules (0.3x0.2cm), where a parasitic cyst with a thick wall formed by several layers was observed microscopically; an outer one formed by connective tissue, an intermediate one formed by the host cells and finally a layer formed by parasitophorous vacuoles and inside abundant bradyzoites in different evolutionary stages. This focal lesion had a discrete infiltration of lymphocytes, plasma cells, and eosinophils with alveolar atelectasis. This lesion was considered an eosinophilic lymphoplasmacytic focal interstitial pneumonia. A nodule was also located in the kidneys and with the same histological characteristics, directly compressing the adjacent tissue, producing necrosis and focal renal atrophy without inflammatory infiltration.

Table 1. Location and frequency of metazoan and protozoan parasites in *Didelphis virginiana* from the state of Colima, Mexico

Parasite	Location site	Frequency %
Nematode		
Didelphostrongylus hayesi	Lung-Bronchi	15 (3/20)
Turgida turgida	Stomach	100 (20/20)
Cruzia sp.	Cecum	80 (16/20)
Viannaia sp.	Small intestine	30 (6/20)
Trichuris didelphis	Cecum	5 (1/20)
Trichostrongylidae	Small intestine	5 (1/20)
Gnathostoma sp.	Stomach	5 (1/20)
Fluke		
Paragonimus mexicanus	Lung	5 (1/20)
Cestode		
Mathevotaenia sp.	Small intestine	5 (1/20)
Protozoa		
Sarcocystis sp.	Small intestine	20 (4/20)
Sarcocystis sp.	Skeletal muscle	30 (6/20)
Besnoitia sp.	Lung and kidney	5 (1/20)

The lesions in the digestive wildly varied, caused by the nematode, cestode, and protozoan parasites. In the case of *Gnathostoma* and *T. turgida*, macroscopically, the stomach showed congestion, abundant mucus on the mucosa, and the presence of nodules of different sizes (3x2cm). The texture of these nodules varied from firm to hard; they were superficially ulcerated and showed the presence of nematodes in the thickened mucosa. Microscopic lesions associated with these two parasites ranged from moderate to severe, focal to nodular. These nodules had an eroded, ulcerated superficial mucosa and showed congestion, hemorrhages, necrosis, dystrophic mineralization, and epithelial hyperplasia with infiltration of eosinophils, lymphocytes, macrophages, plasma cells, epithelioid, and giant cells. They also had a proliferation of fibrous tissue with nematodes and intralesional eggs. This lesion was considered severe eosinophilic granulomatous gastritis (Fig.1-8).

For nematodes and cestodes from the small intestine, *Viannaia* sp., Trichostrongylidae, and *Mathevotaenia* sp., and from the large intestine *Cruzia* sp., and *Trichuris didelphis*, there were mild to moderate, focal, multifocal, and diffuse alterations. The macroscopic lesions on the intestinal mucosa consisted of congestion, epithelial desquamation, abundant mucus, and free parasites in the intestinal lumen. Microscopically, congestion, epithelial and goblet cell hyperplasia, villous atrophy, with the presence of histological sections of parasites with infiltration of eosinophils and few lymphocytes, were observed on the mucosa and submucosa, causing diffuse eosinophilic mucosal enteritis and diffuse eosinophilic mucosal typhlitis (Fig.1-8 and 9-14).

For the protozoan *Sarcocystis* sp., in the small intestine, the macroscopic lesions consisted of congestion and multifocal petechiae. Microscopically congestion, hemorrhages, and necrosis of the tips of the villi were observed, with bradyzoites inside the cytoplasm of the enterocytes and outside them, with infiltration of eosinophils on the mucosa and submucosa causing diffuse eosinophilic mucosal enteritis (Fig.9-14).

The lesion caused by the protozoan parasite in the skeletal muscle system consisted of multiple cysts of *Sarcocystis* sp. in the cytoplasm of the muscle fibers, with different sizes and shapes: round, elongated, and oval, with a continuous thick wall, causing compression of the myofibrils and inside they presented abundant bradyzoites without histological changes.

DISCUSSION

In this research, the most abundant group of parasites in *Didelphis virginiana* were nematodes at 58.33%, followed by protozoa at 25%, whereas cestodes and trematodes were recorded with only one specimen at 8.3%. Results are similar to other investigations regarding the group with the highest presentation. In Mexico, Acosta-Virgen et al. (2015) reported on three species of marsupials six trematodes, two cestodes, three acanthocephalans, and ten nematodes. Aragón-Pech et

al. (2018), in Yucatán, Mexico, in *D. virginiana* reported mainly nemathelminths, followed by protozoans and acanthocephalans. In Brazil, as reported by Zabott et al. (2017) in *Didelphis albiventris,* the frequency was nemathelminths (66%), followed by acanthocephalans (17%) and flatworms (17%). Costa-Neto et al. (2019) in *Didelphis aurita* found that nematodes were the most numerous, followed by Platyhelminthes and a species of the Cestoda class the Acanthocephala phylum. Teodoro et al. (2019) in *D. albiventris* and *D. aurita* found 77.6% for nematode eggs, 34.5% for trematode eggs, and 32.7% for protozoa. In Peru, it has been reported by Chero et al. (2017) in *Didelphis marsupialis* different results where digeneans were the ones with the highest species richness in number and percentage (19.50%), followed by nematodes (17.45%) and acanthocephalans (2.5%). Polo-Gonzales et al. (2019) in *D. marsupialis* and *Didelphis pernigra* reported that nematodes were the most diverse group (85.7%), followed by trematodes (14.3%).

In this study, the most abundant nematodes in *D. virginiana* were *Turgida turgida*, *Cruzia* sp., and *Viannaia* sp., followed by muscle cysts of the protozoan *Sarcocystis* sp. They are also the most frequently reported by Monet-Mendoza et al. (2005), García-Prieto et al. (2012), Acosta-Virgen et al. (2015), and Aragón-Pech et al. (2018) in several states of México. In other countries and marsupials, they have also been recorded, as reported by Jones (2013), in *D. virginiana*, where *T. turgida,* and *Cruzia americana* as the most frequent in southern California, USA. McAllister et al. (2019), in *D. virginiana* collected *Physaloptera* spp., *T. turgida*, and *Cruzia americana* in Oklahoma, USA. Zabott et al. (2017) recorded *T. turgida*, *Cruzia tentaculata* and *Trichuris* sp. in *D. albiventris* in Paraná, Brazil, as the most frequent. Costa-Neto et al. (2019) recorded *Trichuris, Viannaia, C. tentaculata* and *T. turgida* in *D. aurita*, in Brazil. Teodoro et al. (2019) in Brazil, obtained from *D. albiventris* and *D. aurita,* the nematodes *C. tentaculata*, *Trichuris*, and Trichostrongylidae.

Chero et al. (2017) found *Trichuris* and *Viannaia as the* most frequent nematode species in *D. marsupialis* in Peru. Polo-Gonzales et al. (2019) collected the nematodes *C. tentaculata* and *T. turgida* from *D. marsupialis* and *D. pernigra* also in Peru. Fugassa (2020) lists in *D. albiventris*: *T. turgida*, *Trichuris didelphis, Trichuris minuta, C. tentaculata, Gnathostoma turgidum, Didelphostrongylus hayesi, Viannaia hamata, Mathevotaenia, Paragonimus caliensis, Paragonimus mexicanus* and *Trichostrongylus* in Patagonia, Argentina.

The presence of parasites and the cellular response, as well as the eosinophilic and granulomatous lesions found, have been reported in Mexico (Lamothe-Argumedo 1981, Prado-Rebolledo 1995, Monet-Mendoza et al. 2005, García-Márquez et al. 2010, 2014, García-Prieto et al. 2012, López-Caballero et al. 2013, Acosta-Virgen et al. 2015, López-Crespo et al. 2017). These lesions have been reported in *D. marsupialis* in natural and experimental infections (Prestwood 1976, Prestwood et al. 1977) and in *D. virginiana* (Lamberski et al. 2002, Nichelason et al. 2008, Jones 2013) in the United States of America, as well as in *D. aurita* in Brazil (Costa-Neto et al. 2019). Infection with *Besnoitia darlingi* has been reported from *D. virginiana* in several states of the United States of America (Kentucky, Louisiana, Missouri, Illinois, Indiana, Texas, and Kansas City) (Shaw et al. 2009, Houk et al. 2010). *Besnoitia* infections that do not cause disease have been reported, but mortality only in animals with stress, immunosuppression, or concurrent parasitic infections (Ellis et al. 2012). In addition, *P. mexicanus* and *P. caliensis* have also been recorded in Venezuela by Díaz-Marcos et al. (2011), in Costa Rica by Hernández-Chea et al. (2017), and in Colombia by Lenis et al. (2018). The diversity of parasites found in *D. virginiana* is due to its omnivorous eating habits and can consume eggs, larvae, or intermediate hosts. The parasites and co-parasitism found could be explained by the life cycle, abundance and availability of the parasite, intermediate host, and the ecological characteristics of its habitat. Parasitism is a common interaction between *D. virginiana* and its parasites, where the host establishes different types of responses, and the parasites can cause different degrees of injury, from mild to severe. However, threats such as pollution, habitat loss,

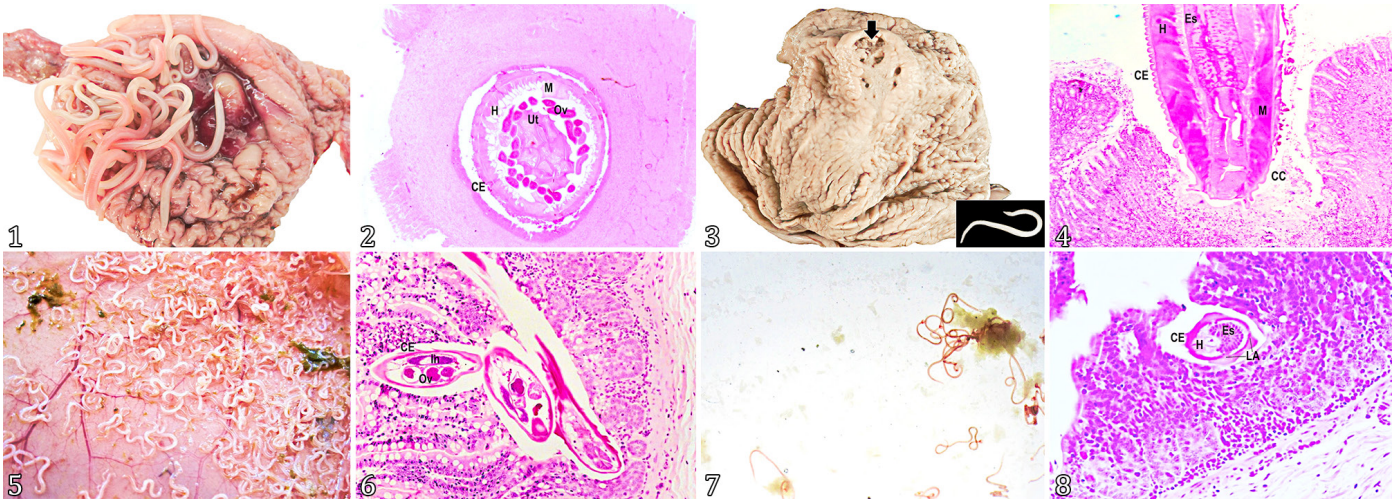
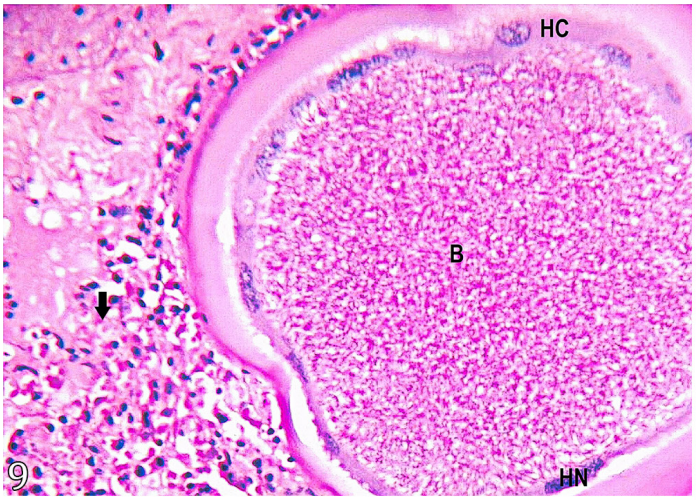
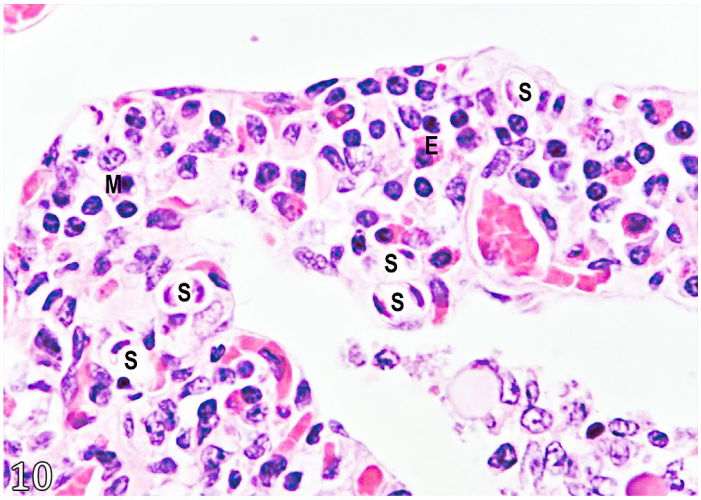
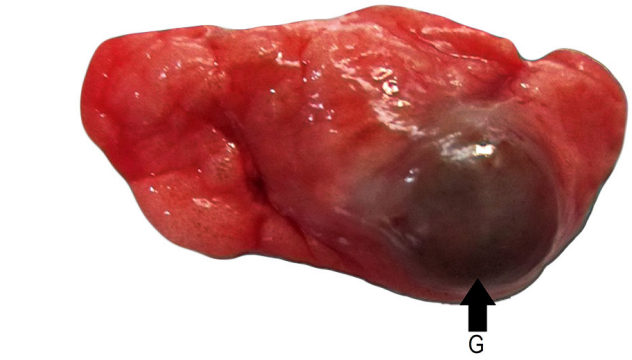
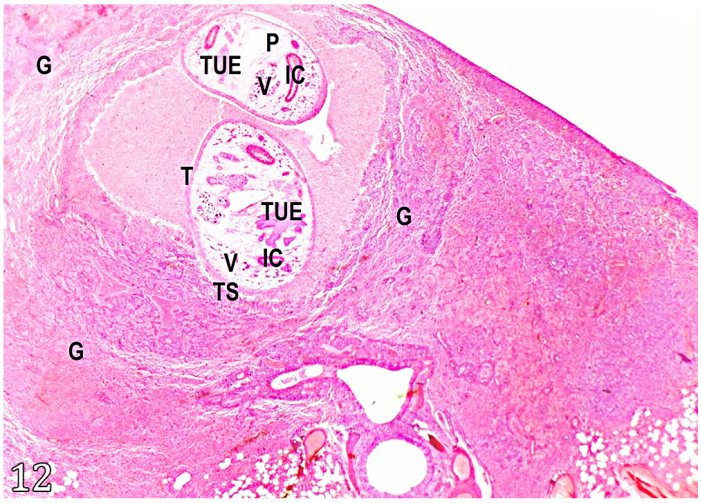


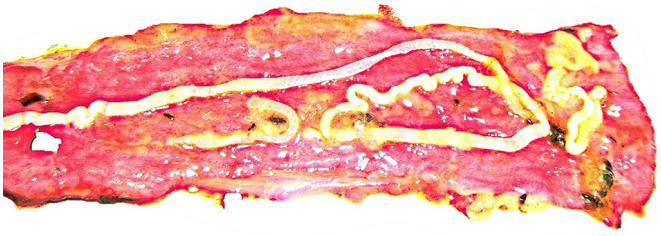
Fig.1-8. Gross and histological characteristics of gastro-intestinal nematodes from opossums. (**1** and **2**) *Turgida turgida* in the gastric mucosa. HE, obj.4x. (**3** and **4**) *Gnathostoma* sp., in the gastric mucosa. HE, obj.10x. (**5** and **6**) *Cruzia* sp., in the cecum mucosa. HE, obj.10x. (**7** and **8**) *Viannaia* sp., in the mucosa of the small intestine. HE, obj.10x. Some histological characteristics in transversal or longitudinal sections of the parasites can be observed: ridges/bumps/spines (CE), cuticular collar (CC), lateral wings (LA), hypodermis (H), esophagus (Es), muscles (M), intestine (In) and sections of ovaries (Ov).











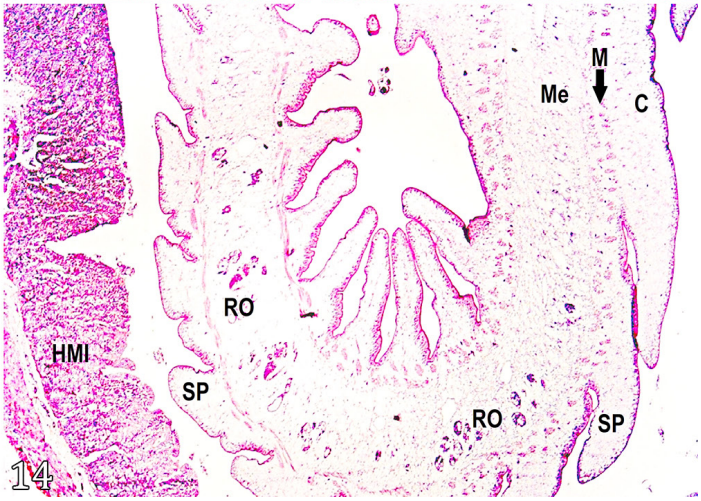


Fig.9-14. (**9**) Histological aspect of a parasitic cyst consistent with *Besnoitia* sp. in the lung of an opossum. Capsule (C), parasitophorous vacuole containing bradyzoites (B), host cell cytoplasm (HC), host cell nucleus (HN). Lymphoplasmacytic inflammatory infiltrate (large arrow). HE, obj.10x. (**10**) Opossum small intestine with numerous sporulated *Sarcocystis* sp., oocysts (S) in the epithelium with mononuclear inflammatory infiltration (M) and eosinophils (E). HE, obj.40x. (**11**) Gross and (**12**) histological appearance of an opossum lung granuloma (G) with two cross sections of *Paragonimus mexicanus*. Histological sections show the integument (T), tegumental spines (TS), parenchyma (P), intestinal caecum (IC), vitellaria (V), testes, and uterus-containing eggs (TUE). HE, obj.4x. (**13**) Small intestine of an opossum showing catarrhal enteritis associated with a corresponding adult cestode with *Mathevotaenia* sp. (**14**) Histological section of *Mathevotaenia* sp., inside the lumen of the small intestine, showing the extensions vellum (V) of the proglottids, muscles (M) that divide the parenchyma into cortical (C) and medullary (Me) regions, segmentation of the proglottids (SP) and the paired reproductive organs (RO). The intestinal mucosa of the host (HMI) has villous atrophy and lymphoplasmacytic and eosinophilic inflammation. HE, obj.10x.

and other factors can cause stress and weaken their immune system. Therefore, these individuals become more susceptible to disease possibly leading to death.

Parasitological studies and their damage to the host are necessary to understand the ecological dynamics in the host-parasite interaction to develop programs for the conservation and protection of the species. The pathological effects of many wild animal parasites are unknown or poorly studied, and most of them coexist in their hosts with other parasites. In this study, the animals presented parasites and co-infections. It is difficult to know about the pathogenic effect of each of them on *D. virginiana;* therefore, it is necessary and essential to understand that these animals play a relevant ecological role in nature, but they are also reservoirs of important parasitic zoonoses in the urban and suburban ecosystems of Mexico such as *P. mexicanus*, *Gnathostoma* and *Trichuris* (Sanyaolu et al. 2016, Muñoz-García et al. 2018, Bezerra-Santos et al. 2020, 2021).

CONCLUSION

This research demonstrated the great diversity of parasites in wild *Didelphis virginiana* opossums in Mexico, as well as the histopathological changes associated with these parasites, presenting high morbidity and no mortality; however, under conditions such as stress, poor nutrition, change of habitat, and concurrent diseases, can even cause the death of the animal. Therefore, histopathological examination is a good indicator of the health status of opossums, and the importance of each lesion will depend on the number of parasites, how it affects the organ's function, and the animal's ability to survive. This study contributes to expanding knowledge about parasitic diseases and their pathologies in the opossums in their natural environment.

Authors' contributions.- All authors participated in the work to a different degree and we assume responsibility for the content of the manuscript.

Jorge L. García V. Fieldwork: Collection of specimens, transport, and conservation. Laboratory work: Necropsies, collection of parasites and tissues, fixation, and processing.

Johnatan A. Ruíz R. Fieldwork: Collection of specimens, transport, and conservation. Laboratory work: Necropsies, collection of parasites and tissues, fixation, and processing.

Luis García P. Parasite collection, fixation, processing, staining, and identification of parasites and interpretation of results.

Cecilia Ramírez H. Laboratory work, fixation and histological processing and interpretation of the results.

Rafael Ramírez R. Laboratory work, fixation and histological processing and interpretation of the results, critical review in Spanish and English.

Rafael J. Macedo B. Fieldwork: Collection of specimens, transport, and conservation. Laboratory work: Necropsies, collection of parasites and tissues, fixation and processing.

Alfonso López M. Design of the project, analysis and interpretation of the results, writing of the article, and critical review in Spanish and English.

Julio Martínez B. Interpretation of the results, writing of the article, and critical review in Spanish and English.

Luis J. García M. Conception and design of the project, field and laboratory work, analysis and interpretation of the results, writing of the article, and critical review.

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