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Prevalence, abundance and intensity of eggs and oocysts of gastrointestinal parasites in the opossum *Didelphis virginiana* Kerr, 1792 in Yucatan, Mexico

R. A. ARAGÓN-PECH[1], H. A. RUIZ-PIÑA[1], R. I. RODRÍGUEZ-VIVAS[2], A. D. CUXIM-KOYOC[1], E. REYES-NOVELO[1]

[1]Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, Av. Itzaes No. 491 por 59 Col. Centro, Mérida, Yucatán, México, C.P. 97000, E-mail: *A11017944@alumnos.uady.mx*, **rpina@correo.uady.mx*, *alan.cuxim.575@gmail.com*, *enrique.reyes@correo.uady.mx*; [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Carretera Mérida-Xmatkuil Km. 15.5, Tizapán, 97100 Mérida, Yucatán, México, E-mail: *rvivas@correo.uady.mx*

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Summary

Virginia opossum, *Didelphis virginiana*, is a synanthropic mammal associated with peridomestic areas of Yucatán, However, little is known about the gastrointestinal parasite infections of this species. The infection prevalence, mean abundance and mean intensity of eggs and oocysts of gastrointestinal parasites, in opossums captured in the peridomestic areas were estimated in six rural localities of Yucatán, Mexico. Eighty-four faecal samples were processed by flotation technique. McMaster test was used to estimate the number of helminth eggs and protozoa oocysts per gram of feces. Seven genera of gastrointestinal parasites were identified, and then infection prevalence was estimated as follows: Protozoa *Eimeria* sp. (51.9 %) and *Sarcocystis* sp. (1 %); nematodes *Ancylostoma* sp. (80.56 %), *Cruzia* sp. (62.04 %), *Trichuris* sp. (60.19 %), *Capillaria* sp. (29.63 %), *Turgida* sp. (23.15 %), *Toxocara* sp. (11.11 %), and *Ascaris* sp. (1.85 %); and one acanthocephalan: *Oligacanthorhynchus* sp. (14.81 %). This is the first study on the diversity of gastrointestinal parasites in Virginia opossums, and first evidence about the potential role of opossums in the transmission of zoonotic gastrointestinal parasites in peridomestic areas of Yucatán, Mexico.
Keywords: Gastrointestinal parasites; *Didelphis virginiana*; Yucatán; Mexico

Introduction

Virginia opossum, *Didelphis virginiana*, is widely distributed across North and Central America and it can be found across a broad range of habitats up from Nearctic in southern Canada to the Neotropics in Costa Rica with the exception of arid zones in Mexico and the United States (Gardner, 2005). Opossums are synanthropic species, able to occupy habitats with high levels of disturbance, and, for this reason they are frequently found in agricultural, urban, and rural areas (Ruiz-Piña & Cruz-Reyes, 2002; Krause & Krause, 2006; Ruiz-Piña, 2010). This characteristic is relevant due to the diversity of parasites and pathogens being capable to infect this species, and suggest a reservoir role for many of them (Acha & Szyfres, 1988).

Few studies have been carried out in Mexico on the occurrence of gastrointestinal parasites (GIP) in *D. virginiana*. Cañeda (1997) recorded 17 parasite species from 10 opossums collected in Veracruz State where the most commonly found species were *Cruzia tentaculata, Turgida turgida, Trichuris didelphis,* and *Oligacanthorhynchus tortuosa*. Monet *et al*. (2005) found 19 helminth taxa (5 digeneans, 1 cestode, 2 acanthocephalans, and 11 nematodes) in *D. virginiana* captured in 10 Mexican states, in which *T. turgida*, and *C. tentaculata* were the most abundant parasites. The most recent study in Mexico, carried out by Acosta-Virgen *et al*. (2015), identified adult parasites in 40 sacrificed opossums from 12 Mexican states. They recorded 21 helminth taxa (6 trematodes, 2 cestodes, 3 acanthocephalans and 10 nematodes), which increased the diversity of intestinal parasite species for *D. virginiana* up to 41.

* – corresponding author

Studies on intestinal parasites of *D. virginiana* from southeastern Mexico are scarce (Cañeda, 1997; Monet *et al*., 2005; Acosta-Virgen *et al*., 2015).

In order to improve the understanding of gastrointestinal parasites infecting of this marsupial species the objective of this study was to evaluate the frequency of GIP infections, as well as assess the mean abundance and mean intensity of eggs and oocysts of GIP identified in feces of synanthropic specimens of *D. virginiana* captured in peridomestic areas of rural dwellings of six localities of northern Yucatán, Mexico.

Material and Methods

Study area

The present study was carried out in six localities: Cacalchén (N20°58'56" W89°13'40"), Homún (N20°44'19" W89°17'06"), Komchén (N21°06'13" W89°39'45"), Motul (N21°05'42" W89°16'59"), Tetiz (N20°57'44" W89°56'02") and Kopomá (N20°38'52" W89°53'55") located in northern Yucatán, Mexico. The altitude ranged between 3 and 20 meters above the sea level. The geology of the region is calcareous (karst) with strong superficial and internal water dissolution. The climate is warm sub-humid, with rains in summer. The native vegetation of the zone is a transition of medium and low tropical deciduous forest, but currently most of the area is covered by secondary vegetation (Flores & Espejel, 1994; Orellana *et al*., 2010). Rural localities ranged between 2000 – 6500 inhabitants. Except for Motul which has 23000 inhabitants, but preserves its rural housing characteristics.

Capture of opossums

The capture of opossums was carried out on monthly basis between July 2015 and January 2016. In each locality, 100 households per month were selected, with authorization of landowners or residents, and the opossums were captured with livetraps (66 × 23 × 23 cm, Tomahawk Live Trap Co.) baited with pineapple and placed in the peridomestic area at each house at dusk. Animals were collected on the following morning. Overall this represented a total of 600 trap/nights of capture effort.

We recorded the sex, age and weight of captured individuals and collected a stool sample. *D. virginiana* were captured and handled under conditions that minimized the stress and employed animal welfare procedures (NOM-062-1999; Sikes, 2016).

Fecal samples

Opossums are mammals that are characterized for their thanatosis behavior, generally described by defecation or urination during handling (Krause & Krause, 2006). Fecal sampling was performed as described by Rodríguez and Cob (2005). Approximately two to three grams of feces were obtained from each individual. Fecal specimens were placed in ziploc bags (12.5 × 8 cm) and/or sterile bottles and then stored in a cooler on ice during transportation to the laboratory. They were kept in refrigeration at 4 °C until their coprological analysis performed within 24 h.

Coproparasitological analysis

The coproparasitological examination consisted of macro and microscopic observations of feces to detect the GIP. A qualitative diagnosis was carried out by the flotation enrichment technique with saturated glucose solution (SSG) described by Rodríguez and Cob (2005). A quantitative diagnosis was made by applying of the modified McMaster technique of Rodríguez and Cob (2005), i.e. adding 1 g of feces and 14 ml of SSG. Fecal samples positive for coccidia for sporulation and identification were incubated for 10 days at 24 °C with 2.5 % potassium dichromate (Duszynski & Wilber, 1997).

Table 1. Infection parameters recorded in 84 fecal samples of *Didelphis virginiana* from six rural localities in northern Yucatán, Mexico.

Parasite	Infected animals (Prevalence, C.I. 95 %)	Mean abundance of eggs per gram (C.I. 95 %)	Mean intensity (C.I. 95 %)
Protozoa			
Eimeria sp.*	38 (45.2 %, 34.3 – 56.5)	18800 (5530 – 66600)	41600 (12700 – 151000)
Nematoda			
Trichuris sp.	49 (58.3 %, 47.1 – 69)	180 (115 – 322)	309 (206 – 531)
Capillaria sp.	24 (28.6 %, 19.2 – 39.5)	38 (22 – 73.8)	133 (87.5 – 227)
Ancylostoma sp.	71 (84.5 %, 75 – 91.5)	563 (43 – 736)	666 (511 – 858)
Cruzia sp.	52 (61.9 %, 50.7 – 72.3)	207 (149 – 291)	334 (255 – 459)
Ascaris sp.	2 (2.4 %, 0.3 – 8.3)	5.36 (0 – 20.8)	225 (100 – 225)
Toxocara sp.	5 (6.0 %, 2.0 – 13.3)	19.6 (5.36 – 48)	330 (160-510)
Turgida sp.	12 (14.3 %, 7.6 – 23.6)	22 (10.7 – 40.5)	154 (100 – 212)
Acantocephala			
Oligacanthorhvnchus sp.	14 (16.7 %, 9.4- – 26.4)	237 (62.5 – 727)	1420 (397 – 3750)

C.I. = confidence intervals. *Mean abundance is in oocysts per gram

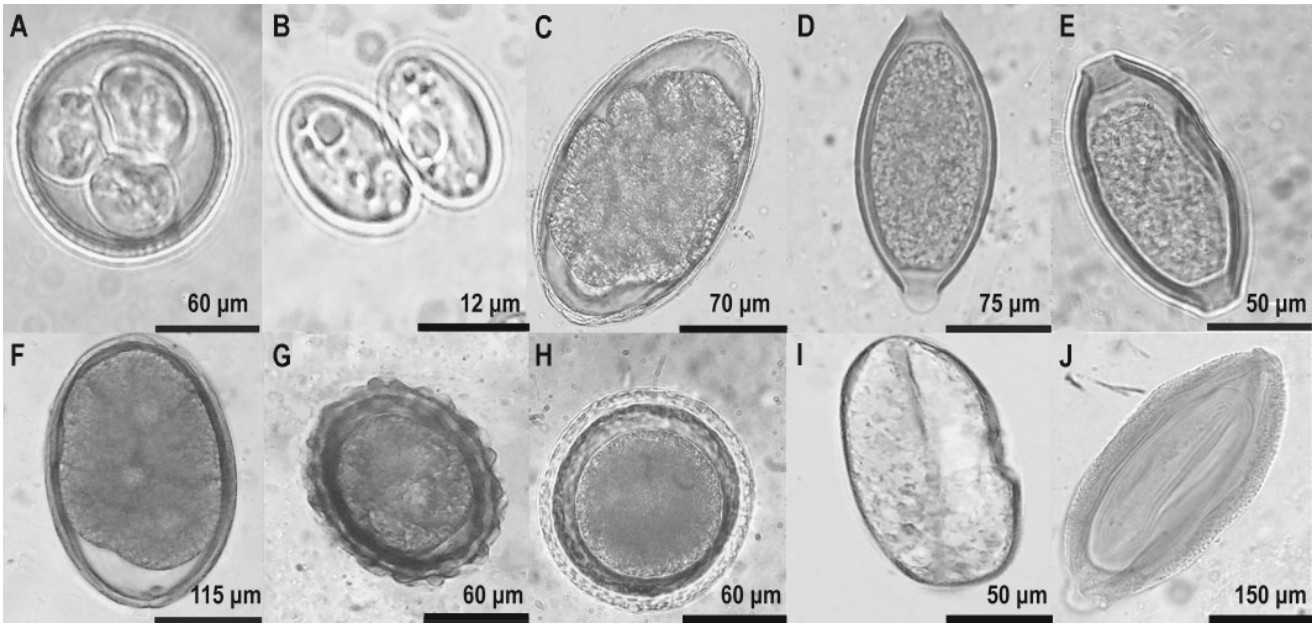

Fig. 1. Helminth eggs found in *Didelphis virginiana* from six rural localities in northern Yucatán, Mexico. **A** – Sporulated oocysts of *Eimeria* sp. (100x); **B** – oocyst of *Sarcocystis* s p. (100x); **C** – egg of *Ancylostoma* sp. (40x); **D** – egg of *Trichuris* sp. (40x); **E** – egg of *Capillaria* sp. (40x); **F** – egg of *Cruzia* sp. (40x); **G** – egg of *Ascaris* sp. (40x); **H** – egg of *Toxocara* sp. (40x); **I** – egg of *Turgida* sp. (40x); **J** – egg of *Oligacanthorhynchus* sp. (40x). The thick black line represents the scale used for each image.

Taxonomic determination

The morphological study of the eggs and oocysts of GIP was performed with light microscopy at 40× and 100× magnification (Zeiss [Axiostar]). Measurements were taken with the aid of a calibrated micrometer eyepiece. All measurements and scales of the images were measured in microns. The taxonomic determination was based on Garcia (2009), Zajac & Conboy (2012), and Bowman (2014). The identification of protozoan genera was based on the morphology of oocysts, their size, and the number of sporocysts (according to Rodríguez & Cob, 2005; Duszynski, 2016). The identification of the acanthocephalans was based on Petrochenko (1956).

Data analysis

For statistical analysis, the parameters prevalence (proportion of infected host with the traditional Clopper-Pearson CI), mean abundance (Bootstrap BCa), and mean intensity (Bootstrap BCa) were used. All summary statistics had 95 % confidence intervals, as proposed by Bush *et al*. (1997) and Rózsa *et al*. (2000), and were estimated with Quantitative Parasitology 3.0 (Reiczigel *et al*., 2013).

Ethical Approval and/or Informed Consent

This work does not involve human or experimentation with animals.

Results

A total of 84 *D. virginiana* were studied for counting the eggs/oocysts of GIP. The diversity of GIP found in *D. virginiana* was composed of two protozoa of the order *Eucoccidiida*, seven nematodes, and one acanthocephalan of the family *Oligacanthorhynchidae* (Table 1). The GIP eggs/oocysts were observed in 100 % of fecal samples (84/84). The oocysts of the protozoa *Eimeria* sp. (42.5 %) and *Sarcocystis* sp. (1.19 %) were found in 58.33 % (49/84) samples (Fig. 1A–B). Eggs belonging to seven genera of nematodes were found: *Ancylostoma* sp. (84.5 %, 71/84, Fig. 1C), *Trichuris* sp. (58.3 %, 49/84, Fig. 1D), *Capillaria* sp. (28.6 %, 24/84, Fig. 1E), *Cruzia* sp. (61.9 %, 52/84, Fig. 1F), *Ascaris* sp. (2.4 %, 2/84, Fig. 1G), *Toxocara* sp. (6 %, 5/84, Fig. 1H), *Turgida* sp. (14.3 %, 12/84, Fig. 1I). Finally, the eggs of the acanthocephalan *Oligacanthorhynchus* sp. (Fig. 1J) were found in 16.70 % (14/84) of positive samples.

Monthly variation in parasite prevalence showed the highest values for November and December, two months after the highest amount of rain registered in the region (Fig. 2).

The co-parasitism was recorded in the 91.6 % of the studied opossums (77/84). Table 2 shows the monthly variation in the prevalence of co-parasitism. Only 8.3 % (7/84) of the studied opossums were infected with a single parasite. However, a co-parasitism of 2 – 7 parasites were recorded (Table 2). The frequency of co-parasitism found in the studied opossums is presented in Table 3.

Discussion

The protozoan *Eimeria* sp. and the nematodes *Ancylostoma* sp., *Ascaris* sp. and *Toxocara* sp. represent new records for *D. virginiana* in Mexico (Cañeda, 1997; Monet *et al*., 2005; Acosta-Virgen *et al*., 2015).

Table 2. Monthly prevalence of co-infection of gastrointestinal parasites in *Didelphis virginiana* studied in peridomiciles from six rural localities in northern Yucatán, Mexico.

Co-infection prevalence (n=77)	2015 July	September	October	November	December	2016 January
2 parasites	21.4	7.1	14.2	14.2	14.2	21.4
3 Parasites	21.7	4.3	13.1	34.7	13.1	13.1
4 Parasites	5.2	26.3	15.7	36.8	15.7	0
5 Parasites	0	20	10	20	50	0
6 Parasites	11.1	0	11.1	44.4	33.3	0
7 Parasites	0	0	25	25	50	0

Among the GIP found in this study, five nematodes with zoonotic potential were recorded: *Ancylostoma* sp., *Toxocara* sp., *Trichuris* sp., *Ascaris* sp., and *Capillaria* sp. The nematode *Ancylostoma* sp. represents the first report for *D. virginiana* in Mexico. Rueda *et al*. (2014) reported a frequency of 60 % (9/15) of *Ancylostoma* sp., in the feces of *D. marsupialis* in Colombia, with a lesser frequency that was found in the present study. In Brazil, Pinto *et al*. (2014) found *Toxocara cati* in the feces of *D. albiventris.*

The presence of *Ancylostoma* sp., and *Toxocara* sp. in the feces of *D. virginiana* could be due to the high abundance of these parasites found in other species of animals such as dogs and cats living in the peridomestic areas of rural households in the studied region (Rodríguez *et al*., 2001; Rodríguez *et al*., 2011; Ortega *et al*., 2015). However, to verify this hypothesis it is necessary to conduct specific studies on the cross-transmission of these parasites between *D. virginiana* and domestic and wild animals.

The nematodes *Trichuris* sp., *Cruzia* sp. and *Turgida* sp. have been previously reported in Yucatán, and are commonly recorded in *D. virginiana* from other Mexican regions (Monet *et al*., 2005; Acosta-Virgen *et al*., 2015).

Due to omnivorous nature of *D. virginiana*, the infection with *Turgida* sp. and *Oligacanthorhynchus* sp. may have been caused due to the ingestion of intermediary hosts. In the case of *Turgida* sp., cockroaches are known to act as intermediate hosts (Anderson, 2000). Also, the myriapods are recognized as intermediate hosts for genus *Oligacanthorhynchus* (Richardson, 2006). These could

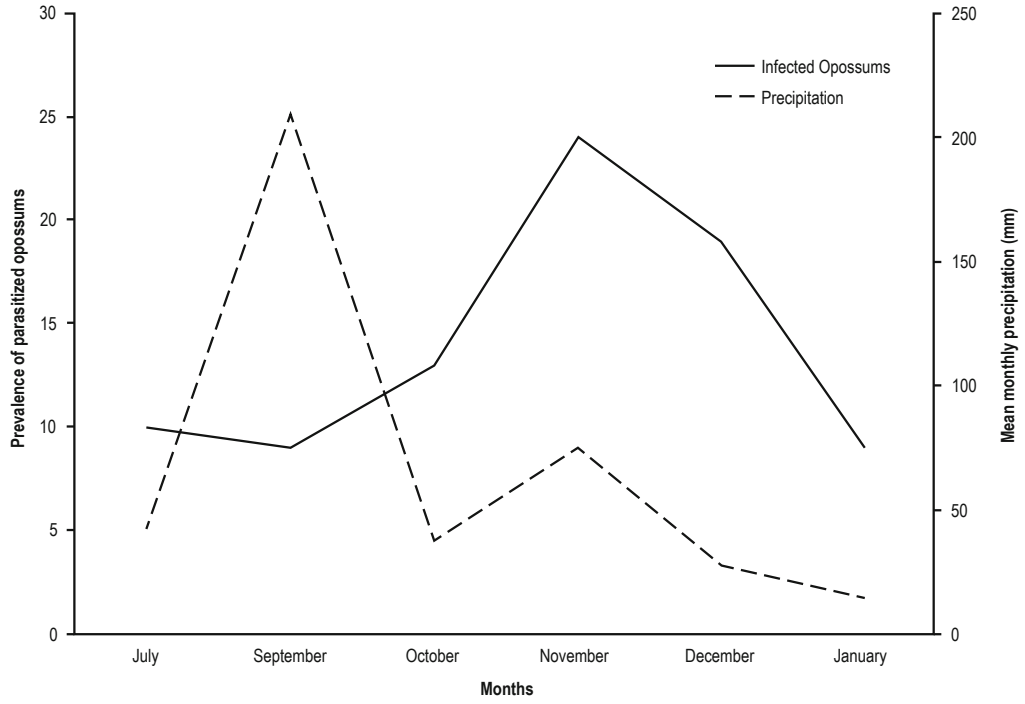

Fig. 2. Mean monthly precipitation registered from six rural localities in northern Yucatán, Mexico and the prevalence of gastrointestinal parasites in *Didelphis virginiana*

Table 3. Frequency of co-parasitism of gastrointestinal parasites for 77 opossum *Didelphis virginiana* from six rural localities in northern Yucatán, Mexico.

Nematoda	**Infected animals (%)**
Ancylostoma + Cruzia	8 (10.39)
Ancylostoma + Trichuris	3 (3.90)
Ancylostoma + Capillaria	1 (1.30)
Ancylostoma + Turgida	1 (1.30)
Capillaria + Turgida	1 (1.30)
Ancylostoma + Cruzia + Trichuris	8 (10.39)
Ancylostoma + Capillaria + Trichuris	2 (2.60)
Ancylostoma + Cruzia + Turgida	1 (1.30)
Ancylostoma + Capillaria + Cruzia + Trichuris	2 (2.60)
Ancylostoma + Capillaria + Cruzia + Toxocara + Trichuris	1 (1.30)
Protozoa + Nematoda	
Eimeria + Ancylostoma	2 (2.60)
Eimeria + Trichuris	2 (2.60)
Eimeria + Ancylostoma + Trichuris	5 (6.49)
Eimeria + Ancylostoma + Cruzia	3 (3.90)
Eimeria + Trichuris + Turgida	1 (1.30)
Eimeria + Ancylostoma + Cruzia + Trichuris	6 (7.79)
Eimeria + Capillaria + Cruzia + Ancylostoma	3 (3.90)
Eimeria + Cruzia + Turgida + Ancylostoma	2 (2.60)
Eimeria + Ancylostoma + Capillaria + Cruzia + Trichuris	2 (2.60)
Eimeria + Ancylostoma + Capillaria + Cruzia + Turgida	2 (2.60)
Eimeria + Ancylostoma + Capillaria + Cruzia + Trichuris + Toxocara	1 (1.30)
Eimeria + Ancylostoma + Capillaria + Cruzia + Trichuris + Turgida	2 (2.60)
Acantocephala +Nematoda + Protozoa	
Oligacanthorhynchus + Trichuris	1 (1.30)
Oligacanthorhynchus + Capillaria	1 (1.30)
Oligacanthorhynchus + Cruzia	1 (1.30)
Oligacanthorhynchus + Cruzia + Ancylostoma	2 (2.60)
Oligacanthorhynchus + Turgida + Ancylostoma	2 (2.60)
Oligacanthorhynchus + Trichuris + Ancylostoma + Eimeria	1 (1.30)
Oligacanthorhynchus + Trichuris + Ancylostoma + Cruzia	3 (3.90)
Oligacanthorhynchus + Capillaria + Cruzia + Trichuris	1 (1.30)
Oligacanthorhynchus + Cruzia + Ancylostoma + Eimeria	1 (1.30)
Oligacanthorhynchus + Ancylostoma + Cruzia + Eimeria + Trichuris	2 (2.60)
*Oligacanthorhynchus + Ancylostoma + Cruzia + Eimeria + Trichuris + Toxocar*a	2 (2.60)
Oligacanthorhynchus + Ancylostoma + Cruzia + Toxocara + Trichuris + Capillaria + Turgida	1 (1.30)

be present in the studied localities, since there are at least 31 of myriapods species distributed in Yucatán (Bueno *et al*., 2004), and during the present study, some species were observed as frequent inhabitants of the peridomestic areas. Future research must consider also the collection and dissection of cockroaches and myriapods. thus confirm the presence of infectious stages of *Turgida* sp. and *Oligacanthorhynchus* sp., and explain the way how they contribute to the life cycle of GIP in *D. virginiana* from Yucatán.

The protozoan *Eimeria* sp. was one of the most frequent and abundant GIP in *D. virginiana*. This protozoan had not been previously reported in *D. virginiana* in Mexico. However, in Yucatán it was found having high frequency, detected in 45.2 % (38/84) of fecal samples, and was present in the six sampled localities. These results are discordant with those reported by Joseph (1974), who reported a lower percentage of infection (13 %, 2/15) in *D. virginiana* individuals infected with *Eimeria indianensis* in the state of Indiana, USA.

The parasites diversity found in *D. virginiana* could be explained as a result of the food source available in the peridomiciles. Due to opossums omnivorous and foraging behaviors, miscellaneous GIP eggs or larvae may be ingested or become exposed through the consumption of intermediate hosts (Krause & Krause, 2006; Ruiz-Piña *et al*., 2013). Another studies have shown that *D. virginiana*, consume feces of other animals (Gibson *et al*., 2003; Livingston *et al*., 2005). This can be important for the transmission of other parasites with direct life cycles. This is extremely relevant in view of the

fact that opossums that inhabit the peridomiciles in Yucatán, interact with dogs, cats, pigs, cows, horses, among other mammals like chickens and other fowl. Domestic animals are susceptible to cross infection with GIP, what may also include many zoonotic diseases (Ruiz-Piña & Reyes-Novelo, 2012) like *Ancylostoma*.

In this context, opossums are frequent visitors and occupants of the peridomiciles in Yucatán, as a result of food and shelter availability in this ecotope (Ruiz-Piña *et al*., 2013). As a result they host the pathogens that circulate between those animals (Ruiz-Piña, 2010). This may represent a potential zoonotic risk to families that inhabit those localities. Primarily, because the use of the peridomicile environment for different activities such as washing clothes, keeping domestic animals, and also as a place for family reunions and recreational activities for children (Pacheco-Castro *et al*., 2013).

The higher prevalence of parasite eggs and co-parasitism in the months of November and December could be explained by parasite's life cycle. Taylor *et al*. (2016) explain that temperature (18 – 26 °C) and humidity (80 – 100 % relative humidity) are the most important factors involved in trichostrongyloids and strongyloids larval survival in the environment. These conditions are typical in Yucatán, during rainy season (June to October), (Orellana *et al*., 2010), and this could also explain that the higher GIP prevalence was recorded after the rainy season. Ruiz-Piña and Cruz-Reyes (2002) and Ruiz-Piña (2007) documented that weaned off juvenile opossums start roaming and looking for food in the peridomiciles at the beginning of the rainy season. So if they get infected with parasite larvae in that time of the year by November/December these population have adult parasites in their digestive tract and excrete eggs through fecal drops.

The present study is the first analysis on GIP diversity that interacts with *D. virginiana* in northern Yucatán, and the presence of this marsupial contributing to the dispersion of GIP with zoonotic potential in the peridomestic zones. In future studies, it would be necessary to apply molecular techniques for the taxonomic identification of GIP. Subsequently, the ecology of transmission and the role of *D. virginiana* in the life cycles of these GIP should be explored.

Conflict of Interest

Authors state no conflict of interest.

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