v. 5, n. 2, 2025

Journal of Agricultural Sciences Research

WHITE-EARED OPOSSUM (DIDELPHIS ALBIVENTRIS): ZOONOTIC RISK FOR ENDOPARASITOSIS IN THE METROPOLITAN AREA OF RECIFE

Acceptance date: 05/02/2025

Vanessa Maranhão Soares
Universidade Maurício de Nassau
Recife – PE
http://lattes.cnpq.br/7582588659600201

Andrea Maria Campos Calado Medina
Universidade Federal Rural de Pernambuco
Recife – PE
http://lattes.cnpq.br/7628998582786353

Abstract: The white-eared opossum (*Didelphis albiventris*) is a marsupial mammal belonging to the Didelphidae family. Due to their high degree of adaptation to the urban environment, they are considered synanthropic and pose a risk to public health. Opossums are susceptible to helminth infections due to their omnivorous diet and can transmit these parasites to humans when they contaminate food and water sources. Knowledge about endoparasitoses in opossums is essential due to their pathogenicity and to adopt measures to prevent infection in humans. The aim of this study was to identify the endoparasites of the white-eared opossum from the Tangará Wildlife Care Center, which belongs to the Pernambuco State Environment Agency (CPRH-PE). The helminths and coccidia were identified by laboratory analysis of the feces using the Willis-Mollay coproparasitological technique. Samples were collected from 70 animals, which were divided by age group. In total, 57.14% of the animals had parasites, but the adult individuals had 100% endoparasitosis. Multiparasitism was also seen in 67.5% of the opossums. The eggs found were from the Strongyloidea and Spiruroidea superfamilies, the Ascaridae and Trichuridae families, *Cruzia sp., c*occidia, *Giardia* sp., *Eimeria* sp. and *Octosporella* spp. *Didelphis albiventris* has a high parasite load and is host to several zoonotic species that have already been reported in the Recife Metropolitan Region. Therefore, its contact with domestic animals and humans can pose a risk to public health.
Keywords: *Didelphis albiventris*, parasites, public health, synanthropic, zoonoses

INTRODUCTION

The white-eared opossum (*Didelphis albiventris*) is a marsupial mammal belonging to the Didelphidae family. It has a very diverse diet that includes vertebrates, invertebrates, fruit, flowers, nectar and tree gum (NASCIMENTO; HORTA, 2014)

Due to their frugivorous and omnivorous diet, the opossums can spread seeds in the environment, which combined with their high capacity to adapt to environments altered by anthropogenic action, play an essential role in the regeneration of vegetation (CACERES, 2002). By feeding on insects and rodents, they act to control their population and also by feeding on carcasses, they clean up the environment, being called "sanitary engineers" of nature (KRAUSE; KRAUSE, 2006). On the other hand, their presence in urban areas causes nuisance to the human population (SOUZA et al., 2012; TEODORO, 2013), leading to physical aggression, run over by vehicles, attack by dogs or electrocussion (SOUZA et al., 2012). As result, a large number of opossums in need of medical care are sent to Wildlife Care Centers (WCC), institutions responsible for receiving, screening and treating wild animals rescued or seized by law enforcement agencies (FELIPPE; ADANIA, 2014).

The opossum is also part of the transmission cycle of several zoonotic diseases in the wild and urban environment, such as salmonellosis, leptospirosis, spotted fever, sarcosporidiosis, leishmaniosis and Chagas disease and can play an important role in transmitting diseases to human populations in urban areas (NASCIMENTO; HORTA, 2014).

With regard to gastrointestinal parasites, due to their omnivorous diet, they are more susceptible to infection and are hosts to various helminths with zoonotic potential, such as *Turgida turgida, Gnathostoma* sp.*, Capillaria* spp.*, Trichuris* sp., *Ancylostoma* sp. and *Toxocara* sp. (CATENACCI et al., 2004; AN-

TUNES, 2005; RIBEIRO et al., 2009; SILVA et al., 2017). Opossums have a high degree of parasitosis, and have been reports of endoparasites being present in 90% of the fecal samples analyzed from free-living animals (SILVA et al., 2017). In addition, the presence of endoparasites with pathogenic potential in opossums is high (96.7%) (ANTUNES, 2005).

Given the role of opossums as hosts and carriers of endoparasites of zoonotic importance, as well as the pathogenicity of these parasites for the species, it is necessary to carry out parasitological studies.

MATHERIAL AND METHODS

Animal data and biological sample collection were performed at Tangará Wildlife Care Center, an institution responsible for receiving, screening, treating, rehabilitating and releasing wild animals, located in the Guabiraba neighborhood in the municipality of Recife in Pernambuco. The research project was authorized by the State Environmental Agency of Pernambuco (CPRH-PE) and the Ethics Committee for the Use of Animals (ECUA) of the Federal University of Pernambuco (UFPE) under registration number 0082/2019.

Data on opossums were collected by analyzing the documents of entry, release, death and medical records of the animals belonging to the Center, recording the date of receiving, age group, history, illness and day of death or release from 2016 to June 2019. However, it wasn't always possible to obtain all the data of the animals received due to a lack of information provided by the people or institutions responsible for delivering the individuals or due to a failure to record. Stool samples were collected from young, sub-adult and adult animals. Neonates were excluded from the collection because the amount of feces produced was insufficient for laboratory analysis.

The animals were restrained using leather gloves as personal protective equipment, with one hand resting on the base of the head and neck and the other on the body, giving stability to the animal's spine. Feces were collected by stimulating the perineal region with circular movements using a clean cloth. This technique was described by Nascimento and Horta (2014) for young animals, but in this study it was used for all age groups. Feces were also collected directly from the box or cage if was only one individual in the place, but preference was given to collection by perineal stimulation. The material was stored in a sterile universal collector and preserved in 10% formaldehyde for later laboratory analysis.

The coproparasitological technique used was the Willis-Mollay fecal flotation using a supersaturated sugar solution (WILLIS, 1921). To produce the solution, 1 kg of sugar was dissolved in 720 ml of water. The samples were analyzed under optical microscope using 10x and 40x objectives. The eggs were identified to the lowest possible taxonomic level.

RESULTS AND DISCUSSION

From 2016 to June 2019, 985 *Didelphis albiventris* were received at the center. This center began receiving wild animals in March 2016, and 154 opossums were admitted that year. In 2017 and 2018, 248 and 346 *Didelphis albiventris* were received respectively. By the first half of 2019, 237 individuals were received. From this data, it can be seen that there has been an increase in the number of opossums admitted to Wildlife Care Centers. Opossums are one of the most common mammals admitted to WCC, both due to rescue activities and the seizure of trafficked animals (PREUSS; SCHAEDLER, 2011; FRANCO et al., 2012; FREITAS, 2014; LACERDA et al., 2014; SILVA, 2015). *Didelphis* sp. are heavily trafficked for their meat (FRANCO et al., 2012; SILVA, 2015).

In addition, because they are animals with a great capacity for adaptation, they can occupy highly urbanized areas, suffering from

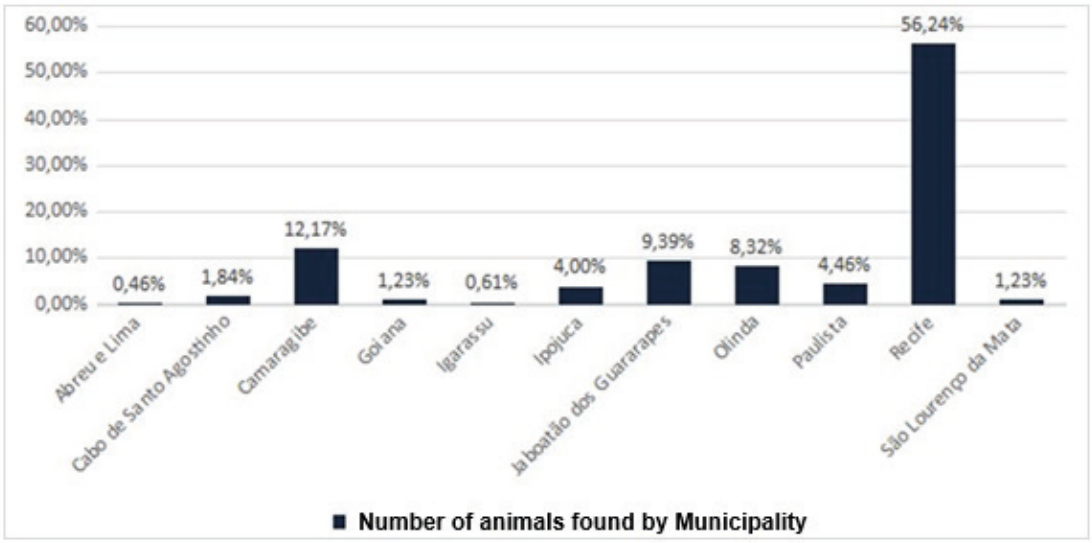
anthropogenic actions such as being run over or physically attacked, and are then sent to WWC (PREUSS; SCHAEDLER, 2011; FREITAS, 2014; SILVA, 2015). The high number of records of these animals can also be explained by the reproductive capacity of opossums, where in one pregnancy a female can give birth to between 5 and 13 young, and can also reproduce throughout the year (NASCIMENTO; HORTA, 2014).

The presence of *D. albiventris* in highly anthropized areas was also observed in this study. The location where the opossums were found was identified in 680 cases. The Metropolitan Region of Recife had the highest presence of animals compared to the other micro-regions of Pernambuco, with 95.44% (649/680) of the individuals coming from this area (Graph 1).

Souza et al. (2012) carried out a survey of requests for opossums to be rescued by the police in Belo Horizonte, Minas Gerais. This study found that there was no correlation between the size of the green area and a greater presence of opossums. Human population density also had no influence on the opossum population. According to IBGE (2017), the municipalities in the Recife Metropolitan Area with the highest population density, in descending order, are Olinda, Recife, Paulista, Camaragibe and Jaboatão dos Guararapes. According to Branco (2015), the municipalities with the largest green area cover are, in descending order, are Igarassu, Camaragibe and Paulista. Therefore, this study also does not observe a correlation between human population density and the size of the green area cover with a greater presence of opossums. However, the municipality of Recife is the basic nucleus of the metropolitan region, accounting for 41% of the population and 49% of the region's GDP (PREFEITURA DO RECIFE, 2019). Despite also being among the municipalities with the largest vegetation cover, deforestation in this area has been increasing over the years (BRANCO, 2015; MAGAROTTO et al., 2018). The destruction of the natural habitat leads animals to adapt to urban conditions (PIEDADE, 2013). According to Rondon (2010), the predominance of the species *Didelphis albiventris* in an environment indicates a high degree of disturbance. These individuals take advantage of the abundance of food wasted by humans. The absence of predators and the presence of many shelters contribute to the permanence of these animals in cities (PIEDADE, 2013). Therefore, these factors may have contributed to the high number of opossums in this municipality. It should also be taken into account that in highly urbanized areas there will be a greater impact on the animals due to run over, physical aggression and attacks by domestic animals, resulting in more opossums being sent to Wild Life Care Centers. According to Nascimento and Horta (2014), orphaned young are one of the main cases in WWC, which is also seen in this study. The age range was recorded in 846 cases, and the majority corresponding to young animals with 84.40% (714/846) of cases, followed by adults with 11.35% (96/846) and sub-adults with 4.26% (36/846).

The percentage of adult females destined for WWC was higher than males, with 69.6%. Many of these females were also carrying young. The weight of their litter reduces the speed of their movements, which makes lactating females more susceptible to attacks by other animals, to being run over and, consequently, resulting in their death; their litter are able to survive because they are protected inside the pouch (SILVA, 2015; COSTA, 2018). Thus, entire litters are sent to the WWC, which explains the high percentage of animals in this age group.

Orphaned young were found near their mothers in 248 cases, and the origin of the mother's death was known in only 44.75% (111/248) of the cases. The causes identified



Graph 1 - Number of *D. albiventris* found per municipality in the Recife Metropolitan Region between 2016 and June 2019

Source: Author (2019)

were dog attacks with 54.05% (60/111), human attacks with 26.12% (29/111), being run over with 18.01% (20/111) and being attacked by cats with 1.8% (2/111). Disregarding the cases of orphaned opossums, a history of 212 individuals was obtained, other animals without a history often arrive in need of medical care but without knowing the origin of the trauma. The main cases were attacks by dogs with 43.87% (93/212), followed by attacks by humans with 14.62% (31/212), rescue with 13.68% (29/212), being run over with 11.79% (25/212), attacks by cats with 8.02% (17/212) and other various causes with 8.02% (17/212).

Abandoned dogs and cats are responsible for preying on a large number of wild animals, especially mammals like rodents and opossums (CAMPOS, 2004; RANGEL; NEIVA, 2013). Domestic animals are considered invasive when introduced into natural environments, often caused by human interventions such as lack of care and allowing them access to the streets (RANGEL; NEIVA, 2013; PEREIRA et al., 2019). Predation on wild animals has a highest impact when it occurs in areas where there is fragmentation and loss of habitat. The interaction between domestic animals and the wild environment also favors the transmission of pathogens between these individuals, domestic species and humans (LESSA et al., 2016).

The genus *Didelphis* sp. has one of the highest rates of road kill reported in the literature, with high numbers in all Brazilian biomes (CIRINO; FREITAS, 2018). Their small and medium body size, nocturnal habits and active movement between forest fragments make vehicle drivers not notice their presence on the roads, making these animals targets for being run over. Their presence in highly anthropized areas is also a predisposing factor (ORLANDIN et al., 2015; COSTA, 2018). Although the reduced mobility of females with young makes them more susceptible to being run over, the rate of males being run over is higher. This is because males move around much more than females during the reproductive period as they look for receptive mates. Females only become more active after weaning their young (SANTANA, 2012; COSTA, 2018).

The historical rescue category consisted of animals that were close to human habitation, with opossums being asked to be removed due to the nuisance of their presence. Opossums have a reputation for smelling bad and transmitting infectious diseases, which is why requests to remove them from the local and physical aggression are common (SOARES et al., 2011; SOUZA et al., 2012).

From the survey, it can be seen that the opossums are completely integrated into the Recife Metropolitan Area, being able to reproduce actively and coming into close contact with domestic animals and humans. As mentioned above, this contact can be worrying due to the possibility of disease transmission.

Regarding the presence of endoparasites in these individuals, 57.14% (40/70) of the animals were positive. Catenacci et al. (2004) found a similar result, with 55% of samples positive. A study carried out in the state of Pernambuco by Ribeiro et al. (2009) found that 75.4% of the samples were positive. Paschoal et al. (2018) and Silva et al. (2017) found 82.3% and 90% of opossums with endoparasites, respectively. Bonfim (2013) and Prado et al. (2018) found the highest rate of parasitism, reporting 100% of animals with endoparasites. Characteristics of the region such as habitat fragmentation and animal density can influence the presence of endoparasites in opossums (CATENACCI et al., 2004). The high density of animals facilitates the process of parasite transmission between individuals, causing an increase in the parasite rate (CATENACCI et al., 2004). Intrinsic factors such as animal immunity and extrinsic factors such as host exposure, seasonality and habitat characteristics such as vegetation, humidity and sun exposure also influence the parasite load (BONFIM, 2013). Therefore, these factors may have contributed to the discrepancy in the results of the parasitological studies carried out by the authors.

The age range of the animals used in the studies also influences the total percentage of parasitized individuals. In this study, young, sub-adult and adult showed 25%, 43.75% and 100% parasitism respectively. Ribeiro et al. (2009) found that 66.7% of young, 73.3% of sub-adult and 80.5% of adult were positive. Teodoro (2013) carried out necropsies on *Didelphis* sp. to check for helminths. In this study, only one young was found to be parasited, with two helminths of the *Cruzia tentaculata* species, and this parasite load was considered to be quite low compared to the other individuals necropsied. According to the author, young animals start exploring the environment from 46 days of age and start eating solid food from 86 days of age, and are considered omnivores after weaning. As mentioned above, the omnivorous diet is a predisposing factor for acquiring endoparasite infections. Thus, young animals have a lower parasite load compared to sub-adults and adults due to the shorter contact time they have with this diet and the contaminated environment.

Other factors could influence the parasite rate of the young animals. A larger body size has a highest contact surface with the environment and a larger living area, resulting in greater exposure to parasites. The sub-adults and adults needs to eat more food, which can increase the possibility of coming into contact with the parasite (ARNEBERG et al., 1998; MORAND; POULIN, 1998). However, Mota (2013) found no correlation between weight and parasite abundance in *D. albiventris*.

Since males have a larger living area and greater weight than females, they are more susceptible (ARNEBERG et al., 1998; MORAND; POULIN, 1998). Another factor associated with the susceptibility of males is due to their higher serum testosterone levels, which has an immunosuppressive effect (FOLSTAD; KARTNER, 1992). This corroborates with Rondon (2010), who observed that males were

more parasitized than females, with 91.8% of parasites compared to 79.5% for females. However, Bonfim (2013) found no correlation between sex and the rate of endoparasitism and the same was observed in this study.

In addition to the lower parasite rate, the young animals also had fewer co-infections, with a maximum of two parasites simultaneously. Co-infection occurred in 67.5% (27/40) of cases. Aragon-Pench et al. (2018) conducted studies with *Didelphis virginiana*, finding 100% of parasitized animals and 91.6% with co-infection, ranging from 2 to 7 endoparasites. Silva et al. (2017) found that 77.5% of the white-eared opossum used in their study had co-infection. Monoparasitism, biparasitism and triparasitism were observed in 22%, 50% and 28% respectively. In the present study, monoparasitism, biparasitism, triparasitism, tetraparasitism and pentaparasitism occurred in 32.5%, 22.5%, 32.5%, 10% and 2.5% of cases respectively. The presence of a greater number of youngs in this study may have contributed to the lower rates of co-infection compared to the other studies.

The diversity of helminth groups in the young animals was lower than in the sub-adults and adults, with only eggs from the Strongyloidea superfamily, the Ascarididae family and coccidia being found. Mota (2013) found a correlation between parasite diversity and larger body size. The longevity of the host is also related to parasite diversity, since animals with longer life spans are more likely to accumulate a richer community of parasites. Nine groups of eggs and oocysts were identified, which belong to the Spiruroidea and Strongyloidea superfamily, the Ascaridae and Trichuridae families, the *Cruzia* sp. genus, *Eimeria* sp. and *Giardia* sp., coccidia and oocysts similar to *Octosporella* spp. The percentage of infected animals per endoparasite group is shown in Graph 2

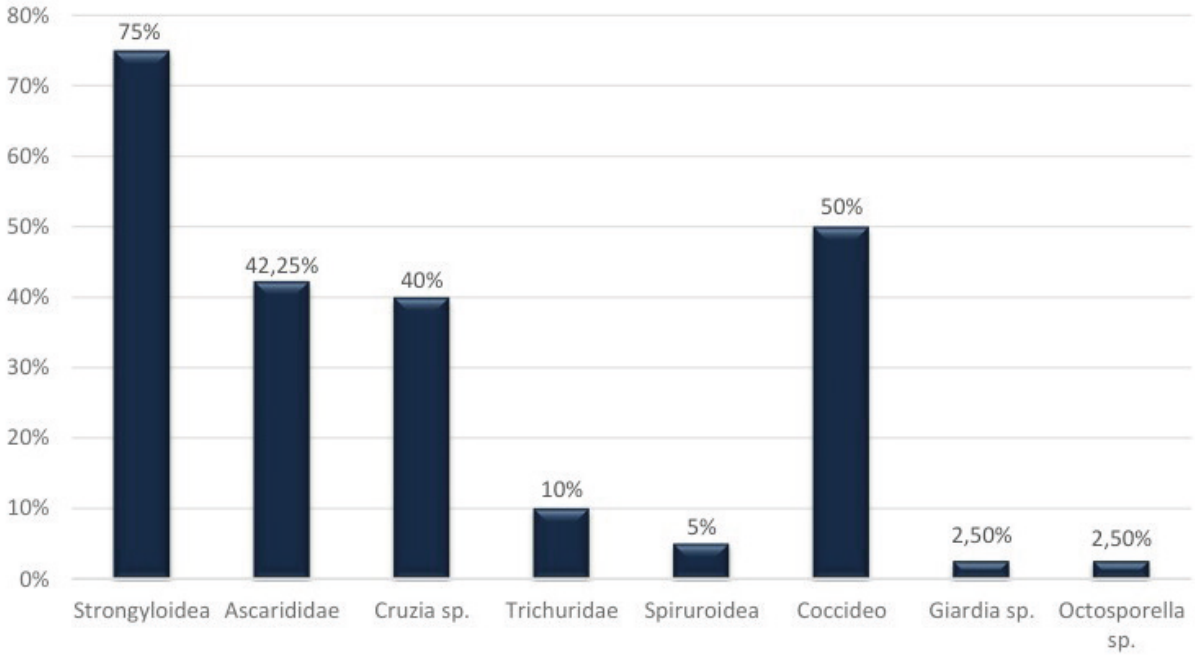
Only nematode helminths were reported in this study, and these are also the most frequently phylum reported in other studies. Ribeiro et al. (2009) found 71.2% of *Didelphis* sp. parasitized by this group. Platyhelminths and acanthocephalans were found separately in only one animal, representing 1.7% of parasitized individuals. Teodoro (2013) found 77.6% of *Didelphis* sp. parasitized with helminths from the Nematoda phylum and 34.5% of the Trematoda class, with no cestodes or acanthocephalans found.

The eggs most commonly found were from the Strongyloidea superfamily, with 75% of the animals parasitized by this group. Prado et al. (2018) found 17.2% of opossums parasitized with eggs from the Strongyloidea superfamily. Bonfim (2013) studied the helminth fauna of *Didelphis albiventris* in two regions of Brasília, finding 66.7% and 88.2% of parasitism in these locations.

Coccidia were found in 50% of the animals. It was possible to see a sporulated oocyst in one individual, and *Eimeria* sp. was identified. Coccidiosis was reported by Rondon (2010), Bonfim (2013), Teodoro (2013) and Paschoal et al. (2018), with 41.3%, 47.1%, 39.6% and 64% of parasitism, respectively.

Eggs from the Ascaridae family represented 42.5% of parasitized individuals. Rondon (2010) found only 3.8% of the opossums parasitized with ascarids. Bonfim (2013) found 33.3% and 11.8% of parasites from this group in *D. albiventris* from different locations.

The genus *Cruzia* sp. was found in 40% of the opossums. *Cruzia* sp. eggs were the most commonly found in *Didelphis albiventris* by Rondon (2010), Teodoro (2013) and Prado et al. (2018), with 76%, 70.6% and 82.7% of parasitized opossums, respectively. Mota (2013) also reported *Cruzia* sp. eggs, but only 10% of the individuals were parasitized with the genus. Studies that carried out necropsies on opossums also reported high parasitism by



Graph 2- Percentage of animals infected by endoparasite group.

Source: Author (2019)

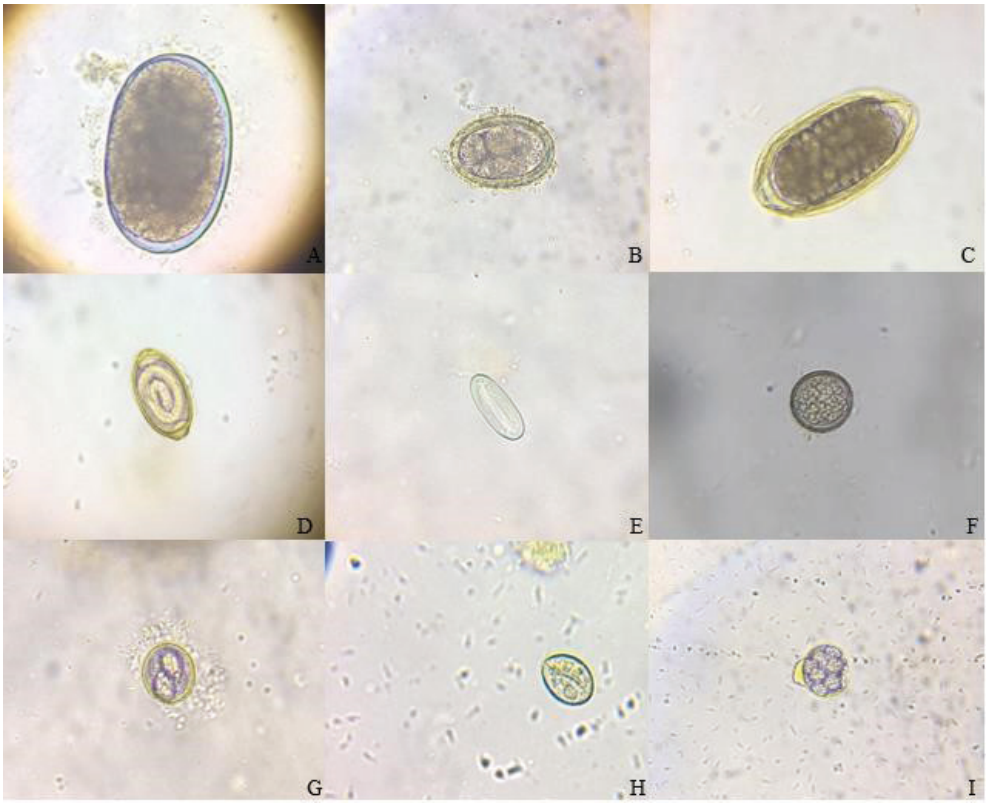


Figure 1 - Eggs found in *Didelphis albiventris*. **A.** Strongyloidea superfamily. **B**. Ascaridae family. **C**. *Cruzia* sp. **D**. Trichuridae family. **E**. Spiruroidea superfamily. **F.** Coccida. **G**. *Eimeria* sp. **H**. *Giardia* sp. **I.** Oocyst similar to *Octosporella* spp

Source: Author (2019)

Cruzia sp. Antunes (2005) found 93.33% of *D. albiventris* parasitized by *Cruzia tentaculata.* Similar results were found by Santa Cruz et al. (1999) and Silva and Costa (1999), who reported 88% and 91% of parasites of this species, respectively.

The Trichuridae family represented 10% of the parasitized animals. Eggs from this family were found by Rondon (2010), Mota (2013) and Teodoro (2013) in 21.2%, 40% and 29.31% of parasitized animals, respectively.

The superfamily Spiruroidea had 5% of parasitized individuals. Eggs from this superfamily were found by Bonfim (2013), Mota (2013) and Teodoro (2013), showing 23.5%, 30% and 27.5% parasitism respectively.

Giardia sp was found in one animal, representing 2.5% of parasitized individuals. Zanette et al. (2008) found *Giardia* sp. in 3 white-eared opossums in Rio Grande do Sul. Melo (2017) also reported the presence of *Giardia* sp. in D. albiventris in the state of Pernambuco.

Oocysts similar to *Octosporella* spp. was found in one animal, representing 2.5% of parasitized individuals. *Octosporella* spp was reported by Teodoro (2013). It is a coccidia belonging to the Eimeriidae family, and this genus has been described in some species of lizards, fish and mammals. However, it seems that this parasite is a protozoan of arthropods and is considered a pseudoparasite of vertebrates. Its presence in animal feces is due to the high resistance of the oocyst wall, which is able to resist passage through the gastrointestinal tract and be eliminated in feces without losing its morphological characteristics (BERTO et al., 2010; MISAEL et al., 2013)

The technique used in this study evaluates the morphology of the eggs and oocysts, with limitations in terms of taxonomic classification, so it is not possible to identify helminths and coccidia at species level due to the fact that their eggs are very similar (BONFIM, 2013; TEODORO, 2013). Despite this, this method is considered to be non-invasive, of low operational cost and quick evaluation, making it ideal for epidemiological and parasitological research on domestic and wild animal populations (MOTA, 2013). Therefore, using this technique, there is no way of identifying the species of opossums endoparasites, and isn't possible to establish which of them are zoonotic. However, studies carried out in the state of Pernambuco have identified endoparasites in *Didelphis* sp. that have zoonotic potential (RIBEIRO et al., 2009; FARIAS et al., 2014; SILVA, 2016; MELO, 2017). The eggs found in *Didelphis albiventris* are shown in Figure 1.

Melo (2017) identified *Entamoeba* sp., *Endolimax nana, Giardia* sp., *Ascaris* sp., *Ancylostoma* sp., *Strongyloides stercolaris* and *Taenia* sp. in the white-eared opossum in the state of Pernambuco, all of these genera and species described are zoonotic. This study also reported the presence of *Giardia* sp. in one animal wich was found in the Boa Viagem neighborhood. A study carried out in the "Entra a Pulso" community, located in this same neighborhood, by Silva (2010) found 44.2% of humans parasitized with this protozoan. Other protozoa found by Silva (2010) and already reported in *D. albiventris* in the state of Pernambuco were *Cryptosporidium* sp., *Entamoeba* sp. and *Endolimax nana.* In Pernambuco, *Cryptosporidium* sp. was found in the white-eared opossum by Ribeiro *et al* (2009), Farias *at al* (2014) and Silva (2016). Silva (2016) found 6 opossums parasitized with the coccidia, with 4 of the animals coming from the Metropolitan Zone of Recife. In this study, 3 infected animals were found close to water sources and reservoirs, posing a risk of contamination of water resources.

The contact of opossums with domestic animals also represents a risk for the transmission of endoparasites to humans (LESSA et al., 2016). According to Melo (2017), when wild animals lose their habitat, they come into con-

tact with domestic animals and humans, becoming reservoirs of these parasites. According to Aguirre (2010), increased contact between humans and domestic animals with wildlife can lead to the introduction of pathogens into wildlife, which can result in high mortality. When wild animals manage to survive, they become reservoirs. An increase in the number of pathogen reservoir species will make it more difficult to implement disease control programs.

As observed during the survey carried out in this study, opossums are in close contact with domestic animals and are negatively affected by them when they are attacked and killed. Santana (2014) carried out a study of endoparasites in canines and felines living in the Metropolitan Zone of Recife with 25% of dogs and 31.4% of cats found to be parasitized. Of the total, 20.2% of dogs were parasitized by *Ancylostoma* sp., 8.7% by *Trichuris* sp. and 6.7% by *Toxocara* sp. In felines, 28.6% were parasitized *by Ancylostoma* sp. and 17.2% by *Toxocara* sp. These three genera have already been reported parasitizing *Didelphis albiventris* by various authors (SANTA CRUZ et al., 1999; CATENACCI et al., 2004; RIBEIRO et al., 2009; RONDON, 2010; PINTO et al., 2012; BONFIM, 2013; MELO, 2017; SILVA et al., 2017; PRADO et al., 2018; TONIN et al., 2018).

CONCLUSION

From the survey carried out, it can be seen that the white-eared opossum is completely integrated into the Metropolitan Zone of Recife, and is able to actively reproduce in this environment, since there is a high number of litters captured in this region. There is also a lot of contact between these individuals and domestic animals, which can result in the transmission of diseases to them and, consequently, to humans. In addition, adult opossums not only showed high parasitaemia but also simultaneously host several endoparasites. Were identified in the white-eared opossum from the Recife Metropolitan Area 9 groups of eggs and oocysts, which belong to the superfamily Spiruroidea and

Strongyloidea, family Ascaridae and Trichuridae, genera *Cruzia* sp., *Eimeria* sp. and *Giardia* sp., coccidia and oocysts similar to *Octosporella* spp. Despite the impossibility of detecting which species are zoonotic, several studies in the literature with *Didelphis sp.* point to the possibility of transmission of zoonoses by these animals.

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