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Research article

Gastrointestinal parasitic nematodes in pet red-footed tortoises (*Chelonoidis carbonaria*) from Grenada, West Indies

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ABSTRACT

This study identifies the endoparasites in Red-footed tortoises (*Chelonoidis carbonaria*) during March 2018 to March 2019 from Grenada, West Indies. Fecal samples from a total of 103 tortoises were collected and examined by simple fecal floatation techniques for parasitic eggs and oocysts. Adult parasites found in the feces were examined under the microscope for identification based on their morphology. Fecal samples for this research were collected from four parishes; St. Andrew, St George, St. John and St. Patrick. The overall prevalence of intestinal parasites among tortoises was 14.6%.

The internal parasites consisted of nematodes belonging to three genera: *Atractis, Labiduris and Proatractis*. The most prevalent nematodes were *Atractis* spp. (86.7%), which included two species: *A. thapari* (7.7%) and *A. marquezi* (15.4%) followed by *Labiduris gulosa* (20%) and *Proatractis parvicapiticaronata* (13.3%). All nematodes of three genera found in red-footed tortoises in Grenada are reported for the first time. They are not zoonotic but are serious pathogens for tortoises.

1. Introduction

Tortoises are land dwelling reptiles in the order Testudines and the family Testudinidae. In various parts of the world, confusion between the terms turtle and tortoise still exists. In North America a turtle is any member of the family Testudinidae, irrespective of their dwelling-on land or in water (Burton and Burton, 2002) whereas, the British usage restricts tortoises to land dwelling members of the order Testudines (Dawkins, 2009). Based on fossils combined with modern (living and extinct) species, 121 tortoise species existed at some point since the beginning of the Pleistocene period however, more than 50% have gone extinct due to human exploitation (Rhodin et al., 2015). Tortoises vary in size and mass depending on the species, from a few centimeters to 2 m in length and weigh from 3 oz to over 900 lbs. Among tortoise species, the Red-footed tortoise, *Chelonoidis carbonaria* (synonym: *Geochelone carbonaria*), has the widest range of habitats, found in countries of South America and in almost all Caribbean Islands, including Grenada. Red-footed tortoises prefer a habitat of seasonal temperature between 20 °C and 30 °C.

Gastrointestinal parasites are commonly found in wild and pet tortoises; broadly, the gastrointestinal parasites found in tortoises belong to nematodes, cestodes, trematodes and protozoa (Rataj et al., 2011) and some parasites have zoonotic potential and may have serious effects in humans (Wolf et al., 2014). Gastrointestinal parasites have been reported in various species of chelonians including; *Geochelone elegans*, *Testudo graeca* and *Testudo horsfieldii* in Iran (Arabkhazaeli et al., 2018); *Stigmochelys sulcata*, *Chelonoidis carbonaria*, *Malachoserus tornieri* and *Pyxis arachnoides* in Germany (Hallinger et al., 2018); *Gopherus polyphemus* in Georgia and South Florida (Huffman et al., 2018; McGuire et al., 2013); *Testudo hermanni*, *T. marginata* and *Agrionemys horsfieldii* in Hungary (Sátorhelyi and Sréter, 1993) and various *Chelonoidis* spp. on the Galápagos islands (Fournié et al., 2015). Helminth infections in chelonians have also been reported in Italy (Papini et al., 2011), Spain (Chávarri et al., 2012) and the United Kingdom (Hedley et al., 2013). Gastrointestinal parasites in red-footed tortoises have been reported in Venezuela (Pérez Mata et al., 2014), Brazil (Leal et al., 2018), and the USA (Rideout et al., 1987), however is a paucity of literature on parasites of red-footed tortoises in the Caribbean region including Grenada.

The aim of the present research is to identify the genera of parasites infecting the intestines of red-footed tortoise in Grenada based on morphological and morphometrical features and estimate the prevalence.

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2. Materials and methods

2.1. Ethical approval

The research project was approved by the Institutional animal care and use committee (IACUC) of St. George's University (IACUC, 18009. 23rd August 2018).

2.2. Consent of tortoise pet owners

Tortoise owners were identified in different parts of Grenada; research plan was explained to them in detail, and a written consent was obtained from those who agreed to participate in the research.

2.3. Study area

Grenada is the southernmost country in the Caribbean Sea with an area of 348.5 km^2. This country with its low hills, small trees, shrubs and tropical climate is most suitable for tortoise habitat. Environmental temperatures vary between 25 °C and 30 °C. The country is comprised of six parishes: St. Andrew, St. David, St. George, St. John, St. Mark and St. Patrick. The terrain throughout the country is similar. Samples submitted for this research were collected from four parishes; St. Andrew, St. George, St. John and St. Patrick.

2.4. Sample collection

A cross sectional study was performed to meet the aim of the research on population of pet tortoises in Grenada. Using the estimated tortoise population in Grenada of 250, sample size was determined using Cochran (1963) formula; $N = \frac{t^2(p)(1-p)}{d^2}$, where t = 1.96, p = estimated prevalence of 50% and d = desired level of precision of 0.05. The calculated sample size was determined to be 384, however a modification to the Cochran's formula using the finite population correction factor for small populations was subsequently utilized; $n = \frac{n_o}{1+\frac{n_o-1}{N}}$, where n_o = Cochran's sample size and N = estimated population, resulting in the new sample size of 152. However, owner consent was not obtained to collect fecal samples from all tortoises and only 103 were collected. Demographic information on location of tortoises in different parishes, gender and age based on observation of tail length, shell shape and owner records were taken at the time of sample collection. Approximately 2–5 g of fecal samples from each tortoise was collected using convenience sampling method for a period of 6 months from September 2018 to March 2019. For collection of feces, a tortoise was put on its back on a hard surface and cloaca was stimulated by gentle finger touch. The tortoise usually voided fresh feces. Fecal samples were placed in sterile vials with 10% formalin and stored until analyzed.

2.5. Sample examination

Centrifugation flotation with zinc sulphate (sp. gr. 1.18) was performed to recover parasitic eggs and larvae for x10 microscopic examination. Adult worms were removed from positive fecal samples using dissection microscopy, rinsed in distilled water and placed on a microscope slide with lacto-phenol to facilitate clearing of the cuticle for identification of morphological features. The identification of eggs and larvae of parasites was made following previous researchers' data (Baker, 1987; Bursey and Flanagan, 2002).

2.6. Statistical analysis

The association between prevalence and demographic factors (age, parish) were evaluated using Chi Square contingency analysis.

3. Results

The endoparasites were identified based on physical characteristics of egg and adult morphology using fecal flotation technique and microscopy of whole specimens.

Of the 103 tortoises examined during the study, 78 were adults ranging from 6 to 70 years in age and 25 were juveniles ranging from 4 months to 5 years in age. Fifteen tortoises (14.6%) were found to be infected with parasitic nematodes from the genera *Proatractis*, *Labiduris* and *Atractis*. According to parishes, the prevalence of endoparasites in St. George parish was 21.2% (11/52), St. Andrew 9% (2/22), St. Patrick 6.3% (1/15), and St. John 7.7% (1/13). The prevalence of parasites and their distribution in various parishes and sex are summarized in Table 1.

The most prevalent parasites were *Atractis* spp. (86.7%) which included the species *A. thapari* (7.7%) and *A. marquezi* (15.4%) followed by *Labiduris gulosa* (20%) and *Proatractis parvicapiticaronata* (13.3%). There were 13 (86.7%) single and 2 (13.3%) mixed infections with endoparasites which consisted of two species in 6.7% and three species in 6.7% of tortoises.

Two types of eggs were found on fecal flotation, based on measurements they were observed to be large and small strongyle type eggs within the range 140–160 × 80–110 μm and 60–88 × 30–44 μm respectively. Identification of nematode genus based on egg diversity was not possible due to the similarity of eggs of strongyle species. Positive fecal samples were examined using a dissecting microscope for the occurrence of nematodes. Nematodes recovered were rinsed in distilled water and mounted in lacto-phenol for microscopic identification.

3.1. General descriptions

3.1.1. Atractis spp. (Nematoda: Atractidae)

The body was small and cylindrical with females ranging from 2.25 – 5.70 mm in length and width of 245.3 μm and males from 2.25 – 3.76 mm in length. The body of the female terminates in a straight, pointy tail (733.6 μm) The esophagus is divided into a thickened, sclerotized anterior portion and a thinner posterior portion that ends in a valvular bulb (Figures 1 and 2). The excretory pore is surrounded by a striated, cuticular pattern surrounding a sucker-like structure (Figure 3). Males contain two unequal length spicules; the left lanceolate (long and thin) and right lagenoid (short and bottle-shaped), in addition to a well-developed

Table 1. Prevalence of endoparasites in Tortoises (*Chelonoidis carbonaria*) from Grenada according to parish and age.

Parish	No. Tested	Positive Percent	Adult		Juvenile	
			Tested	Positive	Tested	Positive
St. Andrew	22	2 (9%)	13	2 (15.4%)	9	0 (0%)
St. George	52	11 (21.2%)	39	8 (20.5%)	13	3 (23.1%)
St. John	13	1 (7.7%)	13	1 (7.7%)	0	0 (0%)
St. Patrick	16	1 (6.3%)	13	1 (7.7%)	3	0 (0%)
Total	103	15 (14.6%)	78	12 (15.4%)	25	3 (12.0%)

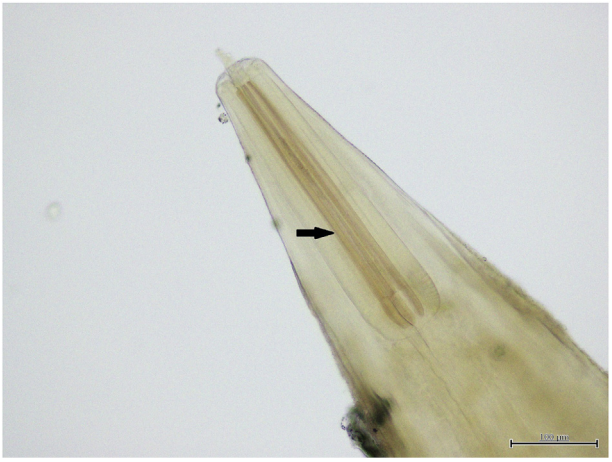

Figure 1. *Atractis* spp. anterior end showing thickened sclerotized anterior esophagus region (arrows).

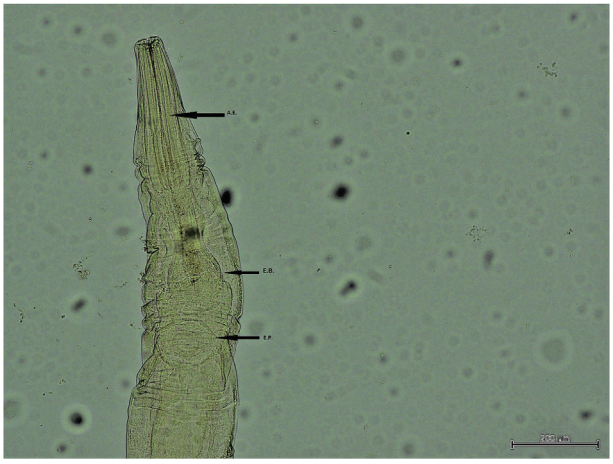

Figure 2. Atractis spp. anterior end showing anterior esophagus (A.E.), esophageal bulb (E.B.) and excretory pore (E.P.).

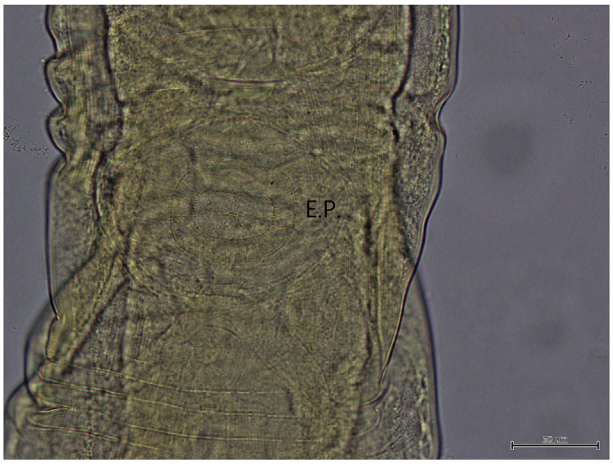

Figure 3. *Atractis* spp. excretory pore.

Figure 4. Male *Atractis* posterior end showing spicules; Left Long lanceolate (LS), Right Short lagenoid (SS) and Gubernaculum (G).

Figure 5. Posterior end of male *Labiduris gulosa* showing spicules and tail spike.

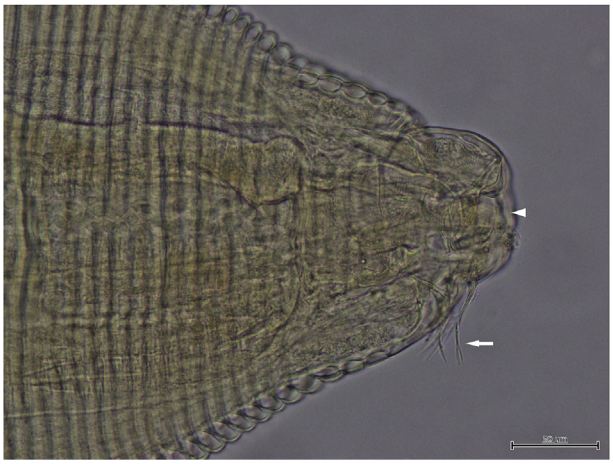

Figure 6. Anterior end of *Labiduris* sp. showing the subventral lips (arrowhead) and fringes (arrow).

Figure 7. *Labiduris gulosa* showing esophagus and esophageal bulb.

gubernaculum (Figure 4). Females were viviparous and produced 1–2 larvae in an advanced stage of development.

3.1.2. *Atractis thapari*

Male: Length 3.9 mm and maximum width 245.3 μm. Esophagus from anterior measured 646.9 μm. Male specimen having left lanceolate spicule measuring 315 μm, right lagenoid spicule 140 μm and gubernaculum 125 μm. The tail was long, tapered to a point at the end and measured 415 μm in length.

3.1.3. *Atractis marquezi*

Male: Length 4.1 mm. Dissimilar spicules, left lanceolate spicule 374.9–394.4 μm, right lagenoid spicule 139.9–145.8 μm and the gubernaculum 125.3–130.3 μm was slightly curved.

3.1.4. *Labiduris gulosa*

Male worms measuring 6.87 mm in length with spicules of similar size 323.9 μ and 324.1 μm and a long tail with a spike (Figure 5). The striated cuticle consists of deep annulations with annules ranging

Figure 8. Anterior end *Proatractis* spp. (a and b) with cuticular fork-like expansions around the oral opening.

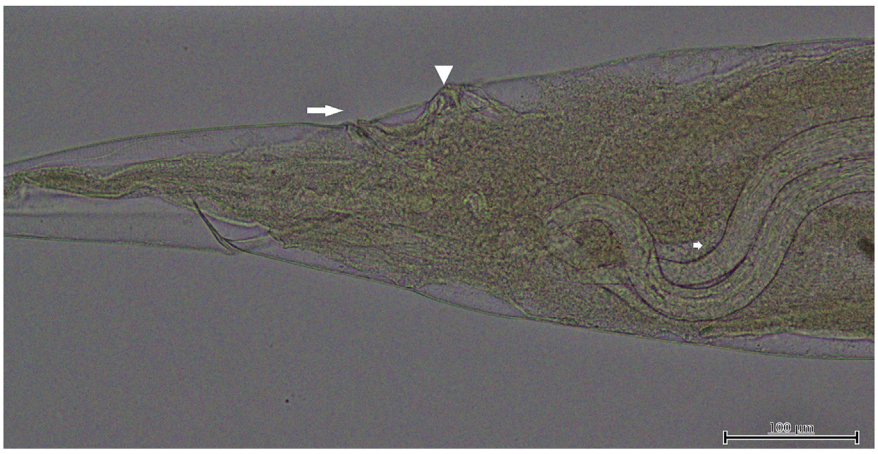

Figure 9. *Proatractis* female posterior end showing the vulva (arrowhead), anus (arrow) and larvae (small arrow).

Figure 10. Viviparous female *Proatractis* containing numerous larvae.

7.04–12.44 µm. Oral opening observed between hypertrophied lobes of subventral lips with lining of buccal cavity projecting through the ventral part of the opening and posteriorly directed fringes along the median edge (Figure 6). Oesophageal isthmus is spherical with a well differentiated bulb and developed valves (Figure 7).

3.1.5. *Proatractis parvicapiticaronata*

Adult males ranging from 2.05 – 2.88 mm and females 2.76–3.34 mm in length. Buccal structures with specialized cuticularized formations and lips with small cuticular fork-like expansions surrounding the oral opening (Figure 8). The esophagus was cylindrical shaped and the length 373–415 µm, with a distinct bulb. The male left spicule and gubernaculum measured 263.9 and 49.2 µm respectively. The female anterior end to vulva measured 2.56 mm and the vulva to posterior end ranged from 800.7 – 956.7 µm. The anus is located posterior to the vulva (Figure 9). Female is viviparous and contained multiple larvae in advanced stage of development (Figures 10 and 11). The tail (261.6–533.4 µm) was long, thin and ended in a pointed end.

4. Discussion

In the present research 14.6% (15/103) tortoises were found infected with intestinal nematodes. The parasites present belonged to three genera: *Atractis*, *Labiduris* and *Proatractis*. The species identified based on morphology were *A. thapari* and *A. marquezi; Labiduris gulosa* and *Proatractis parvicapiticoronata.* Many species of genus *Atractis* have been described infecting tortoises in various parts of the world based on their morphological structure (Bursey and Flanagan, 2002). Infections with *A. marquezi* have been observed in *Chelonoides* spp. of the Galápagos islands (Fournié et al., 2015), and in *C. denticulatus* from Peru (Chávez et al., 2015). *Atractis thapari* has been found parasitizing red-footed tortoises in the state of Piauí, Brazil (Leal et al., 2018). A species of *Atractis* identified only to the generic level was reported from Red footed

Figure 11. Female *Proatractis* giving birth to a larva.

tortoises in Venezuela (Pérez Mata et al., 2014). Nematodes of the genus *Proatractis* were reported in dead red-footed tortoises from the National Zoological Park in the USA (Rideout et al., 1987) and *P. parvicapiticoronata* in the large intestine of the Mexican musk turtle (*Staurotypus triporcatus*) from Veracrus State, Mexico (Caballero, 1971). In the present investigation we also found three red-footed tortoises infected with the nematode *Labiduris gulosa*. Rosa Julca et al. (2014) reported serious pathology in *Chelonoidis denticulata* infected with *Labiduris gulosa* in Peru.

All four of the nematodes found here in feces are viviparous, producing larvae, not eggs and therefore are not represented in fecal flotations. The only parasites found in flotations were two sizes of strongyle eggs, which could not be identified to genus. Pérez Mata et al., (2014) found adult strongyles in *C. carbonaria* from Venezuela, which they identified only to the subfamily level as Sauricolinae. All strongyles previously reported from tortoises belong to the genera *Sauricola* and *Chapiniella* (Baker, 1987).

Prevalence of endoparasite infection in St. George parish was 21.2% (11/52), St. Andrew 9% (2/22), St. Patrick 6.3% (1/15), and St. John 7.7% (1/13). Although the prevalence in St. George parish was greater than other parishes, there was no statistical significance for prevalence of parasites among the parishes ($p > 0.05$, $\chi 2$). In addition, statistical analysis demonstrated that endoparasite prevalence was not influenced by age ($p = 1$, Fisher's exact test). Contrary to our findings, previous researchers (Traversa et al., 2005) found that parasitic infection in tortoises increases with age. Statistical analysis regarding sex could not be performed as sex data was not available for juveniles. The differences in intensity of parasitic infection with age is not well understood.

The nematode parasites of the genera *Proatactis*, *Labiduris* and *Atractis* identified in feces of red-footed tortoises from Grenada are not known to be zoonotic, but have the potential to be serious pathogens for their hosts, particularly since they are viviparous and can multiply to very large numbers. They have been reported as the cause of significant morbidity and mortality in various species of tortoises (Rideout et al., 1987; Rosa Julca et al., 2014). Serious pathological lesions are confined to the large intestines. The mortality and pathological lesions depend mainly on the number of nematodes in the host (Rideout et al., 1987). Contrary to this statement (Pérez Mata et al., 2014) suggested that it is stress and not the amount of parasitic burden responsible for mortality and morbidity. They found no clinical signs in tortoises living in natural habitats with heavy infection. There was no mortality recorded in pet tortoise colonies in our study and no clinical signs of gastrointestinal involvement were observed during fecal collection. Correlation between absence of clinical signs and parasitic burden is not possible as we did not perform quantitative parasitic burdens.

5. Conclusion

Non-zoonotic nematodes (genera *Atractis*, *Labiduris* and *Atractis*) were identified in pet Red-footed tortoises from Grenada. However, these nematodes are considered to be serious pathogens for tortoises and prevention of infection within colonies of pet tortoises is recommended. Awareness of owners with regards to the prevalence of gastrointestinal nematodes and prevention of infection by practicing good hygiene and disposal of feces is advisable. In addition, the incorporation of routine fecal diagnostic test and bi-annual deworming with an approved anthelmintic is also recommended to prevent the transmission of parasites.

Declarations

Author contribution statement

Camille Coomansingh Springer: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mike Kinsella: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Vishakha Vasuki: Performed the experiments; Analyzed and interpreted the data;

Ravindra Nath Sharma: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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