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Internal parasitic burdens in brown rats (*Rattus norvegicus*) from Grenada, West Indies

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ABSTRACT

This study identified the endoparasites in Brown rat (*Rattus norvegicus)* during May to July 2017 in Grenada, West Indies. A total of 162 rats, 76 females and 86 males were trapped from St. George and St. David parishes in Grenada. The collected fecal samples were examined for parasitic eggs and/or oocysts using simple fecal flotation technique. Adult parasites found in the intestinal tract were examined for identification. The overall prevalence of intestinal parasites among rats was 79 %.

Ten helminth species were recovered, several of which were reported for the first time in rodents in Grenada. The internal parasites consist of seven nematodes (*Angiostrongylus* spp., *Nippostrongylus braziliensis*, *Heterakis spumosa*, *Strongyloides ratti*, *Aspiculuris tetraptera*, *Syphacia* spp. and *Protospirura* spp.), one cestode (*Hymenolepsis diminuta*), one acanthocephalan (*Moniliformis moniliformis*) and one protozoa species (*Eimeria* spp.). The most prevalent zoonotic species were *Angiostrongylus* spp. (35.2%), *Hymenolepsis diminuta* (7.4%) and *Moniliformis moniliformis* (3.1%). Several nonzoonotic endoparasites; which included *Nippostrongylus braziliensis* (50.6%), *Heterakis spumosa* (15.4%), *Strongyloides ratti* (43.2%), *Aspiculuris tetraptera* (2.5%), *Syphacia* spp. (1.9%), *Protospirura* spp. (1.2%) and *Eimeria* spp. (4.7%) were also identified. The most prevalent parasites were *Nippostrongylus brasiliensis* (50.6%), *Strongyloides ratti* (43.2%) and *Angiostrongylus spp.* (35.2%). Co-infections occurred with up to six species per rat showing different combinations of parasitic infections.

1. Introduction

Rodents are considered the most commonly occurring mammals on earth, due to their ability to adapt and thrive in almost any location and environment with the only exceptions being the Arctic and Antarctic regions. Around 4 million rodents are born every day, which lead to genetic polymorphism and hence resistance to different groups of rodenticides (Rahdar et al.,2016).

Large populations of rodents can be found wherever human densities are high, such as in cities, suburbs and agricultural areas due to the commensal relationship rodents share with humans for food and shelter. The presence of rodents near humans has been the cause of concern due to the physical damage and economic losses that they inflict to homes and farms. In addition rodents are considered reservoirs of zoonotic pathogens, which are transmitted to humans and cause fatal diseases (Kataranovski et al., 2011). Common zoonotic diseases include: leptospirosis, rat-bite fever, Q-fever and hemorrhagic fever (Coomansingh et al., 2009). The most common endoparasites of zoonotic potential found in the brown rat are *Hymenolepis*, *Trichinella*, *Moniliformis*, *Capillaria*, *Giardia*, and *Balantidium* (Rahdar et al., 2016).

Despite zoonotic potential, Hymenolepis diminuta, only a case of human infection with *H. diminuta* has been previously reported in the Caribbean (Waugh et al., 2006).

There is paucity of literature on endoparasites in brown rats from Caribbean nations. Previous studies in Grenada (Coomansingh et al., 2009) reported 90.9% overall prevalence of endoparasites in brown rats. They examined the rats trapped from all over the Grenada.

The aim of this study was to estimate the prevalence of endoparasite infections in the brown rat (*R. norvegicus*) from two parishes of Grenada, which had more human population compared to the rest of the country. This study was planned after nine years from the first report (Coomansingh et al., 2009).

2. Materials & methods

2.1. Ethics approval

The project entitled 'Detection of zoonotic pathogens in brown rats

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(*R. norvegicus*) in Grenada' was approved by the Institutional Animal Care and Use Committee (IACUC # 16009-R) of the St. George's University, Grenada.

2.2. Study area

Grenada is a 348.5 km^2 volcanic island found at the southern end of the chain of islands in the eastern Caribbean Sea. The mountainous terrain, tropical rainforest vegetation and climate make Grenada an ideal habitat for brown rat populations to thrive. The island consists of six parishes: St. George, St. David, St. Andrew, St. Patrick, St. Mark and St. John. Because of dense human population, the parishes of St. David and St. George were selected for sample collection in this study.

2.3. Collection of rats

Sample size was determined using Cochran (1963) formula; $N = t^2(p)(1\text{-}p)/d^2$. Where $t = 1.96$, p = estimated prevalence and d = desired level of precision. The calculated sample size was found to be 120, based on (90.9%) prevalence of intestinal parasites in rats in Grenada, in a previous study (Coomansingh et al., 2009). We decided to collect 162 rats, more than the estimated sample size. One hundred sixty-two rats were collected live from 1st May to 14th July 2017, using traps (45 cm L x 15 cm W x 15 cm H) baited with either cheese or local fruits. Traps were set out near the human dwellings in the evening and retrieved the next morning. Traps containing rats were covered with a dark cloth and transported to the necropsy laboratory of the School of Veterinary Medicine, St. George's University and were anesthetized using 1–2% isoflurane in oxygen, via portable vet anesthesia machine "isoflurane vaporizer VET CE," manufacturer DRE (Avante Health Solution Company, USA).

2.4. Collection of samples

The gender of rats were noted before anesthesia. Blood was collected from the heart through the thoracic wall and rats were exsanguinated this way.

The abdominal cavity was opened using a surgical blade and a pair of forceps. The entire gastrointestinal tract and the lungs were removed and fixed in 10% buffered formalin for parasitological examination.

2.5. Sample processing

The intestines were cut longitudinally and examined macroscopically for the presence of helminths. Lungs were examined for *Angiostrongylus* spp. Intestinal content was scraped out using a blunt spatula and centrifugation flotation with zinc sulphate (sp. gr. 1.18) was performed to recover parasitic eggs and larvae for microscopic examination. Nematodes found in the intestinal lumen were removed, rinsed with distilled water and placed in lactophenol to clear the cuticle. Acanthocephalans were rinsed, placed in alcohol-glycerin and examined unmounted. The association between prevalence and demographic factors (gender, parish) were evaluated using Chi Square Contingency analysis.

3. Results

The endoparasites were identified on physical character of parasites found in the intestine and morphology of egg/ova examined through flotation technique.

Of the 162 rats sampled during the study, 53% were males and 47 % were females. One hundred twenty-eight rats (79 %) were found to be infected with endoparasites. Ten parasitic species were recovered of which seven were nematode: *Angiostrongylus spp. (35.2%), Nippostrongylus brasiliensis (50.6%), Heterakis spumosa (15.4%), Strongyloides ratti (43.2%), Aspiculuris tetraptera (2.5%), Syphacia spp. (1.9%), Protospirura spp. (1.2%),* one cestode: *Hymenolepis diminuta* (7.4%), one coccidia *Eimeria spp. (4.7%)* and one acanthocephalan *Moniliformis moniliformis* (3.1%).

Infection rate of endoparasites in St George parish was 74.6% and in St David parish 83.7%. There was no statistical significance for prevalence of parasites between the two parishes ($p= >0.05$). In the study, 82.5% males and 75.0% females were positive for endoparasites (Table 1). Statistical analysis demonstrated that endoparasite prevalence was not influenced by gender ($p= >0.05$).

The most prevalent parasites were *Nippostrongylus brasiliensis* (50%) and *Strongyloides ratti* (42.4%) followed by *Angiostrongylus spp.* (35.2%). There were 41 (32 %) single and 87 (68%) mixed infection with endoparasites (Tables 2, 3, 4, 5). Multiple infections were noted up to six helminth species per host. Multiple infections with two species were found in 36.7% of infected rats (Table 3). Three species were found in 23.4%, four parasite species in 6.3 %, five and six parasite species in 0.8 % of the rats (Table 4 and Table 5). Types of polyparasitism was assessed for all rats with multiple infections. They were divided into classes of two, three, four, five and six species, respectively, representing the number of parasite species and specific combination of parasites.

4. Discussion

This study, reports on endoparasite infection in brown rats (*Rattus norvegicus*) and their zoonotic potential in Grenada.

The results of this study demonstrated that the overall prevalence of helminth endoparasites of *R. norvegicus* in Grenada was 79.4 % (n = 162), which is lower than the previous study by Coomansingh et al. (2009), who had reported 90.9% rats infected with endoparasites in Grenada. However, findings of the present research are similar to other prevalence studies: 80% in Malaysia (Mohd Zain et al., 2012), 89.8 % in Thailand (Alexis et al., 2016), 62.5% in Iran (Meshkekar et al., 2014), 68.5% in Serbia (Kataranovski et al., 2011). However, the prevalence rates were relatively higher in Argentina (97.5%; Hancke et al., 2011), India (100%; Sharma et al., 2013), Nigeria (100%; Egbunu and Dada, 2016) and lower in Qatar (35.8%; Abu-Madi et al., 2005), Palestine (58.5%; Al Hindi and Abu-Haddaf, 2013) and Baghdad (55%, Raad, 2010). The reason for variation in the parasites in rat in different areas of the world is not well explained. However, the environmental conditions at different locations is indicated as reason for this variation.

Ten species of helminths were identified in this study. In the Caribbean, similar surveys in Jamaica and Grenada revealed 9 and 6 helminths respectively (Waugh et al., 2006; Coomansingh et al., 2009). Our findings of mixed infections being more common than single infections differs from previous studies conducted in Jamaica (Waugh et al., 2006) and Grenada (Coomansingh et al., 2009) where single infections were more common than mixed infections.

In rodents, the *Angiostrongylus spp.*, *A. cantonensis* and *A. costaricensis* are found in the pulmonary and mesenteric arteries respectively and both

Table 1
Prevalence of endoparasites in Brown Rats from Grenada according to parish and gender.

Parish	No. tested	Positive percent	Male		Female	
			Tested	Positive	Tested	Positive
St. George	82	61 (74.6%)	41	33 (80.5%)	41	25 (68.3%)
St. David	80	67 (83.7%)	45	38 (84.4%)	35	29 (82.9%)
Total	162	128 (79.0%)	86	71 (82.5%)	76	57 (75.0%)

Table 2
Single specie infection of endoparasites in Brown rats from Grenada.

Parasite	A	H.D.	S.R.	N.B.	H.S.	S	E
Number	14	2	9	10	2	1	3

Index: A, *Angiostrongylus* spp.; H.D., *Hymenolepsis diminuta*; S.R., *Strongyloides ratti*; N.B., *Nippostrongylus brasiliensis*; H.S., *Heterakis spumosa*; S, *Syphacia spp.*; E, Eimeria; A.T., *Aspiculuris tetraptera*; M.M., *Moniliformis moniliformis*; P, *Protospirura spp*.

are known to cause disease in other mammal host including dogs, primates and humans. However, in this study the *Angiostrongylus* first stage larvae collected were not differentiated to species level. With regards to the both *Angiostrongylus* spp., *A. cantonensis*, is of a greater concern due to its worldwide distribution and significant public health concern due to its zoonotic threat because it is the major pathogen of human eosinophilic meningitis resulting in central nervous system angiostrongyliasis. Human infections is caused by the consumption of raw or undercooked snails, vegetables contaminated with snail secretions or paratenic hosts. Parasite is endemic to Asia (Taiwan (Tsai et al., 2013), Vietnam (McBride et al., 2017), North and South American countries (Hawaii (Hochberg et al., 2011), Texas (Al Hammoud et al., 2017) and Brazil (Morassutti et al., 2014). Angiostrongyliasis has also been reported in Caribbean islands such as Jamaica (Lindo et al., 2002; Waugh et al., 2005) and Guadeloupe (Dard et al., 2017). The parasite has spread geographically in recent years with the dissemination of one of its intermediate hosts, the giant African land snail (*Achatina fulica)* which is a popular item of food in some countries (Macpherson, 2005) and by ships infested with rats (Wang et al., 2008). It is not known when the parasite entered Grenada, as the first report of its presence was in 2009 (Chikweto et al., 2009). In the present study *Angiostrongylus spp.* was found in 35.2% rats which is within the range found in two previous studies conducted in Grenada. In the first study, Chikweto et al. (2009) reported 23.4% microscopic lesions of *Angiostrongylus spp.* in lung of brown rats in Grenada, where as in a recent study Tiwari et al. (2018) reported 31.8% gross lesions and 39.1% microscopic lesions of *Angiostrongylus spp.* in lung of brown rats. However, as yet no case of Angiostrongyliasis has been reported in humans in Grenada despite the high risk of consuming the infective parasitic stage.

The findings of this study show that the nematode, *Nippostrongylus brasiliensis* was the most common rodent endoparasite found in brown rats in Grenada. This is in agreement with previous studies conducted in Jamaica (Waugh et al., 2006), Grenada (Coomansingh et al., 2009) and Argentina (Hancke et al., 2011). However, it contradicts similar research by Kataranovski et al. (2011) in Serbia and Franssen et al. (2016) in Netherlands who found *Heterakis spumosa* to be most prevalent. Although, studies conducted in Thailand by Alexis et al. (2016) found *Capillaria hepatica* to be most prevalent in rats, no infection with *Capillaria hepatica* was found in the current study in brown rats from Grenada. In contrast to our study, surveys by Raad (2010) in Baghdad, Abu-Madi et al., (2005) in Qatar, Al Hindi and Abu- Haddaf (2013) in Palestine, Mohd Zain et al. (2012) in Malaysia found *Hymenolepsis diminuta* to be the most prevalent intestinal parasite.

The two *Strongyloides* spp. that can infect rodents are *Strongyloides ratti* and *S. venezuelensis,* both of which can occur in wild *R. norvegicus* (Little, 1966). Co-infections of these *Strongyloides* spp. appears to be normal and their geographical distributions overlap. Infection of rats with *S. venezuelensis* was first reported in Venezuela (Brump, 1934) and briefly described, however, it was latter redescribed in New Orleans (Little, 1961). It has also been reported from Brazil (Araujo, 1967), Japan (Hasegawa et al., 1988) and Israel (Wertheim and Lengy, 1964). *S. venezuelensis* is mainly found in warmer regions 35° north and south of the equator, whereas *S. ratti* appears to have a worldwide distribution and has been reported in Jamaica (Waugh et al., 2006), Japan (Shintoku et al., 2005) and England (Fisher and Viney, 1998). Distinction between the two species was made by comparative morphology of the parasitic female, and found to be *S. ratti* as the ovaries lay parallel to the intestines and also there was a mixture of eggs and larvae found in the feces (Little, 1966). In experimental co-infections, there are synergistic effects between two species of parasites, such that the infection success of each species is greater in coinfections compared with single species infections. This might be the reason for a high prevalence of *Nippostrongylus/Strongyloides* type of infection (Viney and Kikuchi, 2017).

The occurrence of several other nonpathogenic parasites with low prevalence included; *Heterakis spumosa*, *Aspiculuris tetraptera*, *Syphacia spp., Protospirura spp. and Eimeria spp.* The soil transmitted ascarid helminth, *Heterakis spumosa*, has been observed in rodents from Sicily (Milazzo et al., 2010), Serbia (Kataranovski et al., 2010), Maryland, USA (Easterbrook et al., 2008) and Brazil (Simões et al., 2016).

The rodent pinworms of the genera *Aspiculuris* and *Syphacia* were

Table 4
Mixed infections of three endoparasites in Brown rats from Grenada.

Parasite combinations	Number
A + N.B. + S.R.	10
A + E + S.R.	1
A + N.B. + H.D.	1
A + N.B. + M.M.	1
A + S.R. + H.S.	3
A + N.B. + H.S.	1
N.B. + S.R. + H.S.	1
N.B. + S.R. + H.S.	5
N.B. + H.D. + H.S.	1
M.M. +N.B. + S.R.	1
N.B. + H.D. + S.R.	3
S.R. + H.D. + H.S.	1
S.R. + A.T. + N.B.	1

Index: A, *Angiostrongylus* spp.; H.D., *Hymenolepsis diminuta*; S.R., *Strongyloides ratti*; N.B., *Nippostrongylus brasiliensis*; H.S., *Heterakis spumosa*; S, *Syphacia spp.*; E, Eimeria; A.T., *Aspiculuris tetraptera*; M.M., *Moniliformis moniliformis*; P, *Protospirura spp*.

Table 5
Mixed infection combinations of four or more endoparasites in Brown rats from Grenada.

Parasite combinations	Number
H.S.+ N.B + S.R. + E	1
A + N.B. + H.S. + S.R.	6
S.R. + M.M. + H.S. + N.B.	1
A + N.B. + H.D. + M.M. + P	1
H.S + P + S.R + E + M.M + N.B.	1

Index: A, *Angiostrongylus* spp.; H.D., *Hymenolepsis diminuta*; S.R., *Strongyloides ratti*; N.B., *Nippostrongylus brasiliensis*; H.S., *Heterakis spumosa*; S, *Syphacia spp.*; E, Eimeria; A.T., *Aspiculuris tetraptera*; M.M., *Moniliformis moniliformis*; P, *Protospirura spp*.

Table 3
Mixed infections of two endoparasites in Brown rats from Grenada.

Parasite combinations	A + N.B.	A + S.R.	A + A.T.	H.S + N.B.	H.D.+E	H.D.+A	N.B.+A.T.	N.B.+S.R.	N.B.+S	N.B.+E	S.R.+A.T.	S.R.+H.S.
Number	11	5	1	2	1	2	1	20	1	1	1	1

Index: A, *Angiostrongylus* spp.; H.D., *Hymenolepsis diminuta*; S.R., *Strongyloides ratti*; N.B., *Nippostrongylus brasiliensis*; H.S., *Heterakis spumosa*; S, *Syphacia spp.*; E, Eimeria; A.T., *Aspiculuris tetraptera*; M.M., *Moniliformis moniliformis*; P, *Protospirura spp*.

found in the rodent population from the Philippines (Jueco and Zabala, 1990) and Brazil (Porta et al., 2014). Our research reported similar prevalence to Jamaica (Waugh et al., 2006) and Iran (Pakdel et al., 2013), but was much lower than that reported in England (Elsheikha et al., 2010).

The spirurid, *Protospirura spp.,* utilizes the roach as a natural intermediate host and has been reported in a broad range of host from areas in Egypt, Africa (Mohamed et al., 1993), Papua New Guinea and Papua Indonesia, Asia (Smales, 2016), Costa Rica in Central America (Campos and Vargas, 1978) and Peru, South America (Sotomayor et al., 2015). However, this is the second report of this parasite from the Caribbean, with the first being by Waugh et al. (2006) in Jamaica.

The protozoan parasite, Eimeria, is common in the small intestines of wild rats and is considered mildly pathogenic in small numbers. This coccidia has been reported in rodents from various parts of the world including Japan (Kasai, 1978), Colombia (Bonfante et al., 1961), Brazil (Chagas et al., 2017) and England (Webster and Macdonald, 1995). However, this study is the first reported finding of coccidia in the rodent population within the Caribbean.

The prevalence of *Hymenolepsis diminuta* reported in this study in comparison to other Caribbean islands, was lower than Puerto Rico (De LeÖN, 1964), but similar to Jamaica (Waugh et al., 2006). Despite the low prevalence reported, *H. diminuta* is still of public health concern due to its ability to infect humans. Although infection is uncommon, human infections have been reported worldwide with reports from; Jamaica (Cohen, 1989), India (Tiwari et al., 2014), Thailand (Wiwanitkit, 2004) and Italy (Marangi et al., 2003). Humans become infected by the accidental ingestion of intermediate host such as fleas, flour beetles and other insects commonly found in grain and cereal containing the cysticercoid larvae. Infection resulting from incidental ingestion of the infective stage are often asymptomatic but heavy infection can result in persistent abdominal pain, diarrhea and anorexia (Ahmad et al., 2017; Alvarez-Fernandez et al., 2012).

Moniliformis moniliformis prevalence within the population of wild rats was low but similar to studies reported from Spain (Galán- Puchades et al., 2018), Malaysia (Mohd Zain et al., 2012) and Jamaica (Waugh et al., 2006). Human infection results from the ingestion of infected insects and sporadic cases of human infections have been reported from Florida (Mathison et al., 2016), Nigeria (Ikeh et al., 1992), Saudi Arabia (Sahar et al., 2010) and Iran (Maraghi et al., 2014). To date, human infections with *M. moniliformis* have not been reported in the Caribbean region.

Our findings indicate that intestinal parasite prevalence was not associated with gender of brown rats which is in line with a study conducted by Franssen et al. (2016) in Netherlands. But these findings contradict with those of Hancke et al. (2011) in Argentina, who found that males have a higher prevalence of endoparasite infection and Waugh et al. (2006) in Jamaica where more females than males were infected. The island of Grenada has a tropical climate and experiences frequent rainfall. It is likely that transmission of the parasites is facilitated by tropical conditions.

5. Conclusion

The majority of rats used in this study were infected with one or more endoparasite. This observation suggests that there may be a symbiotic relationship between the endoparasites and the rodent hosts. In Grenada the Norwegian rats, therefore, pose a potential health risk to humans since some of the parasites they harbor are zoonotic. Based on these findings, it is recommended that rodent control and eradication measures be put into place in order to prevent rodent borne disease outbreaks. Infected rats close to human dwellings may prove a potential source of infection to man and animals. The Grenadian community is advised to maintain hygienic conditions near their dwellings to prevent the proliferation of rats.

Declarations

Author contribution statement

C. 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.

V. Vishakha: Analyzed and interpreted the data; Wrote the paper.

A. Montanez Acuna, E. Armstrong: Performed the experiments; Analyzed and interpreted the data.

R. N. Sharma: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; 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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