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Seroprevalence of Antibodies against *Trypanosoma cruzi* in Brown Rats (*Rattus norvegicus*) from Grenada, West Indies

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ABSTRACT

Chagas disease is an arthropod borne parasitic disease of humans and animals caused by infection with *Trypanosoma cruzi*. Chagas disease is prevalent in Latin America and the Caribbean nations. Rats (Rattus species) are considered a reservoir host in transmission of the disease. The aim of this study was to estimate the prevalence of antibodies against *T. cruzi* in brown rats (*Rattus norvegicus*) from Grenada. A total of 145 rat sera were examined for *T. cruzi* antibodies using a qualitative immunochromatographic screening test: Chagas Stat Pak™ (Chembio Diagnostic System, Inc. Medford NY, USA). A seroprevalence of 10.3% (15/145) for *T. cruzi* antibodies was found. Results from this study indicate a moderate exposure level of *R. norvegicus* to *T. cruzi* in Grenada. Further research to find out the presence of the insect vector near the rat colony and the relationship of reservoir host in disease transmission is indicated.

Keywords: Antibodies, Brown Rat, Grenada, *Trypanosoma cruzi*

Chagas disease, a vector-borne disease, is an important public health problem in Central and South America and in nearly all the countries facing the Caribbean basin (Petana, 1978). Chagas disease is caused by the parasitic protozoan *Trypanosoma cruzi* and is transmitted by blood sucking triatomine bugs. In the mammalian host, *T. cruzi* amastigotes multiply in muscle cells and other nucleated cells of the body. Amastigotes released by the rupture of the cells change into trypomastigotes which circulate in the blood. Trypomastogotes may invade other cells of the host or are ingested by triatomine bugs as they blood feed. Trypomastigotes multiply and undergo metamorphosis in the hind gut of the bug and are passed in the feces (Bowman, 1999). Infection to humans and other animal hosts through infected feces of triatomine bugs is by way of the oral, nasal and conjuctival mucosa or abrasion of the skin. Other routes of transmission to humans are by transfusion of infected blood, transplant of infected organs, or transmission from an infected mother to her child at birth (Alejandro *et al.,* 2013). *T. cruzi* infects many mammalian species as reservoir hosts. Dogs, opossums and rats (Rattus species) are considered the most important reservoir hosts (Alejandro *et al.,* 2013). Chagas disease in humans in South America and Caribbean nations is correlated with infection in dogs (Rosypal *et al.,* 2007, Crisante *et al.,* 2006; Pineda *et al.,* 2011). Chickweto *et al.* (2014) reported 10.5% sero-prevalence of *T. cruzi* in stray and pet dogs in Grenada. An earlier study found 4.3% of pet and stray dogs from Grenada had antibodies to *T. cruzi* (Rosypal *et al.,* 2010).

There is paucity of information on infection in rats from Caribbean nations. Rats were found infected with *T. cruzi* in Mexico (Gurmersindo *et al.,* 2018), Medagascar (Rahelirina *et al.,* 2010), and Venezuela (Herrera *et al.,* 1997). The presence of *T. cruzi* in a wild caught rat was reported from Trinidad (Downs, 1963).

As far as authors are aware, there is no published literature on *T. cruzi* infection in rats from Grenada. The aim of this research was to determine the seroprevalence of *T. cruzi* in brown rats (*R. norvegicus*) in Grenada.

MATERIALS AND METHODS

Ethical approval

The project (Detection of zoonotic pathogens in brown rats (*Rattus norvegicus*) in Grenada) was approved by the Institutional Animal Care and Use Committee (IACUC # 16009-R) of the St. George's University, Grenada.

Study area

Grenada is the southernmost country in the Caribbean Sea with an area of 348.5 Km^2. The country with low hills, small trees, shrubs and tropical climate is most suitable for rat population. The country is comprised of six parishes: St. Patrick, St. Mark, St. Andrew, St. John, St. George and St. David. St. David and St. George parishes, which have a higher human population compared to other 4 parishes, were selected for the study.

Collection of rats

One hundred forty five rats were collected live from 1st May to 14th July 2017, using traps (45cm l × 15cm w × 15 cm h) with cheese and various local fruits as bait. Attempts were made to trap the rats from and near the residential buildings. Traps were placed two days per week in the evening and visited in the morning the next day. Traps with rats were covered with black cloth, transported to the necropsy laboratory of the School of Veterinary Medicine 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).

Collection of samples

The anesthetized rats were examined for their physical health and weighed. Gender was also recorded. Rats below 100g were grouped as young and over 100g as adult following the methodology used by Panti-May *et al.* (2012). Blood was collected from the heart through the thoracic wall and rats were exsanguinated this way.

Sera were separated from the blood by centrifugation at 1500g for 15 minutes at room temperature and stored at -80 °C till tested.

Test method

A rapid immune-chromatographic screening test (Chagas Stat Pak™, Chembio Diagnostic System, Inc. Medford NY, USA)was used for antibody detection in sera of rats. This assay has been used previously for detection of anti- *T. cruzi* antibodies in wildlife, including several rodent species (Charles *et al.,* 2013, Yabsley *et al.,* 2009). Screening was performed on sera of 145 rats according to the manufacturer's directions. Briefly, 5 µl of rat serum was aliquoted onto the sample well of the test device and 6 drops (~240µl) of provided sample diluent was slowly pipetted into the sample well. Results were read after 15 minutes. According to the test procedure, within 15 minutes, a purple control line and a second purple line in the test area indicated that result was positive. The presence of only a single purple line in the control region signified a negative result.

RESULTS AND DISCUSSION

Antibodies to *T. cruzi* were found in 15 rats (15/145) 10.3% (CI 95% from 5.91 to 16.49). The Chagas Stat Pak™ is a qualitative assay, so antibody titers could not be determined. A higher prevalence of antibodies to *T. cruzi* was found in St. David (13.3%) than in St. Georges (7.1%). Male and female rats demonstrated equal prevalence of antibodies (10.0% male and 10.8% female). Among the rats tested, 12.0% of adult rats had antibodies to *T. cruzi*, but none of the young rats tested positive by the Chagas Stat Pak™. The serological results according to parish, sex and age are presented in table 1.

In the present study we found 10.30% seropositive *R. norvegicus* in 2 parishes of Grenada. There was no significant difference in the positive rats between St. George and St. David parish. The seroprevalence of antibodies to *T. cruzi* in rats in Grenada found in the present study was similar to pet and stray dogs (Chikweto *et al.,* 2014), however, in a previous study of dogs Rosypal *et al*. (2010) found 4.3% seroprevalence of *T. cruzi* antibodies in Grenada.

Rats are common reservoir hosts of *T. cruzi* in endemic areas. Gumercindo *et al.* (2018) found slightly higher antibodies (22.70%) of *T. cruzi* in *R. norvegicus* in Western Mexico. Other researchers found 42.90% antibodies positive *R. rattus* in Western Mexico (Martnez-Lbarra and

Table 1: Prevalence of *Trypanosoma cruzi* in brown rats from Grenada according to parish, gender and age

Parish	Tested	Positive (%)	Male		Female		Young		Adult	
			Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)
St. Georges	70	5 (7.1)	35	1 (2.8)	35	4 (11.4)	12	0 (0.0)	57	5 (8.7)
St. David	75	10 (13.3)	45	7 (14.5)	30	3 (10.0)	8	0 (0.0)	68	10 (14.7)
Total	**145**	**15 (10.3)**	**80**	**8 (10.0)**	**65**	**7 (10.8)**	**20**	**0 (0.0)**	**125**	**15 (12.0)**

Villagran, 2009). Carolyn *et al. (*2017) quoted an infection rate ranging from 5% to 57% in *R. rattus* in Latin America. Fifty seven percent of *R. rattus* were demonstrated positive for *T. cruzi* infection in the Republic of Panama (John and Johnson, 1970). We are not comparing our seroprevalence of antibodies to *T. cruzi* in rats with results of previous researchers in different countries. The variation in prevalence in different countries could be due to the various diagnostic tests used. Different techniques were used for the diagnosis of *T. cruzi* by previous researchers, including: direct blood smear microscopy, culture, IHA analysis, complement fixation test, ELISA, and molecular techniques. Although good correlation between the infected vector triatomines and infected rats was shown previously in Mexico (Gurmercindi *et al.,* 2018), it was not possible to determine this correlation in the present study as vector triatomines were not collected and examined for *T. cruzi* infection.

During the present study, we did not find significant differences between young and adults, and between male and female rats positive for *T. cruzi* antibodies. No mention in the literature was found regarding differences in sex among *T. cruzi* infected rats. Previous researchers (Elizabeth *et al.,* 2000; Pascutti *et al.,* 2003) reported young rats (approximately 30 days old) more sensitive than adults. Pascutti *et al.* (2003) showed that increased resistance in adult rats seems to be the result of a more appropriate antibody production.

CONCLUSION AND RECOMMENDATION

Although, no human cases of Chagas disease have been reported in Grenada, the presence of antibodies in rats and dogs found in separate studies in Grenada may suggest a risk factor for humans. The prevention and control of *T. cruzi* in endemic countries is partially through the control of vector triatomines and reservoir hosts. Further research in Grenada is suggested to collect and study the vector triatomines near the population of rats to find out the relationship of infection in rats with vector. The results of this study will help in formulation of prevention and control of Chagas disease policy in Grenada and the region.

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