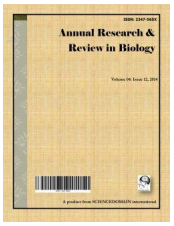

Annual Research & Review in Biology

24(5): 1-6, 2018; Article no.ARRB.39602
ISSN: 2347-565X, NLM ID: 101632869

Cryptosporidium spp.: Serum Antibodies and Coproantigens in Brown Rats (*Rattus norvegicus*) from Grenada, West Indies

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Authors' contributions

This work was carried out in collaboration between all authors. Author RNS was responsible for developing and supervising all steps of this project and wrote the first draft of the manuscript, Author KT and CCS helped with literature search, laboratory analysis and data analysis, Authors KPT, KT, MP, AG and ES managed sample collection and performed ELISA. All authors read and approved the final manuscript.

DOI: 10.9734/ARRB/2018/39602
Editor(s):
(1) Saramma George, Human Physiology, Department of Biomedical Sciences, Faulty of Medicine, University of Botswana, Botswana.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
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(3) J. Siwila-Saasa, University of Zambia, Zambia.
Complete Peer review History: http://www.sciencedomain.org/review-history/23537

Original Research Article

Received 20th November 2017
Accepted 28th February 2018
Published 8th March 2018

ABSTRACT

The aim of the study was to determine the serum antibodies and coproantigens for *Cryptosporidium* spp. in brown rats from Grenada. Intestinal contents from 99 and serum from 169 brown rats (*Rattus norvegicus*) during May to July 2017 were examined for coproantigens and antibodies for *Cryptosporidium* spp. respectively. *Giardia*/*Cryptosporidium* Quick Chek (Tech Lab Inc, USA) was used to detect *Cryptosporidium* coproantigens in intestinal contents. Prevalence of antigens was 2.02%, signifying active infection. Serum anti-*Cryptosporidium* antibodies against *Cryptosporidium* were tested using commercial "qualitative rat *Cryptosporidium* antibody (Anti-CRY) ELISA kit (My BioSource, Santiago, CA, USA). Serum antibodies were present in 29.5% of the rats indicating a heavy exposure of *Cryptosporidium* in brown rats from Grenada. The prevalence rate of antibodies

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in younger rats (up to100g weight) 42.1%, was statistically significant compared to adults (> 100 g) 28.0%. Infected rats in nearby human dwellings may prove a potential *Cryptosporidium* source of infection to man and animals. This is the first report of *Cryptosporidium* infection in brown rats in Grenada, West Indies.

Keywords: Antigen; antibody; Cryptosporidium; grenada; Rattus norvegicus.

1. INTRODUCTION

Cryptosporidium belonging to Apicomplexa phylum, is a coccidian protozoan that infects the gastrointestinal epithelium and rarely respiratory tract of vertebrates; animals, man and birds [1]. Disease caused by *Cryptosporidium* spp. is manifested by diarrhea in animals and man. Severe diarrhea with fatal outcome is observed mostly in children and immunocompromised humans [2]. Direct transmission of the disease is by fecal oral route. Infected hosts excrete infective and resistant oocysts which contaminate the environments. The ingestion of oocyst by susceptible hosts through contaminated water and food is another route of disease transmission.

Rodents were considered most important species in maintenance and transmission of Cryptosporidiosis [3,4]. Among rodents; wild, domestic and laboratory rats have been identified as reservoirs of *Cryptosporidium*. *Cryptosporidium* spp was first detected in rat feces in Japan [5]. Because of the close association of *Rattus norvegicus* (brown rats) with human population and animal houses, *Cryptosporidium* spp. has been widely investigated in brown rats in many countries, including Japan [6,7], England and Wales [8], Warwickshire, UK [9], Egypt [10] and Iran [11].

There is paucity of information on *Cryptosporidium* infection in Caribbean nations. *Cryptosporidium* was diagnosed in livestock (cattle, sheep, goats and pigs) in Trinidad, a neighboring island country of Grenada [12]. In Grenada, Chikweto et al. (personal communication),, in a study of Cryptosporidiosis in small ruminants found a prevalence of 19.5% (59 positive/302 sampled). As far as authors are aware there is no report on *Cryptosporidium* spp. in *Rattus norvegicus* from Caribbean region.

The present study aimed to estimate the prevalence of anti-*Cryptosporidium* serum antibodies and cryptosporidium antigens in fecal samples from *Rattus norvegicus* from Grenada, West Indies.

2. MATERIALS AND METHODS

2.1 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.

2.2 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 and shrubs and tropical climate is most suitable for brown rats. 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 more human population compared to other 4 parishes were selected for the study.

2.3 Collection of Rats

Sample size was determined using formula of Glenn [13]; $N=t^2(p)(1-p)/d^2$. Where t=1.96, p= estimated prevalence and d= desired level of precision. Since prevalence of *Cryptosporidium* spp. in rats for Grenada is unknown, an average of prevalence from other countries was estimated at 10% giving the number of samples to be 138. We decided to collect 170 rats, more than estimated number. Thus, one hundred sixty-nine rats were collected live from 1st May to 14th July 2017, using traps (45cm l x 15cm w x 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 in the evening and visited next day morning. Traps with rats were covered with black cloth and transported to the necropsy laboratory of the School of Veterinary Medicine and transferred to the anesthesia machine. Rats were anesthetized using 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 anesthetized rats were examined for their physical health and weighed. Gender was also recorded. Rats below 100 g were grouped as young and over 100 g as adult. Blood was collected from the heart through the thoracic wall and rats were exsanguinated this way. The abdominal cavity of rats was opened using a surgical blade and a pair of forceps. The intestinal tract with contents were placed into containers with 10% formalin until processing.

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

2.5 Detection of Antigen and Antibody for *Cryptosporidium*

Giardia/Cryptosporidium Quick Chek (Tech Lab Inc, USA) was used to detect antigen for *Cryptosporidium* oocyst according to manufacturer's instructions.

Serum samples were tested for antibodies against *Cryptosporidium* using commercial qualitative rat *Cryptosporidium* antibody (Anti-CRY) ELISA kit (My BioSource, Santiago, CA, USA). ELISA test was performed according to manufacturer's instructions

2.6 Statistical Analysis

The data were analyzed by the statistical methods: Fisher's exact test, using graph pad statistical software (http://www.graphpad.com/quickcalcs/contingency2).

3. RESULTS

Of the 169 tested rats (*Rattus norvegicus*) antibodies for *Cryptosporidium* were detected in 50 rats, with an overall prevalence of 29.58% (95% CI: 22.82 - 37.08). The results for seroprevalence tabulated in Table 1. The difference in positive cases between St. George and St. David was statistically not significant ($p > 0.05$). There was no statistical difference in gender. The seroprevalence was 42.1% in young and 28.0% in adult rats. There was statistically significant difference in age. The young rats were found more infected.

Project was planned to test only 99 rats by Quick Chek for detection of *Cryptosporidium* coproantigens in the intestinal contents. The results are included in Table 2.

4. DISCUSSION

In the current study we found 2.02% coproantigens in intestinal contents using Quick Chek. Comparable to our results, previous researchers [14,15] who, although used different diagnostic techniques also found a low prevalence (between 2.1% and 3.0%) of *Cryptosporidium* spp. in intestinal contents. Others [16] reported a very high prevalence ranging from 63% to 68.1% in brown rats. A moderate prevalence of 11.5% in wild laboratory rats was found (Chaochao et al.) [17]. The wide variation in the prevalence of coproantigens by other researchers is not well explained. However, variation in prevalence could be because of immune status of the host, age and stress from concurrent infections.

Our results of seroprevalence of anti-*Cryptosporidium* antibody in brown rats may not be compared with previous researchers because of different techniques used by them. However, the detection of antibodies provides presumptive evidence of prior exposure [18]. In an experimental study in roof rats, researcher [15] found shedding of oocysts 2-3 post infection (pi), peaked 5-8 pi. and rapidly declined, there after intermittent detection of oocysts until day 60 pi.. These studies support our finding of low prevalence of coproantigens in intestinal contents (2.02%) and high seropositivity (29.5%) amongst the same group of brown rats.

We found 28% male and 32.43% female in St. George parish compared to 29.16% male and 28.88% female in St. Davis parish. The association between sex of the rats and Cryptosporidium spp. infection was statistically not significant ($p>0.05$). Our findings are in agreement with previous researchers [13,14] who reported no significant difference between gender and infection in brown rats. Contrary to our findings there is a report of higher prevalence of cryptosporidiosis in male brown rats [9].

Table1. Prevalence of *Cryptosporidium* antibody (Anti-CRY) 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	76	23 (30.26)	39	11 (28.20)	37	12 (32.43)	12	5 (41.7)	64	18 (28.1)
St. David	93	27 (29.03)	48	14 (29.16)	45	13 (28.88)	7	3 (42.9)	86	24 (27.9)
Total	**169**	**50 (29.58)**	**87**	**25 (28.73)**	**82**	**25 (30.48)**	**19**	**8 (42.1)**	**150**	**42 (28.0)**

Table 2. Prevalence of coproantigen of *Cryptosporidium* oocyst in brown rats from Grenada

Parish	Male		Female	
	Rats examined	Rats infected (%)	Rats examined	Rats infected (%)
St. Georges	23	0 (0.0 %)	19	0 (0.0 %)
St. David	35	1 (2.9%)	22	1 (4.5 %)

The seroprevalence was 42.1% in young and 28.0% in adult rats. The young rats were found more infected. The difference with age was statistically significant ($p<0.05$). Our observation is in agreement with another researcher [9]. The higher incidence in younger rats (< 100g) is not well explained. Development of immunity with age may be indicated for the difference. Further research might help in understanding the difference in infection rates between young and adult rats.

With the use of molecular techniques at least more than 25 species of *Cryptosporidium* found in various vertebrate hosts are recognized [19]. Molecular techniques further helped to identify the genotypes affecting rodents. Genotypes in rodents include rat genotype I-IV, skunk genotype, mouse genotype, chipmunk genotype. Each *Cryptosporidium spp.* or genotypes have different host specificity but at least five types of *Cryptosporidium* infect humans: *C. parvum, C. meliagridis, C. muris, C. andersoni, C. ubiquitum, C. canis* and *C. felis* [20]. The identification of species and genotypes may help in understanding of *Cryptosporidium* zoonotic infections. Further research is warranted at molecular level for the identification of species and genotypes of *Cryptosporidium* spp. in brown rats from Grenada.

5. CONCLUSION

Since rats and humans share common living areas, *Cryptosporidium* infected rats pose a public health problem. The Grenadian community needs to be educated to maintain a proper hygienic condition to prevent the proliferation and survival of rats. This is the first report of *Cryptosporidium* infection in brown rats in Grenada.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the funding of the research from one health one medicine research initiative (OHRI grant 06-14-10) of the St. George's University, Grenada. Technical assistance of Ray Samuel is appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Santin M. Clinical and subclinical infections with *Cryptosporidium* in animals. N.Z. Vet J. 2013;61:1-10.
2. Ng-Hublin JS, Singleton GR, Ryan U. Molecular characterization of *Cryptosporium spp.* in wild rats and mice from rural communities in the Philippines. Inf Genetic and Evolution. 2013;16:5-12.
3. Feng Y. *Cryptosporidium* in wild placental mammals. Int. Parasitol. 2010;124(1):128-137.
4. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: Transmission, detection and identification. Int J Parasitol. 2000;30:1305-1322.
5. Iseki M. Two species of *Cryptosporidium* naturally infecting house rats, *Rattus norvegicus.* Jap J Parasitol. 1986;35:521-526.
6. Kimura A, Edagawa A, Okada K, Takimota A, yonesho S, Karanis P. Detection and genotyping of *Cryptosporidium* from brown rats (*Rattus norvegicus*) captured in an urban area of Japan. Parasitology Research. 2017;100:1417-1420.
7. Miyaji S, Tanikawa T, Shikata JI. Prevalence of *Cryptosporidium* in *Rattus norvegicus* and *Rattus rattus* in Japan. Kiseichugaku Zasshi. 1989;38:368-372.
8. Webster JP. Wild brown rats (*Rattus norvegicus*) as a zoonotic risk on farms in England and Wales. Communicable Disease Report. 1996;6:46-49.
9. Roger JQ, David PC, Peter JH, Anthony PS, Rachel MC, Adam TB, Stephanie AB. The Norway rat as a reservoir host of *Cryptosporidium parvum*. J wildlife Dis. 1999;35:660-670.
10. El-Wahed MMA, Salem GH, El-Assaly TM. The role of wild rats as a reservoir of some internal parasites in Qalyobia Governorate. J Egypt Soc Parasitol. 1999;29:495-503.
11. Nouroozi RV. Prevalence of *Cryptosporidium* in wild brown rats (*Rattus norvegicus*) population at Shoustar, Iran. Internat Electron J of Med. 2016;5:18-22.
12. Adesiyun AA, Kaminjolo JS, Ngeleka M, Mutani A, Borde G, Harewood W, Harper W. A longitudinal study on enteropathogenic infections in livestock in Trinidad. Rev Soc Bras Med Trop. 2001;34:1-7.
13. Glenn ID. Determination of sample size fact sheet PEOD-6, a series of the Program Evaluation and Organization

Development, Florida Cooperative Extension Service Institute; 2002.
14. Jasem S, Mosoud FR, Reza A. Molecular characterization of *Cryptosporidium* spp. in wild rodents of Southeastern Iran using 18s $_r$RNA gene nested -PCR-RELP and sequencing techniques. J Trop Med. 2016; 6834206.
DOI:10: 1155/2016/6834206.
15. Yamoura H, Shirasaka R, Asahi H, Koyama T, M. Motoki M, Ito H. Prevalence of *Cryptosporidium* infection among house rats, *Rattus rattus and Rattus norvegicus*, in Tokyo and experimental Cryptosporidiosis in roof rats. Jap. J. Parasitol. 1990;39:439-444.
16. Webster JP, Macdonald DW. Cryptosporidiosis reservoir in wild brown rats (*Rattus norvegicus*) in the UK. Epidmiol Infect. 1995;115:207-209.
17. Chaochao LV, Longxian Z, Rongjun W, Fuchun J, Sumel Z, Changshen N, Helen W, Chao WF, Xinwel W, Xupeng R, Meng Q, Lihua X. *Cryptosporidium* spp in wild, laboratory and pet rodents in China: prevalence and molecular characterization. Appl Environ Microbiol. 2009;75:7692-7699.
18. Alaa AG, Abdel S, Sobhy MSR, Shaapan R. Cryptosporidiosis in animals and man. Diagnosis. Asian J Epidemiol. 2015;8:84-103.
19. Zahedi A, Paparini A, Jian F, Robertson I, Ryan U. Public health significance of zoonotic *Cryptosporidium* species in wildlife: critical insights into better drinking water management. Int J Parasitol: Parasites and Wildlife. 2016;5:88-109.
20. Xiao L, Fayer R. Molecular characterization of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int J Parsitol. 2008;38:129-1255.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23537