Antibodies Against Seoul Hantavirus in Brown rats (Rattus norvegicus) from Grenada, West Indies

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ABSTRACT

Hantavirus is an emerging zoonotic virus, cause of fatal diseases in humans. Brown rats (Rattus norvegicus) are known reservoir host for Seoul hantavirus. This is the second report of prevalence of antibodies against hantavirus in brown rats in Grenada. Sera from 169 brown rats were tested using ELISA for antibodies against hantavirus. Prevalence of antibodies was found in 47 rats (27.5%). There was no significant difference related to age and sex of seropositive rats. Although no case of hantavirus infection in humans has been recorded in Grenada, the presence of moderate infection in reservoir host should be considered a risk factor for disease transmission in humans.

Keywords: Seoul Hantavirus, antibodies, brown rat, Grenada

Hantaviruses are a globally distributed group of rodent and insectivore borne RNA viruses (Verner-Carlsson et al., 2015). Seoul hantavirus is one of the serotypes of hantavirus. Humans get infected with hantavirus through aerosols of urine and feces from infected rodents. Transmission can also occur by contaminated saliva through bite wound. Human to human transmission is although uncommon, has been described in an outbreak of hantavirus pulmonary syndrome (HPS) in Argentina (Enria and Levis, 2004). In wild rodents, carriers of hantavirus are asymptomatic with lifelong infection and shedding of the virus.

Hantaviruses are known to cause two human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome HPS (Schmaljohn and Hjelle, 1997). Currently, Genus hantavirus includes 23 species. Amongst the known species; hantaa virus, Seoul virus, Dobrava-Belgrade, Saaremaa virus and Puumala virus are known to cause HFRS in Europe and Asia, where as Sin Nombre virus and Andes virus cause HPS in Americas (Jonson et al., 2010). Hantaviruses have specific rodent and insectivore reservoir hosts. Brown rats (Rattus norvegicus) and black rats (Rattus rattus) are specific

reservoir host of Seoul hantavirus. Seoul hantavirus is worldwide in distribution, due to migration of brown rats to all continents following human activities (Lin et al., 2012) Seoul virus has been demonstrated in Rattus species in many countries of the world; a few countries to mention, Baltimore, USA (Childs et al., 1987), Argentina (Cueto et al., 2008), Belgium (Heyman et al., 2009), Japan (Sugiyama et al., 1995), France (Heyman et al., 2004), Indonesia (Angelina et al., 2004), Malaysia (Lam et al., 2001), Cambodia (Reynes et al., 2003), Bangkok (Tantivanich et al., 1992) and China (Gang et al., 2015). Countries of Central and South America (Argentina, Brazil, Chile, Paraguay and Uruguay) had cases of HPS in humans where Sin Nombre species of hantavirus was involved and the deer mouse was identified as reservoir host (James et al., 1999).

There is paucity of report of hantavirus exposure to humans and rats from the Caribbean nations. One report is from the island country of Barbados, neighbor of Grenada, with evidence of exposure with hantavirus in both rats and humans (Groen et al., 2002). There is one report of hantavirus in brown rats from Caribbean Island of Grenada (Lisa et al., 2008). This study was designed to
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determine the seroprevalence of hantavirus in brown rat (Rattus norvegicus) from Grenada, just after 10 years from the first report.

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 km². The country with low hills, small trees, shrubs and tropical climate is most suitable for 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 higher human population compared to the other 4 parishes were selected for the study.

Collection of rats

One hundred sixty-nine rats were collected live from 1st May to 14th July 2017, using traps (45 cm l × 15 cm 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 the morning of the next day. Traps with rats were covered with black cloth and transported to the necropsy laboratory of St. George’s University, School of Veterinary Medicine. Rats 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 and testing

The anesthetized rats were examined for their physical health and weighed. Gender was also recorded. Rats below 100g were grouped as young and those over 100g as adult, following the methodology used by Panti-May et al. 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 until tested.

ELISA test for hantavirus antibodies on sera was performed using “Rat hanta Virus ELISA Kit” from XpressBIO Frederick MD, USA. ELISA was performed following the instructions of manufacturer.

Statistical Analysis

Data was analyzed using a chi-squared (χ²) analysis and stratified by gender, age and parish of rats in Microsoft Excel 2017 software. Statistical significance was set at p=0.05.

RESULTS AND DISCUSSION

Serum antibodies to hantavirus were found in 47 rats out of total 169 tested rats (27.3%). Prevalence of antibodies in St. George was 32.4% and in St David 22.6%. The difference in seroprevalence of hantavirus antibodies between two parish (St. George and St. David) was statistically not significant. Male and female; young and adult rats had similar seroprevalence of antibodies (male 21.8%, female 34.1%; young 21.0%, adults 28.7%). There was no statistical significance between gender and age. The serological results by ELISA according to parish, gender and age are presented in Table 1.

In the present study, seroprevalence of antibodies to hantavirus in brown rats was 27.5%, which did not differ significantly from the previous report (29.3%) by Lisa et al. 10 years before in brown rats from Grenada.

Similar prevalence (28.0%) was reported in Barbados, another Caribbean nation by Groen et al. (2002). The prevalence of serum antibodies to hantavirus in Rattus species varies from 1.45% to 21.6% in different countries: In Xinjiang, Northwest China 15% (gang et al., 2016), in Netherlands 18.75 (Verner-Carlsson et al., 2015), in Kuwait 3.6% (Pacsa et al., 2002), in Vancouver, Canada 1.45% (Himsworth et al., 2015), 21.6% in Northern Island 21.6% (McCaughey et al., 1996), in Vietnam 10.3% (Nguyen et al., 2015). The variation in prevalence of hantavirus positive rats in different part of the world is not well understood. However, landscape composition
and climate are important factors in the ecology of rodent hantavirus ecosystem (Colleen et al., 2010).

We report no significant difference in seroprevalence of hantavirus antibodies in brown rats between two parishes of Grenada (St. George and St David) where the rats were trapped. This finding is in concurrence with Lisa et al. (2008) who also found no difference in seropositive rats among the six parishes of Grenada. Similar terrain and climate in all six parishes of the country might explain the uniformity of seroprevalence of hantavirus antibodies in the entire country.

In the present study, there was no statistically significant difference between sex and age of seropositive rats. The finding is in accordance with Lisa et al. (2008). Our results related to gender difference is in contrast with previous researchers. Padula et al. (2004), Klein et al. (2001), James et al. (1999) found higher antibodies to hantavirus in male rats compared to females. In contrast Jonas et al. (2008) reported higher immunity for hantavirus in females. In the present study, seroprevalence to hantavirus related to age is in contrast to Ella et al. (2004) and James et al. (1999) who found higher antibodies in older brown rats.

Laboratory diagnosis of hantavirus infection is based on serology. A wide array of technologies have been used to detect antibodies to hantaviruses (Zhengiang et al., 2008). ELISA is optimal for specific serological confirmation of hantavirus infections, although antibody responses usually cross-react between different hantaviruses (Lundkvist et al., 1997). We found high number of seropositive R. norvegicus for hantavirus antibodies in Grenada. Since R. norvegicus has been reported to be the main reservoir of Seoul hantavirus in many countries of Asia, Europe, the Americas and Africa (Lin et al., 2012), we expect that Seoul hantavirus is infecting brown rats in Grenada. Further studies may determine the hantavirus species in Grenada.

**CONCLUSION**

This is the second report of hantavirus in brown rats from Grenada. Although, we are unaware of any report on human diseases caused by hantavirus in Grenada, there is a need to raise awareness of hantavirus diseases amongst the Grenadian community. In Grenada, effective preventive measures for human infection should be directed to reduce human exposure to infected rodent host (R. norvegicus) and their excrement. Reduction in population of brown rats should be included amongst other control measures.

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**REFERENCES**


