

Short Communication

Serological evidence of hantavirus infection in an urban area in Mato Grosso State, Brazil

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Abstract

Introduction: In Brazil, Mato Grosso (MT) has the highest number of hantavirus cardiopulmonary syndrome cases. Our study aimed to identify anti-hantavirus antibodies in the sera of patients from Sinop, MT, presenting with acute febrile illness. **Methods:** A retrospective analysis of data for 198 sera samples assessed using enzyme-linked immunosorbent assay (ELISA) was conducted. **Results:** Immunoglobulins G (IgGs) against the hantavirus nucleoprotein were found in 13.6% of the tested sera. No sample had immunoglobulin M (IgM) antibodies to hantavirus. Seropositivity occurred mainly in female residents in urban areas who worked around the household. **Conclusions:** Our findings suggest circulation of hantavirus in Sinop.

Keywords: Zoonosis. Serosurvey study. Hantavirus.

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are emerging zoonotic viruses of great importance for public health. In South America, the rodents of the *Sigmodontinae* subfamily are reservoirs of these viruses. Some hantaviruses infect humans, causing either hemorrhagic fever with renal syndrome (HFRS) and/or hantavirus cardiopulmonary syndrome (HCPS)⁽¹⁾.

The first cases of HCPS diagnosed in Brazil occurred in 1993. Since then, more than 1,920 cases of hantavirus have been notified in all Brazilian regions, with a case fatality rate of up to 39.5%. Mato Grosso (MT) is the leading state for HCPS cases and accounts for 16.1% of the total notified cases in Brazil. In a previous study conducted in the municipality of Sinop, Mato Grosso State, we carried out an epidemiological surveillance for arboviruses in 198 sera samples from patients presenting with acute febrile illness and clinical-epidemiological diagnoses of dengue. The results showed 46 samples positive for arboviruses. However, it was intriguing that more than 70% of the patients did not have dengue or other arbovirus infections that were tested for⁽²⁾. To address the possibility of hantavirus infection, we investigated the presence of antibodies against the hantavirus nucleoprotein in these febrile patients.

Sinop is a municipality located 503km from the capital Cuiabá, in a geographical transition zone between a savannah

and rainforest. The city was founded in 1974 and currently has an estimated population of approximately 130,000 inhabitants with 17.1% of them living in rural areas. The economic activities include an intensive agriculture based on the grain production, animal husbandry, logging, and human services. All of the sera analyzed in this study were part of the serum bank of the Laboratory of Immunology and Molecular Biology of Universidade Federal de Mato Grosso (UFMT), Campus of Sinop, which have been previously described in detail⁽²⁾. At the time of blood collection, the patients filled out a questionnaire that contained their epidemiological data, sex, age, occupation, address and region of birth, as well as information about any contact with rural or forested areas, and contact with rodents. These data were collected to analyze the risk factors associated with infection.

The sera were tested with enzyme-linked immunosorbent assay (ELISA) for the presence of immunoglobulin M (IgM) and immunoglobulins G (IgG) antibodies to hantavirus, by using the recombinant nucleocapsid (N) protein of the Araraquara virus (ARAV), according to the description of Figueiredo et al.⁽³⁾. Samples with an optical density equal to or greater than the average of the negative controls plus three standard deviations at a dilution of 1:400 were considered positives. The positive samples were titrated using serial dilutions (1:200 to 1:3,200) and were tested by using ELISA in order to determine serum antibody titers to hantavirus. The Chi-square test was used to compare the hantavirus antibody status according to sex and age at a significance level of 0.05. Odds ratios corresponding to 95% confidence intervals were calculated for proportions.

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Because of the missing information, the other epidemiological data (occupation, home, the region of birth, and contact with rodents) were analyzed only among the seropositive participants. The statistical analysis was carried out using the R v2.15.1⁽⁴⁾.

Out of the 198 tested samples, 27 (13.6%) had IgG antibodies to hantavirus, at titers of 400 to 3,200. No samples had IgM antibodies to hantavirus. The study participants were between 5 and 64 years old. Furthermore, no significant difference in the seropositivity to hantavirus was observed among various age ranges (**Table 1**). A significant difference in the seropositivity was observed between men (6.5%) and women (20%, $p < 0.01$) (**Table 1**). Most of the seropositive participants were housewives (47.4%) and students (15.8%), and just one was a rural worker (5.3%) (**Table 2**). There was a higher incidence of infection in the seropositive participants living in urban areas (96.3%) than in those living in rural areas (3.7%). These participants are native to different regions of Brazil, including Central West (37.5%), Southeast (31.3%), South (25%), and North (6.3%) (**Table 2**). Fifteen (93.8%) participants reported a previous contact with rodents.

Our results showed a higher rate of seropositivity to hantavirus (13.6%) when compared with other serological studies conducted in all regions of Brazil by using the same methodology (0.6% to 4.7%)^{(5) (6) (7) (8) (9) (10)}. In this study, we used ELISAs that were based on the recombinant N protein of ARAV, despite not having this virus described in MT yet. We observed high antibodies titers in the positive samples (1:400 to 1:3,200). In fact, the N protein induces a strong humoral immune response based on the epitopes that cross-react with members of the *Hantavirus* genus⁽³⁾.

A high seropositivity rate was observed in three studies carried out in rural areas in MT (9.43%, 13%, and 51.1%)^{(11) (12) (13)}. These studies reported an association between the infection and agricultural activities. In our study, seropositivity was significantly associated with women living in urban areas. This finding is unique because previous studies observed a higher proportion of male patients among the HCPS cases, who were probably infected with the hantavirus while performing general farm duties⁽¹⁾. The urbanization that occurred in the city could explain why most seropositive patients were from an urban area. Over the last four decades, extensive native forest areas in Sinop have been deforested for agricultural purposes, especially for soybean and corn production, as well as for urbanization. As a result, there was a disordered growth of human settlements on the outskirts of the city, with the most susceptible people living close to the forests and in rural areas. The majority of the individuals that were analyzed have reported living in these new neighborhoods (data not shown) and having a previous contact with rodents. Therefore, it is possible that they had contact with the natural reservoirs of the hantavirus. In fact, deforestation promotes rodent proliferation, especially those prone to anthropogenic change, such as the rodents belonging to *Sigmodontinae*, thus driving them to invade human dwellings^{(14) (15)}. Humans are typically infected via contaminated aerosolized secretions (feces, urine, and saliva) of the reservoir animals. Considering that 47.4% of the seropositive participants were housewives, primarily responsible for household tasks, it is possible that the practice of activities that allow the dispersion of

TABLE 1 - IgG seropositivity to hantavirus according to age and sex.

Category	Seropositivity positive/total*		OR	95% CI	P**
	n	%			
Age (years)					
≤17	3/13	23.1	1.0	-	-
18-50	20/79	25.3	1.1	0.3-5.5	0.9
≥51	4/14	28.6	1.3	0.2-8.7	0.8
Sex					
male	6/93	6.5	1.0	-	-
female	21/105	20.0	3.5	1.4-10.2	<0.01

IgG: immunoglobulin G; **OR:** odds ratio; **95% CI:** 95% confidence interval. *The variables were analyzed by using only the available data. **p-values were determined via two-tailed chi-square or binomial test for two proportions.

TABLE 2 - Epidemiological data of hantavirus seropositive participants.

Epidemiological data	Positivity/participants*	
	n	%
Occupation		
housewife	9/19	47.4
student	3/19	15.8
retired	1/19	5.3
rural worker	1/19	5.3
other occupations**	5/19	26.3
Home		
rural	1/27	3.7
urban	26/27	96.3
Region of birth		
Central West	6/16	37.5
North	1/16	6.3
South	4/16	25.0
Southeast	5/16	31.3
Contact with rodents		
no	1/16	6.3
yes	15/16	93.8

*The variables were analyzed independently by using only the available data. **Other occupations: receptionist, filling station attendant, woodworker, contractor, and social worker.

aerosols or dust, such as cleaning in the domestic environment, has been the main form of contamination for these women. These activities were reported as risk factors for acquiring the disease⁽¹⁶⁾.

Although the analyzed samples tested positive for IgG, none had IgM. None of the participants reported a history of hantavirus infection; however, they were exposed to the virus, and the majority of patients had mild infections or were asymptomatic. According to Figueiredo et al.⁽¹⁾, there are more

asymptomatic hantavirus infections than clinical. The severity of hantavirus infections can be associated with the virus type involved as well as with the different roles of the pathogenesis⁽¹⁵⁾. The Castelo dos Sonhos virus and Laguna Negra virus are associated with HCPS in both Pará and MT⁽¹³⁾. There are no data regarding the strains circulating in Sinop. However, it is possible that less virulent strains of hantavirus have been causing the disease. Further studies are required to identify hantavirus species and rodent reservoirs in Sinop.

An additional explanation for the seropositivity found in our study could be related to migration. Over the past few decades, Sinop has received a large number of migrants from other parts of the country. Most of the participants, who tested positive in this serosurvey, have migrated from the South (25%) and Southeast (31.3%) regions of Brazil. These two regions together account for more than two-thirds of the HCPS notifications from the country. Therefore, the participants may have been infected with the virus while they were living in those regions. This was also observed in a previous study carried out in MT⁽¹²⁾.

Although the seroprevalence of hantavirus is high, to date, there have been no hantavirus cases reported in Sinop. Over the past decade, the Sinop Municipal Secretary of Health notified 26 allochthonous cases of HCPS. These cases occurred in patients living in the surrounding municipalities. It is possible that most of the infections in Sinop, especially those manifesting as mild forms of the disease, might remain undiagnosed. In dengue-endemic areas, such as Sinop, fever due to other arboviruses or even hantaviruses can be misdiagnosed as dengue based only on clinical-epidemiological data^{(2) (8)}. Taking this into account, it is important to screen for hantavirus in acute febrile illness patients using rapid and sensitive diagnostic methods. Our findings suggest hantavirus circulation in Sinop and emphasize the need to expand the current diagnostic criteria and surveillance programs to improve the understanding of hantavirus dissemination in the region.

ETHICAL CONSIDERATIONS

The study was approved by the Ethical Committee for Human Research of Júlio Müller University Hospital - UFMT (266.568/2013).

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Conflict of interest

The authors declare that there is no conflict of interest.

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