



Letter to Editor/Carta ao Editor

Hantavirus infection in suspected dengue cases from State of Ceará, Brazil

Infecção por hantavírus em casos suspeitos de dengue no Estado do Ceará, Brasil

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Dear Editor:

Human hantavirus infection generally occurs through inhalation of aerosols containing the virus from feces, urine, or saliva of infected rodents¹. The spectrum of clinical symptoms caused by hantaviruses in humans varies from sub-clinical presentations to severe hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the American continent¹. HCPS is an emerging public health problem in Brazil because of the overlap of urban, agricultural, and cattle-raising areas with ecosystems containing several species of *Sigmodontinae* rodents, which are reservoirs of hantaviruses². From 1993 through October 2010, 1,335 cases were reported in Brazil with a case-fatality rate of 39%: 487 in the southern region of Brazil, 399 in the southeast, 336 in the midwest, 82 in the north, and 14 in the northeast. In the northeastern region, hantavirus infections have been reported in the States of Maranhão, Rio Grande do Norte, and Bahia but have not been reported in the northeastern State of Ceará³. Five lineages of hantavirus have been associated with most documented HCPS cases in Brazil: the Juquitiba virus, the Araraquara virus, the Laguna Negra-like virus, the *Castelo dos Sonhos virus*, and the Anajatuba virus. HCPS cases, especially some reported in northeastern Brazil, have probably been caused by other unknown hantaviruses².

Despite efforts to control *Aedes aegypti*, a hyper-endemic situation remains, with large outbreaks by three of the four dengue serotypes. Due to these large outbreaks that occurred in the northeast of Brazil, since 1986, the State of Ceará has begun reporting dengue transmission, with three major outbreaks occurring in 1994, 2001, and 2008⁴.

Dengue cases in State of Ceará have shown a clearly seasonal incidence in the first semester of each year⁵. The same has not been seen for hantavirus infection in the region because there is no study

showing it yet. However, in Uberlândia (State of Minas Gerais, Brazil), Limongi et al., described seasonality for hantavirus infection in the autumn and winter⁶. This seasonality overlaps with dengue circulation.

During the 2008 dengue outbreak in State of Ceará, serum samples from 82 patients with a clinical history of acute fever consistent with dengue were clinically and serologically studied. Serum specimens were assessed for dengue virus antibodies by using a commercial immunoglobulin IgM capture enzyme-linked immunosorbent assay (PanBio ELISA, Ltd., Brisbane, Australia). Dengue virus genome detection by RT-PCR and virus isolation in clinical samples by inoculation into C6/36 cell monolayers were performed, followed by the detection of infection by indirect immunofluorescence assay using anti-dengue hyperimmune mouse ascitic fluids⁷.

Of the 82 patients in the study, 35 (42.6%) had dengue infection, of whom 35 (42.6%) were positive for IgM-ELISA, 4 (4.8%) by RT-PCR, 2 (2.4%) being positive for DENV-2, and 2 (2.4%) for DENV-3. Serological tests for hantavirus were performed in only 72 of the 82 patients because of insufficient sample volume. Among the 10 patients who were not tested for hantavirus, 9 were diagnosed with dengue fever, and 1 patient was negative for dengue in all laboratory tests.

In this study, protocols of IgM-ELISA and IgG-ELISA were performed as previously described by Figueiredo et al., using the recombinant N protein of Araraquara hantavirus as antigen (ARAV rN) produced in *E. coli*⁸. Figueiredo et al., found that ELISA has high sensitivity (97.2%), specificity (100%), positive predictive value (100%), and negative predictive value (98.1%). Thus, this procedure is suitable for the purposes of diagnosing hantavirus infection.

The 72 studied patients were initially screened for hantavirus infection at 1:100 dilution, and all positive results were confirmed in a serial dilution (starting at 1:400) test. One (1.4%) had IgM antibodies to hantavirus, and two (2.8%) other patients had IgG antibodies to hantavirus. The positive sera had shown antibody titers of 1:100 and 1:400. These data corroborate with a study conducted by Campos et al., which demonstrated similar results to ours. Campos et al., found maximum antibody titers of 1:400⁹. Seropositive samples were also tested by an RT-PCR that amplifies part of the small and the medium segments of hantavirus RNA, as previously reported, showing negative results¹⁰. Data from studied patients and test results are shown in **Table 1**.

All the 3 patients seropositive to hantavirus lived in urban areas. The laboratorial profile showed platelet count of <150.000/mm³ (66.6%) and hematocrit level of <50% (100%). One of them,

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TABLE 1 - Characteristics of 3 human cases with serological evidence of human hantavirus infections.

Patient	Gender	Municipality	Age	Sample collection date	Clinical/ outcome	Platelet		Hantavirus RT-PCR	ELISA results hantavirus		ELISA results dengue
						count ($\times 10^3/\mu\text{L}$)	Hematocrit (%)		IgG	IgM	IgM
F.S.P.	Female	Fortaleza	25	August 11	Recovered	111	35.2	Negative	1:100	Negative	Negative
J.G.P.J.	Male	Fortaleza	31	April 27	Recovered	203	45.3	Negative	1:400	Negative	1:100
D.M.D.M.	Male	Fortaleza	10	July 29	Recovered	130	11.6	Negative	Negative	1:100	Negative

ELISA: enzyme-linked immunosorbent assay; **RT-PCR:** reverse transcriptase-polymerase chain reaction; **IgG:** immunoglobulin G; **IgM:** immunoglobulin M.

a 10-year-old child, had acute febrile illness probably caused by hantavirus based on the presence of IgM-specific antibodies in the serum. This patient experienced headache, prostration, and odynophagia, did not have hemorrhagic manifestations, and had decreasing platelet count ($<150,000/\text{mm}^3$) (Table 1). Therefore, regarding this patient, there is no information that could be related to a pulmonary syndrome.

A hantavirus seroepidemiological study conducted in six municipalities State of Maranhão showed a seroprevalence of 4.7% in humans. These data highlight hantavirus circulation in that state. However, in those cases, there were no patients with any symptomatology of hantavirus, which can suggest that moderate or asymptomatic cases of hantavirus infection may be occurring in that region. This study reinforces the suspicion that mild or atypical cases of hantavirus infection are occurring in that region¹¹.

The presence of IgG antibodies to hantavirus in the sera of 2 patients suggests that they were previously infected by hantavirus; however, this does not provide evidence to determine if the virus was the causative agent of the acute febrile illness reported in the present study. One patient (JGPJ), who had IgG antibodies to hantavirus, also showed IgM antibodies to dengue. Data from studied patients and test results are shown in Table 1.

It is well known that during dengue outbreaks, other viral infections are commonly misdiagnosed as dengue. Therefore, cases of hantavirus infection, specially those presenting acute febrile illness, may be confused with dengue. Thus, it is possible that hantavirus infections have been misdiagnosed, and to our knowledge, this is the first evidence of a human infection by hantavirus in the State of Ceará, Brazil. Our data are corroborated by Chioratto et al., who reported that two *Necromys lasiurus*, a well-known hantavirus rodent reservoir, captured in the hills of Ibiapaba in the State of Ceará, had hantavirus antibodies, suggesting that the human population in these areas is at risk of contracting a severe and potentially lethal hantavirus infection¹². Our study supports the suspicion that mild or atypical cases of hantavirus infection are occurring in the region.

The hantavirus from Ceará could be the Anajatuba virus that was associated to human infections in the neighboring State of Maranhão¹¹, and our report shows that it is important to improve epidemiologic surveillance for hantavirus in Ceará. Further studies on hantaviruses are necessary to understand the prevalence of human infections, the clinical presentation of the disease, the virus, and its natural reservoir in State of Ceará.

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