

ANTIBODY LEVELS TO HANTAVIRUS IN INHABITANTS OF WESTERN SANTA CATARINA STATE, BRAZIL

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SUMMARY

Hantavirus cardiopulmonary syndrome (HCPS) is an infectious disease caused by hantaviruses of the family *Bunyaviridae*, and is transmitted by aerosols of excreta of infected rodents. The aim of the present study was to determine antibody levels to hantavirus in the population that lives at frontier of Brazil and Argentina. Participated of the study 405 individuals living in the municipalities of Bandeirante, Santa Helena, Princesa and Tunapolis, state of Santa Catarina, Brazil. IgG antibodies to hantavirus were analyzed in sera by an ELISA that uses a recombinant N protein of Araraquara hantavirus as antigen. The results were also confirmed by immunofluorescent test. Eight individuals showed antibodies to hantavirus (1.97% positivity), with serum titers ranging from 100 to 800. Six seropositives were males, older than 30 years and farmers. Our results reinforce previous data on hantavirus circulation and human infections in the southern border of Brazil with Argentina.

KEYWORDS: Hantavirus; Epidemiology; Brazil; Argentina; Santa Catarina.

INTRODUCTION

Hantavirus is a genus in the family *Bunyaviridae*. These viruses cause two serious illnesses: the hemorrhagic fever with a renal syndrome (HFRS) in Europe and Asia, and the hantavirus cardiopulmonary syndrome (HCPS) in the Americas, which is characterized by respiratory failure, shock and high case fatality^{5,10,14}. Hantaviruses are zoonotic viruses, in general transmitted to humans by inhalation of aerosols of excreta by direct contact with infected rodents, although there are reports documenting the spread of Andes virus from human to human^{19,22}.

In the Americas, hantaviruses have been identified in Argentina, Bolivia, Chile, Costa Rica, Paraguay, Panama, Mexico, Venezuela, Peru, United States and Uruguay². Presently, more than 40 American hantavirus genotypes are known and nearly half of them are pathogenic for humans¹⁰. In Brazil, seven hantavirus genotypes are known. Two of them, Jabora and Rio Mearim virus, were not related to disease in humans. The others, Jiquitiba, Araraquara, Laguna Negra-like, Castelo dos Sonhos, and Anajatuba have been responsible for more than 1440 cases of HPS reported in Brazil since 1993, with a 39% case fatality ratio^{1,10,14}.

HCPS is an emerging public health problem in Brazil that is related to a simplification of ecosystems diminishing biodiversity, agricultural expansion and urban populations²⁰. It increased the density of opportunistic species such as rodents which are reservoirs of hantavirus,

thus increasing the frequency of their interactions with humans, and the occurrence of HCPS¹³.

The state of Santa Catarina has an economy based on agriculture and is presently the second state in number of HCPS reported cases in Brazil, 233 cases, with a 27% case fatality ratio¹. Currently, two known strains circulate in the state: Jiquitiba associated to *Oligoryzomys nigripes* and Jabora related to *Akodon montensis*^{21,28}.

Although, HCPS cases were not reported in the west of the state of Santa Catarina, a previous study in this region showed individuals previously infected by hantavirus in two municipalities²⁷. Thus, this work has been done in order to obtain better information on antibody levels to hantavirus in the west of the state Santa Catarina, at the border with northern Argentina.

METHODS

Studied population: The study was carried out in November of 2011. A number of 405 adult inhabitants of urban and rural areas of four municipalities of the state of Santa Catarina participated of the study: 94 from Bandeirante located at latitude 26°46'07" south and longitude 53°38'18" west, with a population of 2,906; 100 from Princesa located at latitude 26°26'31" south and longitude 53°35'54" west, with a population of 2,758; 96 from Santa Helena located at latitude 26°56'15 south and

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longitude 53°37'09" west, with population of 2,393 and 115 from Tunapolis located at latitude 26°58'08" south and longitude 53°38'21" west and a population of 4,633 individuals. All towns have agricultural economic activity and are located in the border with Argentina (Fig. 1). Serum samples and general information were collected from all participants. This study was approved by the ethics committee and research protocol No. 073/2009 of Universidade do Oeste de Santa Catarina, Brazil.

IgG-ELISA to hantavirus: Serum samples at 1:100 dilution were tested in duplicate by an indirect IgG enzyme linked immunosorbent assay (ELISA), using a N recombinant protein of Araraquara hantavirus

produced in *E. coli* and negative control (a protein extract of *E. coli*, not containing the plasmid encoding the recombinant protein), as previously reported^{7,9}. Values were expressed as the optical density (OD) obtained with Araraquara N antigen minus the OD values for the control antigens. The cut-off value of the test was determined by ODs mean added to three standard deviations of the ODs obtained from at least four negative-control sera. After the test, all positive samples were diluted 1:100 to 1:1,600 and tested by the ELISA in order to determine serum titers to hantavirus^{16,17}.

Immunofluorescence: IgG positive sera in the ELISA were also tested by an indirect immunofluorescence assay (IFA) using Vero E6 cells infected with Rio Mamore hantavirus prefixed in spots slides. Serum samples were diluted 1:100 in saline buffer and the Anti-Human IgG FITCs (Sigma, USA) was diluted 1:2000 in saline buffer. The IFA was performed as previously described^{15,27}.

RESULTS

Sera of eight of the 450 participants of the study presented IgG antibodies to hantavirus (1.97% positivity) at titers of 100 to 800. All positive sera in the ELISA for hantavirus, were also positive by IFA (Table 1). Seropositivity per county was, 2.12% (2/94) in Bandeirante, 1.04% (1/96) in Santa Helena, 2% (2/100) in Princesa and 2.60% (3/115) in Tunapolis.

Among the seropositive participants, seven were more than 30 years old (87.5% of the seropositives), six were men (75%) and also six were farmers (75%), one was a government official and the other was a student. All of them reported contact with wild rodents around their residences (Table 1). Five of the farmer seropositive participants lived in the rural area.

It was not possible to associate previous diseases reported by the eight seropositive participants with hantavirus infection and/or disease.

DISCUSSION

The detection of 1.97% seropositive inhabitants at the four studied municipalities of western Santa Catarina State, shows that hantavirus

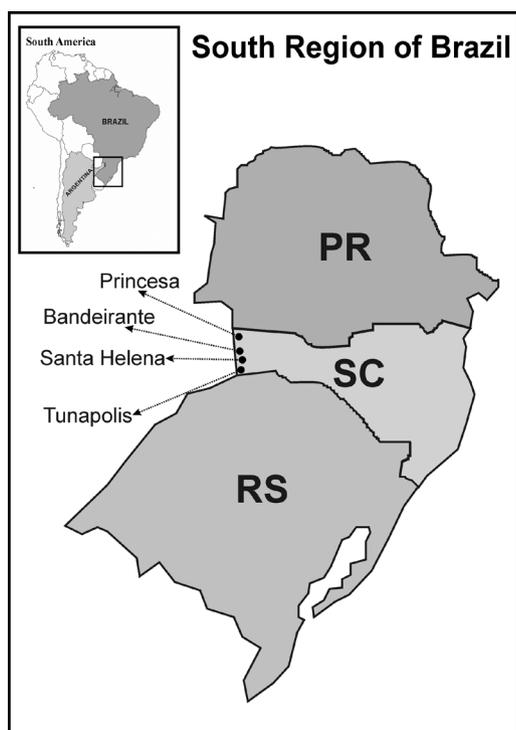


Fig. 1 - Location of four towns participating in the study: Princesa, Bandeirante, Santa Helena and Tunapolis. PR: Parana State; SC: Santa Catarina State; RS: Rio Grande do Sul State.

Table 1
Cases of seroreagents to hantavirus according to city, gender, age, occupation, home, presence of rodent and IgG antibody title

Sample	City	Gender	Age	Occupation	Home	Presence of rodent*	Title
S35	Santa Helena	Male	46 years	Agricultural worker	Rural	yes	200
T1	Tunapólis	Male	61 years	Agricultural worker	Urban	yes	100
T15	Tunapólis	Male	32 years	Agricultural worker	Rural	yes	100
T42	Tunapólis	Male	51 years	Agricultural worker	Rural	yes	200
P5	Princesa	Male	52 years	Agricultural worker	Rural	yes	400
P37	Princesa	Female	51 years	Governmental official	Urban	yes	800
B56	Bandeirante	Female	18 years	Student	Urban	yes	400
B57	Bandeirante	Male	76 years	Agricultural worker	Rural	yes	200

* Related presence of rodent in around the house.

infections are probably occurring in that region. These antibody levels are similar to those reported in other South American studies using IgG-ELISA where seropositivity ranged from 0.2% to 14.3%^{2,6,12,18,24,25,27}. Therefore, hantavirus infections are probably occurring in the studied municipalities despite of no HCPS reported cases. Curiously, close to this region, at the other side of the border, in northeastern Argentina, there is a high number of HCPS reported cases²³.

None of seropositive participants reported previous symptoms compatible with HCPS. It is possible that they have presented an oligosymptomatic infection. The low serum titer positives could be because of long time hantavirus infection⁵. It is also possible that the seropositive participants were infected by a less virulent hantavirus than Jujuitiba, which is supposed to occur in the area. Therefore, Jabora, a hantavirus that has not been reported causing human disease, was also reported in the state of Santa Catarina²¹, and Andes hantavirus have been reported close to the study area in Argentina²³. Further studies looking for virus in acute febrile illness patients and/or in wild rodents could help to know what hantavirus causes human infection in the region^{24,25}.

The hantavirus seropositive participants were mostly (75%) male farmers over 30 years old. It corroborates other South American studies, reporting that this is the highest risk population for hantavirus infections^{4,18,29}. Agricultural workers may have contact with hantavirus infected rodents during their daily tasks and become infected. However, the student and government official could also become infected by hantavirus during recreational activities in the field or in the residence since all seropositives reported contact with wild rodents around their residences^{8,10}.

Currently, two hantavirus genotypes have been reported in the state of Santa Catarina. Jujuitiba-Like virus associated to *Oligoryzomys nigripes* rodent and Jabora virus associated to *Akodon montensis* rodent^{21,28}. In the region where this study was conducted, *Oligoryzomys nigripes* and *Akodon montensis*²⁷ have been notified and at the other side of the border, in the province of Misiones, Argentina, *Oligoryzomys flavescens* rodent, the reservoir of Andes hantavirus, has been reported³.

In short, we show here that human infections by hantavirus occur in western Santa Catarina State at the border Brazil-Argentina and further studies are necessary to improve the study of human disease, an etiological agent and rodent reservoir.

RESUMO

Níveis de anticorpos para hantavírus em habitantes da região Oeste de Santa Catarina, Brasil

Síndrome Cardiopulmonar por Hantavírus (HCPS) é uma doença emergente, causada pelo gênero hantavírus membro da família *Bunyaviridae*, e são transmitidos aos humanos por aerossol de roedores infectados. O objetivo principal deste estudo foi determinar os níveis de anticorpos para hantavírus em uma população de residentes na fronteira do Brasil com a Argentina. Participaram deste estudo 405 indivíduos que moravam nos municípios de Bandeirante, Santa Helena, Princesa e Tunapólis, no estado de Santa Catarina, Brasil. Os anticorpos IgG para hantavírus foram analisados no soro por um ELISA que usa a nucleoproteína recombinante do vírus Araraquara como antígeno,

posteriormente confirmados por imunofluorescência. Oito indivíduos apresentavam anticorpos para hantavírus (1.97% positivo), com título entre 100 a 800. Seis soropositivos foram homens, com idade superior a 30 anos e agricultores. Nossos resultados reforçam a circulação do hantavírus e infecção humana na fronteira do Brasil com a Argentina

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