A retrospective serologic survey of hantavirus infections in the county of Cássia dos Coqueiros, State of São Paulo, Brazil

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INTRODUCTION

Recognized in 1993, hantavirus cardiopulmonary syndrome (HCPS) is an emergent disease reported in many countries of the American continent. Until November 2010, Brazil has the highest number of HCPS patients in the Americas with about 1,300 reported cases1. It is a severe disease that produces pneumonia and shock, and its case fatality rate has reached 39% in Brazil. Thus, HCPS being a recently identified disease, little is known about antibody levels to hantavirus in the general population, as well as how long infections caused by these viruses have occurred in the country. In this study, we present a serologic survey about hantavirus in sera collected from 1987 to 1990 at the county of Cássia dos Coqueiros, State of São Paulo, Brazil, which is located in an area where several cases of the disease have been observed recently.

METHODS

Studied population

Serum samples were collected from 1987 to 1990 from 1,876 urban and rural inhabitants of Cássia dos Coqueiros county, located in the northeast of the State of São Paulo, which is about 360km away from São Paulo City, the state capital. In this region, about 80 HCPS cases were reported, mostly caused by Araraquara virus, which has Necromys lasiurus as rodent-reservoir1.

Participants were included in a population-based survey carried out from 1987 to 1990 to study the distribution of hepatitis B markers. They corresponded to 67% of the total local population at the time and included both urban and rural inhabitants. The samples are currently part of a serum bank in the Laboratory of Serology of the General Hospital of The School of Medicine of Ribeirão Preto, University of São Paulo.

Serologic test

Sera were tested by an indirect IgG-enzyme-linked immunoabsorbent assay (ELISA) using...
the N recombinant protein of \textit{Araraquara} hantavirus as antigen\textsuperscript{3,4}. Briefly, polystyrene microtiter plates (Nunc, Denmark) were coated overnight in a wet chamber at 4°C with 2μg/mL of ARAV N antigen and the respective control. All incubations were conducted at 37°C for 1h, and plates were washed six times with a wash buffer (phosphate-buffered saline [PBS]-0.1% Tween 20) between each step. All reagents were added at volumes of 0.1mL. All serum samples were diluted at 1:200 in dilution buffer (5% skim milk powder in PBS-Tween 20), and double dilutions up to 1:6,400 were added to the antigen-coated wells. Peroxidase-labeled affinity-purified goat antihuman IgG Fc antibody was added, and a specific antibody binding was detected by the addition of 2,2’-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) substrate with absorbance measured at 405nm. Values were expressed as the optical density (OD) obtained with ARAV N antigen minus the OD values for the control antigens. The cut-off value of the test was determined by the mean plus 3 standard deviations of the ODs obtained for at least 4 negative-control serum samples plus 3 standard deviations of the mean.

Data analysis
Possible associations between positive results and some demographic and socioeconomic variables were studied using the Chi-square test, with a level of significance of $p < 0.05$.

Ethical considerations
The samples used for this study were authorized by the Ethics Committee of the same hospital (6998/2007), respecting the confidentiality of the information provided by and derived from the participants.

RESULTS

The 89 positive participants corresponded to a prevalence of 4.7% (CI95%: 3.8%-5.7%), with no difference between sex (Table 1). Similar distribution was observed among the different age ranges, varying from 3.4% to 5.2% ($p = 0.950$).

As seen in Table 2, the prevalence among urban inhabitants was 5.3% (CI95%: 3.7%-6.9%), compared with 4.3% (CI95%: 3.1-5.6) observed among those living in rural areas ($p = 0.336$).

No associations were observed between the presence of antibodies to hantavirus and the variables scholarship, place of birth, and time of residence in Cássia dos Coqueiros.

<table>
<thead>
<tr>
<th>Area of residence</th>
<th>Sex</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>Urban</td>
<td>21</td>
<td>390</td>
</tr>
<tr>
<td>Rural</td>
<td>24</td>
<td>544</td>
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<td>Total</td>
<td>45</td>
<td>934</td>
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DISCUSSION

In 1987, Cássia dos Coqueiros, State of São Paulo, was a small community with 2,871 inhabitants, having an economic activity based on agriculture and cattle raising that occupied 65% of the county area. At that time, there was an intensive farming of \textit{Brachiaria decumbens} grass seeds in Cássia dos Coqueiros. This farming activity demanded hand labor and attracted hundreds of temporary migrants from the neighboring state of Minas Gerais, Brazil. A reliable generalization of the results to the whole community was assured by the fact that this was a population-based survey, which included almost 70% of the inhabitants, both urban and rural. Thus, 4.7% of the population of Cássia dos Coqueiros showed evidence of previous infection by hantavirus in the study period.

In 1999, another serologic survey to hantavirus, using IgG-ELISA, was carried out in three towns at the same region of Cássia dos Coqueiros, showing a 1.2% seropositivity\textsuperscript{5}. In 2003, Campos et al.\textsuperscript{6} performed a serologic survey to hantavirus in Jardimópolis county, also in the same region, showing a 14.3% seropositivity. These different values could be attributed, in part, to the antigens used in the IgG-ELISA performed for the two surveys: a North American N protein antigen of Sin Nombre hantavirus N protein in 1999 and a South American Andes hantavirus N protein in 2003. The lower value found in the present investigation, when compared with the results of the Jardimópolis study, might have been caused by facts other than differences in the laboratory techniques, such as degradation of immunoglobulins in sera stored for more than 20 years. Likewise, it is impossible to completely disregard the circulation of distinct hantavirus in different towns of the region. Remarkably, a recent serologic survey in patients with acute febrile illness from Jardimópolis and Cássia dos Coqueiros, carried out with the same test used in this study (IgG-ELISA with the N recombinant protein of \textit{Araraquara} hantavirus), showed prevalence values of 4.8% and 3.8%, respectively\textsuperscript{7}.

All sera for this serologic survey were collected from 1987 to 1990, and the finding of seropositivity demonstrated that hantavirus infections occurred in the region at least six years before its description as causing human diseases in the Americas. A retrospective study in North America also showed that HCPS occurred there since 1978\textsuperscript{8}.

Before 1987, it was possible that cases of HCPS in Cássia dos Coqueiros were misdiagnosed as severe bacterial pneumonia or septicemia. Evidence of misdiagnosed HCPS was also seen in a study based on fatal cases of severe pneumonia that occurred in the decades of 1980 and 1990 in the City of Ribeirão Preto\textsuperscript{9}. Unfortunately, we do not have any specific information on previous diseases suggestive of HCPS in the participants of this serologic survey. However, it is possible that most of the participants infected with hantavirus observed in this study did not present a severe disease but were oligosymptomatic or even asymptomatic.

### Table 2 - Antibodies to hantavirus according to sex and area of residence.

<table>
<thead>
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\[
\text{pos: positive samples.}
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Similar rates of infection were observed among all age ranges, including children. The absence of a cumulative effect along the age groups was an unexpected finding for which we do not have a consistent explanation. Eventually, it might suggest that these infections occurred in the county in 1987. Indeed, it was corroborated by four HCPS cases detected in Cássia dos Coqueiros after 2000. Only a few serologic surveys reported antibody levels to hantavirus in Brazilian children. In 1997, Mascarenhas-Batista looked for antibodies for Hantaan hantavirus in students from the City of Salvador, State of Bahia, and found the highest levels, 13.2%, among children 10 to 11 years old.

The fact that rural inhabitants did not show differences in prevalence when compared with those living in the city was also an unexpected finding, although infection by hantavirus had been eventually reported among urban dwellers in the same region. The most reasonable explanation for this finding involves some peculiar features of the studied population wherein it is really difficult to precisely separate urban and rural expositions. In fact, many urban inhabitants own small farms close to the city where they maintain agricultural activities, increasing the possibility of contact with wild rodents or their excreta. Additionally, even people living and working in urban areas have access in farms for recreation purposes, such as fishing during weekends. Unfortunately, we do not have any specific information on contact with rodents or previous diseases suggestive of HCPS, as the survey where blood collection originated was designed for getting information on risk factors for hepatitis B only.

Previous reports, before 1993, showed people from other Brazilian regions presenting antibodies to Asian hantavirus. Nevertheless, this study is the first in Brazil to report infections by hantavirus causing HCPS at least 6 years before its recognition.

ACKNOWLEDGMENTS

We acknowledge Mrs. Margarida Maria Passeri do Nascimento in aiding with the serum bank.

FINANCIAL SUPPORT

The Fundação de Amparo à Pesquisa do Estado de São Paulo (2008/50617-6) has supported the serologic tests.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES