Serosurveillance for hantavirus in urban and rural workers in Pernambuco State, Brazil

Serovigilância para hantavírus em trabalhadores urbanos e rurais no Estado de Pernambuco, Brasil

Serovigilancia para hantavirus en trabajadores urbanos y rurales en el Estado de Pernambuco, Brasil

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ABSTRACT

The aim of the present study was to determine the prevalence of antibodies to hantavirus in professionals responsible for urban cleaning (trash collection, weeding and road-sweeping), collectors of recycled material and rural workers in the Municipality of Garanhuns in Pernambuco State, Brazil. Considering the scarcity of data about hantavirus in Pernambuco, a Descriptive, cross-sectional, epidemiological analysis was performed. In total, 156 samples were obtained from workers for an outsourcing company of urban cleaning, collectors of material for recycling and rural workers. The in-house ELISA method was used to detect IgG antibodies to the N protein of the Araraquara hantavirus. The prevalence of antibodies to hantavirus was 1.9% (3/156; C.I. 0.4% -5.5%). Upon completion of titration, two reagent samples were found with titers of 200 (66.7%) and 400 (33.3%). All of the seropositive individuals in the present study were males from urban areas. This was the first study conducted in the Municipality of Garanhuns to report the presence of IgG antibodies to hantavirus in human serum samples. This result identifies the need for special attention on behalf of the epidemiological surveillance authorities in investigations of the occurrence of this disease in non-endemic areas.

Keywords: Diagnosis; Health Profile; Hantavirus Pulmonary Syndrome.

INTRODUCTION

Hantavirus (Bunyaviridae family) is the etiological agent of an acute viral zoonosis which can appear in different clinical forms in human infections: unapparent or sub-clinical; as hemorrhagic fever with renal syndrome (HFRS), which is common in Europe and Asia; or hantavirus cardiopulmonary syndrome (HCPS) which is typical of the Americas1. A new name for these diseases has been proposed, suggesting the use of the term “hantavirus disease” in place of the HFRS/HCPS dichotomy2,3.

In the Americas, hantavirus disease is characterized by a zoonosis associated with wild rodents of the Cricetidae family, which contains the Sigmodontinae (North, Central and South America) and Arvicolinae sub-families (North America)4. Infected rodents eliminate the virus through their urine, feces and saliva. Transmission occurs when susceptible hosts inhale aerosols, containing the pathogenic agent, formed from the dry excreta5.

According to data from the Centers for Disease Control and Prevention (CDC), 616 cases of pulmonary syndrome were reported in the United States up to...
Materials and methods

The present study received approval from the Ethics Committee of the Universidade de Pernambuco (PROPEGE/UPE) by Plataforma Brasil, with CAAE 03798812.3.0000.5207 number, with the opinion number 79157, on November 6, 2012.

A descriptive, cross-sectional, epidemiological analysis was performed in the Municipality of Garanhuns, which has a population of 129,408 inhabitants.1

The sample size was determined by considering an expected prevalence of hantavirus infection of 4.0%, which resulted in a minimum of 60 samples. The level of confidence was set at 95% and the level of statistical error was 5%. As a safety margin, 156 samples were obtained, of which 97 were male and 59 were female. These individuals were professionals who worked in rural and urban areas. In the urban zone, they work for an outsourcing company of urban cleaners (n = 80) and for an association of recycled material collectors (n = 8). In the rural zone, 68 rural workers, who were registered in the family health center in the district of São Pedro in Garanhuns, were selected.

There were no inclusion or exclusion criteria. Volunteers were invited to participate in the research through spontaneous demand. The samples were collected on scheduled days. Before collecting the samples, the aims of the present study were explained clearly and those who agreed to participate signed a statement of consent by resolution 466/12 of the National Health Council. A questionnaire with objective questions was used. It included demographic data (gender, age, address, occupation and length of time doing this activity) as well as questions related to risk factors for infection (presence of rodents at home or in the workplace, garbage disposal, use of protective equipment, such as wearing gloves and boots, accumulation of trash around the house).

In-house ELISA was used to detect IgG antibodies to protein N of the Araraquara hantavirus, as described by Figueiredo et al and adapted by Santos-Júnior. Figureiredo et al used a recombinant protein of the Brazilian Araraquara hantavirus. The samples were tested in duplicate and the positive samples were repeated. After confirmation of the result, they were titrated. Samples were considered positive if they exhibited titration ≥ 200. An aliquot of serum from a patient known to be a reagent to IgG was used as positive control and an aliquot of serum from a patient known to be negative was used as a negative control.

A descriptive statistical analysis was carried out to calculate the relative and absolute frequencies of the results obtained in the serological test. When it was necessary Fisher’s exact test or Pearson’s chi-squared test was used to identify associations between the epidemiological variables and the serological examination of the urban zone participants. This involved univariate analysis of the variables of interest. Epi Info™ v.3.5.2 software was used (CDC).

Results

The prevalence of antibodies to hantavirus was 1.9% (3/156; C.I. 0.4% -5.5%). Upon completion of titration, two reagent samples were found with titers of 200 (66.7%) and one sample with 400 (33.3%).

With regards to the workplace, two of these individuals (66.7%) worked in an outsourcing company of urban cleaners (weeding and company offices) and one (33.3%) collected material for recycling. Thus, 66.7% (2/3) worked in areas of open sewage.

All of the reagents were from urban areas. Regarding the use of protective gear (boots, gloves), 88.2% said they used. The only variable that showed a significant statistical association was the age in the group over 60 years old. The use of protective equipment by the professional was not statistically significant.

Table 1 displays the analysis of the associations between the epidemiological variables studied and the serology for antibodies to hantavirus. It was not possible to study associations for the participants from rural areas since 100% of them were negative.
Table 1 – Distribution of relative and absolute frequencies, according to the result and the variables of the questionnaire applied to urban workers in Garanhuns, Pernambuco State, Brazil

<table>
<thead>
<tr>
<th>Variables</th>
<th>ELISA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reagent Negative</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Absolute frequencies</td>
<td>Relative frequencies (%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>3,7</td>
</tr>
<tr>
<td>Female</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between 15 and 40</td>
<td>2</td>
<td>3,77</td>
</tr>
<tr>
<td>Between 41 and 60</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Over 60</td>
<td>1</td>
<td>33,3</td>
</tr>
<tr>
<td>Have you ever worked in places like ditches,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sewers and terraces†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>7,9</td>
</tr>
<tr>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Have you ever noticed the presence of rodents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in the collection/sweeping areas‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>3,0</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>4,8</td>
</tr>
<tr>
<td>Have you ever been bitten by rodents?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>3,9</td>
</tr>
<tr>
<td>Is there a dumpster or an accumulation of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trash near your residence?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>4,0</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>3,2</td>
</tr>
</tbody>
</table>

* Chi-square. Significant association; † Base used = 86; ‡ Base used = 87; Conventional sign used: – Numeric data not equal to zero due to rounding.

DISCUSSION

The prevalence found in the present study is similar to other studies in the literature, in areas with reported clinical cases, such as the serology performed in 2006 in Uberlândia, Minas Gerais State, which found a prevalence of 3% (12/400)\(^{17}\). An investigation conducted in four cities in Amazonas State between 2007 and 2009 demonstrated a positivity of 0.6% (10/1,731)\(^{18}\). In the City of Ilhéus, Bahia State, blood donors from July 2008 to December 2009 exhibited a prevalence of 0.6% (3/500)\(^{19}\). However, the present study differs from that carried out in Marcelândia, Mato Grosso State, which reported a prevalence of 13% (7/54)\(^{20}\), where cases of HCPS are commonly recorded. The results found in Garanhuns were also similar to the survey conducted in another areas without clinical cases in northeast Brazil, as in Alagoas State. In this region, an epidemiological survey in 2012 demonstrated that 4.0% (4/250) of rural workers in the Municipality of Coruripe exhibited antibodies to Hantavirus\(^{12}\).

In Pernambuco State, a study was conducted with patients suspected of leptospirosis in the Oswaldo Cruz Hospital of the Universidade Federal de Pernambuco in Recife\(^{21}\). The authors reported a prevalence of 9.6%
(15/156) with antibodies to hantavirus, highlighting that two of these patients exhibited IgM antibodies, which is characteristic of acute cases. In a study by Costa et al. with serum samples from professionals working in the handling of rodents for research Yersinia pestis, in the field or in the laboratory, were tested for hantavirus was identified and tested negative in Pernambuco professionals. This study by Hinrichsen et al. and the survey in Garanhuns represent important investigative data for Pernambuco, indicating hantavirus circulating even in areas without clinical cases reported.

The differences between the present study and others conducted in Brazil can be influenced by the ecological conditions in different states, the population studied exposed to different factors (social, environmental, working activities), the methods of diagnosis used in serological surveys, the different cut-off points used and presence of rodent population in the areas studied.

Reservoirs must be present for the agent to be transmitted to humans. Leduc et al. detected antibodies to hantavirus in 6% (2/36) of synanthropic rodents (Rattus rattus and R. norvegicus) captured in the Cities of Recife and Olinda, in Pernambuco. In the same State, no antibodies were detected in 218 rodents captured in the City of Triunfo. According to Raboni et al., the presence of serologically positive rodents by itself does not sufficiently indicate a high-risk area. Other factors must be included in the risk analysis, including the following: seasonality; population density of the rodents and human behavioral habits. Hantavirus should be studied in more detail, as should the relevant ecological and environmental factors, in order to better understand this disease.

With regards to titration, 100% of the reagents exhibited low titers (1:200 and 1:400) in clinically healthy individuals. Frey et al. stated that the clinically unapparent infection varies in different areas and populations in the Americas, proposing two hypotheses: the strains in South America are less pathogenic; there are always at least two factors involved: the nature of the exposure and the genetic make-up of the host population.

Although, no significant associations were found between the hantavirus serology and the variable gender, all of the seropositive subjects in the present study were male. This result is similar to those found in previous studies and could be associated with occupational risks, since men usually perform activities such as food storage (grain), the cleaning of sheds and sites, trash collection and weeding, both in rural and urban areas.

A statistically significant association was found for the variable age in the present study, in the group over 60 years old. However, this result may be related to the low sample size of this group (n = 3).

In the present study, all of the reagents came from urban zones. This is probably due to the fact that these workers were exposed to garbage during their daily activities of sweeping or weeding in open areas. In the rural area studied, the workers did not conduct activities that were considered risk activities for infection: sweeping in dry sheds used to store foodstuffs or in abandoned houses near wild forests; planting and harvesting in areas infested with rodents stated that it is difficult to separate urban and rural exposure because although many of them live in urban areas, have farms or attending rural and leisure activity.

Many studies of hantavirus exposure were conducted with rural workers and less data are available regarding urban workers prevalence. In urban areas, cleaning professionals are exposed to different environmental factors. Their activities, such as sweeping, gathering debris and contact with the wastes are important risk factors that should be considered and investigated, because it allows the contact with aerosolized viral particles (the main form of transmission described in hantavirus).

The hantavirus infection is mainly associated with agricultural activity. A common form of acquiring the disease, especially in small cities, is by cleaning sheds where rural grain is stored. Other forms of transmission include direct contact with infected rodents or recreational activities such as fishing, hunting or camping.

According to Peters and Khan, the occurrence of hantavirus depends on close contact with rodents. Peri-urban areas with large populations and low sanitary conditions generally become infested with rodents that share food and the environment with humans.

In spite of the low prevalence found in the present study, it is suggested that the authorities in epidemiological vigilance pay close attention to new cases of HCPS in the study area, enabling health professionals to make an accurate and reliable diagnosis. Figueiredo et al. stated that in order to control Hantavirus, it is necessary to provide information about the disease to different sections of society, particularly health professionals (doctors, veterinarians, biologists, biomedical professionals and laboratory technicians), political and community leaders, stressing that it is compulsory to report the occurrence of the disease.

Studies of the occurrence of antibodies to hantavirus in rodents have not previously been carried out in the City of Garanhuns. As well as serology, soil and climatic studies with viral isolation are required to identify the ecological variables and the viral variants that circulate in the region. These studies will contribute to a better understanding of the epidemiological chain of hantavirus in that region.

**CONCLUSION**

This was the first study conducted in the Municipality of Garanhuns to report the presence of IgG antibodies to hantavirus in human serum samples and one of the few studies carried out in Pernambuco State. Consequently, the authorities in epidemiological vigilance should pay special attention to the investigation of the occurrence of this disease in the region.
Serovigilância para hantavírus em trabalhadores urbanos e rurais no Estado de Pernambuco, Brasil

RESUMO
O objetivo do presente estudo foi determinar a prevalência de anticorpos para hantavirus em profissionais responsáveis pela limpeza urbana (coletores de lixo, capinadores e varredores de rua), catadores de material reciclado e trabalhadores rurais no Município de Garanhuns, no Estado de Pernambuco, Brasil. Considerando a escassez de dados sobre hantavirus em Pernambuco, foi realizada uma análise descritiva, transversal, epidemiológica. Foram obtidas 156 amostras de trabalhadores de uma empresa de terceirização de limpeza urbana, catadores de material para reciclagem e trabalhadores rurais. O método ELISA foi utilizado para detecção de anticorpos IgG contra a proteína N do hantavirus Araraquara. A prevalência de anticorpos para hantavirus foi de 1,9% (3/156; CI 0,4% -5,5%). Após a conclusão da titulação, duas amostras de reagentes foram encontradas com títulos de 200 (66,7%) e 400 (33,3%). Todos os indivíduos soropositivos deste estudo eram do sexo masculino e de áreas urbanas. Este foi o primeiro estudo realizado no Município de Garanhuns para relatar a presença de anticorpos IgG para hantavirus em amostras de soro humano. Este resultado identifica a necessidade de uma atenção especial das autoridades de vigilância epidemiológica nas investigações da ocorrência desta doença em áreas não endêmicas.

Palavras-chave: Diagnóstico; Perfil de Saúde; Síndrome Pulmonar por Hantavirus.

Serovigilância para hantavirus en trabajadores urbanos y rurales en el Estado de Pernambuco, Brasil

RESUMEN
El objetivo del presente estudio fue determinar la prevalencia de anticuerpos para hantavirus en profesionales responsables por la limpieza urbana (recolectores de basura, desbrozadores y barrenderos), juntapapeles (material reciclado) y trabajadores rurales en el Municipio de Garanhuns, Estado de Pernambuco, Brasil. Considerando la escasez de datos sobre hantavirus en Pernambuco, se realizó un análisis descriptivo, transversal, epidemiológico. Se obtuvieron 156 muestras de trabajadores de una empresa tercerizada de limpieza urbana, juntapapeles y trabajadores rurales. Se utilizó el método ELISA para detección de anticuerpos IgG contra la proteína N del hantavirus Araraquara. La prevalencia de anticuerpos para hantavirus fue de 1,9% (3/156; CI 0,4% -5,5%). Luego de la conclusión de la titulación, dos muestras de reactivos se hallaron con títulos de 200 (66,7%) y 400 (33,3%). Todos los individuos seropositivos de este estudio eran del sexo masculino y de áreas urbanas. Este fue el primer estudio realizado en el Municipio de Garanhuns para relatar la presencia de anticuerpos IgG para hantavirus en muestras de suero humano. Este resultado identifica la necesidad de una especial atención de las autoridades de vigilancia epidemiológica en las investigaciones de la ocurrencia de esta enfermedad en áreas no endémicas.

Palabras clave: Diagnóstico; Perfil de Salud; Síndrome Pulmonar por Hantavirus.

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