

# Seroprevalence of hantavirus and *Yersinia pestis* antibodies in professionals from the Plague Control Program

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## ABSTRACT

**Introduction:** Professionals who handle rodents in the field and in the laboratory are at risk of infection by the microorganisms harbored by these animals. **Methods:** Serum samples from professionals involved in rodent and *Yersinia pestis* handling in field or laboratory work were analyzed to determine hantavirus and plague seroprevalence and to establish a relationship between these activities and reports of illnesses. **Results:** Two individuals had antibodies against hantavirus, and two harbored antibodies against the plague; none of the individuals had experienced an illness related to their duties. **Conclusions:** These results confirm the risks of hantavirus- and plague-related field and laboratory activities and the importance of protective measures for such work.

**Keywords:** Plague. Hantavirus. Zoonoses. Reservoirs. Biosafety.

The plague, which is caused by *Yersinia pestis* infection, has a wide clinical spectrum and usually presents as one of three primary forms: bubonic, septicemic, or pneumonic. Flea bites are the primary transmission mechanism for this microorganism, but infections may occur by other mechanisms, including the inhalation of aerosolized droplets containing *Y. pestis*<sup>1</sup>. In focus areas, pneumonic plague must be differentiated from hantavirus pulmonary syndrome (HPS). Both infections require immediate treatment, with plague infections requiring antimicrobial treatment and eventual supportive measures and HPS requiring intensive care unit (ICU) admission<sup>1,2</sup>.

Hantavirus pulmonary syndrome is caused by a *Bunyaviridae* family virus. The first cases were diagnosed in the USA in 1993 and were initially misdiagnosed as pneumonic plague<sup>2,3</sup>. In the same year in Brazil, cases were detected in Juquitiba in the State of São Paulo<sup>4</sup>. Synanthropic wild rodents are the primary reservoir of HPS. Human exposure usually occurs by inhalation of aerosols containing excreta of rodents infected with hantavirus. However, transmission can also occur through rodent bites and scratches<sup>5</sup>.

In Brazil, the plague and HPS are not endemic to the same areas but the two microorganisms that cause these diseases share the same rodent reservoirs (*Sigmodontinae*)<sup>6,7</sup>. However, no human cases of HPS have been recorded in plague-endemic areas<sup>8</sup>; the few Rio Grande do Norte<sup>9</sup> and Bahia<sup>10</sup> cases of HPS occurred in non-plague-endemic areas. Furthermore, these cases of HPS remain controversial due to their potential misdiagnosis (*Coordenação de Vigilância das Doenças Transmitidas por Vetores e Antropozoonoses, Coordenação Geral de Doenças Transmissíveis, Departamento de Vigilância Epidemiológica, Secretaria de Vigilância em Saúde, Ministério da Saúde - COVEV/CGDT/DEVEP/SVS/MS, personal information*).

Morbimortality analysis indicates that the plague and HPS are occupational diseases<sup>5,11</sup>, and thus Plague Control Program (PCP) professionals require special monitoring due to the transmission mechanisms of these diseases.

To determine the seroprevalences of plague and hantavirus among professionals involved in rodent and *Y. pestis* handling in field or laboratory work, serum samples were obtained during 2007-2010 from field and laboratory staff working at the PCP (**Table 1**) and from individuals at *Serviço de Referência Nacional em Peste* (SRP) from *Centro de Pesquisas Aggeu Magalhães* (CPqAM), *Fundação Oswaldo Cruz* (FIOCRUZ, State of Pernambuco).

An immunoenzymatic indirect test (ELISA) was performed using the EIE IgG HANTEC kit (*Instituto Carlos Chagas, FIOCRUZ, State of Paraná, Brazil*) to detect IgG hantavirus antibodies. The kit was kindly supplied by *Coordenação Geral*

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TABLE 1 - Distribution of professionals by function in the Plague Control Program and study area.

Function	Study area: state and plague foci								
	PE=31			CE=21			RN=11		
	CB	ST	CA	CA	SI	SB	CB	AP	Total=63
Field workers	3	8	0	3 (1)	7 (1)	5	5	3	34 (2)
Health education workers	7	2	5	0	0	0	0	2	16
Laboratory workers	0	3	0	2	1	1	1	0	8
Drivers	0	1	0	1	1	0	0	0	3
Supervisors	1 (1*)	0	1	0	0	0	0	0	2 (1*)
Total	11 (1*)	14	6	6 (1)	9 (1)	6	6	5	63 (3**)

PE: State of Pernambuco; CE: State of Ceará; RN: State of Rio Grande do Norte; CB: Chapada da Borborema; ST: Serra do Triunfo; CA: Chapada do Araripe; SI: Serra da Ibiapaba; SB: Serra de Baturité; AP: Chapada do Apodi; ( ): workers with antibodies to hantavirus; \*workers with antibodies to *Yersinia pestis*; \*\*1 worker with antibodies to *Yersinia pestis*, and 2 workers with antibodies to hantavirus.

de Laboratórios de Saúde Pública/Secretaria de Vigilância Sanitária/Ministério da Saúde (CGLAB/SVS/MS), and the test was performed according to the manufacturer's instructions. The hemagglutination (HA)/hemagglutination inhibition (HI) test was used to detect specific *Y. pestis* anti-capsular protein (F1) antibodies<sup>1</sup>.

An explanation of the study was provided to each participant, informed written consent was obtained from each participant, and a questionnaire addressing work duties, personal protective equipment (PPE) usage, and work-related accidents and illnesses was completed by each participant. The project was approved by the CPqAM Ethics Committee (CEP/CPqAM/FIOCRUZ n. 48/2004).

Of the 63 PCP workers, 28 (44%) had worked for more than 10 years, 13 (21%) had worked 6 to 10 years, 18 (29%) had worked 1 to 5 years, and 4 (6%) had worked less than 1 year. Over 80% reported the use of PPE (boots, overalls, caps, gloves, goggles, and masks); 60% did not use PPE from the beginning of their employment, and 17% still do not use PPE regularly. Accidents such as rodent bites and scratches and scratches from traps were reported by 40% (n=25) of the workers. The average age among the workers was 45 years (27 to > 60). Most of the workers were male (98.4%, n=62); only one was female (1.6%, n=1).

IgG anti-hantavirus antibodies were detected in serum samples from 2 male PCP field workers in the State of Ceará. One of these workers had been active for more than 10 years at the Chapada do Araripe (CE) focus area and reported a rodent bite and the use of PPE only in the last 5 years. The second worker, from the Serra da Ibiapaba (CE) focus area, reported 2 years of work without PPE and no accidents. Although no human cases or illness among the workers was recorded in Ceará, hantavirus antibodies were found in rodents<sup>12</sup>, suggesting virus activity in the area and a risk to the human population.

Seronegativity among professionals from Pernambuco may support the hypothesis of low or even absent virus circulation in that state<sup>13</sup>. In Rio Grande do Norte, hantaviruses have not been investigated in rodents, despite reports of two human cases<sup>9</sup>.

Anti-plague antibodies were found in the serum of an extremely active male professional (supervisor) involved for

over 10 years in many activities in the Chapada Borborema focus area in the State of Pernambuco. The supervisor reported PPE usage over the last 5 years and did not report any occupational accidents or plague-associated events related to his duties in the PCP. Myalgia related to age and the weight of the trapping equipment used in his daily duties was reported in the questionnaire.

A total of 20 sera samples were collected from SRP staff members and coworkers, and 14 samples from individuals that were likely not exposed to hantavirus, who were involved in office and laboratory work (handling *Y. pestis* cultures or DNA), were analyzed for anti-plague antibodies only. A total of 6 samples from individuals involved in laboratory and field work who were exposed to hantavirus and plague were analyzed for both anti-hantavirus and anti-plague antibodies. A female involved in bacteria, reservoir, and vector handling for more than 10 years was positive for antibodies to plague. No accidents or suggestive plague-associated events related to her activities were reported. All other samples were negative.

In Brazil, there has been only one report of *Y. pestis* laboratory-acquired infection resulting in pulmonary infection; this infection occurred before the advent of the use of PPE in the laboratory and field activities, when dangerous practices were routinely performed without appropriate protection<sup>11</sup>. In spite of the knowledge and protective equipment available today, in 2009 a researcher in the USA who manipulated attenuated *Y. pestis* strains died from an infection with the bacterium<sup>14</sup>. There are reports in the USA of the deaths of biologists and other professionals associated with handling wild animals<sup>15</sup>.

In conclusion, the possibility of infection by plague or hantavirus is usually not considered, even in endemic areas. Therefore, the need to follow basic biosafety principles is not perceived. Information on the seroprevalence of specific antibodies against plague and hantavirus provides evidence of exposure to infectious agents and may be related to the lack of observance of personal protective measures such as PPE usage. This information is relevant to occupational risk minimization and to the efficacy assessment of current biosafety practices in

the management of both of these pathogens. In fact, systematic surveys may provide the program with data that will enable the assessment of the risks of professionals and populations in plague-endemic areas.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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