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Maripa virus RNA load and antibody response in hantavirus pulmonary syndrome, French Guiana

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Abstract

We report viral RNA loads and antibody responses in six severe human cases of Maripa virus infection (two favorable outcomes) and monitored both measures during the 6-week course of disease in one non-fatal case. Further research is needed to determine prevalence of this virus and its effect on other hantaviruses.

Keywords:

Hantavirus, Maripa virus, French Guiana

Hantaviruses are members of the genus *Orthohantavirus* (family *Hantaviridae*) and are carried by various rodent species, according to the strain. Humans can be infected by the inhalation of aerosolized virus excreted in the urine or feces of infected rodents. New Word hantaviruses in the Americas cause hantavirus pulmonary syndrome (HPS) in humans, characterized by fever, headache, cough, myalgia and nausea, evolving rapidly to pulmonary edema due to microvascular leakage *(1,2)*. The respiratory insufficiency is associated with death in 26% to 39% of cases, according to the New World hantavirus species *(3,4)*.

Following the identification of Sin Nombre virus (SNV) as the etiological agent of HPS in United States in 1993, many other hantaviruses have been identified in the Americas *(3-6)*. In French Guiana, the first laboratory confirmed case of hantavirus infection was reported in a hospitalized patient in 2008; the complete sequence analysis showed that this was a novel Hantavirus closely related to the Rio Mamore species named Maripa Virus *(7,8)*.

Here, we describe antibody responses to Maripa hantavirus infection and viral RNA loads in the six laboratory-confirmed human cases in French Guiana, measures at admission to the hospital. We also report how these two markers evolved during the course of the disease in the most recent hospitalized case, with favorable clinical outcome.

The study

Since the setting-up of hantavirus diagnostic tools in laboratory of virology at French Guiana‘s Institut Pasteur in 2008, six severe human cases infected by native hantavirus infection were reported. All the patients were male and the mean age was 54.6 years (range 38-71 years). The mean time from the onset of the disease until admission to the hospital was 4.6 days (range 2-7 days). The clinical outcome was favorable for two of the patients; four died (**Table 1**). The clinical and biological parameters of the first five confirmed hantavirus cases have been reported previously *(9)*. The sixth case was a 47-year-old male, presenting fever, cough, myalgia and sweating that had been developing over 6 days. He was admitted to

the Andrée Rosemon General Hospital, Cayenne (French Guiana), on August the 31th 2017. He presented a respiratory failure requiring rapid transfer to the Intensive Care Unit (ICU) for intubation and mechanical ventilation. Thoracic radiography revealed bilateral diffuse alveolar pulmonary infiltrates. The patient remained under mechanical ventilation for 18 days and was discharged from hospital after 23 days with a complete clinical recovery. The clinical symptoms of the patient, and his outdoor activities making the contact with rodents possible, led to suspicion of acute Hantavirus infection confirmed by molecular and serological approaches. The complete RNA coding sequence of the S RNA segment (accession number MG785209) was also generated and compared with those from the five previous Hantavirus cases, showing that it corresponded to a Maripa virus infection (*9*).

Sera provided from the six HPS cases collected on admission to the ICU were subjected to serological IgM and IgG tests and assayed for viral RNA quantification (**Tables 1, 2**). Others seven sequential serum samples provided from patient n°6 (six samples during the hospitalization and the last after discharge) were also tested using the same technical approaches for viral RNA quantification and serological follow-up. Informed consent was obtained from the patients and/or their representatives on admission and before discharge.

All serum samples were assayed by IgM capture and IgG enzyme-linked immunosorbent assays (ELISA) using the protocol described in Ksiasek *et al. (10)*. Samples were tested against SNV antigen and control antigen using 4-fold dilutions, from 1:100 to 1:6400. Due to antibody cross-reactivities, positive ELISA findings with SNV antigens indicated infections with New World hantaviruses. The positive criteria were similar to those described by MacNeil *et al. (11).*

The serological analyses showed that all samples collected at admission had detectable amounts of hantavirus IgM antibodies with minimum IgM titers ≥400 for patients n°1, 2, 4, 5 and 6 and a maximum titer of ≥1600 for patient n°3 (**Table 1**). These data were similar to

those reported in previous work *(11,12)*. Only patient n°5, who died 24 hours after admission, had serum samples positive for hantavirus IgG antibodies (titer ≥6400). Although the time from the onset of disease and sample collection at admission was different for each of the 6 patients, this single positive hantavirus IgG case may be explained in part by the longer viral incubation period, resulting in the induction of IgG before the appearance of symptoms. A previous study reported that the presence of hantavirus IgG during the first week of infection might be a predictor of survival, but we found no evidence supporting this view *(11)*.

To determine the viral RNA load in each serum sample, real-time PCR assay was performed. Each reaction was performed in duplicate. For absolute quantification, the exact number of copies of the gene of interest was calculated using a standard curve established with plasmid DNA at dilutions from 5 to $5x10^7$ copies per mL. The viral RNA loads in samples collected on admission were between 5.8 and 6.6 $\log_{10}$ copies per mL (mean: 6.2 $\log_{10}$ +/- 0.3) (**Table 1**). These values were similar to those observed in patients infected by other hantaviruses, including patients with mild or moderate symptoms *(13–15)*. We also observed that the viral RNA load in the 4 fatal cases was 6.2 log10 copies/ mL, whereas in the 2 nonfatal cases it was 6.1 log10 copies/mL. A correlation between hantavirus RNA loads in the serum during the acute phase of disease and the clinical outcome has been hypothesized (14,15); however, although our study includes only a small number of cases and only severe cases, it provides no evidence supporting this possibility. Presumably, the fatal or nonfatal outcome depends not only on the hantavirus viral load but also on other pathogenic or host factors.

The follow-up of antibodies response and Maripa virus RNA load during the course of disease for patient n°6, from admission on day 7 after the onset of disease until day 46 (**Table 2**). IgM titers were high at admission but decreased to become undetectable by day 46. Conversely, seroconversion (IgM to IgG) was observed between day 7 and day 12; these

hantavirus IgG titers then increased to 4.4 (adjusted sum OD values) by day 46. Likewise, viral RNA load evaluated in these seven sequential samples showed a high value at admission (6.4 $\log_{10}$ copies per mL); seven days later the viral RNA load declined from 6.4 to 4.7 $\log_{10}$ copies per mL. The viral load then remained around 4 $\log_{10}$ copies per mL in samples collected on days 20, 25 and 30. Viral RNA was undetectable on day 46.

Conclusion

Although limited in sample size, this study found similar results for viral load and immune response in the first 6 cases of Maripa virus infection reported in French Guiana after laboratory-based surveillance began in 2008. Further work is needed to determine the overall prevalence of this hantavirus in French Guiana and also the possible undetected mild or moderate cases induced by Maripa virus infection as reported for other New World hantaviruses *(13–15)*. Moreover, it would be informative to determine the infectious potential of the virus in the sequential samples to provide a better understanding of the pathophysiology of this infection. Investigations of the immune response to hantavirus, consequences of different viral loads, and the pathologic characteristics of different hantavirus strains would help identify the determinants of disease outcome.

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Biographical sketch

Dr. MATHEUS is a research assistant at the Institut Pasteur de la Guyane, French Guiana. Her research interests are the diagnosis and pathophysiology of arboviruses, with special interest in hantavirus circulation in French Guiana.

Conflicts of interest

None declared.

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Table 1. Immune response and viral loads on admission in six confirmed hantavirus cases.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Year case reported	2008	2009	2010	2013	2016	2017
Age	38	56	49	67	71	47
Days of disease at admission	7	4	2	4	4	7
SNV IgM	Positive	Positive	Positive	Positive	Positive	Positive
IgM sum OD*	1.02	1.70	4.72	0.92	2.23	1.79
SNV IgG	Negative	Negative	Negative	Negative	Positive	Negative
IgG sum OD*	0.05	0.01	0.01	0.01	2.05	0.73
Viral RNA loads (serum)**	5.8	6.6	6.4	5.9	6.0	6.4
Clinical evolution	Favorable	Death	Death	Death	Death	Favorable

*Adjusted sum OD values (dilution 1:100, 1:400, 1:1600 and 1:6400).

**Virus copy number was determined as $\log_{10}$ copies per mL. Primers and TaqMan® probe for QPCR were the following Maripa_qRT2F GCAGCTGTGTCTACATTGGAGAA, Maripa_qRT2R CCACCAGATCCGCCAACT and Maripa_Probe2 FAM-AAACTTGCAGAACTCA-MGB

Table 2. Monitoring of hantavirus antibodies and viral RNA load in sequential serum samples from patient n°6.

	Days post-symptom onset						
	Day 7	**Day 12**	**Day 15**	**Day 20**	**Day 25**	**Day 30**	**Day 46**
SNV IgM	Positive	Positive	Positive	Positive	Positive	Positive	Negative
IgM sum OD*	1.79	1.56	1.50	1.34	1.01	0.72	0.42
SNV IgG	Negative	Positive	Positive	Positive	Positive	Positive	Positive
IgG sum OD*	0.73	1.83	2.20	3.08	4.21	4.71	4.40
Viral RNA loads **	6.4	5.4	4.7	4.1	4.0	4.1	0

*Adjusted sum OD values (dilution 1:100, 1:400, 1:1600 and 1:6400). **Virus copy number was determined as $logs_{10}$ copies per mL. real time PCR

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