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HAL Authorization

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

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30 **Abstract**

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

35

36 **Keywords:**

37 **Hantavirus, Maripa virus, French Guiana**

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

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

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

54 **The study**

55 Since the setting-up of hantavirus diagnostic tools in laboratory of virology at French
56 Guiana's Institut Pasteur in 2008, six severe human cases infected by native hantavirus
57 infection were reported. All the patients were male and the mean age was 54.6 years (range
58 38-71 years). The mean time from the onset of the disease until admission to the hospital was
59 4.6 days (range 2-7 days). The clinical outcome was favorable for two of the patients; four
60 died (**Table 1**). The clinical and biological parameters of the first five confirmed hantavirus
61 cases have been reported previously (9). The sixth case was a 47-year-old male, presenting
62 fever, cough, myalgia and sweating that had been developing over 6 days. He was admitted to

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

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

79 All serum samples were assayed by IgM capture and IgG enzyme-linked
80 immunosorbent assays (ELISA) using the protocol described in Ksiasek *et al.* (10). Samples
81 were tested against SNV antigen and control antigen using 4-fold dilutions, from 1:100 to
82 1:6400. Due to antibody cross-reactivities, positive ELISA findings with SNV antigens
83 indicated infections with New World hantaviruses. The positive criteria were similar to those
84 described by MacNeil *et al.* (11).

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

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

95 To determine the viral RNA load in each serum sample, real-time PCR assay was
96 performed. Each reaction was performed in duplicate. For absolute quantification, the exact
97 number of copies of the gene of interest was calculated using a standard curve established
98 with plasmid DNA at dilutions from 5 to 5×10^7 copies per mL. The viral RNA loads in
99 samples collected on admission were between 5.8 and 6.6 \log_{10} copies per mL (mean: 6.2
100 \log_{10} +/- 0.3) (**Table 1**). These values were similar to those observed in patients infected by
101 other hantaviruses, including patients with mild or moderate symptoms (13–15). We also
102 observed that the viral RNA load in the 4 fatal cases was 6.2 \log_{10} copies/ mL, whereas in the
103 2 nonfatal cases it was 6.1 \log_{10} copies/mL. A correlation between hantavirus RNA loads in
104 the serum during the acute phase of disease and the clinical outcome has been hypothesized
105 (14,15); however, although our study includes only a small number of cases and only severe
106 cases, it provides no evidence supporting this possibility. Presumably, the fatal or nonfatal
107 outcome depends not only on the hantavirus viral load but also on other pathogenic or host
108 factors.

109 The follow-up of antibodies response and Maripa virus RNA load during the course of
110 disease for patient n°6, from admission on day 7 after the onset of disease until day 46 (**Table**
111 **2**). IgM titers were high at admission but decreased to become undetectable by day 46.
112 Conversely, seroconversion (IgM to IgG) was observed between day 7 and day 12; these

113 hantavirus IgG titers then increased to 4.4 (adjusted sum OD values) by day 46. Likewise,
114 viral RNA load evaluated in these seven sequential samples showed a high value at admission
115 (6.4 log₁₀ copies per mL); seven days later the viral RNA load declined from 6.4 to 4.7 log₁₀
116 copies per mL. The viral load then remained around 4 log₁₀ copies per mL in samples
117 collected on days 20, 25 and 30. Viral RNA was undetectable on day 46.

118

119 **Conclusion**

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

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143

144 **Biographical sketch**

145 Dr. MATHEUS is a research assistant at the Institut Pasteur de la Guyane, French
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147 special interest in hantavirus circulation in French Guiana.

148

149 **Conflicts of interest**

150 None declared.

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

194

	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

195

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

197 **Virus copy number was determined as log₁₀ copies per mL. Primers and TaqMan® probe for QPCR were the following Maripa_qRT2F

198 GCAGCTGTGTCTACATTGGAGAA, Maripa_qRT2R CCACCAGATCCGCCAACT and Maripa_Probe2 FAM-AAACTTGCAGAACTCA-

199 MGB

200

201

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

203

	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

204 *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. real

205

time PCR

