

HANTAVIRUS PULMONARY SYNDROME (HPS) IN GUARIBA, SP, BRAZIL. REPORT OF 2 CASES

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SUMMARY

Human infections caused by a hantavirus were reported in different regions of the State of São Paulo (SP), Brazil during the first six months of 1998. Two cases of fatal pulmonary syndrome occurred in May of 1998 in the City of Guariba, located in the Northeastern Region of SP. Both patients worked in a corn storage barn infested by rodents. These patients, after 2 or 3 days of non-specific febrile illness, developed a severe interstitial pneumonia spreading widely in both lungs, causing respiratory failure and death. At autopsy both patients showed lung interstitial edema with immunoblast-like mononuclear cell infiltrates, consistent with a viral etiology. *Hantavirus* infection was diagnosed by ELISA in both cases and by RT-PCR in one of the patients. Aspects of the clinical presentation, physiopathology and differential diagnosis of *Hantavirus* Pulmonary Syndrome are discussed.

KEYWORDS: Hantavirus pulmonary syndrome; Hantaviruses.

INTRODUCTION

The hantaviruses are viruses of rodents that can occasionally infect humans and cause severe disease with high mortality. In Asia and Europe hantaviruses cause haemorrhagic fever with renal syndrome. Since 1993 hantaviruses have been detected in patients with severe pulmonary syndrome in the Americas²³.

Hantavirus constitutes a genus of the family *Bunyaviridae*. These are spherical enveloped viruses (80 to 120 nm diameter) that possess surface glycoprotein projections²². The viral RNA is tri-segmented and the large (L) and medium (M) segments are of negative polarity, while the small (S) segment is ambisense. The segment L codes for an RNA dependent RNA polymerase, the M segment codes for the envelope surface G1 and G2 glycoproteins, and S codes for the nucleocapsid protein^{22,18}.

The evolution of the American hantaviruses seems to be closely related to evolution of the rodent reservoirs, which are *Rodentia*, *Muridae* of the sub-family *Sigmodontinae*^{23,30}.

A hantavirus was isolated for the first time in Brazil, in 1982, from *Rattus norvegicus* from the State of Pará, in the northern region of the country¹⁶. In 1993, three cases of hantavirus disease were

detected in members of the same family living on a farm, in close contact with wild rodents. These cases occurred in Juquitiba, State of São Paulo, and two of the three patients died with HPS. Between 1993 and 1998, nine other cases were reported in the states of São Paulo, Minas Gerais, and Mato Grosso, Brazil^{13,14}.

We report here two fatal cases of HPS which occurred in the City of Guariba, State of São Paulo (SP), Brazil, in May 1998.

MATERIALS AND METHODS

Guariba is a city of 35,000 inhabitants, most of whom are agricultural workers of the sugar cane industry. The city is located in the southeastern region of Brazil (21° 11'S, 47° 49'W), in the State of SP as shown in Figure 1. This region is characterized by mountain ranges of 400 to 1000m high with valleys in between. The climate is tropical, hot and humid, with a dry winter season, mean temperature of 21.7°C, and 1433mm of rainfall per year. This area is extensively deforested for agriculture and the remaining forested areas are mostly patchy along river borders. Wild rodents are abundant in the rural areas around the City of Guariba, although no information is available on the rodent species, their densities, or habits in these areas.

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Fig. 1 - Localization of the City of Guariba in the State of São Paulo, Brazil.

Hantavirus infection was diagnosed in both patients of Guariba by detection of IgM and IgG specific antibodies to the N protein and G1 glycoprotein of Sin Nombre virus, using an enzyme immunoassay¹⁵. The serologic test was done in the Instituto Adolfo Lutz, São Paulo, SP, Brazil.

Part of the gene of the G2 protein (364-bp amplicom) was amplified from the serum of patient 2 using broad *Hantavirus* primers in a reverse transcription-polymerase chain reaction (RT-PCR)^{11, 26}. This test was done in the Unidade Multidepartamental de Pesquisa em Virologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo.

CLINICAL CASES

Patient 1

This previously healthy 55-year-old white man worked in the rural outskirts of the City of Guariba. The patient developed fever on May 5, 1998 presenting widespread myalgias and malaise. Two days later, the symptoms worsened with development of dyspnea, dry cough. The patient was seen by a doctor, who diagnosed pneumonia. The chest radiograph showed interstitial infiltrates in the lower parts of both lungs and alveolar infiltrates on the right side, as shown in Figure 2. The patient was treated with procaine penicillin, and analgesics. On the next day, his dyspnea worsened and he started to produce copious reddish respiratory secretions. The blood pressure was 70/30 mm Hg, and the hematocrit was 56%. He received about 4,000 ml of fluids intravenously. The patient was transferred to the General Hospital of the Faculty of Medicine of Ribeirão Preto. On arrival he was unconscious, had absent pulses, absent arterial pressure, bilateral mydriasis, and absence of breathing movements. The heart activity returned after cardiopulmonary resuscitation, oro-tracheal intubation and intravenous administration of inotropic agents. The patient remained unstable and acidotic with blood pressure of 70/50 mmHg, pulse rate of 108/min, arterial blood pH of 6.71, and serum bicarbonate of 7.4 mEq/L. The lungs were congested with diffuse pulmonary crackles heard throughout, and the extremities were cyanotic. A large amount of secretion was removed by the oro-tracheal

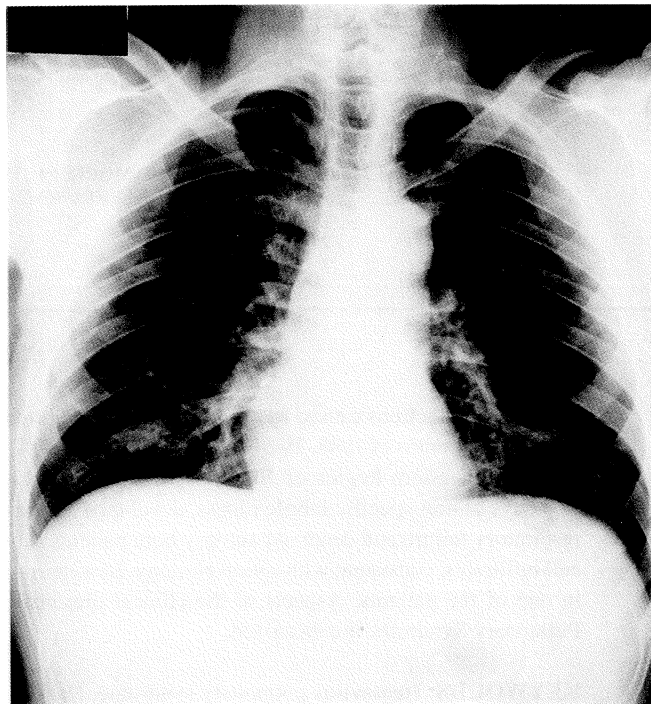


Fig. 2 - Chest radiograph of patient 1 showing interstitial infiltrates in the low part of both lungs and alveolar infiltrates in the right side.

suction. Laboratory analysis revealed a hematocrit of 57%, a leukocyte count of 5,030/mm³ and platelet count of 74,000/mm³. The arterial blood gases showed pCO₂ 60.1 mm Hg; pO₂ 75.3 mmHg; and oxygen saturation, 61.6%. Within 3 hours of transfer the patient had a cardiac arrest and died despite resuscitative attempts.

Autopsy showed a 63Kg man with rubbery voluminous lungs. The lungs weighed approximately twice normal, having massive edema, congestion and multiple hemorrhage areas. Six hundred ml of pleural yellow citrine fluid was collected from both hemithoraces. The microscopy of the lungs showed alveolar spaces distended by edema fluid, containing blood and fibrinous hyaline membranes. There was a moderate mononuclear cell interstitial infiltrate with enlarged alveolar septums as shown in Figure 3-A. Hepatomegaly and splenomegaly were observed. The liver was slightly enlarged and an immunoblast-like mononuclear cell infiltrate was present in the portal spaces, without significant abnormalities of the hepatocytes. The liver sinusoids besides congestion showed focal groupings of immunoblasts as shown in Figure 3-C. The spleen was twice the normal weight and presented abundant immunoblasts in the red pulp.

Patient 2

A previously healthy 38-year-old white male farmer living in the City of Guariba became sick on the May 16, 1998 presenting myalgias, weakness and malaise. Four days later, his symptoms of dry cough and high fever were diagnosed as influenza by his doctor. The disease

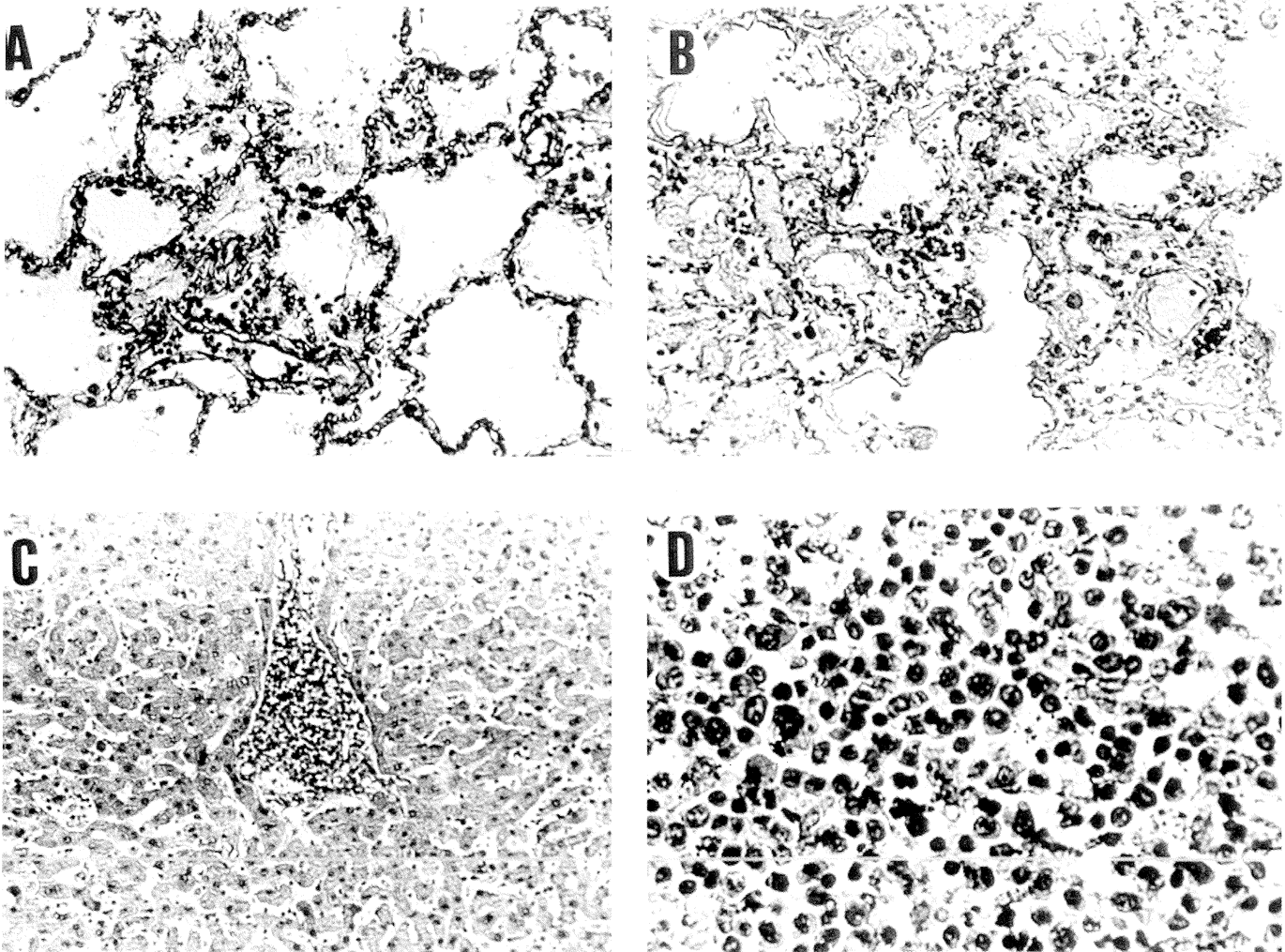


Fig. 3 - A- Microscopical study of the lung tissue of patient 1 showing alveolar spaces distended by liquid edema, containing blood and fibrinous hyaline membranes, and moderate immunoblast interstitial infiltrate in enlarged alveolar septans ; B- Microscopical study of the lung tissue of patient 2 showing the same infiltrate observed in A; C- Microscopical study of the liver tissue of patient 1 showing congestion of sinusoids and focal groupings of immunoblasts; D - Microscopical study of the spleen tissue of patient 2 showing an hiperplasic white pulp and an hiperplasic red pulp containing a large amount of immunoblasts.

progressively worsened with dyspnea at rest, widespread myalgias. The patient was admitted to the local hospital where he looked severely ill, was febrile (axillary temperature 38.6 °C), conscious, dyspneic (40 per minute), red-faced, and cyanotic in his extremities. There were fine rales in the inferior parts of both lungs. The heart was regular at a rate of 120/min, and the blood pressure was 120/80 mmHg. The chest radiograph performed in the same day, demonstrated a diffuse interstitial infiltrate and a normal cardiac silhouette, as shown in Figure 4-A. Laboratory analysis revealed a leukocyte count of 6,600/mm³ with 34% segmented neutrophils, 51% band forms, 3% metamyelocytes, 9% lymphocytes, 3% monocytes, and no eosinophils. The hematocrit was 46% and the platelet count 77,000/mm³. Arterial blood gases while on nasal cannula oxygen therapy showed pH 6.58; pCO₂, 83 mmHg; pO₂, 63 mmHg; HCO₃, 8 mmol/L; and oxygen saturation, 53%.

Because of the possibility of an AIDS associated *Pneumocystis carinii* acute interstitial pneumonia, an HIV serologic test was performed, but was negative. The patient was transferred to the General Hospital in the City of Ribeirão Preto and received the following antimicrobial agents: erythromycin, trimethoprim-sulphamethoxazole, and ceftriaxone. He also received intravenous crystalloid and colloid fluids, and 3 L/min oxygen therapy by nasal cannula. Laboratory analysis revealed a urea of 26 g/dl, creatinine of 0.9 mg/dl, and glucose of 122 mg/dl. Hydroelectrolytic and metabolic disturbances were corrected. Despite treatment, the patient developed a fast and progressively worsening dyspnea, requiring intubation and mechanical ventilation while receiving 100% oxygen. The chest tomography done on May 27 demonstrated diffuse alveolar infiltrates and small bilateral pleural effusions as shown in Figure 5. On the next day the patient presented

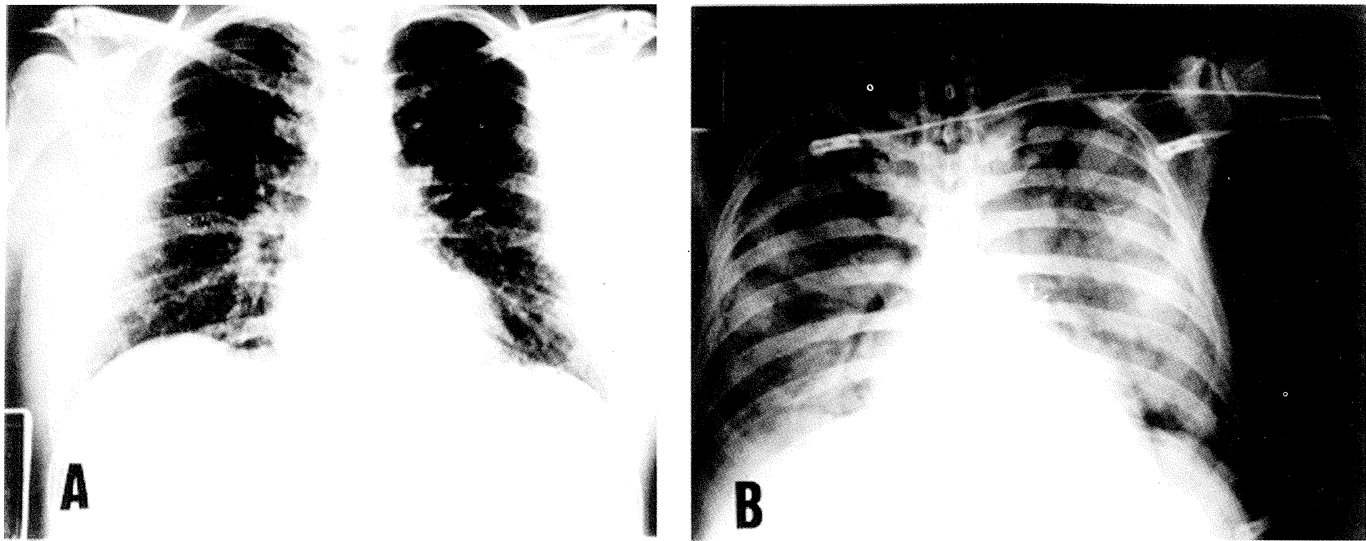


Fig. 4 - A - Chest radiograph of patient 2 showing a diffuse interstitial infiltrate. B - Chest radiograph of patient 2 showing diffuse alveolar floccose infiltrates and a normal cardiac silhouette.

seizures, hypotension, and reddish secretion through the orotracheal tube. Cardiac arrest was twice reverted. The chest radiograph showed diffuse alveolar flocculent infiltrates with a normal cardiac silhouette, as shown in Figure 4-B. The patient died at 3 pm on May 28.

The autopsy showed a 93 kg man with congested lungs. The lungs were edematous, leaked hemorrhagic fluid, and presented approximately twice the normal weight. Microscopy of the lungs showed the same infiltrate observed in the first patient as shown in Figure 3-B. Hepatomegaly and splenomegaly were observed; both organs were twice the normal weight. The liver presented an infiltrate of immunoblasts and steatosis. The spleen white pulp was hyperplastic and the red pulp was also hyperplastic with large numbers of immunoblasts as shown in Figure 3-D.

DISCUSSION

Inhalation of aerosols of excrement of infected rodents are believed to be responsible for *Hantavirus* infections²³. Patient 1 worked for patient 2 on a farm. Both were harvesting corn and storing the crop in a corn barn that was infested by rodents. Probably, that was the place where both infections occurred. Human-to-human is also a possible mechanism of transmission of hantaviruses as reported during an outbreak of HPS in Southern Argentina, in 1996²⁷.

It was not possible to determinate the incubation period of the disease in our 2 patients and, in fact, the HPS incubation period is probably not well known^{4,5,24}. During the person-to-person transmission outbreak of HPS that occurred in Argentina, in 1996, the disease symptoms appeared after 11 to 29 days of incubation period²⁷.

The 2 patients of Guariba presented a severe form of HPS with diffuse alveolar flooding^{4,5,24}. The evolution to death of patient 1 took

3 to 4 days and for patient 2 it took 12 days. It is in agreement with other reports that refer 3 to 12 days as the interval between onset of illness and death²⁰. The two HPS fatal cases occurred in Brazil in 1993, died after 3 to 5 days of disease²⁵. Myalgias, weakness, malaise and respiratory symptoms characterized the prodromic phase of the disease in our patients and nausea or vomiting and diarrhea were not referred⁴. Dry cough started after 2 to 4 days and patient 1 also had dyspnea. The initial chest radiographic abnormalities included interstitial infiltrates in the low part of the lungs, alveolar infiltrates and a normal cardiac silhouette, as shown in Figures 2 and 3A. At this point, pulmonary edema developed rapidly and the patients became hypoxic. The rapid progress of the pulmonary disease of patient 2 can be seen in the radiographs of Figure 3B and Figure 4, showing diffuse alveolar floccose infiltrates and pleural effusion. The patients presented cough and eliminated a rosy abundant secretion suggesting a severe pulmonary edema. Both cases were intubated and mechanically ventilated with 100% oxygen without clinical improvement. They also had tachycardia and the first patient presented hypotension. The patients were intravascularly volume depleted having an increased hematocrit. The clinical presentations and the evolutions of our patients were similar to those reported for the 3 Brazilian patients having HPS in 1993, as well as for the cases occurred during the 1993-94 outbreak of USA^{4,5,24,25}.

The lungs of our patients demonstrated diffuse alveolar edema, hyaline membranes and a lymphocytic interstitial infiltrate. HPS is a fulminant pulmonary syndrome and death probably occurs before the development of extensive hyaline membranes and alveolar lining cell injury²⁰. There was also little immune cell recruitment to the lungs of our patients. The preserved alveolar endothelial and epithelial cells associated to hemoconcentration and pleural effusions suggest a capillary leak syndrome. A high hematocrit value was observed in our

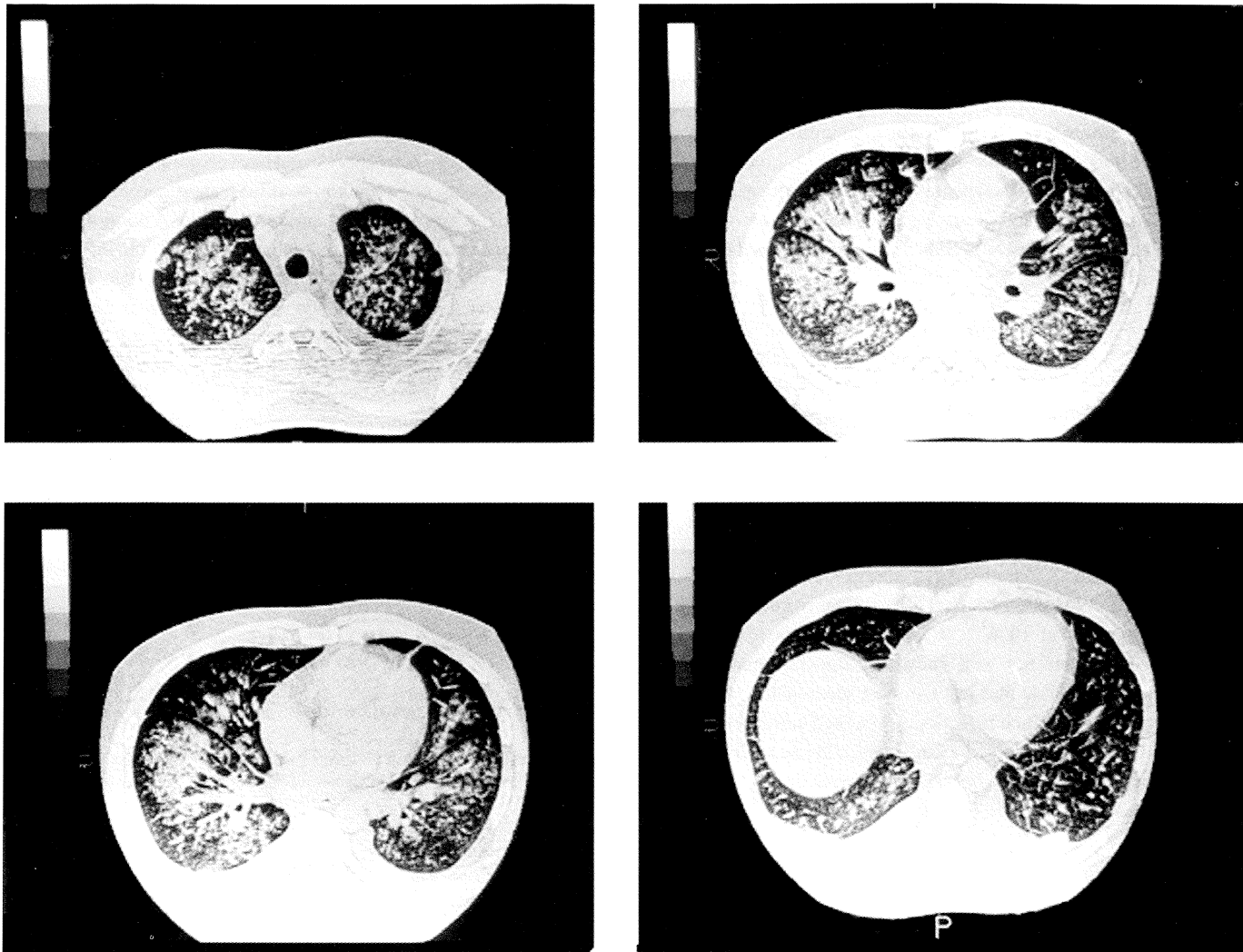


Fig. 5 - Chest tomography of patient 2 showing diffuse alveolar infiltrates and small bilateral pleural effusions.

first patient, suggesting hemoconcentration as a consequence of intravascular liquid leaking.

Virus-like particles at the ultrastructural level and abundant *Hantavirus* antigens have been described in vascular endothelial cells, specially in the lung of patients with severe HPS, suggesting a high virus burden^{9,20}. The hantaviruses invade cells using specific $\beta 3$ integrins as cellular receptors. These integrins are fundamental to maintaining microvascular barrier properties and the virus interactions could propitiate a capillary leak syndrome¹⁰. $\beta 3$ integrins are also present in platelets that become infected with these viruses and lead to thrombocytopenia, as observed in our two patients. The platelet infection probably propitiates virus transport to multiple infection sites and also inhibits platelet aggregation¹⁰. However, hemorrhage was not observed in our patients despite having thrombocytopenia and, in fact, it has been reported as an uncommon phenomenon in HPS²⁰.

Immunoblast-like mononuclear cell infiltrates were observed in the interstitial lung, in the hepatic portal spaces and in the spleens of our two patients. These cells are primarily T cells and their presence associated to activation of the complement cascade, and immune complex detection suggest an involvement of the immune system in the pathogenesis of HPS²⁰. The presence of immunoblast infiltrates in multiple organs and cytokine liberation have been associated with the *Hantavirus* haemorrhagic fever with renal syndrome which occurs in Asia and in Europe²⁵. Other viral diseases such as dengue hemorrhagic fever/dengue shock syndrome are known as capillary leak syndromes associated to cytokine liberation²⁹.

The clinical characteristics of HPS in outbreaks occurred in Argentina included myositis and proteinuria²³. Both HPS patients of Guariba referred myalgias, which could be associated to myositis. However, it was not possible to study specifically myositis and proteinuria in our patients.

The AIDS associated *Pneumocystis carinii* acute interstitial pneumonia, a frequent cause of acute severe bilateral pneumonia in Brazil, does differential diagnosis with HPS⁶. *Pneumocystis carinii* was suspect to cause the patient's pneumonia. Other causes of acute severe bilateral pneumonia in Brazil include influenza A virus and *Mycoplasma pneumoniae*^{17,21}. HPS could also be confused with bacterial infection and sepsis with pulmonary involvement caused by *Staphylococcus aureus* and gram-negative bacilli¹. A less frequent etiologic agent of acute severe bilateral pneumonia is *Legionella pneumophila*⁷.

Our two patients as well as the other HPS Brazilian cases referred contact with wild rodents. The diseases caused by contact with rodents in Brazil include leptospirosis, bubonic plague, and arenaviruses^{3,8,19}. Despite of similar rodent-related transmission and to have the same first symptoms, it is possible to differentiate those diseases of HPS based on the clinical presentations because they generally do not produce pulmonary disease as the main clinical manifestation.

The Brazilian HPS reported cases had a 90% mortality rate. This value is higher than 58% mortality rate observed in the United States during the 1993-1994 outbreak, and 50% mortality rate observed in Southern Argentina in 1996^{2,27}. Considering that it is a recently described disease and not well known by the Brazilian medical community, it is possible that just the most severe HPS cases have been diagnosed and other infections remained unreported. It is corroborated by the diagnosis of 4 infections, done by testing 49 sera of patient contacts to *Hantavirus* antigens, during the HPS outbreak of Juquitiba, in 1993^{14,25}.

Forty percent of the reported HPS Brazilian cases occurred in the State of São Paulo during the first six months of 1998. Environmental or social factors which could be associated to an increased number of HPS in this particular year are unknown²³.

Specific hantaviruses are primarily associated with specific rodent species³⁰. The rodent infection is persistent and infectious hantaviruses can be detectable in different organs even after months of an experimental infection¹². Further studies in Guariba and neighboring cities are necessary in order to determinate the rodent species, and their densities. Serologic and virologic studies in the rodent population should also be carried out in order to know the antibody levels as well as the specific rodent species implicated on this outbreak. A serologic survey should be done in the human population living in Guariba in order to determinate the number of *Hantavirus* infected individuals. It is also important to divulge on hantaviruses and HPS to the Brazilian medical community.

RESUMO

Relato de 2 casos de síndrome pulmonar por hantavirus ocorridos em Guariba, SP, Brasil

Casos de hantavirose foram notificados em diferentes regiões do

Estado de São Paulo (SP), Brasil, durante o primeiro semestre de 1998. Dois casos fatais de síndrome pulmonar ocorreram em maio de 1998 na Cidade de Guariba, localizada na Região Nordeste de SP. Ambos os pacientes trabalhavam no mesmo local, estocando milho em um paiol infestado de roedores. Estes pacientes, após 2 ou 3 dias de doença febril aguda inespecífica, desenvolveram uma grave pneumonia intersticial, que espalhou-se difusamente por ambos os pulmões causando insuficiência respiratória e óbito. À autópsia, ambos os casos apresentavam edema pulmonar intersticial com infiltrado de células mononucleares (imunoblastos) sugestivo de etiologia viral. O diagnóstico laboratorial de hantavirose foi feito pela detecção de anticorpos específicos por ELISA, em ambos os casos e por detecção do genoma viral utilizando a RT-PCR, em um dos pacientes. Aspectos da apresentação clínica, da fisiopatologia e do diagnóstico diferencial desta síndrome pulmonar são discutidos no trabalho.

ACKNOWLEDGEMENTS

We are grateful to Dr. Eurico Arruda and Dr. Robert Shope for review of the manuscript. This work was partially supported by São Paulo State Government Research Agency (FAPESP).

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Received: 21 October 1998.

Accepted: 07 January 1999.

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