

## HANTAVIRUS PULMONARY SYNDROME IN THE PROVINCE OF RIO NEGRO, ARGENTINA, 1993-1996.

Gustavo CANTONI(1), María LAZARO(2), Amanda RESA(3), Odila ARELLANO(4), Ana María AMESTOY(5), Sabrina DE BUNDER(1), Eduardo HERRERO(1), Alicia PEREZ(1) and Edmundo LARRIEU(4).

---

### SUMMARY

Early in 1995 the first case of Hantavirus Pulmonary Syndrome was serologically confirmed in El Bolsón (Province of Río Negro, Argentina), corresponding to the third outbreak reported in Argentina.

A total of 26 cases of HPS related to the Andean region of Río Negro Province, were reported from 1993 to 1996, 17 in El Bolsón, 4 in San Carlos de Bariloche, and 5 in Buenos Aires. The incidence rate was  $5.03 \times 100000$  with a mortality rate of  $51.85 \times 100$ . The occurrence of cases was mainly seasonal, with a significantly greater number in the spring, and the persons affected mainly lived in urban or periurban areas.

In four cases, the affected individuals were members of a couple, spouses or live-in contacts. Seven cases were Health workers (physicians, nurses or administrative staff).

Twelve cases were related among them, due to an outbreak of 80 days. Two of them did not visit the Andean region.

A total of 139 rodents were captured and seven of them, *Olygoryzomys longicaudatus*, were found to be serologically positive. The possibility of infection by contact with rodents or fecal matter is being analyzed and also hypothesis related with interhuman transmission.

**KEYWORDS:** Hantavirus; Outbreak; Epidemiology.

---

### INTRODUCTION

On May 14<sup>th</sup>, 1993 a new Hantavirus disease appeared in the Southwestern States of the U.S., characterized by the predominance of a pulmonary syndrome and its high case-specific death rate. The etiologic agent was identified as a new virus of the genus *Hantaan*, called "Virus without a name"<sup>1,11,12</sup>.

Early in 1995 the first case of Hantavirus Pulmonary Syndrome (HPS) was serologically confirmed in El Bolsón (Province of Río Negro, Argentina), corresponding to the third outbreak reported in Argentina<sup>4,7,8</sup>. Clinical records and samples from patients who presented clinical symptoms compatible with Hantavirus Pulmonary Syndrome during the period of 1993-1994 were analyzed thereafter.

In 1996, there was an outbreak in El Bolsón that affected 12 persons. Other sporadic new cases and cases in San Carlos de Bariloche (Province of Río Negro, Argentina) were also reported. These cases stimulated an epidemiological investigation, and we report here a descriptive analysis of the endemic cases in El Bolsón and Bariloche during the period from 1993 to 1996 and the features of the 1996 outbreak in El Bolsón.

### MATERIALS AND METHODS

#### a) Characteristics of the study area

El Bolsón and Bariloche are located in the west Province of Río Negro, a mountainous region called Cordillera Andino Patagónica. The weather is cold and humid, with a rainfall of 200 to 1000 mm and isotherms of 2°C in winter and 18°C in summer.

---

(1) IV Sanitary Zone, Bariloche, Argentina.

(2) San Carlos de Bariloche Hospital, Bariloche, Argentina.

(3) El Bolsón Hospital, Río Negro, Argentina.

(4) Provincial Council of Public Health, Río Negro, Argentina.

(5) National Institute of Epidemiology, Mar del Plata, Argentina.

Correspondence to: Edmundo Larrieu, Laprida 240, (8500) Viedma, Argentina.

There are also big lakes and mountains. The vegetation is luxuriant, consisting of thick forest, bushy areas mainly with wild rose bushes, brambles, broom, etc.

The areas surrounding Bariloche and especially El Bolsón have rural features due to the woody and bushy thick vegetation occurring even near the urban center.

The agricultural activities include the cultivation of mushrooms, strawberries and hops. In addition, wild fruits are picked to make jams.

San Carlos de Bariloche has a population of 89363. Health Care is provided by a grade VI complexity Hospital, with a staff of 498, which is a reference center and also a regional center receiving patients referred by lower complexity Hospitals. There are also 4 private hospitals equipped with intensive care units.

El Bolsón is located 120 km south of Bariloche, has a population of 14857, and its Health Care is provided by a grade IV complexity Hospital with a staff of 127. Cases which require intensive care are referred to San Carlos de Bariloche. Bariloche and El Bolsón are located approximately 1600 km from Buenos Aires, the capital of Argentina.

#### b) Objectives of the study:

The objectives of the present study were: 1) to describe the frequency and distribution of this disease according to the time (season), place (areas) and persons affected (age, sex, occupation, periods of exposure); 2) to estimate incidence and case-specific death rate; 3) to determine the rodent species that acts as a reservoir of Hantavirus in the region; 4) to interpret the information and elaborate a hypothesis about transmission of the disease.

#### c) Case description

Definition of a case: 1) A Patient with clinical symptoms, i.e., a previously healthy patient who has a febrile syndrome (more than 38° C, myalgias, headaches) followed by respiratory distress and/or a chest X-ray that shows bilateral lung infiltrates. The diagnosis was confirmed by enzyme immunoassay (EIA) at the National Human Viral Diseases Institute "Julio Maiztegui" (INEVH) and by PCR at the National Microbiology Institute "Carlos Malbrán" (INM); 2) Other patients, i.e., serologically positive cases but without respiratory distress.

Clinical records compatible with cases of Hantavirus Pulmonary Syndrome treated in Hospitals of Bariloche and El Bolsón were analyzed and information on cases treated in private clinics of Bariloche and Buenos Aires was collected (always related to the Bariloche/El Bolsón cases). Survivors of the disease and/or relatives of patients were personally interviewed to obtain more information as to their activities during the 60 days prior to the appearance of symptoms compatible with HPS.

#### d) Study of reservoirs

Rodents were captured using 300 Sherman capture traps set in the homes of the patients, in probable areas of infection and in wildlife areas in the district<sup>15</sup>. The species of the captured rodents was determined, blood samples were taken for serologic studies by enzyme immunoassay (EIA) at INEVH and frozen animals were sent to INEVH for PCR studies.

### RESULTS

#### a) Endemic HPS activity

During the period from 1993 to 1996, a total of 26 human cases of HPS were detected in El Bolsón and Bariloche. All cases were serologically confirmed except for two in 1994, from whom no samples were obtained. Twenty-five (96.2%) were symptomatic cases and 1 (3.8%) presenting fever and IgG positivity was assigned to the "other cases" category. Seventeen cases lived in El Bolsón, 4 in San Carlos de Bariloche and 5 in Buenos Aires, all of them linked in different ways to the cases in El Bolsón.

The 1993-1996 incidence was 29 x 100000 inhabitants for El Bolsón and 1 x 100000 inhabitants for San Carlos de Bariloche. Taking into account only those cases living in the area (Table 1), the mortality rate was 53.84% (Table 2).

**TABLE 1**  
Hantavirus pulmonary syndrome: cases and incidence rate in el Bolsón and Bariloche, Río Negro, 1993/1996

Year	El Bolsón N° (X 100000)	Bariloche N° (X 100000)
1993	1(7)	0(0)
1994	2(13)	0(0)
1995	4(27)	0(0)
1996	10(67)	4(4)
Total	17(29)	4(1)

Cases resident in the area

**TABLE 2**  
Hantavirus pulmonary syndrome: case fatality, Río Negro, 1993/1996

Year	Cases	N° of Deaths (%)
1993	1	0(0.00)
1994	2	2(100)
1995	4	2(50.00)
1996	19	10(52.63)
Total	26	14(53.84)

The age of the persons affected ranged from 9 to 70 years, with no sex differences (59% men). In 100 % of the adult patients the predominant signs and symptoms included fever, asthenia, headaches and myalgias followed by respiratory distress. Vomiting, thirst, pharyngitis, abdominal pain and

diarrhea were observed in 84% of the cases. Cough and dyspnea were late symptoms. Clinical analysis showed an increase in hematocrit, leucocytosis with a shift to the left (range 11700-35600) and thrombocytopenia. Complementary analysis by chest X-ray, showed the presence of bilateral interstitial infiltrates as the main indicator.

All cases serologically confirmed as HPS at the INEVH were PCR positive at the Malbrán Institute. The nucleotide sequence of the virus was different from all the sequences of Hantavirus obtained to date, and the agent was classified as the "Andes virus"<sup>7</sup>.

In 1996 serum samples were taken from patient contacts for follow-up and control, and all results were negative. In 3 cases, the initial samples were negative with serologic and PCR positivity occurring later. All 3 patients died within 2/5 days.

The patients admitted to Public Hospitals in Río Negro were treated as follows: A) Patients under observation with febrile syndromes: control of all vital signs 4 times daily, control of diuresis, light diet and oral fluids (intravenous fluids if pills could not be administered); B) Symptomatic patients with fever and/or pains were treated with Paracetamol or Dipirone. Vomiting was treated with Metachlopramide. Highly controlled administration of parenteral fluids and use of an oxygen mask were employed. In 15 cases (55.5%) mechanical respiratory assistance was required.

The biosafety measures applied were based on universal accepted precautions. In all cases patients consigned to intensive care units were not in individual rooms. The appearance of cases was mainly seasonal, with a significantly greater number of cases in spring. (Fig. 1). As to patient occupation, 2 were from rural areas and the rest lived in urban or periurban areas. Five patients

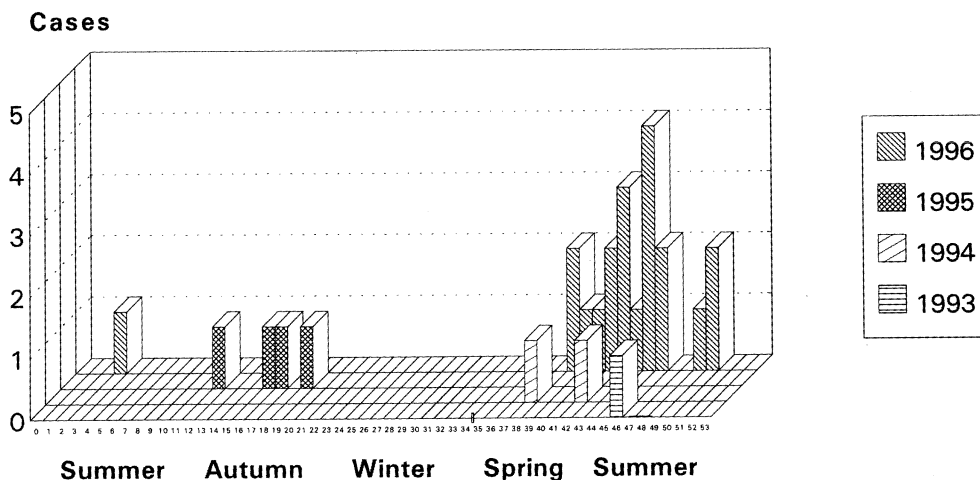
who worked in rural areas were possibly in contact with wild rodents. Four HPS cases later transmitted the disease to their sex partners (Table 3). Seven patients (26.9%) were Health workers (physicians, nurses or administrative employees) in the Hospitals or private clinics of Bariloche (1 case), Bolsón (4 cases) and Buenos Aires (2 cases) who worked with HPS cases related to El Bolsón/Bariloche (Table 4).

The incidence rate during 93/96, taking into account only health workers of El Bolson and Bariloche, was 186 x 100000, reaching 594 x 100000 in 1996. Compared to the general population, the Relative Risk (RR) for Health workers during 1996 was very high (Table 5).

**b) El Bolsón outbreak, September-December 1996:**

Of the 26 patients in the El Bolsón/Bariloche area, 12 (46%) were related. The outbreak took place from 09/22 to 12/14 (Fig. 2). The affected patients were a man (case 1), the physician who attended him (case 2), his mother (case 3), a household employee of the family (case 4), a friend of the man (case 5), the physician who attended them (case 6), the first physician's wife (case 7), a man and relatives of the family who travelled to El Bolsón for the burial of case 1 (cases 8 and 9), the daughter of this couple, who did not travel to El Bolsón but who travelled part of the way back home with them (case 10), a physician who went to El Bolsón to attend case 7 (case 11), and a physician who did not go to El Bolsón but who attended case 7 (case 12).

Cases 1, 3 and 4 shared risky work near the home of case 1. Cases 1, 3, 4, 6, 8 and 9 shared the home of case 1 which had certain limited risks in terms of HPS. Cases 1 and 5 shared an activity in a high risk area far from the home of case 1. Case 10 only had access to risk in a vehicle that has been previously



**1994: Unconfirmed data**

Fig. 1 - Cases of Hantavirus pulmonary syndrome per epidemiological week, Río Negro, 1993-1996.

**TABLE 3**

Number of cases of Hantavirus pulmonary syndrome in sexual partner, Rio Negro, 1993/96

Sexual Partner	Sex During Febril Stage	Diference in Days to Commencement of Symptomatology	Case Fatality Rate
1	Yes	20	50
2	?	27	50
3	Yes	9	100
4	?	3	0
5	No	Sexual partner did not contract the disease	0
6	No	Sexual partner did not contract the disease	0

**TABLE 4**

Number of cases of Hantavirus pulmonary syndrome in general population in the health workers, Río Negro, 1993/96

Year	Cases in the El Bolsón/Bariloche outbreak	Cases in Health Workers (% of the Total)
1993	1	0 (0.0)
1994	2	1(50.0)
1995	4	0(0.0)
1996	19	6(31.5)
Total	26	7 (26.9)

**TABLE 5**

Hantavirus pulmonary syndrome: relative risk for health worker, Río Negro, 1996

Health Worker vs	RR	IC(RR)	P value	AR	REF
General Population					
Bariloche	54	4/705	9.37	179	98%
El Bolsón	50	19/123	69.46	2315	98%
Total	32	14/69	76.88	602	98%

parked at the home of case 1, for several days, used for a trip shared with cases 4, 8 and 9. The risky activity of cases 2, 6, 11 and 12 was the care of patients with HPS. Cases 8, 9, 10, 11 and 12 lived in the city of Buenos Aires.

#### c) Investigation of reservoirs:

A total of 139 rodents were captured, belonging to the following species: *Olygoryzomys longicaudatus* (51%), *Abrothrix longipilis* (41%), *Abrothrix olivaceus* (5%), *Rattus norvegicus* (1%) and *Mus musculus* (2%).

All the rodents captured in the homes of the cases and in risk areas related to the cases were negative to Hantavirus. Seven *Olygoryzomys longicaudatus* were captured in Parque Nacional Lago Puelo (Province of Chubut), 20 km from El Bolsón, and were Elisa and PCR positive to Hantavirus (5% of the captured rodents, 10% of the *O. longicaudatus*, and 100% of the *O. longicaudatus* captured in Lago Puelo).

### DISCUSSION

Since Hantavirus Pulmonary Syndrome has been only recently described, current knowledge about the disease is partial or incomplete. After the description of the first outbreak in the United States in 1993, studies were undertaken with the aim of determining the epidemiology of the disease, including different aspects such as reservoirs, ways of transmission, risk activities, treatment, case specific deaths rates, etc.<sup>2,3,5,6,13,14,15,16</sup>. These studies have defined the Hantavirus Pulmonary Syndrome as a disease caused by variants of the *Hantaan* virus, with an

incubation period of 5 to 45 days, with an eminently pulmonary pathology and a mortality rate of 55%.

Rodents are involved as reservoirs (*Peromyscus maniculatus* in the U.S. was the first reservoir identified) and carry a chronic and asymptomatic infection. It is believed that they transmit the disease to man by shedding virus in their urine, saliva and fecal matter, thus contaminating the environment. The point of entry of the virus in humans is the respiratory system, and aerosolization is the mechanism of infection<sup>1,3,6</sup>.

The clinical symptomatology, laboratory research and X-ray results of HPS cases which appeared in Rio Negro were similar to those described in the U.S.<sup>1,6</sup>. The mortality rates are very high. In the U.S. they tend to decrease, possibly due to the diagnosis of the disease in non-serious cases and to an improvement in treatment due to a better knowledge of the disease.

HPS cases in most areas where the disease has appeared, such as New Mexico (USA), Orán (Argentina) or Paraguay, usually occur in rural populations or among persons who work in rural or agricultural environments. The cleaning of enclosed spaces where there has been contamination by rodents has been defined as a high risk activity<sup>6</sup>.

In the case of El Bolsón/Bariloche (Río Negro, Argentina), the population affected by the disease was basically urban and involved in non-rural activities. The characteristics of these towns, with their strong rural component, may explain this circumstance. In fact, the rodents captured in the very center of El Bolsón and

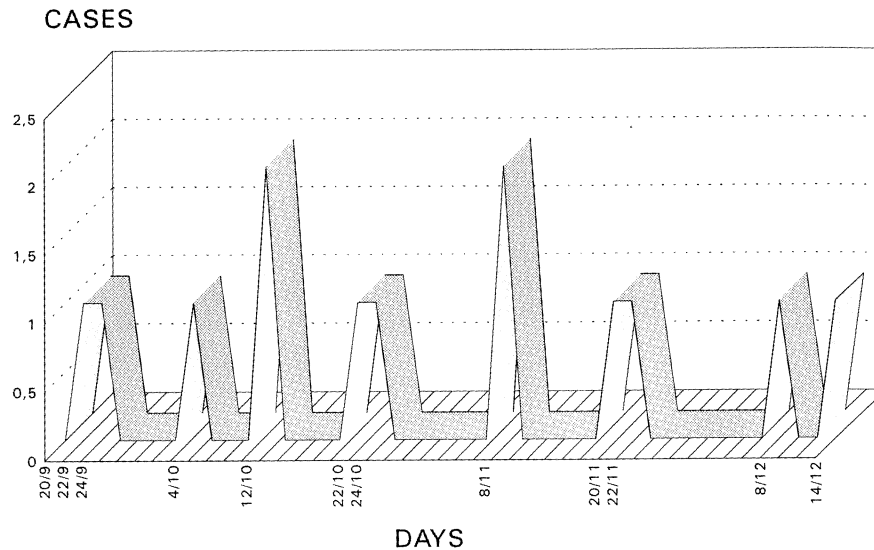


Fig. 2 - Sequence of 12 linked cases in S.P.H. outbreak El Bolsón, September to December, 1996

on the outskirts of Bariloche were mainly wild rodents, mostly *Olygoryzomys longicaudatus*. This is one of the 15 species of wild rodents found in the Andean region of Río Negro, and the most closely related is the *Peromyscus maniculatus* species.

These rodents basically eat fruit and seeds. They live in areas where there is bushy vegetation, such as wild rose bushes and brambles, and where there are no specific plunderers. Their number decreases in winter, they begin to reproduce in spring and reach maximum population levels in summer. Therefore, the occurrence of cases in spring may be explained by the fact that the rodents approach houses when they increase their activities at the end of winter, a time coinciding with lack of food in their natural habitat.

With reference to the mechanisms of transmission, it is clear that in several cases there was a possibility of infection due to access to places contaminated with rodent urine or fecal matter, as also observed in the New Mexico (USA) outbreaks<sup>16</sup>. However, in several cases the information provided did not reveal the existence of a clear contact between the cases and the rodents and their secretions. On the contrary, in two cases the only identifiable risk was the contact with patients with HPS. Also, the significantly high rate of incidence among Health workers at HPS health care centers and the very high Relative Risk of this group when compared to the general population, justifies the hypothesis of person to person transmission mostly by the respiratory route.

Nosocomial studies have ruled out this way of transmission in the U.S., though this hypothesis is considered probable in

Argentina<sup>5,13</sup>. With reference to Health workers, the infection of Laboratory personnel has also been reported<sup>15</sup>.

The hypothesis that sexual transmission could be another way of transmission has been proposed, although the number of cases is small and other routes of transmission may exist. In fact, the occurrence of linked cases, especially in the El Bolsón outbreak, had not been described in the HPS outbreaks in the U.S., Paraguay or Orán.

In view of the present results, it can be seen that serologic studies of persons who had contacts with cases are of limited use. Case-Control studies and Genomic studies seem to be indicated for a better evaluation of the aspects of Hantavirus transmission. Also knowledge about the Biology of the possible reservoirs which exist in the area needs to be expanded<sup>10</sup>. However, taking into account that a future occurrence of endemic or epidemic cases is foreseeable, it seems prudent to increase biosafety measures, including respiratory isolation in the care of cases of HRS in Argentina and also to ensure that the Health Centers promptly act in terms of disinfection and rodent elimination in places or domiciles where outbreaks occur.

## RESUMO

### Síndrome pulmonar por Hantavírus na Província de Rio Negro, Argentina, no período de 1993-1996

Em 1995, o primeiro caso de Síndrome Pulmonar pelo Hantavírus (HPS) foi sorologicamente confirmado em El Bolsón

(Província de Rio Negro, Argentina), correspondendo ao terceiro surto relatado na Argentina.

Um total de 26 casos de HPS relacionados com a região Andina, Província de Rio Negro, foram relatados de 1993 a 1996, 17 em El Bolsón, 4 em São Carlos de Bariloche e 5 em Buenos Aires. O índice de incidência foi de 5,03 x 100.000 com índice de mortalidade de 51,85 x 100. A ocorrência de casos foi principalmente sazonal, com números significativamente maiores na primavera, e as pessoas afetadas viviam principalmente nas áreas urbanas e periurbanas.

Em 4 casos, os indivíduos afetados eram membros de um casal, esposas ou viviam em contato. Sete casos eram trabalhadores da Saúde (médicos, enfermeiras e pessoal administrativo).

Doze casos estavam relacionados entre si, devidos a um surto de 80 dias. Dois deles não visitaram a região Andina.

Um total de 139 roedores foram capturados e sete deles, *Olygoryzomys longicaudatus*, foram sorologicamente positivos. A possibilidade de infecção por contato com roedores ou fezes está sendo analisada e também a hipótese de transmissão interhumana.

#### ACKNOWLEDGEMENTS

We are grateful for the collaboration of Instituto Nacional de Microbiología "Carlos Malbrán", Dirección Nacional de Epidemiología and Instituto Nacional de Enfermedades Virales Humanas "Julio Maiztegui"

#### REFERENCES

1. BUTLER, J. & PETERS, C.- Hantavirus and Hantavirus pulmonary syndrome. *Clin. infect. Dis.*, 19: 387-395, 1994.
2. BUTLER, J. & PETERS, C.- Lessons learned from the Hantaviruses and other hemorrhagic fever viruses. *Amer. J. med. Sci.*, 311: 55-59, 1996.
3. CENTERS FOR DISEASE CONTROL- **Compilation of articles on Hantavirus pulmonary syndrome**: 1993-1994. Atlanta, 1995. (Mimeo).

4. CORTEZ, J.; SUPAGA, M.; CACACE, M.; SEIJO, A. & ENRIA, D.- Síndrome pulmonar por hantavirus y leptospirosis. In: CONGRESO LATINOAMERICANO DE ZOONOSIS, 1, Buenos Aires, 1995. Resúmenes. p.125.
5. ENRIA, D.; PADULA, P.; SEGURA, E. et al.- Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. *Medicina (B. Aires)*, 56: 709-711, 1996.
6. HJELLE, B.; JENISON, S.; GOADE, D.; GREEN, W. & FEDERSON, R.- Hantaviruses: clinical, microbiologic and epidemiologic aspects. *Crit. Rev. clin. Lab. Sci.*, 32: 469-508, 1995.
7. LAZARO, M.; RESA, A.; LEVIS, S. et al.- Distres respiratorio del adulto en El Bolsón. In: CONGRESO LATINOAMERICANO DE ZOONOSIS, 1, Buenos Aires, 1995. Resúmenes. p. 126.
8. LEVIS, S.; CALDERON, G.; BRIGGILER, A. & ENRIA, D.- Infección por un Hantavirus en pobladores y roedores de las Islas Lechiguanas del delta del río Paraná. In: CONGRESO LATINOAMERICANO DE ZOONOSIS, 1, Buenos Aires, 1995. Resúmenes. p. 128.
9. LOPEZ, N.; PADULA, P.; ROSSI, C. & LAZARO, M.- Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. *Virology*, 220: 223-226, 1996.
10. MILLS, J.; CHILDS, J.; KSIAZEK, G. & PETERS, C.- **Methods for trapping and sampling small mammals for virologic testing**. Atlanta, U.S Department of Health and Human Services, Centers for Disease Control and Prevention, 1995.
11. PALMER, D.- The 10 most common questions about Hantavirus. *Infect. Dis. clin. Pract.*, 5: 378-340, 1995.
12. SHEFER, A.; TAPPERO, J.; BRESSE, J. & PETERS, C.- Hantavirus pulmonary syndrome in California: report of two cases and investigation. *Clin. infect. Dis.*, 19: 1105-1109, 1994.
13. VITEJ, C.; BREIMAN, R.; KSIAKEK, T. et al. - Evidence against person-to-person transmission of Hantavirus to health care workers. *Clin. infect. Dis.*, 22: 824-826, 1996.
14. WARNER SCOTT, G.- Hantavirus illness in humans: review and update. *Sth. med. J. (Bgham, Ala.)*, 89: 264-271, 1996.
15. WEISSENBACHER, M.; MERANI, M.; HODARA, V. & VILLAFÁÑE, G.- Hantavirus infection in laboratory personnel and wild rodents in Argentina. *Medicina (B. Aires)*, 50: 43-45, 1990.
16. ZEITZ, P.; BUTLER, J.; CHEEK, J. et al.- A case-control study of Hantavirus pulmonary syndrome during an outbreak in the Southwestern United States. *J.infect. Dis.*, 171: 864-870, 1995.

Recebido para publicação em 20/03/1997

Aceito para publicação em 06/08/1997