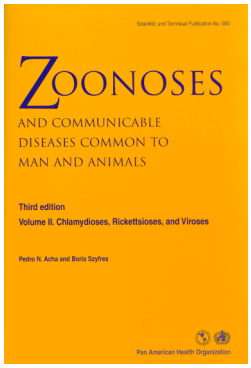


From Zoonoses and Communicable Diseases Common to Man and Animals, Third Edition. Volume II: Chlamydioses, Rickettsioses, and Viroses.
By Pedro N. Acha and Boris Szyfres.
ISBN 92 75 31992 8
www.paho.org/

This section, reproduced in full from the above book, contains information on Virus Etiology, Geographic Distribution and Occurrence, The Disease in Man, The Disease in Animals, Source of Infection and Mode of Transmission, Diagnosis and Control.

ARGENTINE HEMORRHAGIC FEVER

ICD-10 A96.0 Junín hemorrhagic fever

Synonyms: Junín hemorrhagic fever, Junín disease, stubble disease, O'Higgins disease, northwestern Buenos Aires hemorrhagic virosis, endemic-epidemic hemorrhagic virosis.

Etiology: Junín virus, a segmented single-strand RNA genome virus belonging to the genus Arenavirus, family Arenaviridae. The virions of this family, the prototype of which is the lymphocytic choriomeningitis virus, are ovoid or pleomorphic, measuring 110 to 130 nm in diameter (or as much as 300 nm in rare cases), and have a lipoprotein envelope. A characteristic of the family, from which it derives its name, are the sand-like particles seen in the interior of the virion with electron microscopy. The particles come from ribosomes of the parasitized cell which are engulfed by the virion when they are released from the cell.
The Junín virus belongs to the Tacaribe complex (New World arenaviruses), which is composed of the following viruses: Allpahuayo, Amapari, Bear Canyon, Cupixi, Flexal, Guanarito, Junín, Latino, Machupo, Oliveros, Paraná, Pichindé, Pirital, Sabiá, Tacaribe, Tamiami, and Whitewater Arroyo (Charrel *et al.*, 2002). Junín virus, Machupo virus (the agent of Machupo, or Bolivian, hemorrhagic fever) (Weissenbacher and Damonte, 1983), Guanarito virus (the agent of Venezuelan hemorrhagic fever), and Sabiá virus (the agent of Brazilian hemorrhagic fever) are pathogenic for humans. Laboratory infections have been produced with the Pichindé and Tacaribe viruses (Johnson, 1981).
Viruses of the Tacaribe complex share an antigenic affinity with the Old World arenaviruses, genus Arenavirus, which include the agents of Lassa fever in Africa and lymphocytic choriomeningitis in the Americas and Europe.
With the exception of Tacaribe virus, for which bats are the reservoir, the reservoirs of the arenaviruses are rodents, which carry a persistent infection.

Geographic Distribution and Occurrence: Argentine hemorrhagic fever (AHF) is found over a large area of Argentina's humid pampas, where the main agricultural crops are corn and other grains. The endemic area is inhabited by a population of more than 1 million and covers approximately 120,000 km^2 in portions of the following provinces: Buenos Aires (northwest), Córdoba (southeast), Santa Fe (south), and La Pampa (east). Recent studies have shown that the virus is active outside its known endemic areas, as indicated by the fact that it was isolated from the field mouse *Akodon azarae* in the village of Pila located in southwest Buenos Aires Province. Antibodies were also found in 2 of Pila's 449 inhabitants, although no human cases of the disease were confirmed (Weissenbacher *et al.,* 1983a). The first epidemics occurred in 1953 and 1954, and the etiologic agent was first isolated in 1958. Since then there have been annual epidemics of varying intensity. Over a 23-year period (1958-1980) more than 18,000 clinically confirmed cases of Argentine hemorrhagic fever were reported, with a case fatality rate of

between 10% and 15% in untreated cases. The number of cases peaked every two or three years, and the largest epidemic was in 1964. The last four years of this period saw a downward trend, from 989 cases in 1977 to 161 in 1980 (Pan American Sanitary Bureau, 1982). During the 1981-1983 period, there was an average of 302 cases a year (Argentina, Ministry of Social Welfare, 1981-1983). In 1990, there were 727 cases; in 1991, 154 cases; and in 1992, only 2 cases. More of these cases occurred in the province of Córdoba than in the province of Buenos Aires (Argentina, Ministry of Public Health and Social Action, 1992). In a survey of the rural population in these two provinces conducted 14 years after the first appearance of AHF, neutralizing antibodies were found in 12% of the Cordoba inhabitants (7.6% with clinical disease and 4.4% with subclinical infection) and 11.6% of those in the province of Buenos Aires (9.7% with clinical disease and 1.9% with subclinical infection) (Weissenbacher *et al.,* 1983b).
The disease mainly affects the rural population, and in particular those involved in the harvesting of corn and other grains, who are mostly male migrant workers. This trend coincides with the higher incidence of AHF in adult males. Most of the cases occur in autumn, between April and July, with the highest number usually in May. This seasonal distribution coincides with an increase in agricultural activities that facilitate contact with the rodent reservoirs of the virus, whose population also peaks at the same time of year.

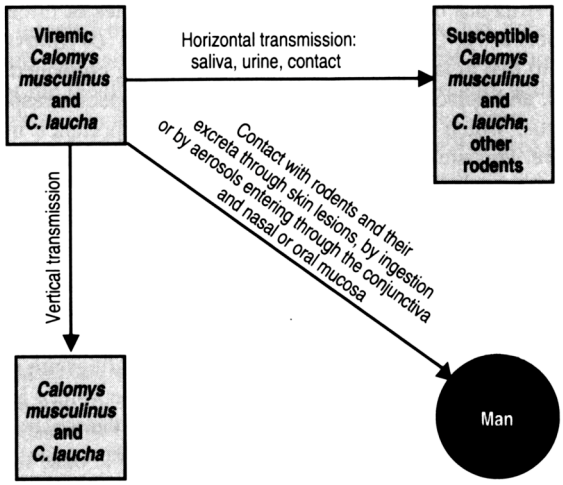
The Disease in Man: The incubation period is from 10 to 16 days. The symptoms are similar to those of Machupo hemorrhagic fever, and their severity varies. The disease has an insidious onset. Its clinical manifestations are fever, malaise, chills, fatigue, dizziness, cephalalgia, and dorsalgia. Most patients experience conjunctival congestion, retro-orbital pain, epigastralgia, halitosis, nausea, vomiting, and constipation or diarrhea. Other symptoms are increased vascularization of the soft palate, axillary and inguinal adenopathy, petechiae on the skin and palate, and a congestive halo on the gums. Leukopenia, thrombocytopenia, albuminuria, and cylindruria are always present. The fever is constant and lasts for five to eight days. The symptoms that appear after day four include epistaxis, gingival hemorrhaging, slowed mental response, unsteady gait, hypotension (in 75% of patients), bradycardia, muscular hypotonia, and osteotendinous hyporeflexia.
In its mild form, the disease lasts about six days. In contrast, serious hemorrhagic cases are marked by hematemesis and melena, as well as more pronounced epistaxis and gingival hemorrhaging. When neurologic symptoms are predominant, the patient manifests muscular tremors in the tongue and hands, confusion or excitability, and sometimes tonic-clonic convulsive seizures. The intermediate forms are the most common and are seen in about 60% of patients. Convalescence lasts for several weeks, and sequelae are rare. After an apparent recovery, some patients develop a cerebellar syndrome, which clears up after several days without further consequences. In a group of 130 laboratory-confirmed patients, 12 (9%) died (Argentina, Ministry of Social Welfare, 1971-1974). In untreated cases, the administration of human plasma made it possible to reduce the case fatality rate from 15%-20% to less than 3% (Carballal *et al.,* 1991).

The Disease in Animals: As with the other arenavirus infections, rodents are the reservoir for maintenance of the Junín virus in nature (the exception is Tacaribe virus, for which bats are the reservoir). The main hosts of the Junín virus are the cricetid rodents *Calomys musculinus*, *C. laucha*, and *Akodon azarae*. Experimental inoculation of *C. musculinus* and *C. laucha* with field strains of Junín virus showed that the infection in those animals was asymptomatic, regardless of the age of the animal, the route of administration, or the amount of virus injected (Sabattini *et al.,* 1977). Experimental.infection with *Akodon* produced symptoms only when this animal was inoculated during the first week of life (Weissenbacher and Damonte, 1983).

Source of Infection and Mode of Transmission (Figure 9): The risk is not the same throughout the endemic area. Between 1965 and 1974, a total of 8,728 cases were reported, and 3,075 (35%) of these were from the parish of Pergamino in the province of Buenos Aires. The vicinity within which most of these 3,075 cases were contracted represents a very small portion of the entire area affected by hemorrhagic fever. The disease is more prevalent in males than females (4:1) and it occurs especially

among rural laborers. The seasonal changes parallel the variations in the rodent population and also the degree to which workers are exposed to the predominant rodent species. A sizable outbreak occurred in this same area in 1977; from April to June, more than 300 cases were reported (Bond, 1977).

Figure 9: Argentine hemorrhagic fever. Possible transmission cycle of the Junín virus.



As mentioned earlier, the epidemic curve follows the variation in the density of the rodent population: the highest incidence occurs in autumn (April to July), which corresponds to the peak number of rodents, and the drop in human cases during the winter coincides with the sharp decline in the rodent population.

The Junín virus has been isolated from several species of cricetids, including *A. azarae*, *A. obscurus*, *C. musculinus*, *C. laucha*, and *Oryzomys nigripes*. These rodents tend to live in tall brush along fences enclosing cultivated fields, roadsides, stream banks, and railroad tracks. The various rodent species respond differently to the Junín virus, which may suggest their relative importance in maintaining the agent in nature. Persistent viremia was observed in specimens of *C. musculinus* that were captured, released, and then recaptured twice or three times at intervals of up to 55 days. It was verified that under natural conditions they shed the virus through their urine. Experimental inoculation of newborns confirmed that this rodent is chronically infected; although it does not manifest clinical symptoms, it has persistent viremia and sheds the virus through buccopharyngeal secretions and urine. When complement-fixing antibodies are present in adult animals, the infection produces viremia and viruria of shorter duration. These findings are similar to observations with *C. callosus*, which is considered the main reservoir of Machupo virus, the agent of Machupo hemorrhagic fever. It has also been demonstrated that the infection in *C. musculinus* is transmitted both vertically and horizontally (Sabattini *et al.,* 1977). Although the population of this cricetid declines sharply in winter, the virus survives in nature because of the persistent viremia that is characteristically seen in this species. From the trapping of rodents in the endemic areas of Cordoba Province, it was learned that *C. musculinus* was the most abundant species, and the virus was isolated from a very high proportion of the specimens caught. In a study involving the capture, release, and recapture of *C. musculinus* in the south of Santa Fe Province and the north of Buenos Aires Province, it was shown that the animals which were antigen-positive in the enzyme-linked immunosorbent assay (ELISA) were predominantly males (76%). In light of the greater mobility of males and their increased likelihood of being wounded, this result suggests that the primary route of virus transmission is horizontal (Mills *et al*., 1992). Since *C. musculinus* prefer to live along the borders of cultivated fields, researchers contend that humans contract the infection in these areas more frequently than in the fields as such. The virus was also isolated from 4 out of 40 captured *Akodon*, but their scant numbers in cultivated fields would indicate that this rodent plays a limited role in the epidemiology of AHF (Sabattini *et al.,* 1977).

It is believed that the emergence and subsequent expansion of AHF has been due to disruptions in the

environment generated by the cultivation of grains that favor the *Calomys* populations (Villafañe *et al.,* 1977).
The type of crop under cultivation is also an important factor in the ecology of the virus. Rodent densities, especially those of *Calomys* populations, are lower in soybean fields compared with corn and sunflower fields. In areas where the cultivation of soybeans has increased, there has been a reduction in the cases of AHF (Kravetz *et al.,* 1981, cited in Weissenbacher and Damonte, 1983).
Although the virus has been isolated from mites found on the rodents, it has not been shown that these parasites can transmit the virus, and so far they are not considered to play a role in the ecology of the virus or the epidemiology of the disease. The fact that the virus can be isolated from oral swabs and the urine of *Calomys* indicates that these secretions are the main sources of the virus in the transmission of infection to other members of the species, and perhaps to other rodent species with which they come in contact. It has also been possible to demonstrate transmission from a mother to her litter and between animals placed in the same cage. There is no doubt that *Calomys* plays an important role in the natural cycle of the virus.
Man becomes infected by contact with infected rodents and their excreta. The routes of penetration in man may be through skin lesions, the ingestion of contarninated products, or the inhalation of aerosols that come in contact with the conjunctiva and the oral or nasal mucosa. These portals of entry have been corroborated in the laboratory. Human-to-human transmission is uncommon, but precautions should be taken. As in the case of Machupo hemorrhagic fever, intimate contact may lead to contagion, since viremic patients can have hemorrhages, and the virus has been isolated from pharyngeal swabs and the urine of patients.

Diagnosis: At one time, AHF was known as the "rubber stamp disease" because its signs and symptoms made it easy to diagnose. However, subsequent studies have shown that only 60% of cases can be correctly diagnosed on the basis of clinical examination alone. Presumptive diagnosis is based on the patient's epidemiologic history (i.e., occupation as a migrant worker or residence in an endemic area) and laboratory analyses, which consist of identifying leukoplaquetopenia, round cells in the urine, and inversion of the ratio of CD4+ ("helper") lymphocytes to CD8+ cytotoxic suppressors (Carballal *et al*., 1991). Up until 1965, virologic diagnosis of the disease was only attempted in a few of the cases identified. During the period 1965-1974, diagnosis was confirmed by virologic studies in 64% of the cases reported. Specific diagnosis is accomplished by isolating the virus or using serologic tests on acute- and convalescent-phase sera. It can also be isolated by inoculating the blood of febrile patients or autopsy material intracerebrally in suckling mice, or intraperitoneally or intramuscularly in guinea pigs, and observing whether the animals develop hemorrhagic lesions similar to those seen in man. The virus can also be isolated by culturing patient blood on monolayer Vero cells, in which case the virus produces a cytopathogenic effect, and by demonstrating the presence of viral antigen with immunoperoxidase stain (Lascano *et* al., 1981). This procedure, which gives results in two to eight days, is more rapid than inoculation in laboratory animals. The serologic tests used most often are complement fixation, serum neutralization, and indirect immunofluorescence. The complement fixation test is the least sensitive, and the antibodies that it detects appear late and disappear rapidly. The serum neutralization test is the most specific, detecting antibodies at three to four weeks after onset of the disease, while the indirect immunofluorescence test gives the earliest results and is rapid, economical, and simple to perform (Sarnoilovich *et al.,* 1983). In a group of 50 individuals who had had AHF between 1 and 14 years earlier, the indirect irnmunofluorescence test detected antibodies in 88% and the serum neutralization test in 96%, while the complement fixation test found antibodies in only 30% of the persons studied (Darnilano *et al.,* 1983).
The most commonly used tests are ELISA and indirect immunofluorescence, because they are capable of detecting IgM and IgG (Carballal *et* al., 1991). Reverse-transcription--polymerase chain reaction (RT-PCR) techniques for diagnosing AHF have also been developed (Lozano *et al.,* 1993; Lozano *et al.,* 1995).

Control: In Bolivia, it was shown that an urban epidemic of hemorrhagic fever caused by the Machupo virus could be checked by controlling rodents that had acquired peridomestic habits. However, application

of this measure would be very difficult and costly in the agricultural areas of Argentina. Instead, hopes are directed toward the development of a safe and effective vaccine. The effort to obtain an inactivated vaccine appears to have been abandoned, and greater attention is now being given to live attenuated vaccines. Vaccines based on attenuated strains of XJC13, XJO, and Candid #1 have been developed and tested in laboratory animals. The preparation based on the XJC13 strain was administered to a total of 636 human volunteers, in which it induced a subclinical infection or mild clinical symptoms, and neutralizing antibodies lasted from seven to nine years in 90% of the subjects. Intracerebral inoculation of the strain in guinea pigs and *Cebus* monkeys produced neurovirulence, but in the marmoset *Callithrix jacchus*, which is considered a reliable animal model for AHF, the strain provided good protection (Weissenbacher and Damonte, 1983).

The vaccine based on the attenuated Candid #1 strain offers the greatest promise in terms of being both safe and effective. Trials conducted in laboratory animals have shown high seroconversion titers, very good protection against virulent strains, and no neurovirulence in monkeys. When this vaccine was given to human volunteers, there were no complications whatsoever and the levels of seroconversion were satisfactory (Barrera Oro, J., personal communication, March 1986; Barrera Oro and McKee, 1991; McKee *et al.,* 1993). This vaccine was the subject of a cooperative international project. Starting with larger-scale preclinical trials, its safety and immunogenicity were confirmed in 300 volunteers in Argentina and the United States. Next, a random, double-blind, placebo-controlled prospective study was undertaken in 1988-1990 with 6,500 volunteers from 41 localities in the endemic area. When the results clearly demonstrated the efficacy of the vaccine, the immunization project was extended to 100,844 persons. No adverse reactions have been observed; the duration of immunity and persistence of antibodies have yet to be determined (World Health Organization, 1993).

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