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EPIDEMIOLOGY OF ARGENTINE HEMORRHAGIC FEVER

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Present knowledge points to horizontal transmission as the most significant mechanism for Junín virus maintenance in the main natural reservoirs, namely *Calomys musculinus* and *Calomys laucha.*

The existence of naturally infected *Akodon azarae,* both within and outside the endemic area, as well as the finding that other species, ecologically and phylogenetically related to the main reservoirs, such as *Akodon molinae* and *Calomys callidus,* can experimentally develop persistent infections with virus shedding through fauces, suggest a potential role for these cricetids as alternative reservoirs.

Furthermore, since those rodents inhabit the borders of the area in which Argentine Hemorrhagic fever is endemic, the risk of spread of this area is to be expected.

Whether the establishment of Junín virus persistence in *Calomys musculinus* and other reservoirs depends on viral or host factors, such as a selective defect in L3T4$^+$ lymphocytes as recently shown for mice, remains to be explored.

INTRODUCTION

Argentine Hemorrhagic Fever (AHF), is an endemoepidemic disease affecting one of the richest farming of Argentina. Junín virus (JV), the etiological agent of this disease, is a member of the Tacaribe Complex within the Arenaviridae family.

Arenaviruses have been extensively studied because of their remarkable capacity to induce persistent infections in natural reservoirs and severe immunopathology in experimental models, either by T-cell mediated mechanisms in acute infections and/or by immunocomplexes in persistent infections (6, 47). Both conditions are of considerable interest in human pathology since these mechanisms are ascribed with increasing frequency to viral infections (100).

JV was isolated from blood and organs of AHF patients in Junín city, independently by Parodi and Pirosky in 1958, and the virus was recovered from different species of wild rodents belonging to the *Calomys, Akodon* and *Muroidea* genera a few years later (72).

Here, we have attempted to review the literature on epidemiological aspects of AHF and JV reservoirs.

SOME FEATURES OF ARENAVIRUS INFECTION IN WILD RODENTS

The Arenaviridae family includes 2 groups: a) The Old World arenaviruses, Lymphocytic choriomeningitis virus (LCM) and the African arenaviruses, Lassa, Mopeia, Ippy and Mobala viruses, and b) The New World arenaviruses, or Tacaribe Complex viruses, which include Tacaribe, Machupo, Junín, Amaparí, Paraná, Latino, Tamiami, Pichindé and Flexal viruses.

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Arenaviruses are maintained in nature by persistent infection in wild rodent reservoirs, with the sole exception of Tacaribe virus, which was recovered from fruit bats (*Artibeus* sp.).

Although the dissemination of virus from rodents to other species is unusual, human infection, both in nature with LCM, JV, Lassa, Machupo or Flexal and in the laboratory with LCM, Junín, Lassa, Pichindé or Tacaribe, have been documented (41).

According to their capacity to induce disease in humans, arenaviruses are classified as pathogenic or non-pathogenic. The human-pathogen arenaviruses are: JV, agent of Argentine hemorrhagic fever; Machupo, agent of Bolivian hemorrhagic fever; Lassa, agent of African hemorrhagic fevers and LCM, responsible for mild or inapparent infections.

LCM virus persists in *Mus musculus* (domestic mice), thus explaining its world-wide distribution. In contrast, the remaining pathogenic arenaviruses persist in wild cricetids whose habitats are restricted, thus explaining the appearance of disease only in circumscribed areas.

JV reservoirs are cricetids belonging to the *Calomys* and *Akodon* genera, the most important being *Calomys musculinus.*

Machupo virus persist in *Calomys callosus* and Lassa viruses in *Mastomys natalensis.*

The principal mechanism for infection maintenance in wild rodents seems to be horizontal transmission, due to virus shedding through saliva and urine. Prenatal vertical transmission has been confirmed only for LCM virus, while for the remaining arenaviruses, transplacental and/or sexual cell passage in wild reservoirs have not yet been clearly established (41, 61).

Experimental arenavirus infection in laboratory-bred wild rodents may present a diversity of patterns according to both virus and host. As for the former, strain, passage history, dose and inoculation route are all crucial. As far as the host is concerned, genus, species, genotype, age, degree of viral antigen expression in certain target organs and ability to mount an adequate immune response are particularly relevant to the outcome of the infection.

The most widely studied model of arenavirus infection is LCM virus in mice. Neonatal or uterine LCM inoculation in *Mus musculus* leads to persistent infection featuring virus detection during the animal's entire life. Traditionally, virus persistence has been attributed to a lack of immune response, a finding regarded as the paradigm of of immunological tolerance. However, it was later demonstrated that LCM-carrier mice exhibit renal lesions in adult life due to immuno-complex deposition indicating a humoral immune response (reviewed in 47 and 6).

The recent development of hybridization techniques has been able to demonstrate larger amounts of LCM viral nucleic acids in persistently infected animals with respect to acutely infected animals. This accumulation of virus nucleic acids suggests incomplete or defective virus formation « in vivo » (36).

During LCM persistence, although antibodies to viral proteins are produced, the virus-specific H_2-restricted cytotoxic T lymphocyte (CTL) response is lower than that occurring in acute infections. This selective defect is thought to be one of the reasons for the establishment of the persistent state (102, 67).

When adult *Mus musculus* are inoculated with LCM virus, an acute infection is produced, the outcome of which depends on the dose, inoculation route, viral strain tropism, passage history and even on the mouse strain. The infection may lead to death from immunopathological tissue damage, or to remission with viral clearance. Clearance is mainly dependent on virus-specific, H_2-restricted CTLs, and the failure to generate this response allows the establishment of viral persistence (67, 2).

Whether this mechanism also operates in the remaining arenaviruses and their natural reservoirs has not yet been clearly demonstrated.

Machupo virus does not induce acute disease in suckling or adult *Calomys callosus,* although animals can be infected by intranasal (IN) or parenteral routes. Adults may develop either a persistent infection, featuring splenomegaly, anemia, retarded growth, shorter life-span and reduced fertility, or an immune response with neutralizing antibodies, fostering viral clearance.

Suckling *Calomys callosus* inoculated with Carvallo strain invariably produced chronic infections with viremia and virus shedding through urine and saliva. The growth of chronically infected rodents was retarded (99). Coincidently, similar findings were shown for mice persistently infected with LCM, which was attributed to viral interference with synthesis of growth hormone (66).

When a high dilution of Machupo was used, 90% of adults developed persistent infections with high viremia. The percentage decreased to 50% if larger doses of virus were inoculated (99). Remarkably, the ratio of viremic animals to responders was changed for a constant virus dose by cross-breeding studies, suggesting genetic host factors involved in determining the response. The infertility observed and the detection of virus in ovaries lead to the hypothesis that Machupo virus may regulate the size of *Calomys callosus* population. If epizootics occur among rodents genetically dominant for the viremic response, an increase in the number of infertile females should follow resulting in a population crash. However, there is no evidence that this occurs in nature.

Human infections, when correlated with high rodent densities and prevalence of virus in *C. callosus*, were much higher in those areas (60).

The first studies in Sierra Leone shown that Lassa virus spreads by enzoonotic transmission in *Mastomys natalensis*. However, it was later shown that *Rattus rattus* and *Mus minutoides* in the Benue-Plateau and Eastern North Eastern status of Nigeria were also infected (64).

Usually, man becomes infected with Lassa by contact with infected rodents although secondary transmission between persons is frequent, especially within domiciliary groups or in common environmental exposure, e.g. in the hospital. Lassa virus apparently causes overt disease only in man. In contrast, *Mastomys natalensis* develops persistent infections, regardless of age, with continuous virus shedding through saliva and urine.

Suckling animals show infectious virus and viral antigens in spleen, liver, kidney and lung, although no significant histopathological changes were observed. In adults, a tropism for spleen and lymph nodes was detected together with signs of meningoencephalitis (86).

Of the arenaviruses non-pathogenic to man, Tamiami is the only one that has been well studied in its natural reservoir *Sigmodon hispidus*. Suckling animals develop a persistent infection with marked viral lymphoreticular tropism (65).

JUNIN VIRUS, ETIOLOGICAL AGENT FOR ARGENTINE HEMORRHAGIC FEVER

JV shares the main features of the Arenaviridae family, including a single strand RNA genome, round, oval or pleomorphic particles measuring 50-300 nm, containing a variable number of electron-dense ribosome-like granules; lipoprotein envelope originating by budding from the plasma membrane of the infected cell; spikes on this envelope, the presence of which is controversial in JV; and maintenance in nature by persistence in wild rodent reservoirs.

The biochemical properties and replicative cycle of JV, as well as the pathology in experimental infections, have been reviewed in (98).

JV genome is made up by 2 large species of RNA, 33 and 25 S (2.4×10^6 and 1.3×10^6, respectively) and 3 small pieces of RNA of 4, 5 and 5.5 S. Two additional 28 and 18 RNAs, probably of cellular origin, were also found (39).

Six structural viral polypeptides, with MW of 91 KD (VP1), 72 KD (VP2), 64 KD (VP3), 52 KD (VP4), 38 KD (VP5) and 25 KD (VP6), were found in purified virions analyzed by SDS-PAGE. VP3 and VP5 are major components. VP1, VP2, VP4 and VP5 are glycosylated and recently called G91, G72, G52, and G38. NP64 is assumed to be the nucleoprotein and G38 is the major envelope glycoprotein responsible for the induction of neutralizing antibodies (57).

As a member of the Tacaribe complex within the Arenaviridae family, JV strongly cross-reacts with other Tacaribe complex viruses by complement fixation (CF) and immunofluorescence (IF). It reacts weakly with the Old World Arenaviruses LCM, Lassa and Mozambique viruses (101). In spite of the antigenic distance, LCM and Junín can interact; LCM was isolated from *Mus musculus* captured in the AHF endemic area (78) and non-homologous reassortants between LCM and JV were recently shown by mixed infection in tissue culture with ts mutants (75).

Within the Tacaribe complex, the closest relationship has been found between JV and Tacaribe. Tacaribe has not been shown to be pathogenic for guinea pigs, marmosets or man. In contrast, Tacaribe infection induces, in guinea pigs or primates, heterologous neutralizing antibodies and full and long-lasting protection against JV (89). Recently, it has been suggested that JV and Tacaribe share at least one antigenic determinant in their surface glycoproteins (28). Those findings, together with the lack of both overt immunopathologic disease and viral persistence in Tacaribe-infected guinea pigs, support the use of Tacaribe virus as a potential heterologous vaccine against AHF (15).

JV multiplies in cells of different origin, although replication is usually poor and no cytopathic effect (CPE) is detected. However, in some subline Vero and MRC-5 cells, a characteristic CPE is produced and suitable plaque assays have been developed.

Two types of particles are produced: UV-sensitive plaque-forming units and CPE-producer particles and UV-resistant interfering particles (IP), which are neutralized by anti-Junín immune sera. IP particle synthesis is favored in cells infected at high moi or when serial passages of undiluted virus are performed. IP appear to modulate CPE in cells and it has been suggested that they could play a role in the establishment of viral persistence both «in vivo» and «in vitro» (27).

Persistent JV infections « in vitro » have been produced in several cell lines of different origins including human cells such as MRC-5 (reviewed in 98).

THE ENDEMIC AREA FOR ARGENTINE HEMORRHAGIC FEVER

The AHF endemic area occupies a large agricultural and cattle-raising region, near the main cities of the country. The disease was first described in 1955 as a clinical entity in epidemic outbreaks in Bragado city (Buenos Aires province), although it had been reported as early as 1943. The etiologic agent was isolated for the first time in 1958 (72).

In 1958-62, the endemic area was restricted to 16,000 km² with an estimated population of roughly 268,000 inhabitants in the northwest of Buenos Aires province. A gradual spread has been observed over the last two decades with a five-fold increase. Latest surveys indicate an area of more than 120,000 km² with an exposed population of 1,500 000 (Fig. 1) (52).

At present, the endemic area occupies the north-west of Buenos Aires province, the south of Santa Fé and the south-east of Córdoba provinces (Fig. 2). Epidemic outbreaks peaking in late autumn, coinciding with the corn harvest, have been recorded without interruption since 1958. These peaks are closely related to population dynamics of JV-bearing rodents in nature (49).

The high prevalence among male rural workers from 20 to 50 years of age is due to a greater exposure. From 1958 to 1987 the total number of AHF cases reached roughly 21,000, with an annual mean of 360 cases for the past 5 years (Fig. 3). (Maiztegui J.I. personal communication).

The changing incidence of the disease reported for the different endemic areas (Fig. 2) is, as yet, unexplained (52).

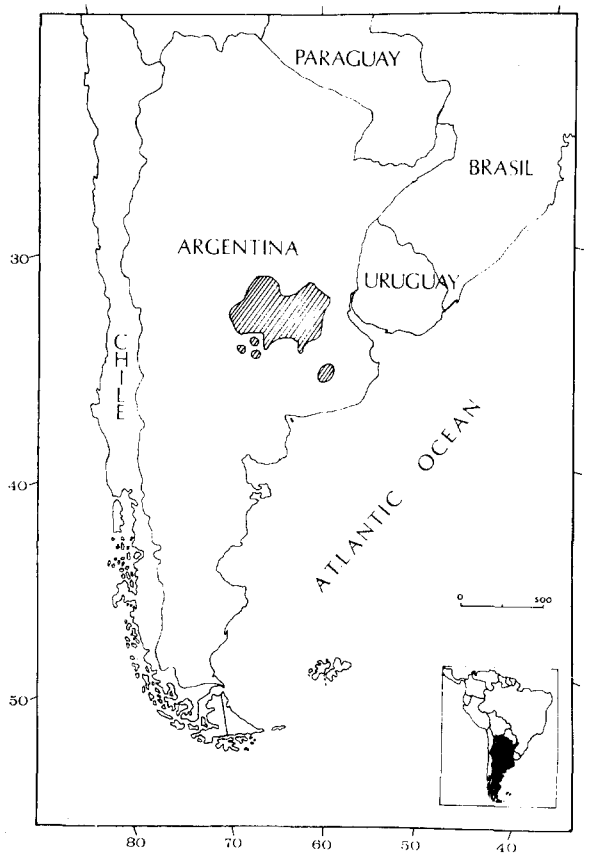

Figure 1. - Map of Argentina showing the Argentine Hemorrhagic Fever endemic area.

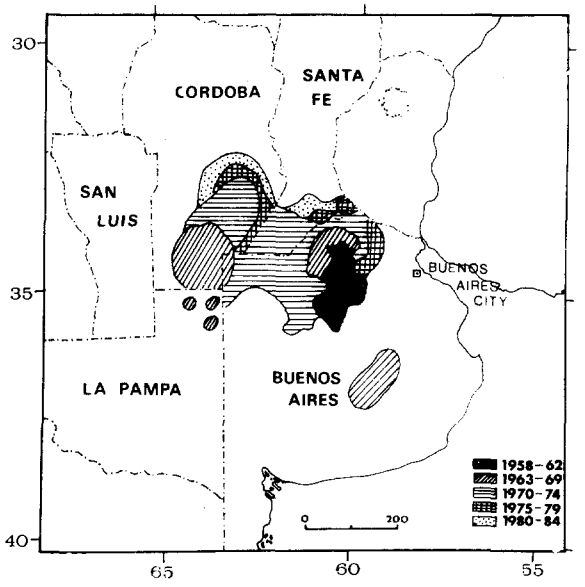

Figure 2. - Progressive extension of the Argentine Hemorrhagic Fever endemic area.

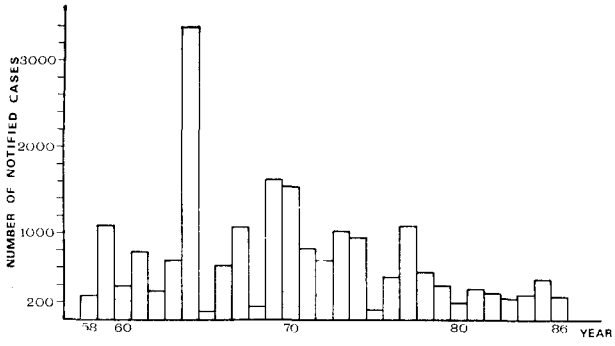

Figure 3. - Annual distribution of Argentine Hemorrhagic Fever cases.

Seroepidemiological surveys showed that inapparent or subclinical JV infections occur in the endemic area in approximately 4% of residents, while 96% of the local population remains susceptible to infection (93). The reasons for inapparent infection are still obscure since the existence of JV strains having lower virulence for man is not supported by available evidence. However, opportunities for virus transmission from rodent to man may change; for example, in initial outbreaks, the disease primarily affected people working in the corn harvest. Coincidently, cornfields were shown to have higher rodent densities than soybean fields (43).

On the basis of this finding, it was suggested that soybean cultivation should be favored over corn and sunflower cultivation in order to set up an ecological control for AHF (7). However, the association between crops and incidence of the

disease has not yet been clarified for the different endemic areas (52).

Since the endemic area is defined by the presence of clinical cases, there may be regions, as yet undescribed, with JV-infected cricetids. A shift in crop management in these areas or other factors may increase human exposure, thus leading to the appearance of clinical cases. This hypothesis was confirmed in 1983 by the detection of JV activity in the Pila locality, 280 Km south west of the endemic area by virus isolation from two wild *Akodon* and demonstration of neutralizing antibodies in two local inhabitants with no previous history of the disease (94).

Furthermore, there may be other species, different from the main reservoirs which could also play a role in the maintenance of the virus in nature. The finding that *Akodon molinae*, and *Calomys callidus*, although not yet described as virus carriers in nature, can be experimentally infected and develop persistent infections with virus shedding through saliva, supports this hypothesis.

JUNIN VIRUS ISOLATION FROM NATURE

In the endemic area the most commonly captured rodents are *Calomys musculinus*, *Calomys laucha*, *Akodon azarae*, *Bolomys obscurus* and *Oryzomys flavescens*, all belonging to the Cricetidae family, and *Mus musculus*, a member of Muridae.

Naturally JV-infected species include *C. musculinus*, *C. laucha*, *A. azarae* and *M. musculus*. In the endemic area of Cordoba province, JV was recovered from fauces from 28% of the *C. musculinus*, 20% of the *C. laucha* and to a lesser extent from *A. azarae* and *M. musculus* (79). These figures highlight the importance of *Calomys* for JV maintenance in nature.

However, the search for naturally infected cricetids in regions surrounding the endemic area is still under way. The few studies conducted thus far have yielded positive results.

JV was isolated from wild rodents in areas far removed from the known endemic AHF zone. In 1961, Parodi et al. recovered JV from a single rodent captured in the region of Pila (Buenos Aires province). Remarkably enough, immunofluorescent and neutralizing antibodies were detected in two local inhabitants and JV was recovered from two *Akodon* out of 200 cricetids captured, 20 years later (94). Therefore, it seems that JV is not restricted to the known AHF area and the future appearance of clinical cases in hitherto unrecognized zones is to be expected.

ECOLOGICAL BEHAVIOUR OF RODENT POPULATIONS

The AHF endemic area is disturbed with continual changes throughout the year, related to agricultural activities. Rodent populations are distributed according to their habitat preferences, population strategies and synchronization of their biological cycles with the crops.

The main JV-bearing species in nature, *Calomys musculinus* and *Calomys laucha*, occupy croplands subject to continual disturbances which these species are able to tolerate since they make low demands on habitat quality.

On the other hand, less disturbed environments, such as field borders, where autocthonous vegetation was been preserved, are inhabited by other species having higher habitat demands, preferring greater green coverage, a group including *A. azarae* and *B. obscurus* (31).

The reproductive period of all these cricetids extends from midspring to early autumn. Lower densities are recorded in spring and early summer, then increase exponentially to drop in July-August (mid-late winter) (19).

Since JV-infected *Calomys musculinus* and *Calomys laucha* are found in areas devoted to farming and rodent density peaks during autumn harvest, they are the most important species in the trasmission of JV infection to rural workers.

Every 3 or 4 years, long-term populational cycles of rodents are detected. Thus, *Calomys* invasions were shown in 1964, 1967, 1974 and 1977 in various areas of Buenos Aires province. This finding coincided with the greater number of human AHF cases reported for those years (52). (See Fig. 3).

Several proposals have been made to control AHF spread. One is to fence in *Calomys* populations to foster the development of the *Akodon* genus (43). However, this suggestion would be feasible only if *Akodon* were not allowed to replace *Calomys* in the AHF epidemiological chain, a risk which cannot be ruled out.

Another proposal, based on the fact that *Calomys* density is greater on ungrazed or corn-sown land, is to encourgae soybean production over that of corn, as well as intensive field pasturage before sowing (7).

THE HUMAN DISEASE

Routes of infection

JV gains entry to the human body by cutaneous (skin abrasions) and/or mucosal (conjunctival, oral and/or nasal) routes by inhaling contaminated dust in the field during manual or mechanized harvest or plowing. Such routes of entry

have been documented by both accidental laboratory infections (90) and experimental infections in animal models (81). Interhuman transmission, although infrequent, has also been described between couples (53).

Usually, human infection with wild strains leads to clinical disease; however, subclinical and inapparent infections have been described both in the endemic area (2.5 to 4.7%) and in 20% of laboratory-acquired infections, detected by the presence of antibodies in the absence of overt disease (93, 90).

Whether inapparent infections are due to the existence of lower-virulence strains or to other factors, such as differences in susceptibility mediated by histocompatibility antigens or to variations in the immune response, is, as yet, unknown.

Finally, persistent JV infections have been described in cell culture (98), in wild reservoirs (79) and also in some experimental models, such as guinea pigs (55), rats (97) or congenitally athymic mice (92, 96, 71).

However, no persistent infections have yet been demonstrated in man.

Clinical features

Clinical forms of the disease are abortive (2%), mild (20%), common (70%), severe (5%) and mixed (3%).

The main features of the common forms are as follow: after an 8-12 day incubation period, fever, asthenia, dizziness, retro-ocular and muscular pain, lymph node enlargement and cutaneous and pharyngeal petechiae appear. Pronunced leukothrombocytepenia, bone marrow depression and impaired platelet survival are usually found. Hemorrhagic dyathesis, although extremely variable in severity, is practically constant and various degrees of myocardial, renal, liver and CNS involvement may be observed (49). Albuminuria, oliguria, hyaline and granular casts, together with round or so-called « Milani cells » in urine are the main manifestations of renal involvement. Moderate bradycardia, hypotension and, in severe cases, a clinical picture resembling a « shock syndrome » are observed. Neurological signs and symptoms (tremor, ataxia, extrapyramidalism) suggest diffuse CNS involvement, with absence of meningeal signs (77). Acute illness lasts for ten days and roughly 90% of cases recover without sequelae after a 2-3 week convalescence. Some 10-16% of all cases prove fatal after 2 weeks of evolution with severe CNS involvement whose physiopathology is as yet unknown.

Mortality was drastically reduced to 1-2% with the introduction of antibody-rich convalescent plasma for the treatment (51). However, 8-10% of treated patients show CNS involvement such as ataxia, nystagmus, cerebellar tremors and gait lateralization, attributable to immunopathological mechanisms during convalescence (35).

Diagnosis and immune response

Diagnosis is confirmed either by virus isolation from blood or organs or by serological conversion. Complement fixing (CF) and neutralizing antibodies appear in late convalescence, 4-6 weeks after onset (49).

A depression of both E and EAC rosette-forming lymphocytes has been observed during the acute stage. Thus, it was suggested that immunodepression caused the virus may account for the delay in antibody production (1).

Recently, during the acute stage, a significant decrease in numbers of $OKT4^+$ peripheral lymphocytes has been reported which returned to normal levels in convalescence. Cytotoxic suppresor lymphocyte number remained unchanged (35).

However, by employing indirect IF on persistently infected cells, circulating antibodies could be detected by the time of clinical improvement, significantly sooner than by means of CF or neutralinzing antibody tests. Thus, in patients who recovered from the disease, IF antibodies were detected in IgM or IgG in 6 cases, in IgA and IgM in 3 cases and only in IgA in 2 cases. In contrast, no CF, neutralizing or even IF antibodies could be detected in fatal cases (23).

In summary, the correlation between appearance of IF antibodies and clinical improvement suggests a beneficial role of antibodies in this infection. Previously, it had been speculated that antiviral immunocomplexes could play a role in the pathogenesis of AHF. If this were the case, the detection of antibodies when clinical improvement is evident may correspond to the switch to the antibody excess zone. Nevertheless, neither immunopathological nor complement (C) studies suggests the participation of systemic immunocomplexes in the pathogenesis of the disease in humans (29).

Therefore, the above-mentioned studies established the first rational basis for the use of convalescent plasma as the main therapeutic procedure for AHF. In addition, the long-lasting detection of IF antibodies permitted their use in epidemiological surveys in endemic areas (93, 94).

Pathology

In spite of the fact that more than 30 years have elapsed since the first description of the disease, studies on physiopathology are scarce.

JV replicates in spleen, bone marrow, lung and liver, and viremia is constant and long- lasting during the acute febrile period (49).

In a study of 12 fatal cases, generalized hemorrhoges, hypoplasia of bone marrow, reticulum cell hyperplasia in lymph nodes and acidophylic bodies with focal liver necrosis were the most significative findings. Papillary and tubular necrosis with hemorrhages in kidney and myocarditis were less frequently found (32). However, except for acidophylic bodies, none of these lesions are specific of viral infection. In half of cases, superimposed bacterial infections are observed in lung. Lymphohemopoietic tissue is intensively affected. High JV titers, lymphoid necrosis and acute and transient inhibition of hemopoiesis is observed. The recovery of bone marrow function occurs in those patients who achieved clinical remission (63).

With the exception of lymphohemopoietic tissue, the observed alterations appear to be secondary to generalized hemorrhages, to capillary lesions, to bacterial infections and/or to the «shock syndrome» (49).

In five fatal cases studied by IF and electron microscopy (EM), JV antigens were observed in reticular cells of hemopoietic tissue, in small foci of tubule cells in kidneys and in scattered hepatocytes. Virus- like particles and cytopathic effect were detected in the same cells exhibiting viral antigens (50).

These findings, together with the absence of of IGs and C3 deposits in glomeruli or in vascular kidney structures, suggested a direct cytopathic effect of JV (20).

However, it was recently postulated that some alterations observed in severe AHF forms may be associated to high levels of interferon (48).

As for the hemorrhagic syndrome, activation of coagulation (63) and C (29) were demonstrated although they were not shown to be directly related to the severity of the disease. The existence of intravascular coagulation was initially postulated, but further studies have shown that such findings were rarely found in AHF (63, 62).

CNS involvement, present in different degrees in most cases, has not yet been clearly understood. Severe clinical neurologic signs are obvious in fatal cases, though, no significant histological alterations were found (44). This points to a major difference with the mouse model in which a defined encephalitis is constantly observed (82).

Attempts to isolate JV from the CNS of three fatal cases failed although the coexistence of viral antigens and IgG, IgM and IgA was detected in capillary brain vessels (21).

Therefore, further studies are required to define whether locally formed immuno-complexes may play a role in the pathogenesis of JV infection in the human brain either in acute fatal cases or in the late neurological syndrome observed with significantly higher incidence in immune plasma-treated patients.

Treatment

The present treatment consists in the administration of human immune plasma obtained from convalescents of AHF. When treatment was given before the 8th day of clinical disease, mortality was reduced from 15-20% to less than 3% (51).

Initially, a fixed volume of 500 ml was administered. This dose was later modified in consideration of the variation in neutralizing antibody titers in immune plasma from different donors. Thus, therapeutic units are defined by considering neutralizing antibody titer, volume and body weight of the patient. According to results of a prospective study, the administration of 3000 therapeutic units/kg is recommended (34).

Immune plasma treatment is associated with the appearance of a late neurologic syndrome in 8-10% of cases. This syndrome is rarely severe and its physiopathology is unknown, although an immune-mediated mechanism with persistence of JV antigens in brain has been suggested (35). This hypothesis is supported by experimental studies on *Cebus apella* infected with JV (12, 16).

Ribavirine was shown to reduce morbidity and mortality induced by the XJ strain of JV in the marmoset *Callithrix jacchus* (95). This drug has been useful in patients infected with Lassa virus but, to our knowledge, it has not yet been tested in AHF patients.

THE REPRODUCTION OF THE HUMAN DISEASE IN ANIMAL MODELS

Studies with the XJ prototype strain in guinea pigs and primates

The XJ prototype strain of JV, isolated from a human being in 1958, is lethal for guinea pigs, marmosets, rhesus monkeys, suckling mice, hamsters and rats but not for adult mice or rats (98). This strain readily induces persistent infections in genetically athymic mice (92, 96) and in a low percentage of euthymic rats (97). However, the reproduction of the hemorrhagic picture resembling the human disease is only found in guinea pigs (88), marmosets (91), or rhesus monkeys (58) while in suckling rodents, a lethal encephalitis is regularly observed. In mice, this encephalitis was shown to be T cell- mediated (82, 71).

Mongrel or Hartley guinea pigs regularly develop a fatal disease 10-18 days post-infection (PI), depending on the dose, but regardless of the route of inoculation (intranasal (IN), cutaneous, intramuscular (IM), intraperitoneal (IP) or intracerebral (IC)).

Fever, weight loss, petechiae in skin and hemorrhages in organs are usually found (40). Pronounced leukopenia and thrombocytopenia are

regularly observed. The decrease in platelet count, detected before the appearance of changes in clotting factors, suggests that it may be related to an altered production, probably induced by infection of megakariocytes (68). Changes in clotting factors and C components are present simultaneously, suggesting a unique triggering mechanism.

The presence of fibrin monomers in plasma and the pattern of decrease in coagulation factors together with histological studies do not support the existence of intravascular coagulation in the guinea pig (62). Activation of C through the classical pathway has been observed, but evidence pointing towards C activation by lysosomal extract-like substances, unrelated to immuno-complexes, has also been reported (74).

Viremia is present from the 3rd day PI and lasts until death. Viral excretion through fauces is also long-lasting.

Peak infectivity titers are found first in hemopoietic organs and later in lung and adrenals. No virus can be recovered from brain unless a viral strain highly passaged in guinea pig is used (69). In this case, an enhanced neurotropism, with neurologic signs, viral isolation and antigen detection in brain has been reported after peripheral inoculation, thus suggesting selection of viral subpopulations with high neurotropism (70).

A severe and progressive necrosis of all lymphohemopoietic organs is regularly found (10). An interstitial pneumonitis and focal lung hemorrhages (24), necrotic foci, focal hemorrhage areas in liver, adrenal gland and digestive tract, are frequently found. In brain no inflammatory phenomena are observed (33).

Large amounts of JV antigens in hemopoietic organs, lung, and adrenal gland, and scattered antigen foci in kidney and liver can be detected. By EM, CPE was observed in bone marrow, kidney and lung. Typical JV viral particles within the megakariocytes were described for the first time in this model (9). The correlation between CPE and viral antigen expression in organs, in the absence of inflammatory phenomena or evidence of immune-complexes deposition, suggest a direct CPE of the XJ strain in guinea pig.

Both primary and secondary humoral immune response to heterologous antigens as well as tuberculin reactions were markedly depressed (11).

A noticeable decrease in percentage and absolute T lymphocyte numbers, in peripheral blood, spleen, and lymph node were observed.

On the contrary, no modification in B lymphocytes were found. These findings strongly suggest that cell-mediated immunity is markedly impaired in the guinea pig infected with the XJ strain (11). These animals died without detectable CF, neutralizing or IF antibodies (69). High levels of interferon were also detected in infected guinea pigs (30).

When XJ-infected guinea pigs received homologous immune sera treatment 24 h before or up to 5 days PI, a drop in mortality rate from 100 to 40% is observed together with lower degrees of immunodepression and no increase of serum proteolytic enzymes (87, 8, 37).

Studies on South-American primates reported that New World primates, such as *Saimiri scireus, Alouatta caraya,* and *Aotus trivirgatus,* develop only inapparent infections (98). However, the marmoset *Callithrix jacchus* infected with XJ strain reproduces both hemorrhagic and neurological signs of AHF. Mortality is 100% and animals exhibit anemia, leukopenia and thrombocytopenia, multiple hemorrhages and marked meningoencephalitis with extensive brain necrosis and demyelinization. Virus can be isolated in high titers from blood, brain and hemopoietic tissue, and JV antigens can be shown in those organs (91).

Cebus apella inoculated IM with XJ develops an inapparent infection with transient viremia, virus in saliva and leuko-thrombocytopenia followed by high antibody titers. These features are similar to the mild AHF forms in humans. However, one out of four inoculated *Cebus* developed a late neurologic syndrome with deposits of Igs and C3 in capillary brain vessels (12).

Other human pathogenic JV strains also induce inapparent infections in *Cebus apella,* although the histopathological changes observed in brain suggest that JV is neuroinvasive for this primate by peripheral route (58). In contrast, rhesus monkeys develop hemorrhagic disease after inoculation with human pathogenic JV strains (59).

Other JV strains

Guinea pigs are good virulence markers for JV. Most viral strains isolated from patients are lethal for guinea pigs and induce a hemorrhagic disease like the XJ prototype strain, which was isolated from a patient but which had undergone a high number of passages in guinea pigs and mice.

However, strains with moderate (MC2) or attenuated pathogenicity for guinea pigs have been described. Although characterization of biological, antigenic and biochemical properties of JV isolates from humans are scarce, a certain correlation was observed for six JV isolates between the human disease and the experimental infection in guinea pigs or rhesus monkeys. For example, some strains recovered from fatal cases with hemorrhagic forms induced hemorrhagic disease in guinea pigs and rhesus monkeys, while others obtained from patients with fatal neurologic disease induced mainly neurologic signs and brain viral titers in those animal models. These results suggest an heterogeneity of naturally occurring JV strains (92) which needs to be further studied.

Attenuated JV strains have great importance with regards to their use in immunization procedures. XJ-Clone 3 was the first attenuated strain obtained in the laboratory by cloning, in MA111 cells, a JV strain isolated from a patient. XJ-Clone 3 is attenuated for guinea pigs and marmosets and protects them from a lethal JV challenge. In 1968 this strain was employed in a pioneer study of human immunization. It showed no pathogenic effect on vaccinees and produced high and long-lasting neutralizing antibodies (76). However, due to the strain's unclear passage history, to the vaccine substrate — suckling mouse brain — and to other factors, it was abandoned and efforts were made to obtain new attenuated strains.

In 1982, another strain called Candid 1, was obtained and proposed as a vaccine candidate. Candid 1 induces inapparent infection with viremia in rhesus monkeys and guinea pigs and protects them from challenge with human pathogenic strains.

Rhesus monkeys inoculated with Candid 1 by intrathalamic, intraspinal and IM routes show significantly milder lesions in the CNS than monkeys inoculated with virulent JV strains (73).

Since Candid 1 was highly attenuated for guinea pigs and monkeys and has complied with the preclinical requirements of the Food and Drug Administration (USA), it was assayed in North American volunteers. No adverse effects and low neutralizing antibody titers were obtained. Similar results were obtained in Argentinian volunteers, and Candid 1 is currently being field-tested in Argentina (54).

However, the demonstration of high risk properties for the attenuated XJ Clone 3 strain such as transplacental passage (4), persistence in guinea pig brain after peripheral inoculation (3), induction of both immuno-complexes in neurons and persistence of viral antigen in brain of the subhuman primate *Cebus apella* (16, 17) as well as in *Akodon molinae* (14), suggest the need to carefully rule out these adverse effects in human vaccines. Furthermore, the finding of tropism modification of JV (70, 5) for the CNS in guinea pigs points to the necessity to control the possible reversion to neurovirulence in attenuated JV strains used for vaccines.

The search for inactivated JV antigens has rended inconclusive results with regards to protection, in spite of the induction of neutralizing antibodies (13). However, new generation techniques, such as subviral components, recombinant or anti-idiotype vaccines, have just started to be explored.

The mouse model

The response of immunocompetent mice to JV infection is age-dependent. Suckling animals infected by IC route with either pathogenic or attenuated JV strains develop invariable 100% mortality 2 weeks after infection with clear neurologic signs, meningoencephalomyelitis (82) and presence of large amounts of viral antigen in cortical neurons, meninges and choroid plexus, with absence of circulating antibodies (22).

Considerable evidence suggests that the T cell response is responsible for CNS damage in suckling mice since protection and absence of lesions were recorded in infected animals after thymectomy or treatment with immunosuppressors or in congenitally athymic mice (nu/nu). These animals, whether adult or suckling, develop persistent asymptomatic infections with high brain virus titers and absence of lesions (92, 96).

In contrast, adult immunocompetent adult mice are slightly sensitive, mortality is low (8%), brain viral titers are nil or scarce and a humoral immune response is promptly detected (38).

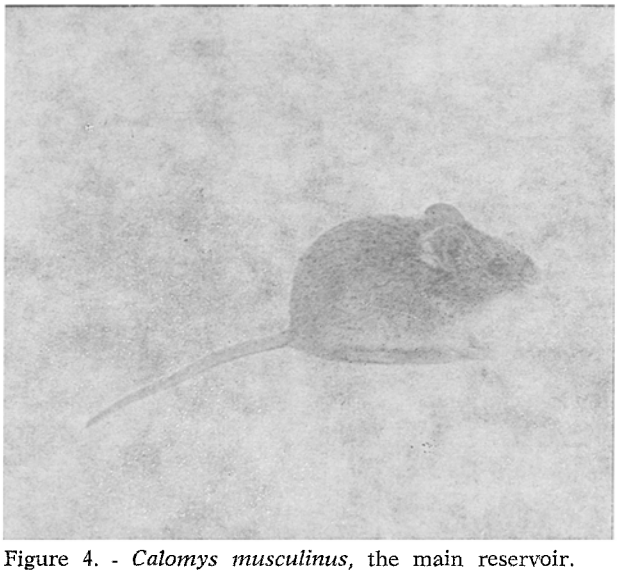

Figure 4. - *Calomys musculinus*, the main reservoir.

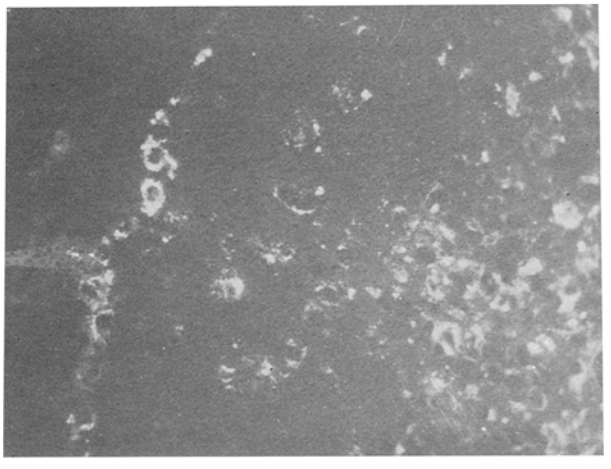

Figure 5. - Junín antigen in cells of the brain. Suckling mice infected with the XJ-Clone 3 strain at 9 days PI Indirect IF. X400.

These results of infection in mice with either pathogenic or attenuated JV strains are the opposite of those reported for LCM virus, where most strains induce encephalitis in adults and persistent infection in suckling animals. The reasons for this reverse response of the same host to infection with LCM and JV have not yet been clarified.

As also reported for LCM, available evidence points to a relevant role for T lymphocytes both in clearance and/or CNS damage in JV infected mice since the persistent asymptomatic state of the JV infected nu/nu mice can be readily switched in a lethal infection with brain damage and decrease of viral titers after transfer of virus-specific T lymphocytes (71).

These studies on immunodeficient mice allowed a selective defect in L3T4+ subpopulations to be postulated as the reason for the establishment of the persistent state in the mouse infected with JV.

The correlation detected between the early infection of thymus and bone marrow and the establishment of persistent JV infection in mice and rats also support this hypothesis (18).

However, further studies are essential to determine whether the same mechanism is operative in the main reservoir *Calomys musculinus*.

EXPERIMENTAL INFECTION OF LABORATORY BRED WILD RODENTS

Viral recovery from specimens captured in endemic areas has shown that *Calomys musculinus* and *Calomys laucha* are probably the most important wild reservoirs (79). *Akodon azarae* has also been found to be naturally infected both within (79) and outside the AHF endemic area (94).

Unfortunately, studies on experimental JV infection in laboratory-bred reservoirs with both wild and laboratory JV strains are still fragmentary and incomplete.

Available data are summarized below and shown in Table 1.

Calomys musculinus and laucha

Wild JV strains

Suckling *C. musculinus* infected with the wild JV strain CbaAN 9446 by IC or IN route develops a persistent, asymptomatic infection with life-long viremia and virus shedding through saliva and urine (79), like the naturally infected *C. musculinus* captured in endemic area.

Salivary glands have been shown to be heavily infected without histologic lesions and high viral titers can be detected in saliva (56).

However, in *C. musculinus* infected at birth with CbaAN 9446 by IN route, a selective mortality period was recently recorded 1-2 months after infection (85). Surviving animals show a decreased reproductive efficiency, manifested by low percentage of pregnancy and lower litter number, as formerly demonstrated for *Calomys callosus* infected with Machupo virus (99).

JV horizontal transmission, primarily through saliva, was experimentally shown when *C. musculinus* were in close contact. Post-natal vertical transmission during lactation has also been observed in *C. musculinus*, most likely by viral transfer to newborn from maternal saliva and/or milk

TABLE 1. — Experimental infection of suckling cricetids with wild or laboratory Junín virus strains.

Species	Inoculation route	Wild strains Mortality	Persistence	references	Laboratory strains Mortality*	Persistence	references
Calomys	IC	—	+	(79)	95%	?	Vitullo, unpublished
musculinus	IN	—**	+	(79)	70%	?	» »
	IP	ND	ND	—	50%	+	(46)
Calomys	IC	—	+	(80)	95%	—	Vitullo, unpublished
laucha	IN	—	+	(80)	70%	—	» »
Akodon	IC	—	?	(80)	98%	?	Videla, unpublished
azarae	IN	—	?	(80)	25%	+	» »
Akodon molinae	IC	ND	ND	—	60%	+	(14)
Akodon dolores	IC	ND	ND	—	60%	?	Videla, unpublished
Calomys	IC	ND	ND	—	17%	?	(83)
callidus	IN	ND	ND	—	70%	+	(83)

IC: intracerebral; IN: intranasal; IP: intraperitoneal; ND: not done.
*: mortality is preceded by neurologic disease.
**: a late high mortality period without neurologic disease was observed around 1 month post-infection (85)
?: Although no persistence was recorded by direct isolation in suckling mice, persistence can not be ruled out since no high sensitivity techniques such as co-culture were not yet employed.

(79). No conclusive data are yet available on prenatal JV transmission, whether transplacental or by germinal cells, as shown for LCM virus (47, 6).

Studies on survival and reproduction of JV-infected *C. musculinus* strongly suggest that vertical transmission « *per se* » is insufficient to overcome the effects of viral infection upon survival and reproduction of the host. Therefore, horizontal transmission is considered the main mechanism for maintenance of JV infection in nature (85).

Attenuated strains

The XJ-Clone 3 strain of JV, though attenuated for guinea pigs, proves neurotropic and neurovirulent for rats (98), mice (82), *Cebus apella* (16) and also for both suckling and adult *C. musculinus*.

Suckling *C. musculinus* inoculated with XJ-clone 3 by IP route show 80% morbidity and 50% mortality. Survivors, as well those lacking signs of disease throughout, develop a persistent infection featuring high brain viral titers (46). These chronically infected *C. musculinus* exhibit meningoencephalitis regardless of the presence of neutralizing antibodies (45).

Remarkably, virus recovered from brain or blood of persistently infected *C. musculinus* was only partially neutralized by serum from the same animal, whereas the latter completely neutralized the original infectious strain. The recovery of these variants from chronically infected animals may explain the inability of the humoral response alone to complete viral clearance from the host (Lampuri J S, 1985, personal commun.).

Antithymocyte serum administration during the first days of life, in XJ-Clone 3-infected *C. musculinus*, lowers mortality and delays the time of death versus non-immunodepressed controls, indicating that cellular immunity seems to play a role in the acute infection (25).

IN or IC inoculation of XJ-Clone 3 in suckling *Calomys* also induces an acute infection, with high morbidity, 95% mortality and high virus titers in brain. Although viral persistence cannot be excluded, no virus in brain was detected in survivors by conventional methods (suckling mice isolation) and neutralizing antibodies were present (Vitullo A. et al., unpublished results).

In contrast to the high susceptibility of suckling *C. musculinus*, adults prove resistant to XJ Clone 3 administered by IP route.

JV replicates in peritoneal macrophages from suckling animals both « in vivo » and « in vitro », whereas it only replicates in macrophages from adult rodents « in vitro » (26). This finding could explain the lack of susceptibility of adult *Calomys* to infection. However, when a natural route of infection is employed (IN), XJ-Clone 3 induces in adult *Calomys* 50% mortality with brain virus recovery and meningoencephalitis even in the presence of neutralizing antibodies (Laguens R.P. et al. Personal communication).

Furthermore, adult females in contact with their XJ-Clone 3 infected litters, develop, in 26% of cases, persistent infections with neurological signs and virus recovery from brain. (Lampuri J.S., personal communication).

Recently, a correlation has been found between the estrus and susceptibility of *Calomys* females to persistent infection. (Merani M.S., unpublished results) although further studies are needed to determine the importance of hormonal changes for the establishment of persistent JV infection.

Studies performed with XJ-Clone 3 in *C. musculinus* suggest that there may exist in nature JV strains with different degrees of pathogenicity for the wild reservoirs. However, the factors responsible for viral persistence, related either to the virus or to the host, remain to be explored.

Akodon azarae

Suckling *A. azarae* inoculated with the wild CbaAN 9446 strain either by IC or IN route show no morbidity or mortality, though a transient viremia and circulating antibodies were detected (79, 80). In contrast, the XJ-Clone 3 strain proves neurovirulent with mortality reaching 98% when inoculated IC, but a long-lasting infection with viral recovery and/or antigen detection in the CNS up to 2 months PI was detected after IN inoculation (Videla C., et al unpublished results).

Adult *A. azarae* inoculated with wild strains or with XJ-Clone 3 by various routes present no mortality, no infectivity in organs or fauces and all animals develop a rapid humoral immune response.

Other wild cricetids

Experimental infection in rodents phylogenetically and ecologically related to the main reservoirs is of great interest since it enables alternative hosts for virus maintenance to be identified.

Two other species belonging to the *Akodon* genus, namely *Akodon molinae* and *Akodon dolores*, which inhabit the borders of the known AHF endemic area, were studied. Both suckling *A. molinae* and *A. dolores* are susceptible to the XJ-Clone 3 strain of JV.

Suckling *A. molinae*, IC-inoculated with XJ-Clone 3, showed 60% mortality. From the CNS of survivors, virus could be recovered up to 2 months PI and antigen detected up to 6 months together with immunocomplexes in neurons of healthy animals (14).

Likewise inoculated, *A. dolores* presented similar morbidity and mortality. Although JV was isolated from CNS during acute stages, no per-

sistent infection could be detected in survivors by conventional methods (suckling mouse isolation).

In contrast, infection of adult *A. molinae* and *A. dolores* with XJ-Clone 3 induces no morbidity or mortality and no infectivity in fauces, blood or organs although animals develop a humoral immune response (Videla C., unpublished results).

The *Calomys callidus* species has been recently proposed as a full species on the basis of cytotaxonomical studies (84). Although its geographical distribution has not yet been fully studied, animals have been captured in the endemic areas in Cordoba province, the Argentinian Mesopotamia and the south of Santa Fé Province.

Both suckling and adult *C. callidus* infected with XJ-Clone 3 by IN or IC route are susceptible, and virus shedding through fauces has been detected.

Inoculated by IN route, suckling *C. callidus* present 70% mortality, while survivors develop long-lasting infections with virus recovery from brain and salivary gland up to 2 months PI. Females can be infected and develop persistent infections by contact with their infected litters (83).

The fact that the above species are capable of persistent JV infection and long-lasting virus shedding should not be taken to mean that they are actually reservoirs for JV in nature, but they certainly pose a potential risk, which deserves further attention.

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