



HAL
open science

Zoonotic aspects of arenavirus infections

R.N. Charrel, X. de Lamballerie

► **To cite this version:**

R.N. Charrel, X. de Lamballerie. Zoonotic aspects of arenavirus infections. *Veterinary Microbiology*, 2010, 140 (3-4), pp.213. 10.1016/j.vetmic.2009.08.027 . hal-00556057

HAL Id: hal-00556057

<https://hal.science/hal-00556057>

Submitted on 15 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Zoonotic aspects of arenavirus infections

Authors: R.N. Charrel, X. de Lamballerie

PII: S0378-1135(09)00399-X
DOI: doi:10.1016/j.vetmic.2009.08.027
Reference: VETMIC 4555



To appear in: *VETMIC*

Received date: 3-7-2009
Revised date: 3-8-2009
Accepted date: 21-8-2009

Please cite this article as: Charrel, R.N., de Lamballerie, X., Zoonotic aspects of arenavirus infections, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.08.027

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ZOONOTIC ASPECTS OF ARENAVIRUS INFECTIONS.

R.N. Charrel*, X. de Lamballerie

Unité des Virus Emergents, UMR190 "Emergence des pathologies virales" Université de la Méditerranée & Institut de Recherche pour le Développement. Faculté de Médecine. Marseille, France.

E-mail: remi.charrel@univmed.fr

Telephone: +33 491 324420, FAX: +33 491 324421

Short summary

To date, the International Committee for Taxonomy of Viruses recognizes that the family *Arenaviridae* contains a unique genus *Arenavirus* that includes 22 viral species. There are 9 additional arenaviruses that either have been discovered recently, or which taxonomic status remains pending. Arenaviruses have been classified according to their antigenic properties into two groups, the Lassa-Lymphocytic choriomeningitis (LCM) serocomplex and the Tacaribe serocomplex which has been further divided into four evolutionary lineages. Each arenavirus is more or less tightly associated with a mammal host. The distribution of the host dictates the distribution of the virus. Humans may become infected by arenaviruses through direct contact with infected rodents, including bites, or through inhalation of infectious rodent excreta and secreta. Lassa, Junin, Machupo, Guanarito, and Sabia viruses are known to cause a severe hemorrhagic fever, in western Africa, Argentina, Bolivia, Venezuela, and Brazil, respectively. Infection by LCM virus can result in acute central nervous system disease, congenital malformations, and infection in organ transplantation recipients. Detection of arenaviruses in their animal host can be achieved by virus isolation, and has recently taken advantage of PCR-based techniques. The approach based on consensus degenerate primers has shown efficient for both detection of known arenaviruses, and discovery of new arenaviruses.

Introduction

The eight edition of the Report of the International Committee for Taxonomy of Viruses (ICTV) states that the family *Arenaviridae* contains a unique genus *Arenavirus* that includes 22 viral species (**Salvato et al., 2005**). New arenaviruses were discovered recently and will be discussed (**Table**). Virions are spherical to pleomorphic with a diameter of 50–300 nm (average diameter for spherical particles is 120 nm). They possess a dense lipid-containing envelope proteins covered with 8-10 nm long club-shaped projections. Ribosomes (20-25 nm) may be present in the viral particles, and are responsible for their sandy appearance when viewed by electron microscopy, hence the name arenavirus (*arena*: sand, Latin). Arenaviruses are enveloped single-stranded RNA viruses, with a genome consisting of two RNA segments, designated large (L) and small (S). Each RNA segment encodes 2 non-overlapping reading frames of opposite orientations. The S RNA segment (~3,400 nucleotides) encodes the structural nucleoprotein, and the glycoprotein precursor secondarily cleaved into the envelope proteins G1 and G2. The L RNA segment (~7,200 nucleotides) encodes the viral polymerase and the Z protein, a zinc-binding matrix protein. The genes on both S and L segments are separated by an intergenic non coding region with the potential of forming one or more hairpin configurations. The 5' and 3' untranslated terminal sequences of each RNA segment possess a relatively conserved reverse complementary sequence spanning 19 nucleotides at each extremity.

The arenaviruses have been classified according to their antigenic properties into two groups: the Tacaribe serocomplex (including viruses indigenous to the New world) and the Lassa-Lymphocytic choriomeningitis serocomplex (including the viruses indigenous to Africa and the ubiquitous lymphocytic

choriomeningitis virus (LCMV), recognized as the Old world group) (**Salvato et al., 2005**). Nucleocapsid antigens are shared by most arenaviruses, and quantitative relationships show the basic split between African viruses and those in the Western Hemisphere. Individual viruses are immunologically distinct by neutralization assays, which depend on the specificity of epitopes contained in the envelope glycoproteins (**Salvato et al., 2005**). Each virus species is more or less tightly associated with a rodent species (the only exception known at this time is Tacaribe virus which is associated with a species of bat) (**Childs & Peters, 1993**). Accordingly the distribution of the host dictates the distribution of the virus. LCMV is the only arenavirus to exhibit a worldwide distribution due to its association with *Mus musculus*. Other arenaviruses are distributed either in the New World or in Africa.

History

The family prototype, LCMV, was first isolated in 1933 during serial passage in monkeys of human material obtained from a fatal infection in the first documented epidemic of St. Louis encephalitis (**Armstrong and Lillie, 1934**). Lassa, Junin, Machupo, Guanarito, and Sabia viruses are known to cause a severe hemorrhagic fever, in western Africa, Argentina, Bolivia, Venezuela, and Brazil, respectively (**Peters et al., 1996**), and were first recovered during investigations of outbreaks in 1958 (**Parodi et al., 1958**), in 1963 (**Johnson et al., 1965**), 1969 (**Buckley et al., 1970**), 1989 (**Salas et al., 1991**), and of a single case in 1990 (**Lisieux et al., 1994**), respectively. They are included in the Category A Pathogen List as defined by the CDC, and listed as Biosafety Level 4 (BSL-4) agents. LCMV, Lassa, and related viruses from the Old World are associated with rodents from the family Muridae, subfamily Murinae. New World arenaviruses are associated with New World rodents in the family Muridae, subfamily Sigmodontinae (**Wilson and Reeder, 2005**). The relative correspondence between the phylogeny of the hosts and of the viruses has suggested a long association also known as cospeciation or coevolution with rodents (**Gonzalez et al., 1986, Bowen et al., 1997**). However, this concept remains controversial due to the limited information in terms of dating the phenomenon and understanding the mechanisms involved. For example rates of evolution estimated for LCMV appear to contradict estimated evolution rates of viruses and rodents by several orders of magnitude. Moreover, Tacaribe virus has been isolated from bats, not rodents, and is not known to be a chronic, inapparent infection of rodents.

Newly discovered arenaviruses

Chapare virus was recently isolated from a unique fatal human case of hemorrhagic fever in the Chapare River region close to Cochabamba in Bolivia (**Delgado et al., 2008**). There is currently no information concerning the rodent host of Chapare virus.

Morogoro virus was isolated from *Mastomys sp* trapped in Tanzania (**Vieth et al., 2007**). This virus is most closely related to Mopeia virus regardless of the gene used for analysis (**Charrel et al., 2008**). Dandenong virus was identified via a metagenomic approach, and subsequently isolated from tissue specimens collected from a fatal case of infection in a patient who underwent kidney transplantation in Australia from a donor who spent a 3-month visit in the former Yugoslavia, where he had traveled in rural areas (**Palacios et al., 2008**). Genetic distance analyses suggested that the taxonomic status of Dandenong virus requires additional investigations for its resolution.

For Catarina, Skinner Tank, Tonto Creek and Big Brushy Tank viruses, recently discovered in the United States of America, complete sequences are only available for the NP and GPC genes at present (**Cajimat et al., 2007; Cajimat et al., 2008; Milazzo et al., 2008**). Their taxonomic classification is currently under investigation at the ICTV. These four viruses were isolated from *Neotoma* rodents. They appear most closely related to Whitewater Arroyo virus. As in the case of their close relatives, North American Whitewater Arroyo, Tamiami, and Bear Canyon virus, they demonstrate a discrepant grouping with clade A and clade B viruses depending on which structural protein (NP or signal peptide/G2) is used for analysis. This suggests that these seven viruses are derived from a common ancestor.

Kodoko virus was detected, but not isolated, in tissues of *Mus Nannomys minutoides* trapped in Guinea. Partial sequence analysis indicates that Kodoko virus roots LCMV (**Lecompte et al., 2007**). This partial information tends to support the consideration of Kodoko virus as a new species. However, this must be confirmed by virus isolation, complete genome sequencing and identification of the natural host. Partial sequences of Pinhal virus (*Calomys tener* from Brazil) NP protein were obtained from Genbank with minimal information. Using these partial NP sequences for comparison, Pinhal virus is most closely related to Oliveros virus (19-20%) (**Charrel et al., 2008**).

Evolutionary relationships

Genetic studies recently revealed congruency with serological delineation (**Figure**). Phylogenetic analyses were historically based on sequence representing a partial region of the NP gene (**Bowen et al., 1996; Bowen et al., 1997**). The genus was divided into two main groups corresponding to the Old World and the New World viruses. The Old World (Lassa-LCM serocomplex) lineage comprises seven viruses: LCM, Lassa, Mopeia, Mobala, Ippy, Morogoro and Kodoko and is distantly rooted to the three New World (Tacaribe serocomplex) lineages, designated A, B, and C. Lineage A includes five South American viruses, Pirital, Pichinde, Flexal, Parana, Allpahuayo. Lineage B includes eight South American viruses including Sabia, Junin, Machupo, Guanarito, Amapari, Tacaribe, Cupixi and Chapare viruses. Lineage C comprises two South American viruses: Oliveros and Latino. Phylogenetic studies conducted with complete S RNA sequences recently demonstrated that discrepancies observed in the topology of phylograms reconstructed from nucleoprotein and glycoprotein genes are attributed to the recombinant nature of the S RNA segment of the three North American viruses: Whitewater Arroyo, Tamiami, and Bear Canyon (**Charrel et al., 2002; Charrel et al., 2003**). The recent discovery of additional North American arenaviruses, Skinner Tank, Catarina, Tonto Creek and Big Brushy Tank viruses (isolated from *Neotoma* rodents) confirmed this finding (**Cajimat et al., 2007; Cajimat et al., 2008; Milazzo et al., 2008**), and indicated that these 2 viruses underwent the same evolutionary mechanism (**Figure**).

Phylogenetic analyses were progressively updated with the availability of complete sequences for the L RNA segment. The most comprehensive study, based on the comparative analysis of complete sequences of the four genes for the largest set of viruses, was published recently (**Charrel et al., 2008**). The delineation into an Old World group and a New World group, which was subsequently divided into three clades corresponding to the antigenic findings, was confirmed. Comparative analysis of the NP and GPC sequences showed that the discrepant position observed in the two genomic regions respectively for the viruses indigenous to North America (Tamiami, Whitewater Arroyo, Bear Canyon, and the more recently discovered Skinner Tank, Catarina, Tonto Creek and Big Brushy Tank viruses) is due to the recombinant nature of the S RNA segment of these viruses, as reported previously (**Charrel et al., 2001**). No recombination was observed in the L RNA segment. From this dataset and analysis, it appeared that no arenavirus genome was the result of a reassortment (also known as intersegmental recombination) mechanism.

Disease in animals and man, therapy and diagnostic

Humans may become infected by arenaviruses through direct contact with infected rodents, including bites, or through inhalation of infectious rodent excreta and secretions. The domestic and peridomestic behavior of several species of rodent reservoir hosts is a major contributing factor facilitating viral transmission from rodent to human. However, in most cases, transmission of arenaviruses to humans occurs following recreational or agricultural incursions into environments providing critical habitat for rodent hosts. Additionally, professionals handling infected rodents in the field or laboratory are at

increased risk of infection (**Sewell, 1995**). Mostly, modifications of the environment due either to human activities (modern farming practices), or natural ecological changes (flooding, storms) have been implicated in the emergence of human disease caused by arenaviruses resulting from changes in behaviour of the reservoir host.

To date, 7 arenaviruses are associated with human disease with different clinical pictures. Lassa, Junin, Machupo, Guanarito, Sabia and Chapare viruses are known to cause a severe hemorrhagic fever, in western Africa, Argentina, Bolivia, Venezuela, Brazil, and Bolivia respectively (**Peters et al., 1996; Delgado et al., 2008**). Infection by LCM virus can result in acute central nervous system disease and congenital malformations (**Barton and Hyndman, 2000; Barton et al., 1993**); it has recently been described as an important cause of fatal infection in organ transplantation recipients, and immunocompetent patients (**CDC, 2005; Fischer et al., 2006; Amman et al., 2007; Charrel et al., 2006; Emonet et al., 2007, Palacios et al., 2008**). Very little is known about the health consequences of infection with the other arenaviruses. Flexal and Tacaribe viruses have caused febrile illness in laboratory workers (**Peters et al., 1996**). Whitewater Arroyo virus may have been associated with 3 fatal cases of infection in California (**CDC, 2000**). Exposure to Pichinde virus has resulted in numerous seroconversions among humans without any noticeable clinical significance (**Buchmeier, unpublished results**). Tacaribe virus is believed to have caused a single case of a febrile disease with mild CNS symptomatology (**J. Casals, unpublished data**).

A live attenuated Junin virus vaccine (Candid#1) was produced. Its efficacy was proven in a double-blind trial in 15,000 agricultural workers at risk to natural infection in Argentina. Subsequently, more than 100,000 people were immunized in Argentina. A prospective study conducted over two epidemic seasons among 6,500 male agricultural workers in Argentina showed that Candid #1 vaccine efficacy was greater or equal to 84%, and no serious adverse effects were detected (**Maiztegui et al., 1998**). Development of Lassa virus vaccine was attempted during the last 30 years. The most advanced projects include (i) a replication-competent vaccine based on attenuated recombinant vesicular stomatitis virus vectors expressing the Lassa viral glycoprotein showed that a single intramuscular vaccination elicited a protective immune response in nonhuman primates against a lethal challenge (**Geisbert et al., 2005**); (ii) ML29, a recombinant Lassa/Mopeia virus vaccine which replication is attenuated in guinea pigs and nonhuman primates, and demonstrated protection against Lassa virus challenge in guinea pigs, Rhesus macaques (**Lukashevich et al., 2008**); (iii) a yellow fever 17D vaccine expressing Lassa virus glycoprotein precursor protected guinea pigs against fatal Lassa fever (**Bredenbeek et al., 2006**).

Recently, a recombinant LCMV/Vesiculovirus vaccine which is attenuated, prevents lethal challenge with LCMV in mice, elicits rapid and long-lived cell-mediated immunity against lethal challenge with wild-type LCMV, confers rapid and long-lived cell-mediated protection against overwhelming systemic infection and liver disease, presents no detectable gain in pathogenicity after propagation in immunodeficient hosts (**Bergthaler et al., 2007**).

Antivirals

No antiviral is approved and commercially available for arenaviruses, except for ribavirin, which proved to be efficient if treatment was administered at the early stage of Lassa fever (**McCormick et al., 1986**). There is however an intense activity centered on molecules with potential antiviral activity against arenaviruses. To date, the most promising molecules are those which interfere with the membrane fusion through the interaction of the G2 fusion subunit with the signal peptide due to IC50 below 10nM (**Larson et al., 2008; York et al., 2008**).

Immune plasma therapy

For Junin virus infection, immune plasma therapy at an early stage showed efficient by reducing the mortality rate from 30% to 1% (**Maiztegui et al., 1979**).

Diagnosics and detection of arenaviruses in clinical and environmental samples

In the animal reservoir, detection of antibodies specific for arenaviruses is poorly indicative since they do not systematically correlate with the presence or absence of live virus. Depending on the virus, the mode, and age at the time of infection, the mammalian host may be persistently infected regardless of the presence or absence of antibodies. Alternatively, an efficient immune response may result in the eradication of the virus. Therefore, serology can be indicative of the circulation of arenaviruses for a given population in a specific region, and thus can be used as an indicator for further investigation of virus circulation by molecular means. Therefore, the following paragraph will focus on the molecular methods that can be used to detect arenaviruses in animal samples.

The detection of arenaviruses in animal specimens can be achieved by techniques based on consensus primer sequences or by assays that target specifically one virus. The latter require good knowledge of the genetic heterogeneity within a given species in order to avoid false negative results due to unaddressed mismatches in the primers or probes. Therefore, in the absence of this knowledge for most species, the consensus primer approach is preferred. Moreover, the frequent circulation of two or more arenaviruses in the same geographic region has encouraged the development of assays that amplify all recognized arenaviruses by combination of degenerate primers located in the conserved regions of the genome (**Lozano et al., 1997; Bowen et al., 1996**). However, there are no reports of the use of these strategies in practical situations. A few years ago, the diagnostic strategy based on degenerate primers was hampered by the lack of genomic data in regions other than the nucleoprotein gene (**Bowen et al., 1996; Bowen et al., 1997**). Nowadays, complete sequence data have been made available for all arenaviruses. However, degenerate primer strategies have not been fully reevaluated in light of these new data. This needs to be accomplished to enable identification of alternative genomic regions that can reveal relationships between both identified and as yet unidentified arenaviruses in human and animal samples. Recent discoveries of novel arenaviruses relied on the use of primers, broadly reactive for viruses within the genus, located in the L RNA segment. Kodoko virus and Morogoro virus were identified by using such an approach (**Lecompte et al., 2007; Vieth et al., 2007**). Recent advances in high-throughput methods, based on pyrosequencing technology, have proven to be efficient at identifying a novel arenavirus in clinical samples with no previous evidence of the presence of this virus (**Palacios et al., 2008**).

As described above, the limited amount of knowledge on genetic diversity with a given species should be taken into account when designing diagnostic assays intended to detect specifically one arenavirus. For instance, new strains of LCMV were shown to be sufficiently divergent to hamper detection by the Taqman probe, due to mismatches. In the paper from McCausland et al, the two primers described for a real-time RT-PCR assay using the SybrGreen technology presented respectively 6 and 6 mismatches when aligned against an alignment of 6 LCMV strains (Armstrong, WE, CH, Traub, Pasteur, Marseille)(**McCausland et al., 2008**). Based on the same alignment, the four primers designed by Mclver et al showed up to 6 mismatches in the sequence, and 6 mismatches in the TaqMan probe; such rates of mismatch will undoubtedly have a negative impact on the detection of LCMV RNA in clinical or environmental samples (**Mclver et al., 2005**). To date, the assay described in Emonet et al (2007) based on SybrGreen technology is the only one to use primers designed to match all 6 previously listed sequences of LCMV (**Emonet et al., 2007**). However, it has been inadequately evaluated, and merits further investigation to determine its sensitivity and specificity. Such evaluation will be rendered necessary whenever a new strain of LCMV is isolated and sequenced. To date, there is insufficient knowledge of genetic heterogeneity within the species LCMV. Accordingly, it is advocated to combine the two approaches.

RT-PCR is to date the only method available for rapid diagnosis of Junin virus infection. For these reasons, several RT-PCR assays have been developed (**Lozano et al., 1995; Lozano et al., 1993; Bockstahler et al., 1992**). However, these techniques have never been employed practically in an epidemic situation to manage the outbreak, or more specifically to detect Junin virus infection at an early stage in order to initiate the immune plasma therapy that reduced the mortality rate from 30% to 1% (**Maiztegui et al., 1979**). More recently, a real time RT-PCR assay, with a 0.5 TCID₅₀ detection limit, has been reported (**Vieth et al., 2005**). However, it has not been used in a clinical or environmental situation. In the same study, an assay specific for Guanarito virus was designed and detected 5 TCID₅₀.

Lassa virus is also a prominent threat outside the area of endemicity with several imported cases in Europe (**Gunther et al., 2000; Cummins, 1990; Van der Heide et al., 1982**), Japan (**Hirabayashi et al., 1988**), the United States (**Holmes et al., 1990**), and the Middle East (**Schlaeffer et al., 1988**). RT-PCR diagnostic assays have been developed but never used for the early detection of human cases in epidemic situations in Africa (**Demby et al., 1994; Trappier et al., 1993; Lukenheimer et al., 1990**). More recently, a previously reported PCR assay was adapted to the real-time SybrGreen format and was tested with patient samples (**Drosten et al., 2002**); the detection limit was evaluated at 2245 genome-equivalent per ml of serum whilst the viral load in patients is generally very high ($> 10^5$ genome-equivalent per ml of serum). This assay has been compared to an L-assay based on 7 human specimens, and both tests showed a similar detection rate (**Vieth et al., 2007**). The first quality assurance study on the rapid detection of Lassa virus RNA included 24 participant laboratories from 17 countries; tenfold dilutions of Lassa virus inactivated genetic material were detected at a rate varying from 21 to 85% depending on the concentration in the sample (**Niedrig et al., 2004**). Of interest is the fact that specimens containing high concentrations of viral RNA may produce false negative results due to inhibition of the enzymatic reactions. To date there is no study addressing the presence and prevalence of RT and PCR inhibitors depending on the organ used for analysis. This would be of utmost interest to deploy a strategy adapted to virus search. Meanwhile, it is therefore proposed that clinical specimens should be “spiked” with an internal control positive sample to monitor enzymatic reaction efficacy. Alternatively the test samples should be serially diluted to alleviate the problem of inhibition of enzymatic reactions in mammalian tissues. This point is pivotal to avoid underestimation of virus prevalence due to technical artifacts.

Conclusions and future prospects

During the last 3 years, the discovery of 9 new arenaviruses, of which 2 were human pathogens, strongly suggests that many more arenaviruses may be discovered in the future. As new technologies begin to impact on virus discovery it is now becoming clear that a large number of viruses remain to be unearthed as recently suggested (**Pybus et al., 2002**). As illustrated above, the quest for new arenaviruses requires the combination of traditional virological approaches with innovative technologies such as large scale sequencing and extensive bioinformatic capacities.

Acknowledgements

We thank Stephan Günther for sharing results before publication. This work was partly supported by the Rivigene EU project.

Conflict of interest statement

None.

References

- Amman, B.R., Pavlin, B.I., Albariño, C.G., Comer, J.A., Erickson, B.R., Oliver, J.B., Sealy, T.K., Vincent, M.J., Nichol, S.T., Paddock, C.D., Tumpey, A.J., Wagoner, K.D., Glauer, R.D., Smith, K.A., Winpisinger, K.A., Parsely, M.S., Wyrick, P., Hannafin, C.H., Bandy, U., Zaki, S., Rollin, P.E., Ksiazek, T.G., 2007. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. *Emerg. Infect. Dis.* 13, 719-725.
- Armstrong, C., Lillie, R.D., 1934. Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St Louis encephalitis epidemic. *Public Health Rep.* 49, 1019-1027
- Barton, L.L., Hyndman, N.J., 2000. Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. *Pediatrics* 105, E35.
- Barton, L.L., Budd, S.C., Morfitt, W.S., Peters, C.J., Ksiazek, T.G., Schindler, R.F., Yoshino, M.T., 1993. Congenital lymphocytic choriomeningitis virus infection in twins. *Pediatr. Infect. Dis. J.* 12, 942-946.
- Bergthaler, A., Gerber, N.U., Merkler, D., Horvath, E., de la Torre, J.C., Pinschewer, D.D., 2006. Envelope exchange for the generation of live-attenuated arenavirus vaccines. *PLoS Pathog.* 2, e51.
- Bockstahler, L.E., Carney, P.G., Bushar, G., Sagripanti, J.L., 1992. Detection of Junin virus by the polymerase chain reaction. *J. Virol. Methods* 39, 231-235.
- Bowen, M.D., Peters, C.J., Nichol, S.T., 1997. Phylogenetic analysis of the Arenaviridae: patterns of virus evolution and evidence for cospeciation between arenaviruses and their rodent hosts. *Mol. Phylogenet. Evol.* 8, 301-316.
- Bowen, M.D., Peters, C.J., Nichol, S.T., 1996. The phylogeny of New World (Tacaribe complex) arenaviruses. *Virology* 219, 285-290.
- Bredenbeek, P.J., Molenkamp, R., Spaan, W.J., Deubel, V., Marianneau, P., Salvato, M.S., Moshkoff, D., Zapata, J., Tikhonov, I., Patterson, J., Carrion, R., Ticer, A., Brasky, K., Lukashevich, I.S., 2006. A recombinant Yellow Fever 17D vaccine expressing Lassa virus glycoproteins. *Virology* 345; 299-304.
- Buckley, S.M., Casals, J., 1970. Lassa fever: a new virus disease of man from West Africa. III. Isolation and characterization of the virus. *Am. J. Trop. Med. Hyg.* 19, 680-691
- Cajimat, M.N., Milazzo, M.L., Borchert, J.N., Abbott, K.D., Bradley, R.D., Fulhorst, C.F., 2008. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the Mexican woodrat (*Neotoma mexicana*). *Virus Res.* 133, 211-217.
- Cajimat, M.N., Milazzo, M.L., Bradley, R.D., Fulhorst, C.F., 2007. Catarina virus, an arenaviral species principally associated with *Neotoma micropus* (southern plains woodrat) in Texas. *Am. J. Trop. Med. Hyg.* 77, 732-736.
- Centers for Disease Control and Prevention, 2000. Fatal illnesses associated with a New World arenavirus – California, 1999-2000. *MMWR Morb. Mortal. Wkly. Rep.* 49, 709-711.

Centers for Disease Control and Prevention, 2005. Lymphocytic choriomeningitis virus infection in organ transplant recipients--Massachusetts, Rhode Island, 2005. *MMWR Morb. Mortal. Wkly. Rep.* 54, 537-539.

Charrel, R.N., de Lamballerie, X., Fulhorst, C.F., 2001. The Whitewater Arroyo virus: natural evidence for genetic recombination among Tacaribe serocomplex viruses (family Arenaviridae). *Virology* 283, 161-166.

Charrel, R.N., Feldmann, H., Fulhorst, C.F., Khelifa, R., de Chesse, R., de Lamballerie, X., 2002. Phylogeny of New World arenaviruses based on the complete coding sequences of the small genomic segment identified an evolutionary lineage produced by intrasegmental recombination. *Biochem. Biophys. Res. Commun.* 296, 1118-1124.

Charrel, R.N., Lemasson, J.J., Garbutt, M., Khelifa, R., De Micco, P., Feldmann, H., de Lamballerie, X., 2003. New insights into the evolutionary relationships between arenaviruses provided by comparative analysis of small and large segment sequences. *Virology* 317, 191-196.

Charrel, R.N., Retornaz, K., Emonet, S., Noel, G., Chaumoitre, K., Minodier, P., Girard, N., Garnier, J.M., de Lamballerie, X., 2006. Acquired hydrocephalus caused by a variant lymphocytic choriomeningitis virus. *Arch. Intern. Med.* 166, 2044-2046.

Charrel, R.N., de Lamballerie, X., Emonet, S., 2008. Phylogeny of the genus Arenavirus. *Curr. Opin. Microbiol.* 11, 362-368.

Childs, J.E., Peters, C.J., 1993. Ecology and epidemiology of arenaviruses and their hosts. In: Salvato, M.S. (Ed), *The Arenaviruses*, Plenum Press, New York.

Cummins, D., 1990. Lassa fever. *Br. J. Hosp. Med.* 43, 186-192.

Delgado, S., Erickson, B.R., Agudo, R., Blair, P.J., Vallejo, E., Albariño, C.G., Vargas, J., Comer, J.A., Rollin, P.E., Ksiazek, T.G., Olson, J.G., Nichol, S.T., 2008. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog.* 4, e1000047.

Demby, A.H., Chamberlain, J., Brown, D.W., Clegg, C.S., 1994. Early diagnosis of Lassa fever by reverse transcription-PCR. *J. Clin. Microbiol.* 32, 2898-2903.

Drosten, C., Götting, S., Schilling, S., Asper, M., Panning, M., Schmitz, H., Günther, S., 2002. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *Clin. Microbiol.* 40, 2323-2330.

Emonet, S., Retornaz, K., Gonzalez, J.P., de Lamballerie, X., Charrel, R.N., 2007. Mouse-to-human transmission of variant lymphocytic choriomeningitis virus. *Emerg. Infect. Dis.* 13, 472-475.

Fischer, S.A., Graham, M.B., Kuehnert, M.J., Kotton, C.N., Srinivasan, A., Marty, F.M., Comer, J.A., Guarner, J., Paddock, C.D., DeMeo, D.L., Shieh, W.J., Erickson, B.R., Bandy, U., DeMaria, A. Jr., Davis, J.P., Delmonico, F.L., Pavlin, B., Likos, A., Vincent, M.J., Sealy, T.K., Goldsmith, C.S., Jernigan, D.B., Rollin, P.E., Packard, M.M., Patel, M., Rowland, C., Helfand, R.F., Nichol, S.T., Fishman, J.A., Ksiazek, T., Zaki, S.R., LCMV in Transplant Recipients Investigation Team, 2006. Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N. Engl. J. Med.* 354, 2235-2249.

- Geisbert, T.W., Jones, S., Fritz, E.A., Shurtleff, A.C., Geisbert, J.B., Liebscher, R., Grolla, A., Ströher, U., Fernando, L., Daddario, K.M., Guttieri, M.C., Mothé, B.R., Larsen, T., Hensley, L.E., Jahrling, P.B., Feldmann, H., 2005. Development of a new vaccine for the prevention of Lassa fever. *PLoS Med.* 2, 183.
- Gonzalez, J.P., Georges, A.J., Kiley, M.P., Meunier, D.M.Y., Peters, C.J., McCormick, J.B., 1986. Evolutionary biology of a Lassa virus complex. *Med. Microbiol. Immunol.* 175, 157–159
- Gunther, S., Emmerich, P., Laue, T., Kuhle, O., Asper, M., Jung, A., Grewing, T., Ter Meulen, J., Schmitz, H., 2000. Imported lassa fever in Germany: molecular characterization of a new lassa virus strain. *Emerg. Infect. Dis.* 6, 466–476
- Hirabayashi, Y., Oka, S., Goto, H., Shimada, K., Kurata, T., Fisher-Hoch, S.P., McCormick, J.B., 1988. An imported case of Lassa fever with late appearance of polyserositis. *J. Infect. Dis.* 158, 872–875
- Holmes, G.P., McCormick, J.B., Trock, S.C., Chase, R.A., Lewis, S.M., Mason, C.A., Hall, P.A., Brammer, L.S., Perez-Oroz, G.I., McDonnell, M.K., et al., 1990. Lassa fever in the United States. Investigation of a case and new guidelines for management. *N. Engl. J. Med.* 323, 1120-1123.
- Johnson, K.M., Wiebenga, N.H., Mackenzie, R.B., Kuns, M.L., Tauraso, N.M., Shelekov, A., Webb, P.A., Justines, G., Beye, H.K., 1965. Virus isolations from human cases of hemorrhagic fever in Bolivia. *Proc. Soc. Exp. Biol. Med.* 118, 113–118
- Larson, R.A., Dai, D., Hosack, V.T., Tan, Y., Bolken, T.C., Hruby, D.E., Amberg, S.M., 2008. Identification of a broad-spectrum arenavirus entry inhibitor. *J. Virol.* Aug 20.
- Lecompte, E., ter Meulen, J., Emonet, S., Daffis, S., Charrel, R.N., 2007. Genetic identification of Kodoko virus, a novel arenavirus of the African pigmy mouse (*Mus Nannomys minutoides*) in West Africa. *Virology* 364, 178-183.
- Lisieux, T., Coimbra, M., Nassar, E.S., Burattini, M.N., de Souza, L.T., Ferreira, I., Rocco, I.M., da Rosa, A.P., Vasconcelos, P.F., Pinheiro, F.P., 1994. New arenavirus isolated in Brazil. *Lancet* 343, 391-392.
- Lozano, M.E., Enria, D., Maiztegui, J.I., Grau, O., Romanowski, V., 1995. Rapid diagnosis of Argentine hemorrhagic fever by reverse transcriptase PCR-based assay. *J. Clin. Microbiol.* 33, 1327-1332.
- Lozano, M.E., Posik, D.M., Albarino, C.G., Schujman, G., Ghiringhelli, P.D., Calderon, G., Sabattini, M., Romanowski, V., 1997. Characterization of arenaviruses using a family-specific primer set for RT-PCR amplification and RFLP analysis: Its potential use for detection of uncharacterized arenaviruses. *Virus Res.* 49, 79-89.
- Lozano, M.E., Ghiringhelli, P.D., Romanowski, V., Grau, O., 1993. A simple nucleic acid amplification assay for the rapid detection of Junin virus in whole blood samples. *Virus Res.* 27, 37–53
- Lukashevich, I.S., Carrion, R. Jr., Salvato, M.S., Mansfield, K., Brasky, K., Zapata, J., Cairo, C., Goicochea, M., Hoosien, G.E., Ticer, A., Bryant, J., Davis, H., Hammamieh, R., Mayda, M., Jett, M., Patterson, J., 2008. Safety, immunogenicity, and efficacy of the ML29 reassortant vaccine for Lassa fever in small non-human primates. *Vaccine* 26, 5246-5254.

Lunkenheimer, K., Hufert, F.T., Schmitz, H., 1990. Detection of Lassa virus RNA in specimens from patients with Lassa fever by using the polymerase chain reaction. *J. Clin. Microbiol.* 28, 2689-2692.

Maiztegui, J.I., Fernandez, N.J. de Damilano; A.J., 1979. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet* 2, 1216-1217.

Maiztegui, J.I., McKee, K.T. Jr., Barrera Oro, J.G., Harrison, L.H., Gibbs, P.H., Feuillade, M.R., Enria, D.A., Briggiler, A.M., Levis, S.C., Ambrosio, A.M., Halsey, N.A., Peters, C.J., 1998. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J. Infect. Dis.* 177, 277-283.

McCausland, M.M., Crotty, S., 2008. Quantitative PCR technique for detecting lymphocytic choriomeningitis virus in vivo. *J. Virol. Methods* 147, 167-176.

McCormick, J.B., King, I.J., Webb, P.A., Scribner, C.L., Craven, R.B., Johnson, K.M., Elliott, L.H., Belmont-Williams, R., 1986. Lassa fever. Effective therapy with ribavirin. *N. Engl. J. Med.* 314, 20-26.

Mclver, C.J., Jacques, C.F., Chow, S.S., Munro, S.C., Scott, G.M., Roberts, J.A., Craig, M.E., Rawlinson, W.D., 2005. Development of multiplex PCRs for detection of common viral pathogens and agents of congenital infections. *J. Clin. Microbiol.* 43:5102-5110.

Milazzo, M.L., Cajimat, M.N., Haynie, M.L., Abbott, K.D., Bradley, R.D., Fulhorst, C.F., 2008. Diversity among tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the white-throated woodrat (*Neotoma albigula*) in the southwestern United States. *Vector Borne Zoonotic Dis.* 8, 523-540.

Niedrig, M., Schmitz, H., Becker, S., Günther, S., ter Meulen, J., Meyer, H., Ellerbrok, H., Nitsche, A., Gelderblom, H.R., Drosten, C., 2004. First international quality assurance study on the rapid detection of viral agents of bioterrorism. *J. Clin. Microbiol.* 42:1753-1755.

Palacios, G., Druce, J., Du, L., Tran, T., Birch, C., Briese, T., Conlan, S., Quan, P.L., Hui, J., Marshall, J., Simons, J.F., Egholm, M., Paddock, C.D., Shieh, W.J., Goldsmith, C.S., Zaki, S.R., Catton, M., Lipkin, W.I., 2008. A new arenavirus in a cluster of fatal transplant-associated diseases. *N. Engl. J. Med.* 358, 991-998.

Parodi, A.S., Greenway, D.J., Rugiero, H.R., Frigerio, M., De La Barrera, J.M., Mettler, N., Garzon, F., Boxaca, M., Guerrero, L., Nota, N., 1958. Concerning the epidemic outbreak in Junin. *Dia Med.* 30, 2300-2301.

Peters, C.J., Buchmeier, M., Rollin, P.E., Ksiazek, T.G., 1996. Arenaviruses. In: Fields, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Melnick, J.L., Monath, T.P., Roizman, R., Straus, S.E. (Eds), *Fields Virology*, Lippincott-Raven Publishers.

Pybus, O.G., Rambaut, A., Holmes, E.C., Harvey, P.H., 2002. New inferences from tree shape: numbers of missing taxa and population growth rates. *Syst. Biol.* 51, 881-888.

Salas, R., de Manzione, N., Tesh, R.B., Rico-Hesse, R., Shope, R.E., Betancourt, A., Godoy, O., Bruzual, R., Pacheco, M.E., Ramos, B. et al., 1991. Venezuelan hemorrhagic fever. *Lancet* 338, 1033-1036

Salvato, M.S., Clegg, J.C.S., Buchmeier, M.J., Charrel, R.N., Gonzalez, J.P., Lukashevich, I.S., Peters, C.J., Rico-Hesse, R., Romanowski, V., 2005. Family Arenaviridae. In: Van Regenmortel, M.H.V., Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A., Virus Taxonomy, Eight report of the International Committee on Taxonomy of Viruses. Academic Press

Schlaeffer, F., Bar-Lavie, Y., Sikuler, E., Alkan, M., Keynan, A., 1988. Evidence against high contagiousness of Lassa fever. *Trans. R. Soc. Trop. Med. Hyg.* 82, 311

Sewell, D.L., 1995. Laboratory-associated infections and biosafety. *Clin. Microbiol. Rev.* 8, 389–405

Trappier, S.G., Conaty, A.L., Farrar, B.B., Auperin, D.D., McCormick, J.B., Fisher-Hoch, S.P., 1993. Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection. *Am. J. Trop. Med. Hyg.* 49, 214-221.

Van der Heide, R.M., 1982. A patient with Lassa fever from Burkina Faso (formerly Upper Volta), diagnosed in the Netherlands. *Ned. T. Geneesk.* 126, 1-7.

Vieth, S., Drosten, C., Charrel, R., Feldmann, H., Günther, S., 2005. Establishment of conventional and fluorescence resonance energy transfer-based real-time PCR assays for detection of pathogenic New World arenaviruses. *J. Clin. Virol.* 32, 229-35.

Vieth, S., Drosten, C., Lenz, O., Vincent, M., Omilabu, S., Hass, M., Becker-Ziaja, B., ter Meulen, J., Nichol, S.T., Schmitz, H., Günther, S., 2007. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. *Trans. R. Soc. Trop. Med. Hyg.* 101, 1253-1264.

Wilson, D.E., Reeder, D.M., 2005. *Mammal Species of the World*, 2nd edn. Johns Hopkins University Press, Baltimore

York, J., Dai, D., Amberg, S.M., Nunberg, J.H., 2008. pH-induced activation of arenavirus membrane fusion is antagonized by small-molecule inhibitors. *J. Virol.* Sep 3.

Legends

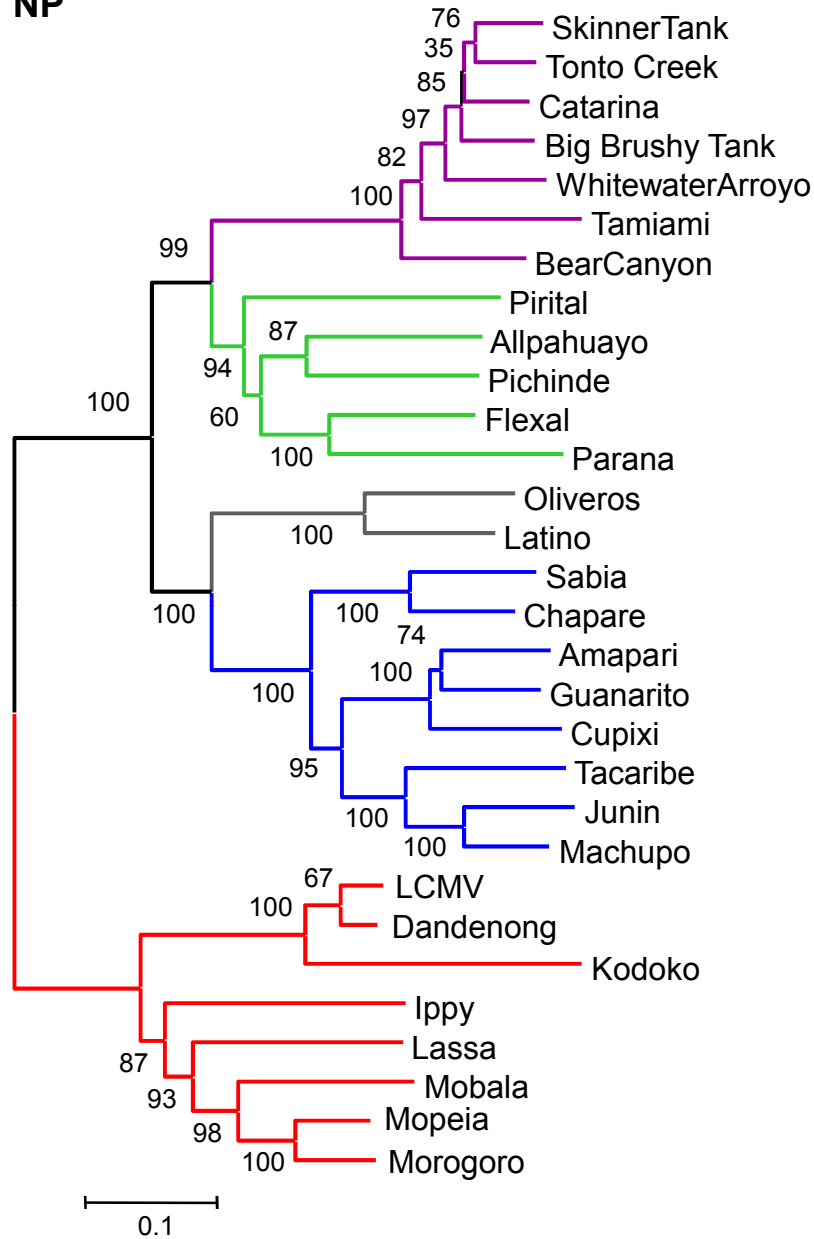
Table

List of arenavirus species and newly discovered arenavirus not yet classified, and respective characteristics.

Figure

Phylogenetic relationships between arenaviruses within evolutionary lineages. Red, Old world viruses; Green, lineage A new world viruses, Blue, lineage B new world viruses; Grey, lineage C new world viruses; Purple, recombinant new world viruses. Left panel, phylogram based on nucleoprotein amino acid sequences, right panel, phylogram based on concatenated signal peptide and glycoprotein 2 amino acid sequences. The neighbor-joining, poisson and bootstrapping (200 pseudoreplications) algorithms were computed by MEGA 2 software package.

NP



SP-G2

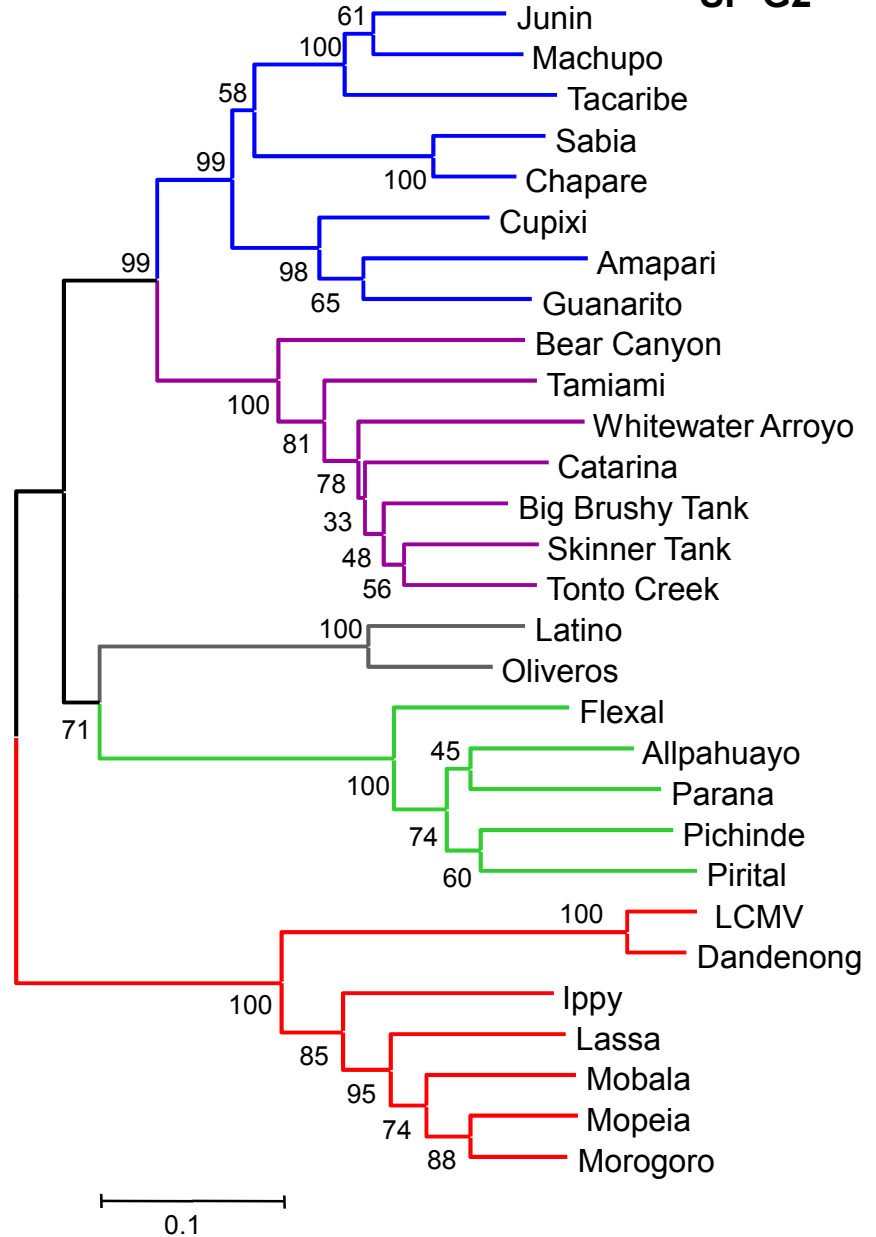


Table 1. List of arenaviruses.

virus	acronym	lineage	country	(possible) host	BSL	S RNA Number of complete(partial) sequences**	L RNA Number of complete(partial) sequences
Allpahuayo	ALLV	NW-A	Peru	<i>Oecomys bicolor</i> , <i>Oecomys paricola</i>	2	2(0)	1(0)
Amapari	AMAV	NW-B	Brazil	<i>Oryzomys goeldi</i> , <i>Neacomys guianae</i>	2	1(0)	1(0)
Bear Canyon	BCNV	NW-Rec	USA	<i>Peromyscus californicus</i> , <i>Neotoma macrotis</i>	2	3(4)	2(0)
Catarina	na	NW-Rec	USA	<i>Neotoma micropus</i>	2	2(0)	0(0)
Chapare	na	NW-B	Bolivia	unknown	NC	1(0)	1(0)
Cupixi	CPXV	NW-B	Brazil	<i>Oryzomys capito</i>	2	1(0)	1(0)
Dandenong	na	OW	Australia	Unknown	NC	1(0)	1(0)
Flexal	FLEV	NW-A	Brazil	<i>Oryzomys spp</i>	3	2(0)	1(0)
Guanarito	GTOV	NW-B	Venezuela	<i>Zygodontomys brevicauda</i> , <i>Sigmodon alstoni</i>	4	2(33)	1(12)
Ippy	IPPYV	OW	Central African Republic	<i>Arvicanthus spp</i>	2	1(0)	1(0)
Junin	JUNV	NW-B	Argentina	<i>Callomys musculus</i>	4	5(83)	5(1)
Kodoko	na	OW	Guinea	<i>Mus Nannomys minutoides</i>	NC	0(2)	0(2)
Lassa	LASV	OW	West Africa	<i>Mastomys natalensis</i>	4	11(95)	6(11)
Latino	LATV	NW-C	Bolivia	<i>Callomys callosus</i>	2	1(0)	1(0)
LCM*	LCMV	OW	ubiquitous	<i>Mus musculus</i> , <i>M domesticus</i>	2/3	9(25)	8(5)
Machupo	MACV	NW-B	Bolivia	<i>Callomys callosus</i>	4	9(20)	3(8)
Mobala	MOBV	OW	Central African Republic	<i>Praomys spp.</i>	2	1(3)	1(2)
Mopeia	MOPV	OW	Mozambique, Zimbabwe	<i>Mastomys natalensis</i>	2	3(0)	2(1)
Morogoro	na	OW	Tanzania	<i>Mastomys sp.</i>	NC	0(0)	0(0)
Oliveros	OLVV	NW-C	Argentina	<i>Bolomys spp</i>	2	1(0)	1(0)
Pampa	na	NW-C	Argentina	<i>Bolomys spp</i>	2	0(1)	0(0)
Parana	PARV	NW-A	Paraguay	<i>Oryzomys buccinatus</i>	2	1(0)	1(0)
Pichinde	PICV	NW-A	Colombia	<i>Oryzomys albigularis</i>	2	3(2)	3(0)
Pinhal	na	NW-C	Brazil	<i>Calomys tener</i>	NC	0(5)	0(0)
Pirital	PIRV	NW-A	Venezuela	<i>Sigmodon alstoni</i>	2	1(28)	1(4)
Sabia	SABV	NW-B	Brazil	unknown	4	1(0)	1(0)
Skinner Tank	na	NW-Rec	USA	<i>Neotoma mexicana</i>	NC	1(0)	0(0)
Tonto Creek	na	NW-Rec		<i>Neotoma spp.</i>	NC	0(0)	0(0)
Tacaribe	TCRV	NW-B	Trinidad	<i>Artibeus bat</i>	2	1(0)	1(0)
Tamiami	TAMV	NW-Rec	USA	<i>Sigmodon hispidus</i>	3	1(0)	1(0)
Whitewater Arroyo	WWAV	NW-Rec	USA	<i>Neotoma spp.</i>	2	5(11)	1(0)

*, Lymphocytic choriomeningitis virus, ** available in Genbank in December 2008.