Correlation Coefficients Describing the Relationship between Shape and Size for 2710 Particles LCMV, Grouped By Size Range of Arena Virus

Author's Details:

[1] **Abdullah Sethar D.V.M., M. Sc (Honors)** from Pakistan and Ph.D from England, UK. Deputy Project Director, Sindh Agricultural Growth Project (Livestock Component) World Bank Assisted, Government of Sindh, Hyderabad-Sindh, [2] **Dr. Benjamin W. Neuman,** School of Biological Sciences, University of Reading, UK. [3]**Dr. Gul Hassan Sethar**- Consultant PhysicianAl- Amiri Hospital Kuwait city, Kuwait [4]**Dr. Nargis Khan** - Senior Registrar Medicine Department Dow University of Health Sciences, Karachi [5]**M.Ali Bhatti,** Assistant Professor IBA-Sukkur

Abstract: *LCMV is best treated as two population one is small viruses that are unnaturally round and large viruses that are the same as vesicles. We also found that shape of small arenavirus particles is significantly rounder than shape of vesicles. Therefore: Protein interactions of Arenaviruses make small particles round. Large particles are elliptical due to insufficient interaction of protein. Future work now focuses on variation in the GP, NP, and Z that correlates with changes in size and shape*.
Key Words: Correlation coefficients describing the relationship

Introduction: LCMV native and Fusion-activated LCMV (see fig 35) from the same preparation and then took its average size and average ratio to do the coefficient correlation (r). We then selected both sets of data of shape and GP% of each of them to whether GP% affects the shape and size of the particles. We discovered by doing correlation coefficient that fusion activation strongly reduces the association between shape and size. This might be due to changes upon fusion activation by acidic pH or Lithium chloride as GP-1 dissociates from the virion causing particles to appear "spikeless",GP-2 changes shape and appears longer is no longer visible near the viral membrane and NP remains inside the virion. We further hypothesized that GP has important role in forming the shape of virus particle.

Data analysis shows that small LCMV particles are significantly rounder than vesicles as P value is = .0001 by ANOVA (Please see fig 33).The shape of vesicles and large LCMV particles are not significantly different (ANOVA, P = .29). Therefore interactions among LCMV proteins are qualitatively different on small and large LCMV particles. We hypothesized that small LCMV are more infectious as compare to large ones whereas large LCMV are less infectious. We will carry out molecular investigation for this hypothesis in future.

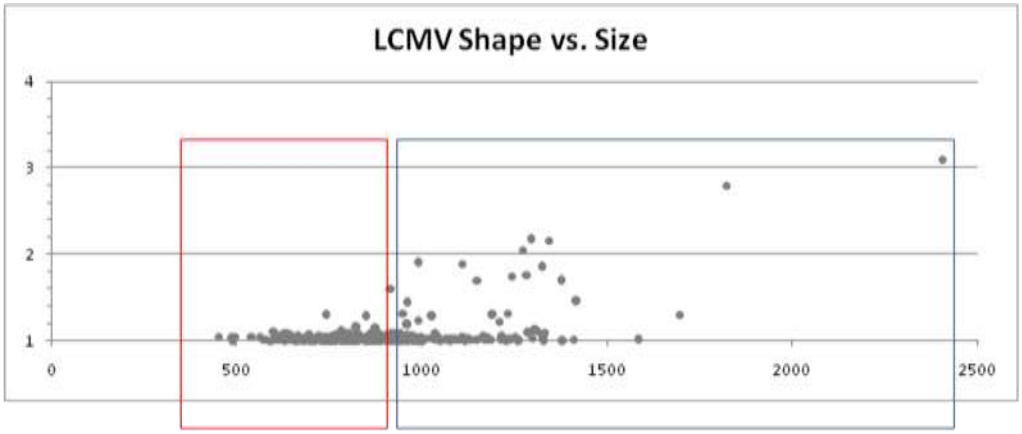

Figure 26. Scatter plots showing the separation of two population a sensible dividing point.

We compared small and large LCMV particles separately to all the vesicles found that small LCMV particles are significantly rounder than vesicles (see Figure 26). As far as small and large particles contain the same protein but the outcome of protein interaction is very different on small and large particles.

We therefore concluded that: Shape of LCMV overall is significantly different from shape of vesicles, LCMV is best treated as two populations :small viruses that are unnaturally round and large viruses that are the same shape as vesicles, Shape of small LCMV particles is significantly rounder than shape of vesicles and therefore, Protein interaction of LCMV make small particles round. We hypothesize that due to insufficient interaction between large particles they are elliptical and future work will now focus on variation in the GP, NP, and Z that correlates with changes in size and shape.

We measured longest and shortest diameter of each particles LCMV, PICV, TCRV and DOPC (see Fig 27) from the same preparation and then took its average size and average shape to do the coefficient correlation (r). We then selected both sets of data of shape and size of each of particle of LCMV, TCRV, PICV and DOPC to see whether or not affects the shape and size of the particles. We

discovered by doing correlation coefficient that (r) LCMV 0.40 PICV 0.06 TCRV 0.02 and DOPC -0.03. Larger size correlates more elliptical shape for Arenaviruses .Shape and size of vesicles are not correlated (see Fig 27)

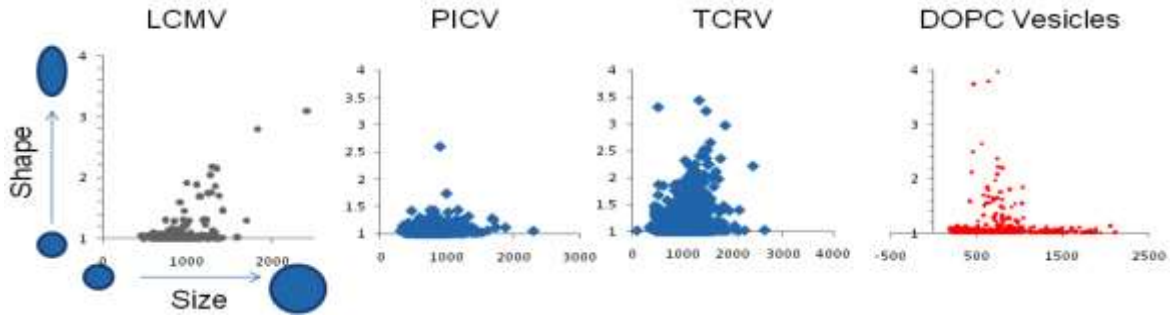

Figure 27. Scatter plots showing the relationship between size and shape for three types of arenavirus and for empty DOPC vesicles.

We measured longest and shortest diameter of each particles LCMV native and Fusion-activated LCMV (see fig 35) from the same preparation and then took its average size and average ratio to do the coefficient correlation (r). We then selected both sets of data of shape and GP% of each of them to whether GP% affects the shape and size of the particles. We discovered by doing correlation coefficient that fusion activation strongly reduces the association between shape and size. This might be due to changes upon fusion activation by acidic pH or Lithium chloride as GP-1 dissociates from the virion causing particles to appear "spikeless",GP-2 changes shape and appears longer is no longer visible near the viral membrane and NP remains inside the virion. We further hypothesized that GP has important role in forming the shape of virus particle.

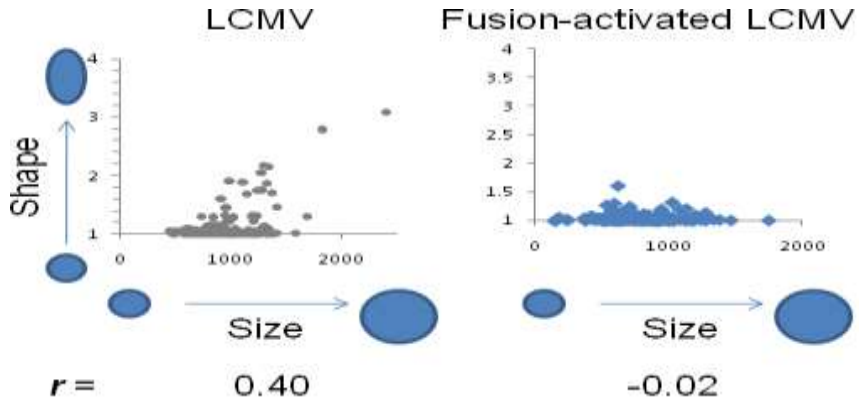

Figure 28. Scatter plots showing the shape and size of LCMV and fusion-activated LCMV particles from the same preparation. Correlation coefficients (r) between shape and size are shown below.

We then investigated whether the relationship between size and shape affected by fusion activation. Changes upon fusion activation by acidic pH or Lithium chloride include GP-1 dissociates from the virion causing particles to appear "spikeless", GP-2 changes shape and appears longer, Z is no longer visible near the viral membrane and NP remains inside the virion. We found that Correlation Coefficient (Pearson r)If r is near then It means -1 means inverse correlation (both change proportionately, but in opposite directions) if it is 0 means not correlated and +1means values are correlated (both change proportionately) so here we see in LCMV native value is (r) 0.04 whereas in case of fusion-activated LCMVs its value is -0.02 which means not correlated. From this data we concluded that fusion activation strongly reduces the association between shape and size (see Fig 28).

We next compared the shape of Arenaviruses and vesicles to see the variance in between the shape and size of virus particle and to find an answer of proteins role in making shape of virus particles. We concluded that concluded shape of arenaviruses is significantly different from shape of vesicles, but significantly less so after fusion activation (see Fig 29). Therefore we concluded that proteins of arenavirus make particles slightly more round (on average), but fusion activated particles almost revert to shape of vesicles.

	Average Shape	
LCMV native (n = 2302)	1.07	P < 0.003
fusion (n = 286)	1.05	P = 0.05
PICV (n = 1127)	1.03	P < 0.001
TCRV (n = 1997)	1.08	P < 0.001
Vesicles (n = 617)	1.09	

Figure 29. P value is comparing the different type of particle to vesicles

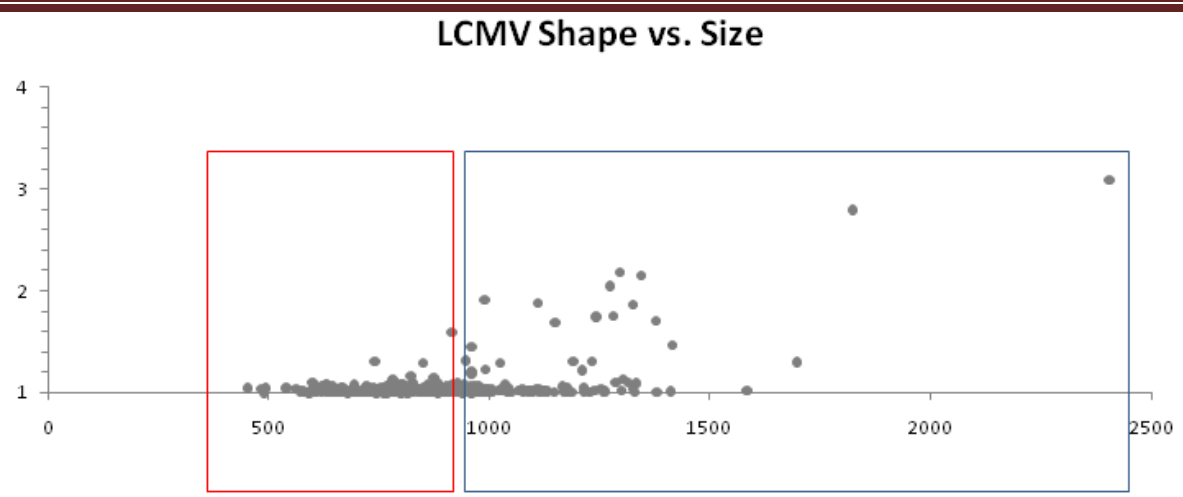

Figure 30. Comparison of the shape of vesicles of two LCMV populations showing scatter plots. Data is the same as presented in Fig. 26.

Small LCMV particles are significantly rounder than vesicle (ANOVA, P = .0001). The shape of vesicles and large LCMV particles are not significantly different (ANOVA, P = .29). Therefore interactions among LCMV proteins are qualitatively different on small and large LCMV particles. We therefore hypothesized that small LCMV are more infectious as compare to large ones whereas large LCMV are less infectious.

We also investigated how arenavirus protein-protein interactions contribute to shape by counting GPs and calculated percentage GP and measured fullness of virions appear in the particles and measuring the correlation between GP coverage, shape, size and fullness. Fullness was calculated as the ratio of core (NP) to edge (lipid) brightness for each virion. Here we concluded that percent GP coverage is not consistently correlated with any other parameters (see Fig 31).

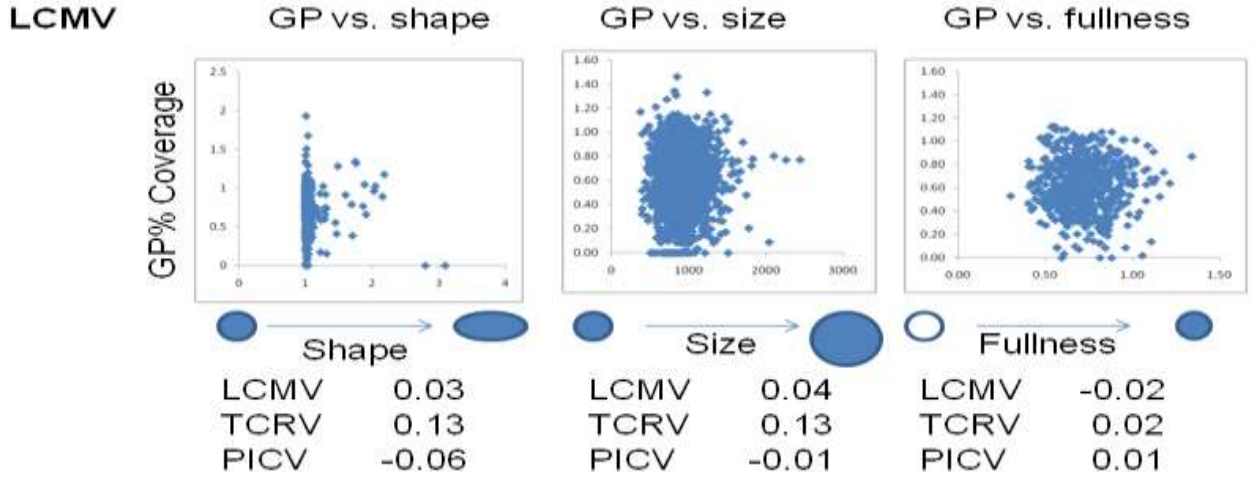

Figure 31. Scatter chart showing percentage GP vs Shape, size and fullness.

We also investigated that relationship of ribonucleoprotein density in the virion core to size, shape and GP coverage and concluded that fullness is consistently correlated with virus shape, but not with size or amount of GP (see Fig 32).

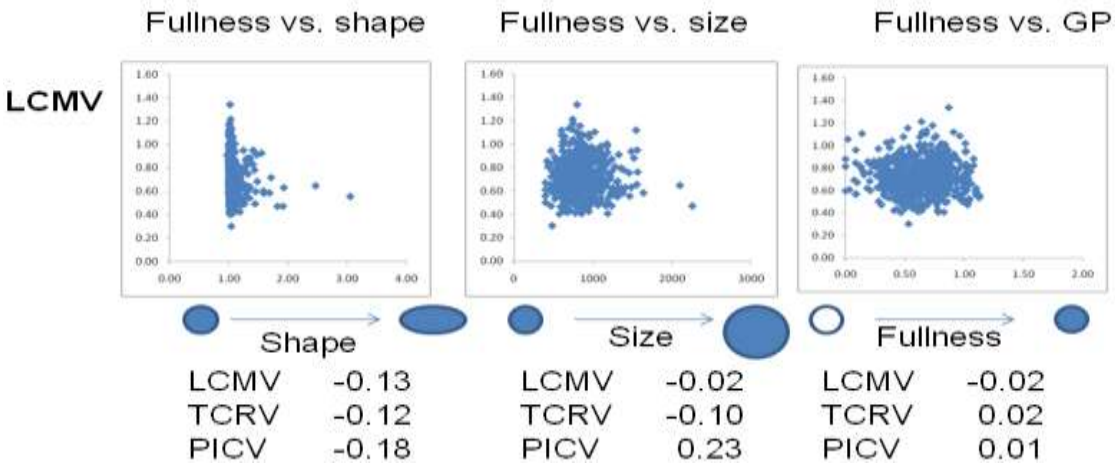

Figure 32. Relationship of ribonucleoprotein density in the virion core to size, shape and GP coverage. Here we concluded that fullness is consistently correlated with virus shape, but not with size or amount of GP.

We selected GP and Picked GPs of LCMV (See Fig 33) and JUNV (see Fig 34)and did Eman NR classes to see how they appear in virion. Then we looked in to Junin virus as well. We will measure protein spacing from class averages to investigate and compare the protein size and shape of each of species of arenavirus.

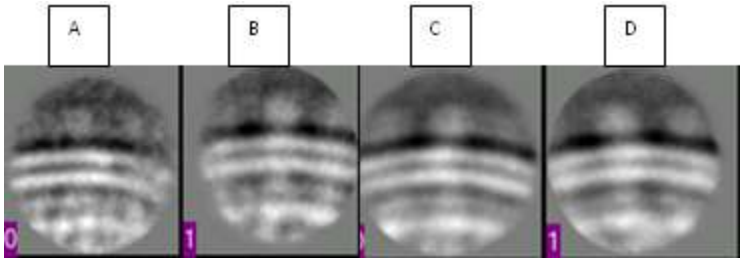

Figure 33. Surface features as well as inner densities. (A and B) Class averages of LCMV showing GP, LB, Z and NP attached from Top to bottom of the NR class generated images. (C and D) same as of A and B but different group of Classes.

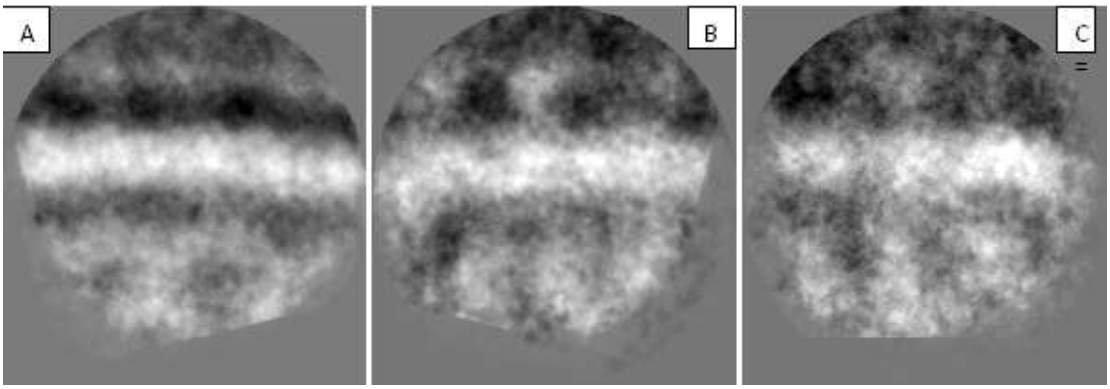

Figure 34. First structural analysis on the Junin virus GP, NP and Z (A, B and C) From top to bottom NR class generated image of GP, Lipid Bilayer(LB),Z and NP attached in the virion.

We have concluded that the shape of Arenavirus overall is significantly different from shape of vesicles. LCMV is best treated as two population one is small viruses that are unnaturally round and large viruses that are the same as vesicles. We also found that shape of small arenavirus particles is significantly rounder than shape of vesicles. Therefore: Protein interactions of Arenaviruses make small particles round. Large particles are elliptical due to insufficient interaction of protein. Future work now focuses on variation in the GP, NP, and Z that correlates with changes in size and shape.

A final model of LCMV virion is presented which shows GP on top attached to lipid bilayer. Ring finger Z protein is attached with lipid bilayer and with Nucleoprotein. L and S segments are inside of virion attached with NP (Please see Fig 35).

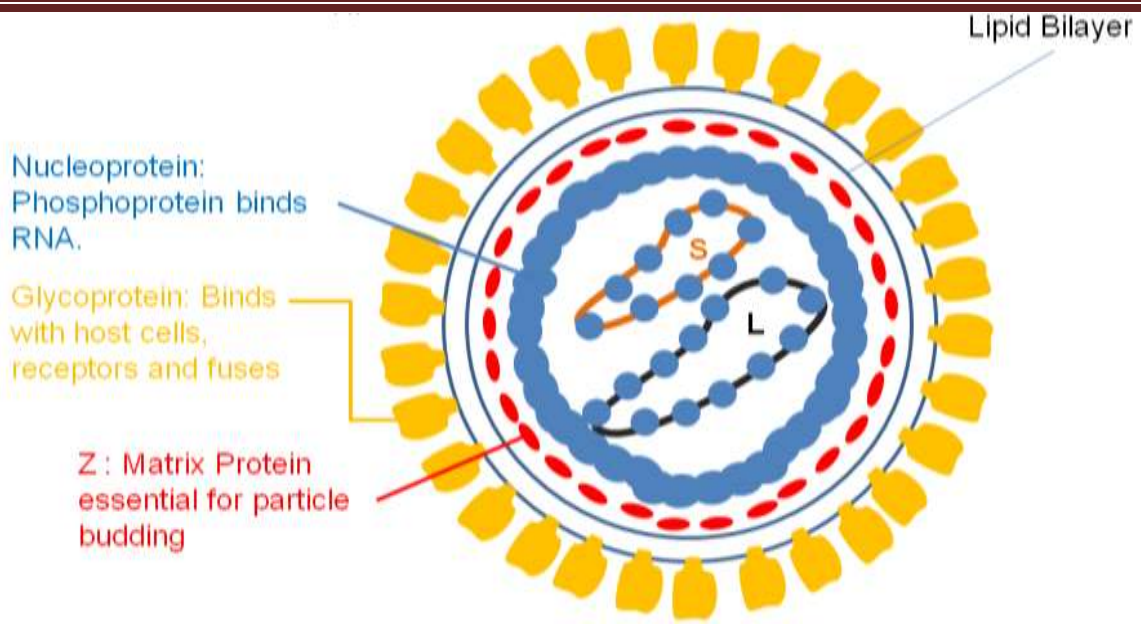

Figure 35. Final model of LCMV virion showing protein assembly.

Future work:

We have a plan to trap more mice and rats from different locations in Sindh province of Pakistan. We will trap them from agricultural land, residential areas, poultry farms and food godowns for collection of tissue (liver and spleen) samples. All collected samples will then be stored at Central Veterinary Diagnostic and Research Laboratory (CVDL) Sindh, Tandojam, Pakistan for dispatching to here in our lab G-70 for further analyzes.

Conclusion

After completion of remaining measurement and GP counting and GP selecting we will analyze that data by using Eman program and ANOVA. We will also make viruses, grow them and separate them by size using column chromatography. We will then examine the ratio of particles to infectious units to determine how rigid, stable and infectious these particles are. We will collect blood and tissue samples from rodents from different parts of Sindh province of Pakistan as part of our epidemiological work to find a scientific evidence of disease incidence and distribution caused by Arenaviruses and Toroviruses. We will make VLPs of arenaviruses to present their structure which will help in development of antiviral drugs. Giving up-to-date data and scientific evidence and distribution of both of the viruses will also help livestock and fisheries department′s planning and development directorate to formulate and devise a control strategy to from further spread of arenavirus-like diseases among livestock and aseptic meningitis among human population regionally and globally.

References:

1. Auperin, D. D., and J. B. McCormick. 1989. Nucleotide sequence of the Lassa virus (Josiah strain) S genome RNA and amino acid sequence comparison of the N and GPC proteins to other arenaviruses. Virology 168:421-5.
2. Auperin, D. D., V. Romanowski, M. Galinski, and D. H. Bishop. 1984. Sequencing studies of pichinde arenavirus S RNA indicate a novel coding strategy, an ambisense viral S RNA. J Virol 52:897-904.
3. Auperin, D. D., D. R. Sasso, and J. B. McCormick. 1986. Nucleotide sequence of the glycoprotein gene and intergenic region of the Lassa virus S genome RNA. Virology 154:155-67.
4. Borden, K. L., E. J. Campbell Dwyer, and M. S. Salvato. 1998. An arenavirus RING (zinc-binding) protein binds the oncoprotein promyelocyte leukemia protein (PML) and relocates PML nuclear bodies to the cytoplasm. J Virol 72:758-66.
5. Borrow, P., and M. B. Oldstone. 1994. Mechanism of lymphocytic choriomeningitis virus entry into cells. Virology 198:1-9.
6. Buchmeier, M. J. 2002. Arenaviruses: protein structure and function. Curr Top Microbiol Immunol 262:159-73.
7. Calain, P., and L. Roux. 1993. The rule of six, a basic feature for efficient replication of Sendai virus defective interfering RNA. J Virol 67:4822-30.
8. Carter, M. F., N. Biswal, and W. E. Rawls. 1973. Characterization of nucleic acid of pichinde virus. J Virol 11:61-8.
9. Charrel, R. N., and X. de Lamballerie. 2009. Zoonotic aspects of arenavirus infections. Vet Microbiol.
10. Childs JE, P. C. 1993. Ecology and Epidemiology of arenavirus and their hosts, p. 331-384. *In* M. S. Salvato (ed.), The Arenavirus. plenum Press, New York.

11. Conzelmann, K. K. 1996. Genetic manipulation of non-segmented negative-strand RNA viruses. J Gen Virol 77 (Pt 3):381-9.
12. Downs, W. G., L. Spence, and J. G. Borghans. 1963. Arthropod-Borne Encephalitis Viruses in the West Indies Area. V. A Serological Survey of Cura Cao, N.W.I. Trop Geogr Med 15:237-42.
13. Eichler, R., O. Lenz, T. Strecker, M. Eickmann, H. D. Klenk, and W. Garten. 2003. Identification of Lassa virus glycoprotein signal peptide as a trans-acting maturation factor. EMBO Rep 4:1084-8.
14. Eichler, R., O. Lenz, T. Strecker, and W. Garten. 2003. Signal peptide of Lassa virus glycoprotein GP-C exhibits an unusual length. FEBS Lett 538:203-6.
15. Francis, S. J., and P. J. Southern. 1988. Molecular analysis of viral RNAs in mice persistently infected with lymphocytic choriomeningitis virus. J Virol 62:1251-7.
16. Froeschke, M., M. Basler, M. Groettrup, and B. Dobberstein. 2003. Long-lived signal peptide of lymphocytic choriomeningitis virus glycoprotein pGP-C. J Biol Chem 278:41914-20.
17. Fuerst, T. R., E. G. Niles, F. W. Studier, and B. Moss. 1986. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. Proc Natl Acad Sci U S A 83:8122-6.
18. Fuller-Pace, F. V., and P. J. Southern. 1989. Detection of virus-specific RNA-dependent RNA polymerase activity in extracts from cells infected with lymphocytic choriomeningitis virus: in vitro synthesis of full-length viral RNA species. J Virol 63:1938-44.
19. Fuller-Pace, F. V., and P. J. Southern. 1988. Temporal analysis of transcription and replication during acute infection with lymphocytic choriomeningitis virus. Virology 162:260-3.
20. Gallaher, W. R., C. DiSimone, and M. J. Buchmeier. 2001. The viral transmembrane superfamily: possible divergence of Arenavirus and Filovirus glycoproteins from a common RNA virus ancestor. BMC Microbiol 1:1.
21. Garcin, D., S. Rochat, and D. Kolakofsky. 1993. The Tacaribe arenavirus small zinc finger protein is required for both mRNA synthesis and genome replication. J Virol 67:807-12.
22. Iapalucci, S., N. Lopez, and M. T. Franze-Fernandez. 1991. The 3' end termini of the Tacaribe arenavirus subgenomic RNAs. Virology 182:269-78.
23. Ludtke, S. J., P. R. Baldwin, and W. Chiu. 1999. EMAN: semiautomated software for high-resolution single-particle reconstructions. J Struct Biol 128:82-97.
24. Nathaanson, N. 1997. Viral pathogenesis.
25. Neuman, B. W., B. D. Adair, M. Yeager, and M. J. Buchmeier. 2008. Purification and electron cryomicroscopy of coronavirus particles. Methods Mol Biol 454:129-36.
26. Neuman, B. W., B. D. Adair, C. Yoshioka, J. D. Quispe, G. Orca, P. Kuhn, R. A. Milligan, M. Yeager, and M. J. Buchmeier. 2006. Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy. J Virol 80:7918-28.
27. Oldstone, M. B., R. Ahmed, J. Byrne, M. J. Buchmeier, Y. Riviere, and P., and S. 41:70–74. 1985. Virus and immune responses: lymphocytic choriomeningitis virus as a prototype model of viral pathogenesis. Br. Med. Bull 41:70-74.
28. Riviere, Y., R. Ahmed, P. J. Southern, M. J. Buchmeier, F. J. Dutko, and M. B. Oldstone. 1985. The S RNA segment of lymphocytic choriomeningitis virus codes for the nucleoprotein and glycoproteins 1 and 2. J Virol 53:966-8.
29. Romanowski, V., and D. H. Bishop. 1985. Conserved sequences and coding of two strains of lymphocytic choriomeningitis virus (WE and ARM) and Pichinde arenavirus. Virus Res 2:35-51.
30. Salazar-Bravo, J., L. A. Ruedas, and T. L. Yates. 2002 Mammalian reservoirs of arenavirus. Curr Top Microbiol Immunol 262:25-63.
31. Salvato, M., Clegg JCS,Buchmeier MJ,Charrel RN, Gonzales JP,Lukashevish IS, Peters CJ, Rico-Hesse R, Romanoswski V (ed.). 2005. Family Arenaviridae, vol. Eigth, Los Angless.
32. Salvato, M., E. Shimomaye, and M. B. Oldstone. 1989. The primary structure of the lymphocytic choriomeningitis virus L gene encodes a putative RNA polymerase. Virology 169:377-84.
33. Salvato, M. S., and E. M. Shimomaye. 1989. The completed sequence of lymphocytic choriomeningitis virus reveals a unique RNA structure and a gene for a zinc finger protein. Virology 173:1-10.
34. Southern, P. J., M. K. Singh, Y. Riviere, D. R. Jacoby, M. J. Buchmeier, and M. B. Oldstone. 1987. Molecular characterization of the genomic S RNA segment from lymphocytic choriomeningitis virus. Virology 157:145-55.
35. Suloway, C., J. Pulokas, D. Fellmann, A. Cheng, F. Guerra, J. Quispe, S. Stagg, C. S. Potter, and B. Carragher. 2005. Automated molecular microscopy: the new Leginon system. J Struct Biol 151:41-60.
36. Trapido, H., and C. Sanmartin. 1971. Pichinde virus, a new virus of the Tacaribe group from Colombia. Am J Trop Med Hyg 20:631-41.
37. Wattem, R. 2004. Junin Virus. Virginia Informatics Institute Verginia Tech.
38. Wilson, S. M., and J. C. Clegg. 1991. Sequence analysis of the S RNA of the African arenavirus Mopeia: an unusual secondary structure feature in the intergenic region. Virology 180:543-52.