Role of Epidemiology of Arena Viruses

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Abstract: *The current research investigates the Epidemiology of Arenavirus .The results shows that infections follow a typical epidemic curve with a 1-2 week latent period, as shown for the case of Lujo virus which was identified in South Africa, September – October 2008 (see Fig 12). Source: Special Pathogens Unit and Epidemiology Division, NICD; Gauteng Provincial Outbreak Response Team and partners; SA-FELTP residents; Department of Anatomical Pathology, University of the Witwatersrand and the National Health Laboratory Service*

Key Words: *Role,Epidemiology , Arenaviruses,*

Introduction:

The presence of arenavirus is worldwide but some of arenavirus species are confined to countries or particular geographical locations. It is because the previous studies have been only carried out in those areas where it caused a major problem in human population. The epidemiology of disease depends on the patterns of infection in the reservoir hosts and on the factors that bring man in contact with the rodents. Tacaribe virus was isolated from 11 of 398 fruit bats in Trinidad. The isolation occurred during a 3 year period, but experimental infection of bats and later attempts to isolate the virus failed, so the actual reservoir remains in doubt (12).

All arenavirus form stable, infectious aerosols and have been important causes of laboratory infections and deaths. Human pathogens must be handled with care, and the VHF viruses (Lassa, Junin, Machupo, and Guanarito) are manipulated under BSL-4 containment.

Some of figures (Fig. 10 and Fig 11) given below also shows geographical distribution of various species of arenaviruses. Our studies of their presence in Sindh Pakistan will also significantly contribute towards their persistence in that part of the world which lack any evidence of arenavirus presence.

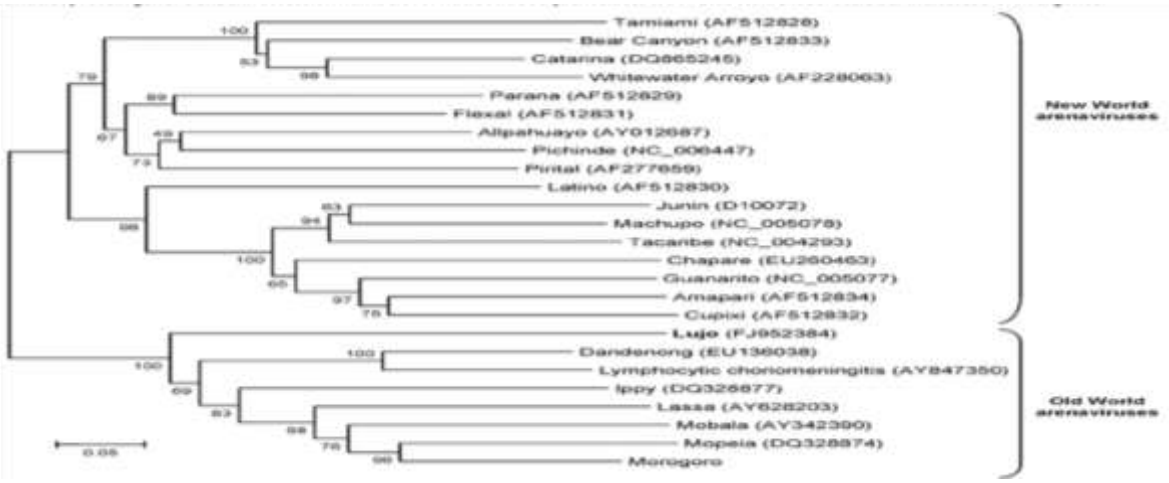

Figure 10. Neighbor-joining tree reconstructed by using bootstrap analysis with 1,000 pseudoreplicate datasets showing the phylogenetic relationship of known arenaviruses (data derived from GenBank) to the novel Lujo arenavirus from southern Africa (boldface), inferred from a 619-nt region of the 5´ end of the nucleoprotein gene. GenBank accession numbers for nucleotide sequence data are shown on the tree. Scale bar indicates 5% divergence.

Figure 11 . Gegraphical map showing the some of species of arenavirus with their host and year of their first isolation.

Arenavirus infections follow a typical epidemic curve with a 1-2 week latent period, as shown for the case of Lujo virus which was identified in South Africa, September – October 2008 (see Fig 12). Source: Special Pathogens Unit and Epidemiology Division, NICD; Gauteng Provincial Outbreak Response Team and partners; SA-FELTP residents; Department of Anatomical Pathology, University of the Witwatersrand and the National Health Laboratory Service.

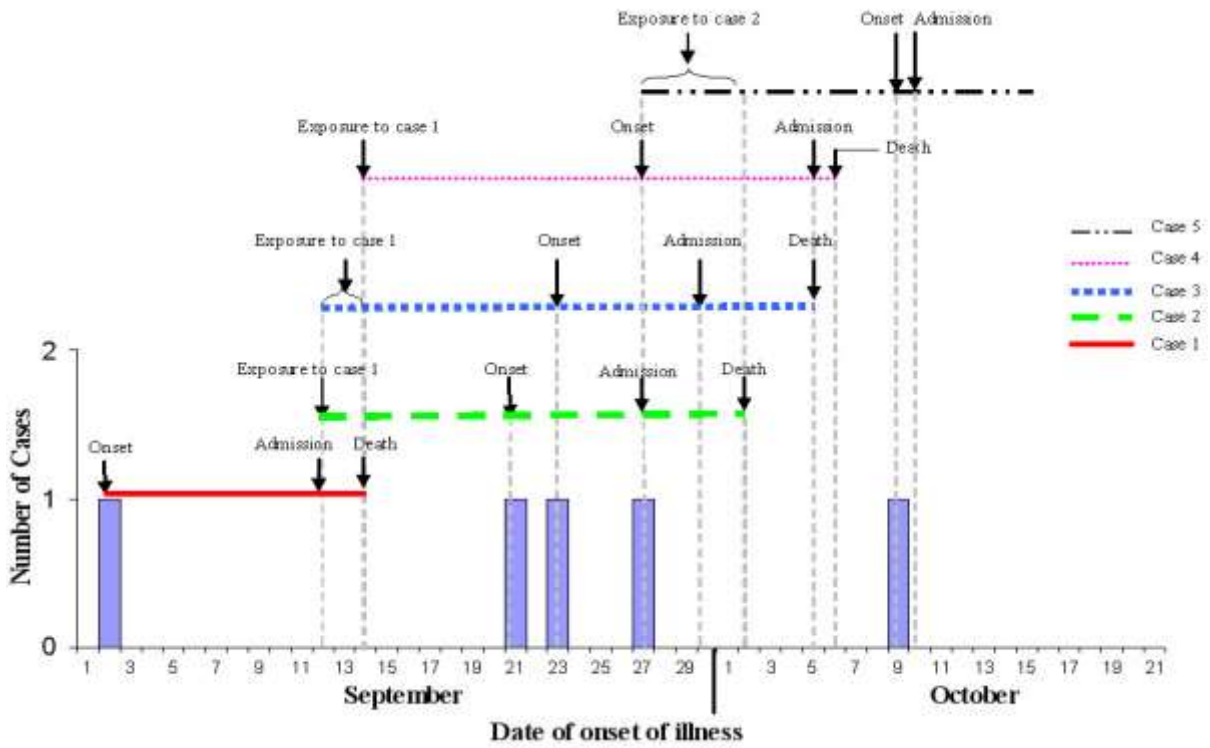

Figure 12. Distribution of arenavirus cases in a two month period in according to hospital records.

Procedure for baiting and capturing rodents:

There are different procedures for baiting the rodents in Sindh Pakistan. We bought a cage made of iron and put into a tomatoes and pieces of vegetable just to attract the rodent to enter into cage. Rodents were trapped live. We captured 22 rodents in September and October, 2009 from different places of Shikarpur and Nawabshah districts of Sindh for organs/tissue samples.

Figure 14. A digital image of Wild Rodents laying on dressing table for laparotomy.

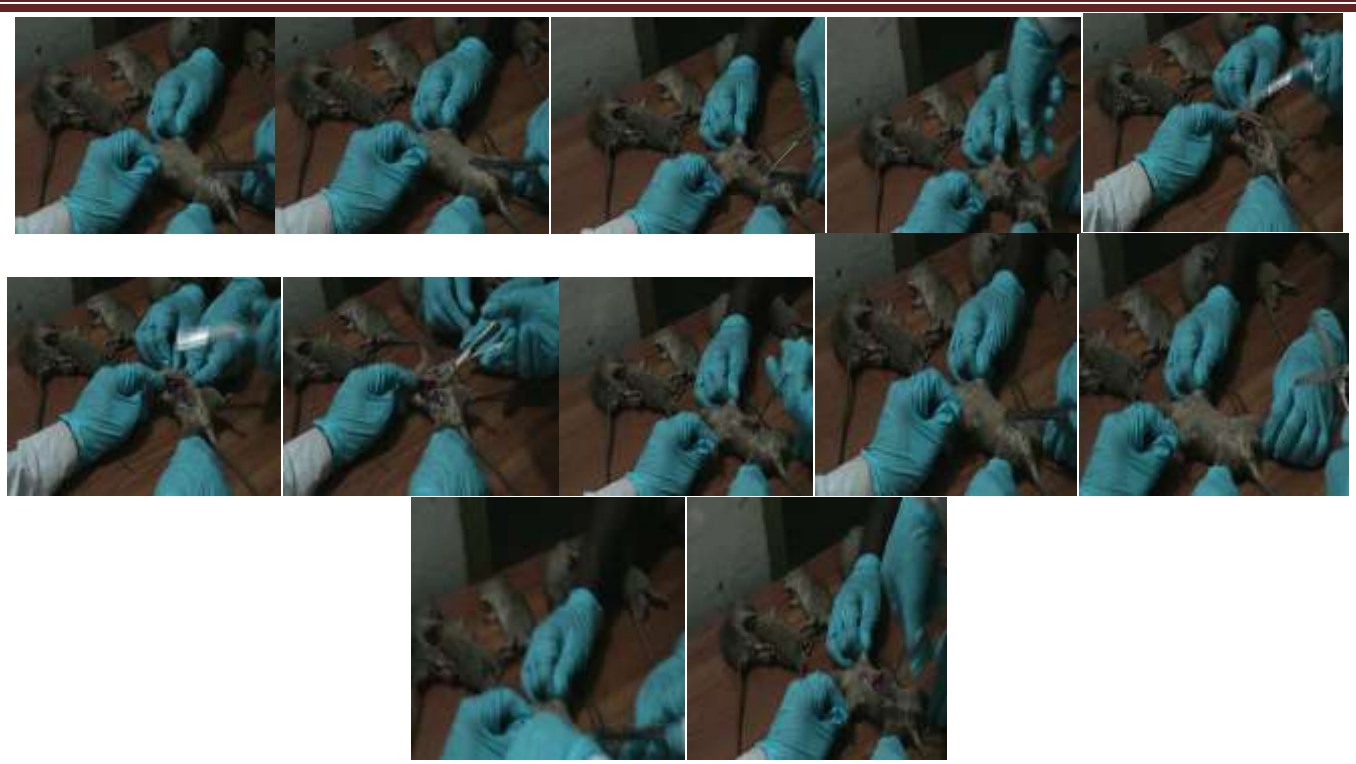

Figure 15.Dissection of wild rodents for the collection of tissue samples in Shikarpur and Nawabshah Districts of Sindh, Pakistan.

Figure 16 .UK rats dissection being done by Dr Ben Neuman, Gabriella Kiss, Abdullah and Dorin Ile. Rats were kindly provided by Dr Colin Prescott and Dr Alexander Stuart of Plant Sciences Department of Reading University.

We have collected tissue samples (spleen and liver) from 22 wild rodents (see Fig 14 and 15) in Pakistan and 40 from UK rats (see fig 16) in collaboration with Dr Colin Prescott and Dr Alexander, Plant Sciences Department of Reading University. We have purified RNA from all 62 samples and have reverse transcribed 12 samples and also did the PCR. Our future work is to complete RT-PCR with multiple sets of degenerate arenavirus primers and collect more samples from Sindh Pakistan and UK rates from different location.

Procedure of the collection of organs for RNA extraction:

We took each mouse from the cage one by one by covering their head with the piece of cloth. Mice were euthanized by cervical dislocation (see Fig 14). We took dead mice to the table, performed a laparotomy and then collected tissue samples from liver and spleen of each mouse. Tissue samples were stored in tubes with 1 ml Trizol. Before dispatching them to our virology lab in UK samples were stored in the freezer. Samples were transported by courier service to the UK with frozen gel blocks to keep the temperature cool. Upon arrival all the samples were kept in -80 freezers for RNA extraction and RT-PCR.

Homogenization:

We homogenized tissue samples in 1ml of Trizol reagent of per 50-100 mg of tissues using a glass-Teflon and then stored back in -80 for RT-PCR.

RNA Extraction:

We extracted Ribonucleic acid (RNA) from all of 62 (22 from Pakistan and 40 from UK rats)tissue samples .After homogenization we spined it at 14000RPM for 10 minute at 4˚C .After that transferred supernatant to new clean tube and discarded pellet . We also discarded any fatty material at top. We added Trizol to make the volume 1ml in each of the tube and then added 200µl of chloroform per 1ml Trizol and vertexed it for 15 seconds and spined at 14000RPM for 15minutes at 4˚C. We carefully transferred the top (aqueous) to a new tube and then added 500µl of isopropanol per tube and mixed it by inverting for about 15 times and then spined at 14000 RPM for 10 minutes at 4˚C. After centrifugation we dumped and discarded supernatant and let the pellet to dry by inverting tube on paper towel in the virus hood and then resuspended the pellet in RNAs free water.

Before resuspended the pellet in RNAs free water we smelt the pellet and resuspended those who were found to have phenol scent then proceed resuspend pellet in ~500 micro liters of RNAs free water and then added 500µl chloroform and then vertexed for 15 seconds so to mix it thoroughly then centrifuged it at 13000RPM for 5 minutes at 4˚C. We took aqueous layer into a new tube and then added 500µl isopropanol to aqueous layer and mixed by inverting and centrifuged at 13000RPM for 15 minutes at 4˚C. After that we found white pellet

without phenol scent. Pellets were then resuspended into RNAs free water and stored in -80˚C freezer for Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) (see Fig 17).

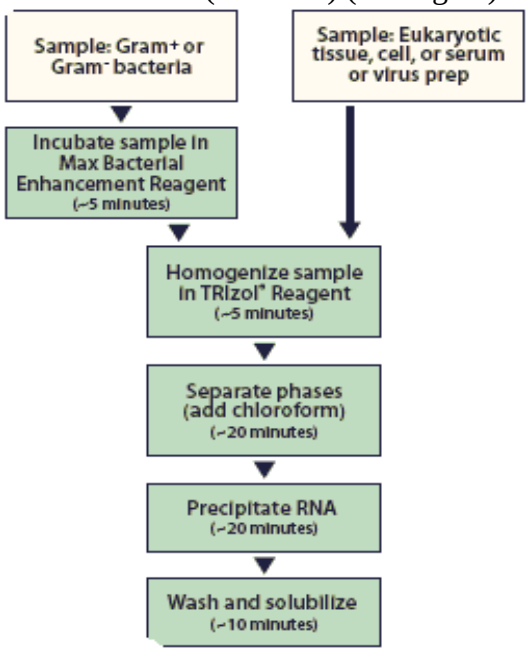

Figure 17. Schematic showing the method for purification of total RNA from tissue samples which are stored in Trizol.

Table 4.Oligonucleotide Primers of Arena viruses for RT-PCR.

Oligonucleotide primer name	**Sequence**
Arena s RVS	CAA TGC CCA GCY TGA CAA T
Arena S FWD	AGT GCY TGC ACA ACA GCG TTT
All arena RT	CGC ACM GGG GAT CCT AGG C
Z150R	SRA ACC ARC ANS WYY TRC A
Z150LCMR	CTG TCG ANY CTT TGC CRC A
L3200R	TTG GNG ANT CNT CTG AAT AGA AAC A
L2000F	ATN AAA TGT TTT GAN AAG TT
L2000R	AAC TTN TCA AAA CAT TTN AT
L1700F	TTY TAY KSD GAY CCH AAR MGR T
L1700R	AYC KYT TDG GRT CHS MRT ARA A
L2100F	ACW GAY CAR ATH AAR TGY TTY GAR AA
L2100R	TCW GGN GTY TCY TTN GTN A
L3300R	TTN SHH CCH ACY TGY TCY TTR TA
L3600F	GAY CAY WSH AAR TGG GGN CC
L3800R	TGN ARD ATN CCY TGN CCC WTR TC

Table 5. PCR Pairs used for amplification of arenavirus.

Primer 1	Primer 2	Amplicon (bp)
All RT	Z150R	210
All RT	Z150LCMR	210
3200RL	2000FL	1066
2000RL	L1700F	463
3200RL	L2100F	463
L2100R	L1700F	1100
L3800R	L3600F	430
Arena S Fwd	S RNS	270
All RT	Z150R	Z150LCMR

The above (see tables 3, 4 and 5) show the nomenclature, sequences and amplicon sizes expected from the amplification of novel sequences using degenerate RT-PCR oligos that reflect the natural sequence variation observed among known arenaviruses for detection of any of arenavirus species.

Procedure for Reverse Transcription:

Primers Used in Reverse Transcriptase (RT):

The primers used in RT were All Arenavirus RT, L2000F, L3600F, L3200R and L1700R to transcribe RNA to cDNA for polymerase chain reaction (PCR).

SUPERSCRIPT II RNase H- Reverse Transcriptase:

RNA was reverse transcribed to cDNA by using following this method. A 20-µl reaction volume was used for 1 ng–5 µg of total RNA or 1–500 ng of mRNA and then added the following components to a nuclease-free microcentrifuge tube Oligo(dT)12-18 (500 µg/ml) or 1 µl,50–250 ng primer 1 and primer 2 mole gene-specific primer (GSP)1 ng to 5 µg total RNA or x µl 1–500 ng of mRNA,1 µl dNTP Mix (10 mM each) 1 µl Sterile, distilled water to 12 µl. The mixture was heated to 65°C for 5 min and quick chill on ice then collected the contents of the tube by brief centrifugation and added 5X First-Strand Buffer 4 µl 0.1 M DTT 2 µl RNase (40 units/µl) and then 1 µl RNase free water was added using <50 ng starting RNA. Then the contents of the tube were mixed gently. Then GSP (Gene Specific Primers) were added (See Tables 4 and 5), incubated at 42°C for 2 min.After that 1 µl (200 units) of SuperScript™ II RT was added and mixed by pipetting gently up and down. The amount of SuperScript™ IIRT to 0.25 µl (50 units) was added and added sterile, distilled water to a 20-µl final volume and then incubated at 42°C for 50 min. After that the reaction was inactivated by heating at 70°C for 15 min and yielded cDNA which were then used as a template for amplification in PCR.

Procedure for PCR:

Master Mixture for 12 PCR samples: We prepared a mixture by adding 162.5 µl of master mix 6.5µl primer 1,6.5 µl primer 2 and 141.75 µl water .

PCR:

We prepared a vials for PCR by adding 1µl of cDNA and 24 µl of master mix (GoTaq Green Master Mix) 0.5 µl Primer 1, 0.5 µl primer 2, 1 µl template cDNA and 10.5 µl nuclease free water and then mixed with rotor .All the above 36 vials were then placed in PCR machine according to following time, temperature and rounds as given in table.

Table 7. PCR protocol.

95˚C	95˚C	55˚C	72˚C	72˚C	
3 Minutes	00:30 Minutes	00:30 Minutes	1:00 Minutes	3 Minutes	
	40 cycles				4˚C

Results :

Detection of arenaviruses in the wild:

Collection of samples from rodents from Pakistan:

I went to Nawabshah and Shikarpur districts of Sindh province, Pakistan for the collection of samples from rodents (mouse).Twenty two samples were collected and dispatched to Laboratory for further investigation whether or not LCMV is present in the liver and spleen of mouse species generally found in Sindh Province of Pakistan.

RT- PCR results:

We did three sets of PCRs with the same 12 samples using the primers pairs listed in table 6 and results see Fig 18.

Table 8. Pairs of primers used in PCR.

Pair A	2 Primers	L2000R	L1700F	463
Pair B	2 Primers	L2100R	L2000F	430
Pair C	2 Primers	L3800R	L3600F	270

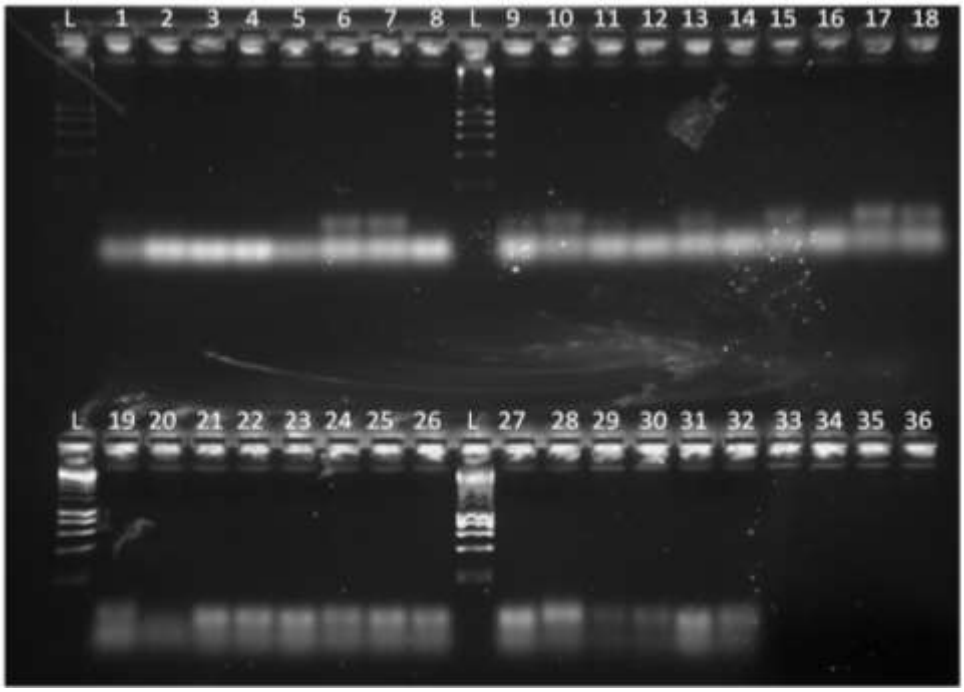

Figure 18. PCR results of 12 samples with different primers.

Results: The expected PCR product was not amplified from these samples.

We repeated the two sets of PCRs with the same 12 samples using the primers pairs listed in table 9.

Table 9. Pairs of primers of arenavirus.

Pair A	3 Primers	All Arena RT	Z150R	Z150LCMR
Pair B	2 Primers	L3200R	L2000F	

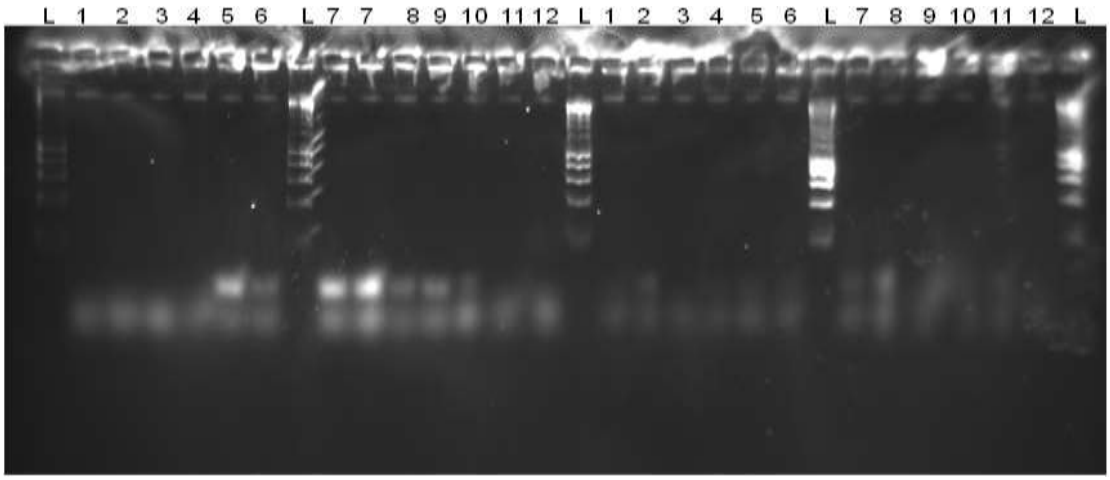

Figure 19. Results for the same rat samples as shown in Fig. 18, but using different primers. Numbers refer to samples from specific rats.

Results: The expected PCR product was not amplified from the same samples with different primers. From this we concluded that either the hosts were not infected with any arenavirus species, or there may have been flaws in the purification or amplification procedures. To address this possibility, we will be performing additional controls in future experiments.

Measurement and analysis of arenavirus structure:

I have measured the LCMV, PICV, TCRV particles. I have counted and selected the GP of LCMV particles. I am still measuring and counting the GP of rest of the particles of arenavirus species as mentioned in Table 10 and Table 11.

Table 10. Number of arenavirus particles, glycoproteins and vesicles analyzed to date.

Name of Virus	Particles measured 2x	GP counted	GP selected from virion	Remarks
LCMV-FA[1]	341	NA[2]	239	GP is not visible after fusion activated
LCMV	2302	33844	637	GP selected represents less than one-tenth of the total available number
PICV	1127	8589	477	GP needs to be counted and selected
TCRV	1997	15722	534	
JUNV	19	316	8	A few JUNV particles have been imaged
Total	5786	58471	1895	

[1]Fusion-activated LCMV

[2]In the case of LCMV-FA, not available.

TABLE 11. Number of vesicles measured found in arenavirus particles, glycoproteins analyzed to date.

Name of virus where from vesicles measured	Particles measured 2x
LCMV-FA[1]	17
FLCMV	29
PICV	2
TCRV	10
JUNV	6
DOPC[2]	617

[1]Fusion-activated LCMV

[2]Measurement of synthetic DOPC-containing vesicles

We have taken Cryo-EM images of Lymphocytic Choriomeningitis Virus (LCMV; see Figure 20). Most of them are round and variation in their shape and size. Here you can see proteins are visible in virus particles i.e. GP, NP & Z protein.

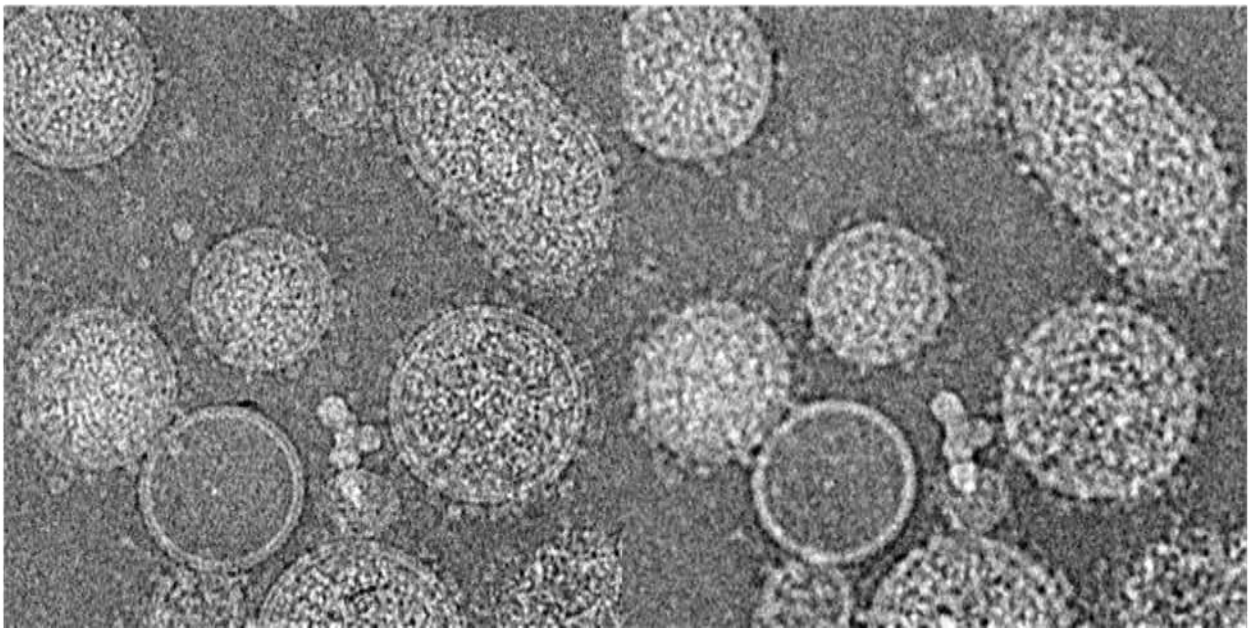

Figure 20. Images of LCMV particles and empty exosomal vesicles that were purified together Images of LCMV recorded near (left) and far (right) from focus.

Conclusion

Most smaller particles are round and large particles often appear to be elliptical and stretched (see Figure 20). We wanted to investigate why some of them are round and some of them are elliptical .To answer this question, we first tested whether small particles are significantly rounder than large particles by comparing shape of LCMV and shape of vesicles under the same condition. An empty vesicle can be seen amongst LCMV particles in Figure 21. Our method of measuring particle shape and size is shown in the same Figure 21 (left).

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