Filoviridae
Ebola virus

History:

In 1967 (31) cases of hemorrhagic fever with 7 death occurred among laboratory workers in Marburg, Germmany, Belgrade and Yugoslavia, who were processing kidneys from African green monkeys (*Ceropithecus aethiops*) that had been imported from Uganda. The new virus with very long, filamentous virions was isolated from the patients and monkeys it was named **Marburg virus**.

Later a virus morphologically identical to but antigenically distinct from Marburg virus was isolated from patients in Zaire and Sudan it was named **Ebola virus**. In 1989 and 1990 several shipments of monkeys imported from the Philippines into the United State were found to have been infected with filovirus morphologically identical and serologically related to Ebola virus.

Properties of Filoviridae:

- **Virions** of the family filo (threadlike) are very long filamentous rods with 80 nm. Virion contains seven proteins.
- **The genome** is single molecule of minus sense ssRNA 19 Kb in size
- **The nucleocapsid** helical and contains L, NP, VP3 and VP30.
- **Viral replication** in the cytoplasm of host cells is marked by the formation of large inclusion bodies and maturation occurs via budding from plasma membrane.
- **Lipid bilayer envelope** covered with peplomers surrounding the nucleocapsid. The sensitivity of filovirus to lipid solvent and inactivating agents resembles that of other enveloped RNA viruses. They retain infectivity at room temperature for several days.

Viral Replication:

Virions appear to enter cells by endocytosis and replication occurs in the cytoplasm in which nucleocapsid accumulate to form prominent inclusion bodies. Maturation occurs by budding through plasma membranes. The minus sense RNA contains seven open reading frames which code for seven known structural proteins, there is conserved transcriptional stop , start signals and a highly conserved intergenic sequence of five nucleotides at the genes boundaries.

Pathogenesis and Clinical features:

Infected patients have highest case fatality rates, sever hemorrhagic manifestations and liver necrosis. There is an early leukopenia, neutrophilia with little monocyte infiltration in site of parenchymal necrosis in liver but without evidence of disseminated intravascular clotting. Antigen is localized in the liver, spleen, kidney and adrenal glands when virus can also be seen by electron microscopy.

Infections of humans with Ebola viruses cause similar syndromes:

- Sever hemorrhagic
- Vomiting
- Abdominal pain
- Myalgia
- Pharyngitis
- Conjunctivitis
- Proteinuria
- The onset is sudden and progress to prostration, hypotension and rapid death.

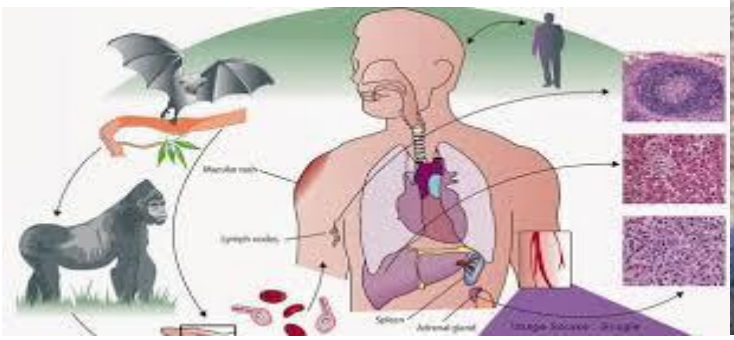
Laboratory Diagnosis:

Note: the filovirus are classified as Biosafety Level 4 pathogens and must be handled in maximum security laboratories.

- Filoviruses can be isolated from the blood during the febrile phase and most strain grow well in Vero cell.
- The key to identification is the distinctive morphology by electron microscopy.
- Direct immunofluorescence can be used to identify viral Ag in cultured cells.
- Indirect immunofluorescence can be used for serological tests.

Epidemiology:

Viruses are transmissible to humans from primates. In all outbreaks secondary spread between humans appears to have been principally due to contact with body fluids from an acute case, although respiratory infection may also occur, outbreaks spread was largely within hospitals owing to the reuse of blood contaminated syringes and/or needles.



Prevention and Control:

- In 1990 following local, national and international primates transport and import protocols to prevent filovirus infection in workers were improved.
- If filovirus cases are suspected laboratory diagnosis in central lab such as the Centers for Disease Control in the United States.
- If human cases are diagnosed they should be treated by isolation with barrier nursing and careful attention to prevent nosocomial or respiratory spread.

Flavivirus
Yellow fever

Yellow fever (yellow jack, yellow plague and bronze john) is a viral disease of typically short duration caused by yellow fever virus, the virus is an RNA virus of the genus Flavivirus. Two genotypes have been identified I and II, genotype I has been divided into five subclasses A through E.

Epidemiology:

Yellow fever is common in tropical and subtropical areas of south America and Africa. An estimated about 90% of infections occur on the African continent. Yellow fever has never occurred in Asia this may be due to that the mosquito in the east are less able to transmit the virus and the immunity present in the populations because of other diseases caused by related viruses (for example: dengue).

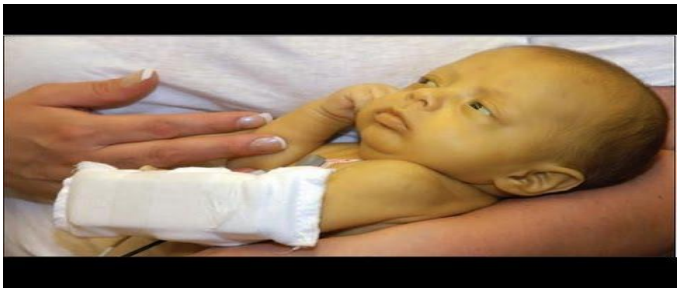
Transmission: It is spread by the bite of infected female mosquito (*Aedes aegypti*).

Pathogenesis:

After transmission from mosquito the viruses replicate in the lymph nodes and infected dendritic cells from there they reach the liver and infect hepatocytes via kupffer cells which lead to eosinophilic degradation of these cells and release of cytokines. Apoptotic masses known as councilman bodies appear in the cytoplasm of hepatocytes.

Fatality may occur when cytokine storm, shock and multiple organ failure follow.

Symptoms:



- Fever
- Chills
- Loss of appetite
- Nausea
- Muscle pain in the back
- Headaches

All these symptoms improve within five days. In about 15% of people within a day improve the fever comes back, abdominal pain occurs and liver damage begins causing yellow skin. The risk of bleeding with kidney problems is also increased.

Diagnosis:

- Clinically diagnosis made on the basis of symptoms.
- To confirm a suspected case, blood sample testing with Real time polymerase chain reaction is required until to 10 days after illness.
- Virus isolation in cell culture using blood plasma this take one to four weeks.
- ELISA for specific IgM and specific IgG titer.
- Liver biopsy to detect viral Ag.

Prevention and Control:

- Use of insect repellent as vector control
- Wear proper clothing to reduce mosquito bites
- Vaccination is recommended for those traveling to affected areas. The WHO recommends routine vaccinations for people living in affected areas between th 9^{th} and 12^{th} months of birth.

Arenaviridae
Lassa virus

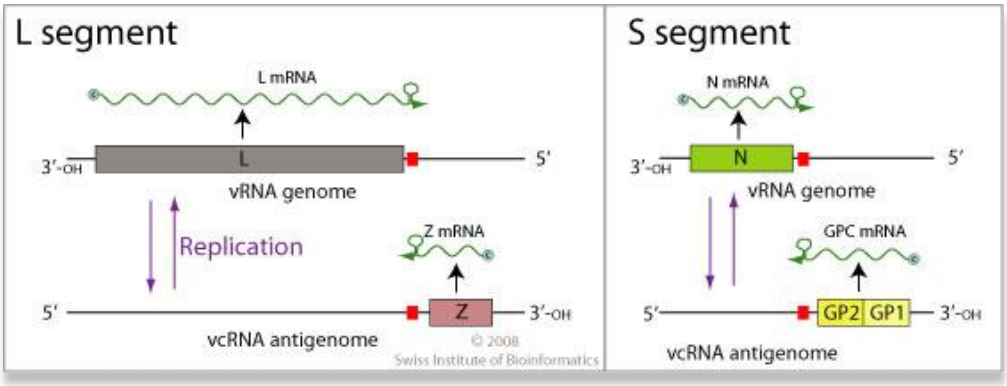
History:

The prototype arenavirus is lymphocytic choriomeningitis virus which produce inapparent lifelong infection in mice and occasionally transmitted to humans causes disease ranging from mild fever to meningitis.

First isolated over 60 years ago. In 1969 another arenavirus made newspaper headlines when nurse from a mission in Lassa, Nigeria had died in the hospital, the virus was isolated from her blood by virologist at Yale University. Lassa virus like a number of other arenaviruses isolated from humans during outbreaks of hemorrhagic fever in South America occurs as a lifelong infection in its natural rodent host.

Properties of Arenaviridae:

- The family Arenaviridae comprise a single genus Arenavirus which is divided inti two serogroups or complexes:
  1- The Old World (LCM-LAS complex) arenaviruses contains lymphocytic choriomeningitis (LCM) virus and Lassa virus (African Arenavirus).
  2- The New World serogroup (Tacaribe complex) comprises the South American arenavirus Tacaribe, Junin, Machupo and Guaranito.
- Spherical enveloped virion and virion contain nonfunctional ribosomes.
- Lipoprotein envelope covered with glycoprotein peplomers.
- The genome has *ambisense* arrangement : the genome comprises two linear segments of ssRNA L and S, 7.2 and 3.4 kb respectively, each segment forms a circle by hydrogen bonding of its ends conserved nucleotide sequences at the 3 end of each RNA. Most of the genome is minus sense but the 5 half of 5 segment and a short sequence at the 5 end of the L segment are of plus sense.



- Replication occurs in the cytoplasm.
- Genetic reassortment occurs during replication.
- Maturation by budding from plasma membrane.

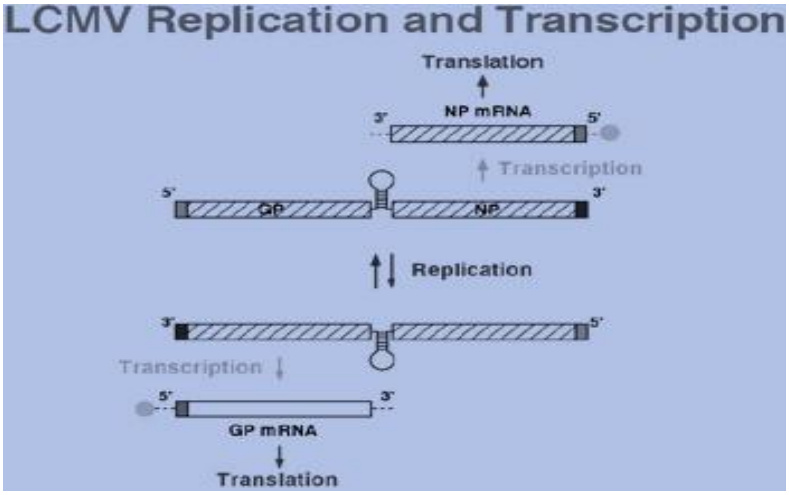
Viral Replication:

Arenaviruses grow to high titer in cell culture, replicating in the cytoplasm and maturing by budding from the plasma membrane. They have limited lytic capacity leading to produce defective interfering particles (DI) in cultures.

After entry and uncoating of the virion , subgenomic mRNA encoding the nucleoprotein (N) is transcribed by the virion transcriptase from the minus sense 3 half of ambisense S segment of the genome. A short hairpin configuration in the intergenic region in the middle of the S gene segment is serve as the transcription termination signal. The mRNA appears to derive its 5 cap snatching from heterogeneous cellular RNA. Subgenomic mRNA encoding the transcriptase (L) that transcribed from the minus sense 3 portion of L segment of the genome. Translation of both N and L proteins is required prior to replication step which required for the synthesis of full length complementary copies of L and S segments. The glycoprotein (G) and the zinc- binding protein (Z) be transcribed from the end of S and L genome segments respectively.

Budding of the virion occurs from the plasma membrane and when a cell is infected with two different arenavirus species genetic reassortment regularly emerge.

So, Coding, transcription, translation and replication strategy of S RNA indicating the sequence of events necessary to obtain the S- coded gene products and there are similar strategies for the transcription of the ambisense L RNA species.



Clinical stages of Lassa fever:

The clinical course of Lassa fever indistinguishable from those of febrile illnesses such as malaria and other viral hemorrhagic fever such as Ebola. However, clinical course of Lassa fever has 4 stages:

- **Stage 1:** (1-3 days) general weakness and malaise, high fever > 39^0C, constant with peaks of 40-41^0C.
- **Stage 2:** (4-7 days) sore throat with white exudative patches very common headache, back chest, side or abdominal pain, conjunctivitis, nausea, vomiting, diarrhea, proteinuria with low blood pressure < 100 mmHg.
- **Stage 3:** (after 7 days) Facial edema, convulsions, mucosal bleeding (mouth, eyes, nose), internal bleeding confusion or disorientation.
- **Stage 4:** (after 14 days) Coma and death.

Laboratory Diagnosis:

Biosafety Level 4 containment is required for lab diagnosis. Specimen (blood, CSF, autopsy) transport in keeping with national and international regulations. Diagnosis is usually based on:

A. Serology to demonstrate antibodies of IgM.
B. Indirect immunofluorescence (IFA) using spot slides of virus infected acetone fixed Vero E6 cell monolayers
C. Enzyme immunoassay (EIA)
D. Polymerase Chain Reaction assays.

Prevention and Treatment:

- Rodent control by trapping and poisoning
- A live attenuated Junin virus vaccine and a vaccinia recombinant carrying Lassa virus glycoprotein gene
- Isolation of barrier nursing are required to prevent nosocomial spread to other patients and nursing staff
- Fluid, electrolyte and osmotic balances need correction and management of hypotension and shock
- Ribavirin administered intravenously and orally
- High titer convalescent phase plasma within the first week of the illness

Epidemiology:

Lassa fever, a viral hemorrhagic fever transmitted by rat, is endemic in West Africa and may kill tens of thousands of people each year. Peak incidence was thought to be in the dry seasons (January to March) and wet season (May to November). The natural host for the virus is multimammate rats (*Mastomys natalensis)* distributed widely through west, central and east Africa.

The natural history of the human disease is determined by the pathogenicity of the virus, the geographic distribution, habitat of rodent reservoir host and the nature of human-rodent interaction. However, Lassa and LCM are subclinical, Machupo induce hemolytic anemia and splenomegaly while Junin induce fetal death in rodent.