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Hemorrhagic Fever Viruses with Emphasis on Ebola

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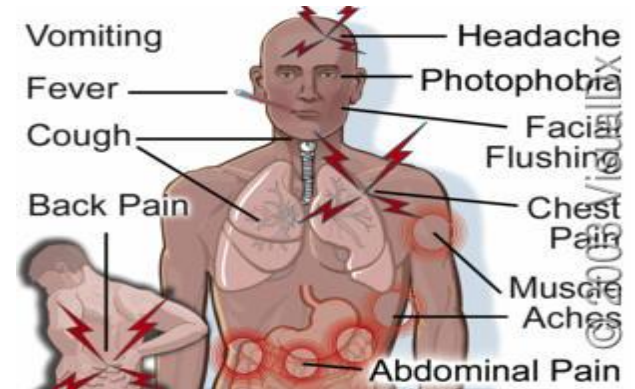
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Definitions

Viral hemorrhagic fever (VHF)

- Acute, febrile, multisystemic illness characterized by malaise, myalgia, prostration, and bleeding diathesis.
- Etiology - lipid-enveloped, single-stranded, RNA viruses in the Filoviridae, Arenaviridae, Bunyaviridae, and Flaviviridae families.



Definitions

Hemorrhagic fever virus (HFV) is a term used to generically identify those agents that cause VHF.

Ebola virus disease (EVD) is clinical term used in current epidemic, etiology Zaire ebolavirus.



Etiologic Agents of VHF

Family

Genus

Species

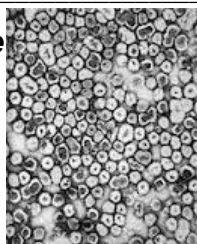
Arenaviridae



Arenavirus

Lassa (Old World)
Junin, *Machupo*, *Guanarito*, *Sabia* (New World)

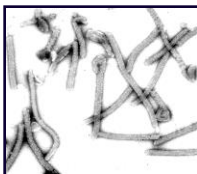
Bunyaviridae



Nairovirus *Phlebovirus* *Hantavirus*

Crimean-Congo hemorrhagic fever
Rift Valley fever
Hantaan, *Seoul*, *Puumala*, *Dobrava-Belgrade* (Old World)
Sin Nombre, *Andes* (New World)

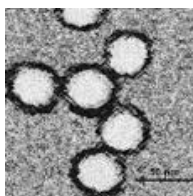
Filoviridae



Ebolavirus *Marburgvirus*

Zaire, *Sudan*, *Tai Forest*, *Reston*, *Bundibugyo*
Marburg marburgvirus

Flaviviridae



Flavivirus

Omsk HF
Kyasanur forest disease
Dengue
Yellow fever

Etiologic Agents of VHFs

VIRUS

Mortality Rate

Ebola Zaire

75-90%

Marburg

25-90%

Lassa

15-20% of hospitalized

Crimean-Congo hemorrhagic fever

30%

Rift Valley fever

50% of patients with hemorrhagic form

Arenaviridae

- Natural reservoir includes several species of mice and rats
- Direct contact with rodent feces and urine
- Exposure to rodents caught in agricultural machinery
- Secondary **person-to-person** (blood, sexual contact, urine, pharyngeal secretions) and nosocomial transmission
- Contaminated food or water
- Aerosol

Mastomys sp. - Lassa reservoir



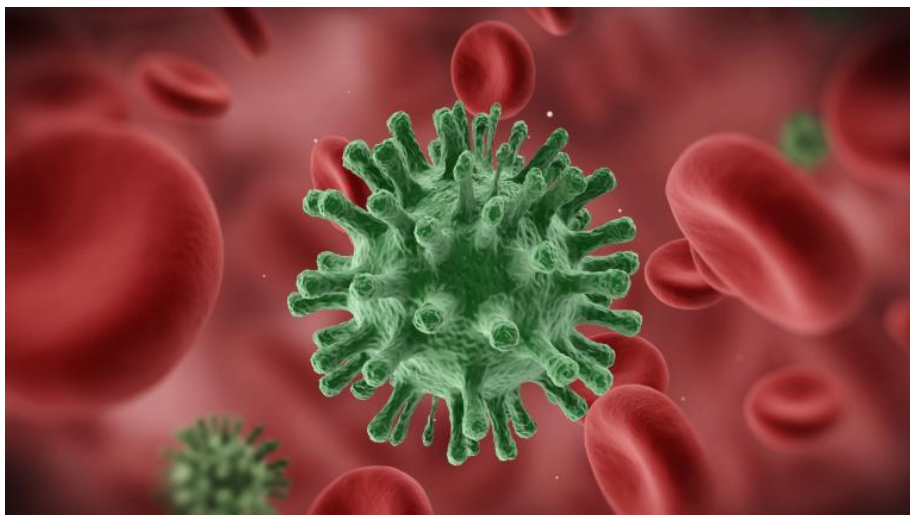


Arenaviridae

- **Lassa virus** was found in Nigeria in 1969 (2 missionary nurses died in Nigeria)
 - estimated 100-300k cases per year in West Africa and approximately 5000 deaths
 - 80% of human cases are asymptomatic
 - 1% case fatality rate; up to 15% among those hospitalized

Reservoir includes several species of mice and rats

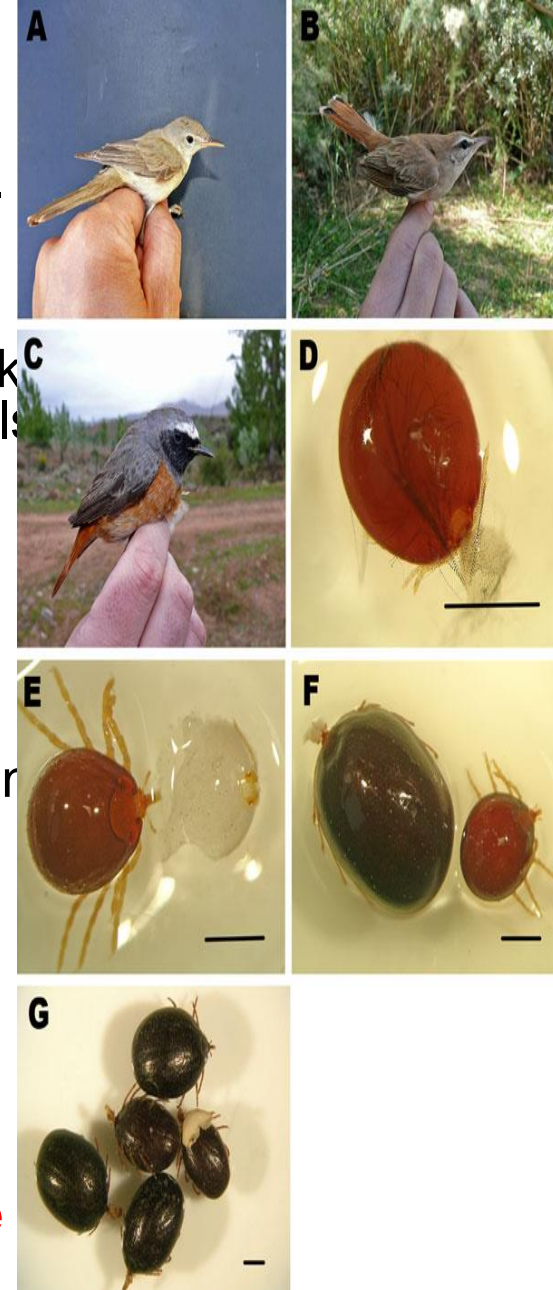
- Direct contact/Aerosol exposure with rodent feces and urine
- Exposure to rodents caught in agricultural machinery
- Contaminated food or water
- Secondary person-to-person (blood, sexual contact, urine, pharyngeal secretions) and nosocomial transmission



CCHF (*Bunyaviridae*)

Crimean-Congo Hemorrhagic Fever

- CCHF is a zoonotic disease that is transmitted by ticks and infects a wide range of domestic and wild animals
- Humans contract the disease from handling infected livestock (slaughtering), direct contact with blood, or from tick bites
- 2008-2009 Increased numbers of cases particularly in Russia and Central Asia
 - Turkey: >50 deaths since Jan 2009
 - Iran: 8 deaths since Jan 2009
 - Pakistan: 38 confirmed cases in 2012
 - **U.S. Soldier in Afghanistan: Died Sep 09 in Landstuhl, Germany secondary to a tick bite**
 - **UK traveler returning from Kabul – died in the UK October 2012**





Rift Valley Fever (Bunyavirus)

- A zoonotic disease transmitted by several species of **mosquitoes**
- Humans are infected during epizootics of the disease through **mosquito bites**, handling infected tissues (**animal slaughter**), and possibly through the **ingestion** of raw milk. **Aerosol** transmission has also led to infection in laboratory workers.
- In humans, no symptoms to mild illness but can progress to hemorrhagic fever (1% fatality rate)
- **Retinitis leading to blindness is the most common complication associated with RVF in humans (1-10%)**
- First cases outside Africa In September 2000 in Saudi Arabia and subsequently, Yemen.
- South Africa: Feb 2010
 - Department of Health of South Africa reported 172 cases and 15 deaths



Bird et.al. Rift Valley Fever Virus
Vaccine.J Virol. Dec 2011

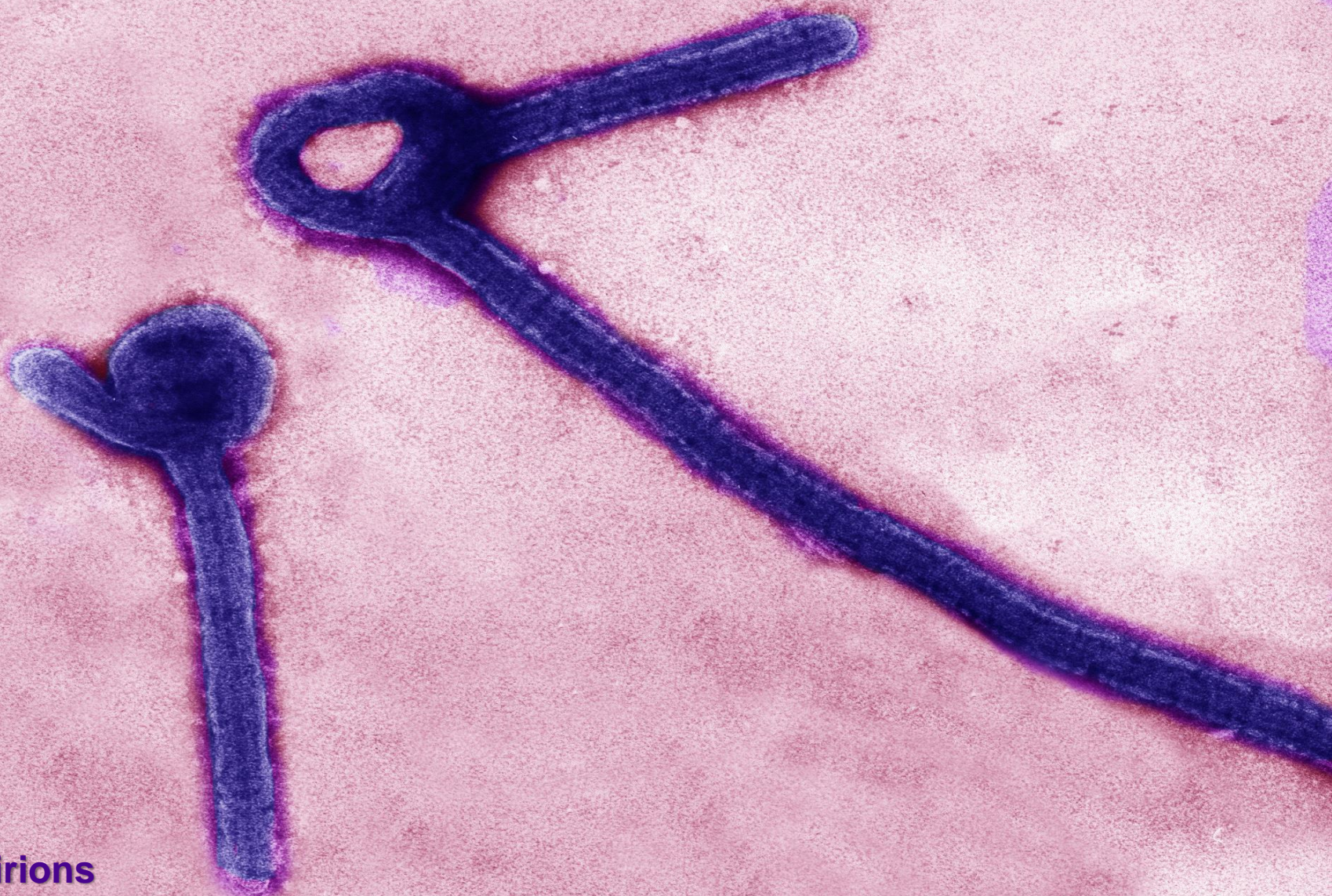
- RVF (*Bunyaviridae*) can have major societal impacts, including significant economic losses and trade reductions.
- RVFV causes significant disease in sheep, cattle, camels, and goats.
- The most notable RVF epizootic occurred in Kenya in 1950-1951, resulting in the **death of an estimated 100,000 sheep**.

Yellow Fever (Flaviviridae)

- Yellow fever virus is found in tropical and subtropical areas in South America and Africa.
- Illness ranges in severity from a self-limited febrile illness to severe liver disease with bleeding.
- Steps to prevent yellow fever virus infection include using insect repellent, wearing protective clothing, and getting vaccinated.



Filoviridae



Ebola virions

Image Courtesy Pathology Division USAMRIID

Marburg virus (Filoviridae)

- **One species (Marburg *marburgvirus*)** with recognized strains such as Musoke, Ravn, Popp, etc.
- First discovered in 1967 in a Marburg, GE laboratory using infected African green monkey tissue from Uganda.
- 1998-2000 outbreak - Democratic Republic of Congo with a fatality rate of 83%.
- 2004-2005 outbreak - Angola between with a fatality rate of 90%.
- 2005-current sporadic outbreaks in Africa. Many of the outbreaks started with male mine workers working in bat-infested mines.

Fruit bat reservoir???
Rousettus aegyptiacus



The Egyptian (African) fruit bat is a cave-dwelling bat widely distributed across Africa.

Ebola virus (Filoviridae)

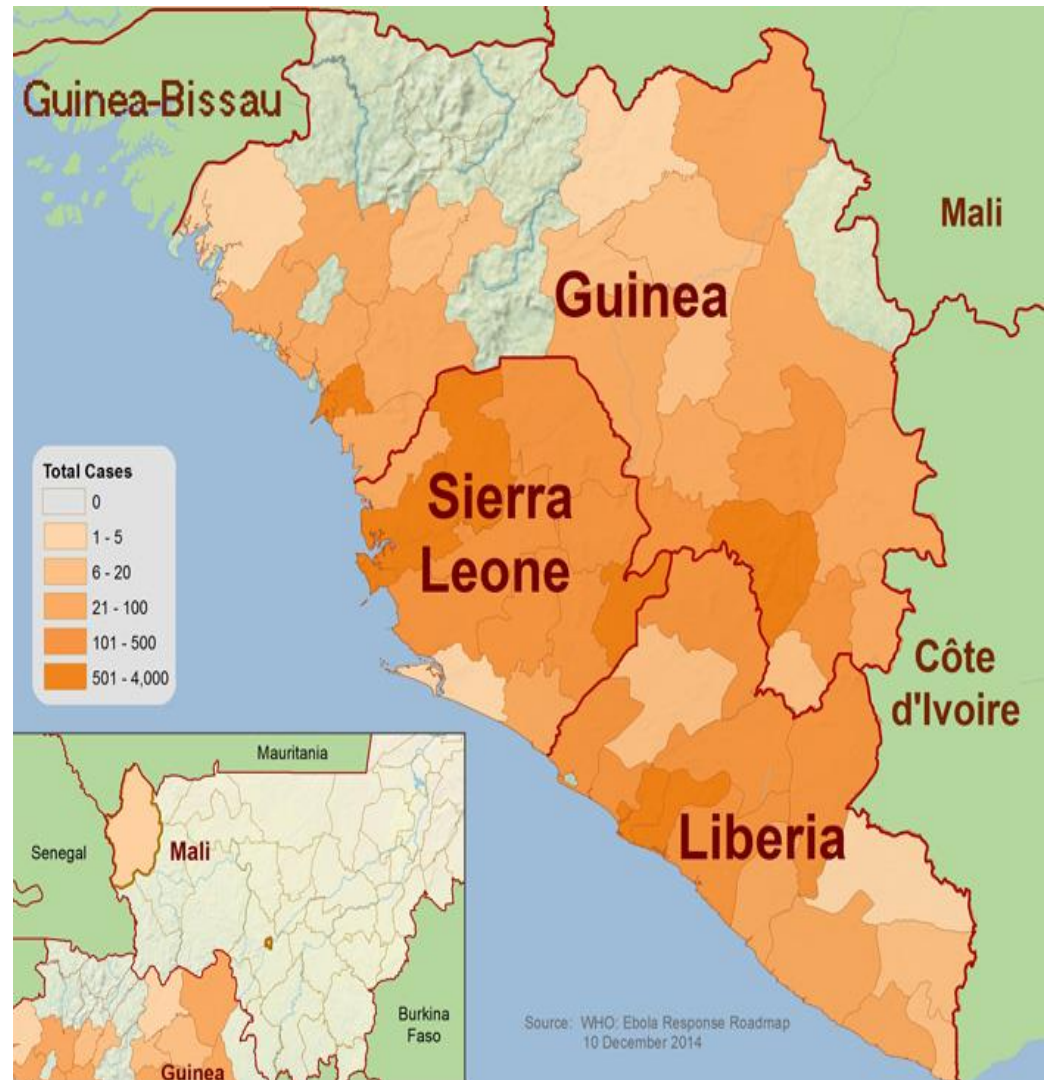


- Five species of Ebola - each with one or more strains
 - *Zaire* , *Sudan* , *Bundibugyo* , *Tai Forest* , *Reston*
- First discovered in 1976 with separate outbreaks of strain ***Zaire*** (318 cases / **88% mortality**) & strain *Sudan* (284 cases / **53% mortality**)
- Strain ***Zaire*** in Kikwit, Democratic Republic of Congo (DRC) in 1995 (315 cases / **81% mortality**)
- Strain ***Sudan*** in Uganda in 2000-2001 (425 cases / **53% mortality**)
- The 2014 Ebola epidemic (pandemic) is the largest in history - strain ***Zaire***.

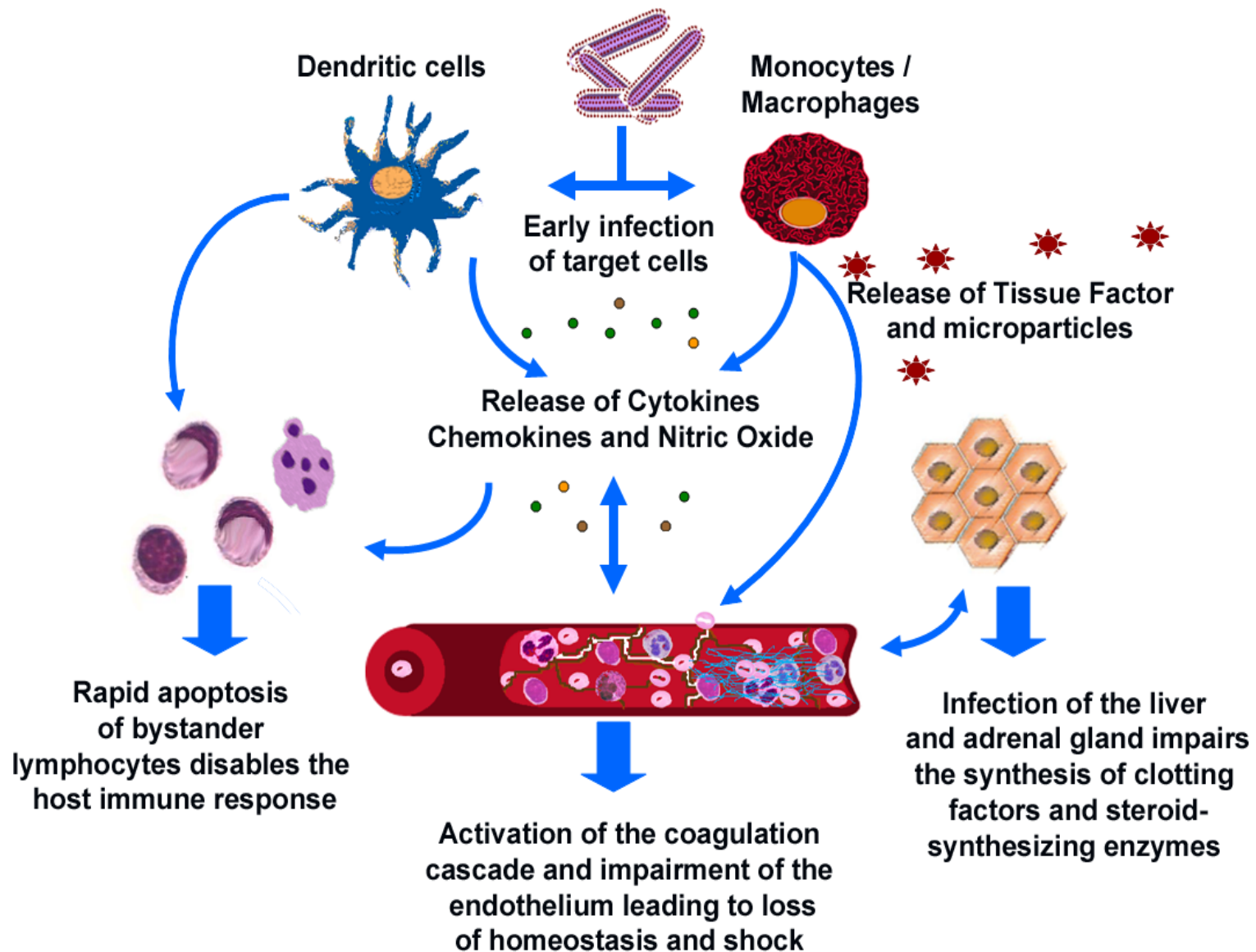
Ebola Virus Disease (EVD) Epidemic

(Nov 2014)

- Infected: 18,603
- Deaths: 6,915
- Lab confirmed: 11,807
- 55-60% mortality



Model of Ebola Pathogenesis



Clinical Pathology EVD

- Profound dehydration
- Leukopenia +/- neutrophilia
- Reduced RBC; some have hemoconcentration (dehydration)
- Thrombocytopenia or abnormal platelet function
- Elevated liver enzymes (ALT / AST)
- Prothrombin time, activated partial thromboplastin time (APTT) and bleeding time are prolonged
- Disseminated intravascular coagulation (DIC); have elevated d-dimers (FDP's) and decreased fibrinogen
- Hypoalbuminemia, decreased globulins, decreased total protein (dehydration may alter)
- Azotemia- elevated BUN and Creatinine (pre-renal)
- Acidosis
- Altered electrolytes (V/D and dehydration)

Ebola virus(Filoviridae)



- Four species of fruit bats carry Ebola virus and *MAY* be the host reservoir: *Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*, and *Rousettus aegyptiacus*.
- Direct contact with blood, secretions, or tissues of humans and nonhuman primates (NHP); eating of infected bush meat(?); EBOV genetic material identified in NHP (chimps, gorillas, etc.), antelopes, porcupines, rodents, dogs, and pigs.
- Nosocomial contact: Needlestick injuries, contaminated syringes, etc.
- Direct contact with the body during burial ceremonies or handling of bodies can play a significant role in transmission.
- Mucosal exposure
 - demonstrated in NHPs

Towner JS, Pourrut X, Albarino CG, Nkogue CN, Bird BH, et al (2007) Marburg Virus Infection Detected in a Common African Bat. PLoS ONE 2(8): e764. doi:10.0371/journal.pone.0000764



Clinical Features/Symptoms in the Current Outbreak in West Africa

- Fever (87.1%)
- Fatigue (76.4%)
- Loss of appetite (64.5%)
- Vomiting (67.6%)
- Diarrhea (65.6%)
- Headache (53.4%)
- Abdominal pain (38.9%)
- Cough (29.6%)
- Unexplained bleeding (18% - blood in stool, gums, vomit, cough, epistaxis)
- Rash (5.8%)

Clinical Features/Symptoms in the Current Outbreak in West Africa

- Average Incubation period (time between infection and onset of symptoms) is **11.4 days**
- Average interval from symptom onset to hospitalization is **.3 to 9.7 days**
- Average interval from hospital admission to death is **0-10 days**
- Average interval from hospital admission to discharge is **5.7-17.9 days**
- Fatality rate for civilians: **70.8%** (when using definitive outcome data)
- Fatality rate for health care workers: **56.1%** in Guinea to **80%** in Liberia

Diagnosis of EBOV

- Virus isolation or virus neutralization from blood, serum or tissue biopsy is **Gold Standard**
- Real Time - polymerase chain reaction (PCR) from blood
 - **Increasingly important tool**
- Rapid ELISA techniques most often used (sandwich assay)
 - Antigen or Ab capture detection
 - IgM (test of choice for Hantaviridae, yellow fever, & Dengue) or IgG antibody capture
- Serology on paired sera
- Electron microscopy can provide definitive evidence
- Immunohistochemistry (IHC) & in situ hybridization (ISH) of infected tissues
 - Formalin-fixed tissue
 - CDC has developed a skin biopsy procedure for detection of EBOV using IHC

Medical Management

The foundation of treatment is supportive care

- Hemodynamic resuscitation & monitoring
- **Careful management of fluid and electrolytes, blood pressure, and circulatory volume**
 - Use of colloid: Usually fluid of choice
 - Hemodialysis or hemofiltration as needed
 - Esp. HFRS patients
- Vasopressors and cardiogenic drugs (some cases do not respond to i.v. fluids)
- Cautious sedation and analgesia

Medical Management Challenge

- DIC may be important in some VHF_s (RVF, CCHF, EVD)
- Coagulation studies and clinical judgment as guide
 - Replacement of coagulation factors / cofactors
 - Platelet transfusions
- No aspirin, NSAIDs, anticoagulant therapies, or IM injections

Ethical considerations for use of unregistered interventions for Ebola viral disease

Report of an advisory panel to World Health Organization

- 11 August 2014, WHO panel reached consensus that it is ethical to offer unproven interventions with as yet unknown efficacy and adverse effects, as potential treatment or prevention.
- There was unanimous agreement that there is a moral duty to also evaluate these interventions (for treatment or prevention) in the best possible clinical trials under the circumstances in order to definitively prove their safety and efficacy or provide evidence to stop their utilization. Ongoing evaluation should guide future interventions.

Report of an advisory panel to World Health Organization

- Panel identified areas that need more detailed analysis and discussion, such as:
 1. ethical ways to gather data while striving to provide optimal care under the prevailing circumstances;
 2. ethical criteria to prioritize the use of unregistered experimental therapies and vaccines;
 3. ethical criteria for achieving fair distribution in communities and among countries, in the face of a growing number of possible new interventions, none of which is likely to meet demand in the short term.

Experimental Antiviral Therapies Filoviruses

- Immune (convalescent) plasma
- Phosphorodiamidate morpholino oligomers (PMO's)

USAMRIID Evaluating leading Ebola medical countermeasure candidates

- Therapeutics
 - zMAPP antibodies
 - Oral favipiravir (T-705) - In Phase III clinical trials for influenza
 - BCX4430 – IND to be filed Oct 2014
 - AL -8176 – In Phase II clinical Trials for Respiratory Syncytial Virus
- Vaccines
 - rVSV – Phase I scheduled for Oct 2014
 - ChAd3 – In Phase I clinical trial
 - Oral rAd5-EBOV – completed Phase I for influenza; IND for EVD indication to be filed Dec 2014
 - Nano Virus Like Particle
 - DNA-based

Dr. Bruce Ribner's treatment of Dr. Kent Brantly

- Fluid/Electrolyte replacement from vomiting/diarrhea (Sodium and K⁺ were low)
- Replacement of proteins (Plasma? or Colloids?) to combat the tissue edema
- Platelet replacement (when platelet count is low and there is bleeding)

“The focus should remain on aggressive intensive care and the ability to correct abnormalities metabolically, rather than receiving any magic vaccine or product that may or may not improve survival.”

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Questions/Discussion

References

Allela L, et.al. Ebola Virus Antibody Prevalence in Dogs and Human Risk, *Emerging Infectious Diseases*. Vol. 11, No. 3, March 2005.

Bishop BM. Potential and emerging treatment options for ebola virus disease. *Anal of Pharmacology*. December 2014. DOI: 10.1177/106002801456122.7

Casillas AM, et al. A current review of ebola virus: Pathogenesis, clinical presentation and diagnostic assessment. *Biological Research for Nursing*. Vol. 4, No. 4, April 2003, 268-275.

Goeijenbier M, et.al. Ebola virus disease: a review on epidemiology, symptoms, treatment and pathogenesis. *Netherlands Journal of Medicine*. Vol. 72, No. 9 November 2014.

Olival KJ, et.al. Ebola Virus Antibodies in Fruit Bats, Bangladesh. *Emerging Infectious Diseases*. Vol. 19, No. 2, February 2013.

World Health Organization. Report of an advisor panel to WHO. www.who.int 11 August 2014.

WHO Ebola Response Team. Ebola Virus Disease in West Africa-The first 9 months of the epidemic and forward projections. *New England Journal of Medicine*. Sep 2014 DOI:10.1056/NEJMoa1411100.