Hemorrhagic Fever Viruses
with Emphasis on Ebola

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Disclaimer

• The opinions, interpretations, conclusions, and views expressed herein are those of the author(s) and do not reflect the official policy of the Department of the Army, the Department of Defense, or the U.S. Government.

• Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.
• Definitions
• Hemorrhagic Fever Viruses
• Arenavirus
• Bunyavirus
• Flavivirus
• Filovirus
  1. Marburg
  2. Ebola

Pathogenesis
Current Epidemic/Pandemic
Clinical Features
Diagnosis
Treatment
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
- **Genus**: Arenavirus  
- **Species**:
  - Junin, Machupo, Guanarito (New World)
  - Lassa (Old World)

### Bunyaviridae
- **Nairovirus**: Crimean-Congo hemorrhagic fever
- **Phlebovirus**:
  - Rift Valley fever
- **Hantavirus**:
  - Hantaan, Seoul, Puumala, Dobrava-Belgrade (Old World)
  - Sin Nombre, Andes (New World)

### Filoviridae
- **Ebola**:
  - Zaire, Sudan, Tai Forest, Reston, Bundibugyo

- **Marburgvirus**

### Flaviviridae
- **Flavivirus**:
  - Omsk HF, Kyasanur forest disease
  - Dengue
  - Yellow fever
# Etiologic Agents of VHF

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>Mortality Rate</th>
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<tbody>
<tr>
<td><em>Ebola Zaire</em></td>
<td>75-90%</td>
</tr>
<tr>
<td><em>Marburg</em></td>
<td>25-90%</td>
</tr>
<tr>
<td><em>Lassa</em></td>
<td>15-20% of hospitalized</td>
</tr>
<tr>
<td><em>Crimean-Congo hemorrhagic fever</em></td>
<td>30%</td>
</tr>
<tr>
<td><em>Rift Valley fever</em></td>
<td>50% of patients with hemorrhagic form</td>
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</tbody>
</table>
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

Palomar et al. Crimean Congo Hemorrhagic Fever virus in ticks from migratory birds, Morocco. EID Vol 19, Number 2, Feb 2013
Rift Valley Fever (Bunya virus)

- 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
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.
• Infected: 18,603
• Deaths: 6,915
• Lab confirmed: 11,807
• 55-60% mortality
Model of Ebola Pathogenesis

Dendritic cells

Early infection of target cells

Release of Cytokines, Chemokines, and Nitric Oxide

Monocytes / Macrophages

Release of Tissue Factor and microparticles

Rapid apoptosis of bystander lymphocytes disables the host immune response

Activation of the coagulation cascade and impairment of the endothelium leading to loss of homeostasis and shock

Infection of the liver and adrenal gland impairs the synthesis of clotting factors and steroid-synthesizing enzymes
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 plays a significant role in transmission.

- Mucosal exposure – demonstrated in NHPs

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

NEJM, 1 October 2014
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
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 cardiotonic drugs (some cases do not respond to i.v. fluids)
• Cautious sedation and analgesia
Medical Management Challenge

• DIC may be important in some VHF (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.
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 morpolino 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.”

Q&A from Scientific American 28 August 2014
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Questions/Discussion
References


