Ebola Virus Outbreaks - Lessons Learned

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2018 Ebola Outbreak, Bikoro - DRC


2018 Ebola Outbreak, Bikoro - DRC

Ebola Virus Outbreaks - Lessons Learned

- Ebola (Ebola virus disease (EVD), Ebola hemorrhagic fever (EHF))
  - Filovirus - Filoviridae family
  - Order - Mononegavirales
  - Outbreak in West Africa in 2014 - concerns worldwide
- First known case of Ebola virus, occurred in 1976, in South Sudan, then part of Sudan
- This filovirus first surfaced in 1967 in Marburg, Germany, Frankfurt (Yugoslavia), Serbia
- Fatal hemorrhagic fever - laboratory employees in Marburg, later Belgrade
- The Marburg cases - Behringwerke, produced sera and vaccines
- Frankfurt outbreak - Paul Ehrlich Institute, control institute for sera and vaccine production
- Belgrade - Institute acquired of Institute for Immunology and Virology, Tatar, now Institute of Virology, Vaccines, and Seres, Tatar
- The veterinarian’s wife also became infected while caring for him
- Both recovered from the disease

Common link filovirus outbreak - Marburg, Frankfurt, and Belgrade
- Green African (Cercopithecus aethiops) monkeys, imported from Uganda

Primary function of these institutes was production and safety testing of live poliomyelitis vaccine
- Infected employees of these institutes, had direct contact with organs, blood, and cell cultures from these monkeys
- These monkeys were primarily used for the production of kidney cell cultures, which is essential for propagation of vaccine strains
- Controversy - health status of monkeys in Africa, and Europe

- Typical route of transportation - Cairo, Egypt, then to Europe
- Six-Day War (June 5-10) 1967
- Sinai versus Egypt, Jordan, and Syria
- One consignment of 100 monkeys transported to Germany via London-Heathrow
- 180 monkeys arrived Heathrow a day earlier, proceeded Moscow, Russia
- Three of the 100 monkeys - German consignment escaped while in London
Controversy on health status of monkeys in Africa, and Europe

- Next day, 97 of the 100 monkeys - Dusseldorf, Germany in two shipments
  - 20 monkeys to Frankfurt, 77 to Marburg, and 6 to Biberach
- 3 monkeys that escaped were caught, harm no one, sent to Frankfurt
- The Belgrade outbreak animals - other consignments from Uganda
- One consignment was sent to Belgrade through Munich
- Two through London to Munich, then to Belgrade
- Yugoslavian veterinarian infected had performed necropsies, for cause of death

- At Heathrow - monkeys housed in African-South American Room of the Royal Society for the Prevention of Cruelty to Animals
- Using monkeys - close proximity to Indian birds, and animals from several countries
- London, two-day delay airport employees on strike
- Animals caged
  - Contact with South American finches (family Frigillidae)
- Two langur monkeys from Ceylon (now Sri Lanka) from shipment to the Netherlands
- One langur died of an unknown cause
- Rhesus macaques (Macaca mulatta) monkeys caged in adjacent room
- While infection could have originated from Uganda, monkeys might have acquired an infectious agent from the finches or the langur monkeys
- One langur monkey that survived was later tested negative for antibodies new virus

Ugandan Veterinarian later reported the same individual captured the African monkeys in Sese Island, Lake Victoria
- Monkeys sent to Entebbe, and sick monkeys removed from the consignment
- Sick monkeys diverted to a small island in Lake Victoria
- It was later noted they were running short of animals, hunters sent out to this area to recapture the monkeys
- Examinations of the animals Entebbe - frequently rushed, not thorough
- Investigations later concluded the veterinarian’s accounts were obscure
- Others have reported that these monkeys were captured from Lake Kyoga, then sent to homesteads, before being their departure to Europe
Ebola Virus Outbreaks - Lessons Learned

<table>
<thead>
<tr>
<th>Year</th>
<th>Location(s)</th>
<th>Deaths</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>Marburg, Belgrade</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>1975</td>
<td>Johannesburg, SA</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1980</td>
<td>Kenya</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1990</td>
<td>Kenya</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1995</td>
<td>Ebola West Asia</td>
<td>128</td>
<td>154</td>
</tr>
<tr>
<td>2003</td>
<td>Uige, Angola</td>
<td>297</td>
<td>352</td>
</tr>
<tr>
<td>2007</td>
<td>Ugand</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2008</td>
<td>Durba, DRC</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2014</td>
<td>Uganda</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>2017</td>
<td>Uganda</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>377</td>
<td>469</td>
</tr>
</tbody>
</table>

Ebola Virus Disease (EVD) - Ebola Hemorrhagic Fever (EHF)

- Apart from the Marburg virus, Ebola virus is another member of the Filoviridae family.
- Viral disease of primates as well as humans, associated with Ebola virus.
- High rate of mortality, ranging from 25% - 90%.
- High rate of morbidity and lack of appropriate therapy.
- Public health concern.
- Likely candidate virus for bioterrorism.
- Acquired via contact with body fluids or blood from infected animals or humans.
- Fruit bats have been implicated and are the reservoir for this virus.
- Fruit bats may infect humans directly.
- Fruit bats infect animals such as chimpanzees, then humans that eat these animals.

- First case - 1976, Nzara, Sudan, now South Sudan.
- WHO workers unable to properly identify the disease.
- 284 infected in Sudan outbreak, with 151 deaths.
- Naming - same year, in Yambuku, Democratic Republic of Congo (DRC), then Zaire.
- American and Belgian scientists.
- Scientists concerned (stigmatize the town by naming it Yambuku virus).
- Scientists came up with the name Ebola (Ebola River).
- Small map of Zaire, not realizing the river was 151 Km (93.8 miles) from Yambuku.
- 318 cases in the Democratic Republic of Congo outbreak, with 280 fatalities.
- Two outbreaks initially thought to be connected.
- Distance between Nzara, South Sudan and Yambuku, DRC, is 1088 Km (676 miles).
- Subsequently confirmed these were distinct Ebola viruses: SEBOV, and ZEBOV.
## Ebola Virus Outbreaks - Lessons Learned

<table>
<thead>
<tr>
<th>Year</th>
<th>Virus Type</th>
<th>Country</th>
<th>Total Cases</th>
<th>Total Deaths</th>
<th>Fatality Rate</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>SUDV</td>
<td>South Sudan</td>
<td>284/151</td>
<td>0</td>
<td>53%</td>
<td>Occurred in Nzara</td>
</tr>
<tr>
<td>1976</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>318/280</td>
<td>0</td>
<td>88%</td>
<td>Occurred in Yambuku</td>
</tr>
<tr>
<td>1976</td>
<td>EBOV/SUDV</td>
<td>United Kingdom</td>
<td>1/0</td>
<td>0</td>
<td>0%</td>
<td>Accidental contamination needle stick</td>
</tr>
<tr>
<td>1977</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>1/1</td>
<td>0</td>
<td>100%</td>
<td>Occurred in Tandala</td>
</tr>
<tr>
<td>1979</td>
<td>SUDV</td>
<td>South Sudan</td>
<td>34/22</td>
<td>0</td>
<td>64.7%</td>
<td>Recurrent outbreak at Nzara</td>
</tr>
<tr>
<td>1989-1990</td>
<td>RESTV</td>
<td>Philippines</td>
<td>3/0</td>
<td>0</td>
<td>0%</td>
<td>High mortality in cynomolgus monkeys at facility which exports monkeys to the US</td>
</tr>
<tr>
<td>1990</td>
<td>RESTV</td>
<td>United States</td>
<td>0/0</td>
<td>0</td>
<td>0%</td>
<td>Occurred in cynomolgus monkeys in Reston, Virginia, and Philadelphia, Pennsylvania</td>
</tr>
<tr>
<td>1990</td>
<td>RESTV</td>
<td>United States</td>
<td>0/0</td>
<td>0</td>
<td>0%</td>
<td>Occurred in cynomolgus monkeys in Reston, Virginia, and Alice, Texas</td>
</tr>
<tr>
<td>1992</td>
<td>RESTV</td>
<td>Italy</td>
<td>0/0</td>
<td>0</td>
<td>0%</td>
<td>Sienna monkeys imported from the Philippines</td>
</tr>
<tr>
<td>1994</td>
<td>EBOV</td>
<td>Gabon</td>
<td>52/31</td>
<td>0</td>
<td>59.6%</td>
<td>Occurred in Mkouka gold mining camps</td>
</tr>
<tr>
<td>1994</td>
<td>TAFV</td>
<td>Ivory Coast</td>
<td>1/0</td>
<td>0</td>
<td>0%</td>
<td>Tai National Park chimpanzees</td>
</tr>
<tr>
<td>1995</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>315/254</td>
<td>0</td>
<td>80.6%</td>
<td>Occurred in Kikwit. 7 of 8 patients treated with convalescent patient blood transfusions survived.</td>
</tr>
</tbody>
</table>

[Source: http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html](http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html)
[Source: http://www.cdc.gov/vhf/ebola/outbreaks/history/summaries.html](http://www.cdc.gov/vhf/ebola/outbreaks/history/summaries.html)
[Source: http://apps.who.int/iris/bitstream/10665/155082/1/roadmapsitrep_11March2015_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/155082/1/roadmapsitrep_11March2015_eng.pdf?ua=1)
Locations of Ebolavirus infections and outbreaks

(A) Africa
- Ebola outbreaks associated with three central African species of Ebolavirus, Zaire Ebola virus (ZEBOV), Sudan Ebola virus (SEBOV), and Bundibugyo Ebola virus (BEBOV).
- The Tai Forest region in Côte d’Ivoire is the only case of the species Côte d’Ivoire Ebola virus (CIEBOV).

(B) Reston ebolavirus REBOV has been introduced several times through imported macaques into USA from 1989 to 1996 (Philadelphia, PA; Reston, VA; San Antonio, TX).

(C) Italy (Siena) in 1992.

(D) Source of all cases of REBOV a primate export facility in the Philippines (Ferlite farm). Animals of this farm have been diagnosed with REBOV infection several times in the 1990s. REBOV detected in pigs on two farms in the Philippines (Pangasinan, Bulacan).

DRC = Democratic Republic of the Congo.

Ebola Virus Outbreaks - Lessons Learned

Ebola virus disease (EVD)
- Next Outbreak - approximately twenty years later
- DRC – Kikwit, early 1995
- Diagnosis of shigellosis initially made – patients with bloody diarrhea
- Blood samples from 14 patients were sent to the Centers for Disease Control, Atlanta, GA, USA, on May 4, 1995, and a diagnosis of Ebola was confirmed on May 9, 1995.

Year | Virus | Country | Cases/Deaths | % Fatality | Comments
--- | --- | --- | --- | --- | ---
1996 | EBOV | Gabon | 37/21 | 56.7% | Occurred in Mayibout where hunters had eaten a dead chimpanzee
1996-1997 | EBOV | Gabon | 60/45 | 75% | Occurred on Booue
1996 | EBOV | South Africa | 2/1 | 50% | Infected Medical worker travelled from Gabon to Johannesburg, nurse taking care of this patient became infected and died
1996 | RESTV | United States | 0/0 | 0% | Occurred in cynomolgus monkeys in Alice, Texas
1996 | RESTV | Philippines | 0/0 | 0% | Occurred at a monkey export facility
1996 | EBOV | Russia | 1/1 | 100% | Occurred at a laboratory
2000-2001 | SUDV | Uganda | 425/224 | 52.3% | Occurred in Gulu, Masindi, and Mbarara
2001-2002 | EBOV | Gabon / Republic of Congo | 122/96 | 78.6% | Occurred on the border of Gabon and Republic of Congo

http://www.who.int/mediacentre/factsheets/fs103/en/
http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html
http://www.cdc.gov/vhf/ebola/outbreaks/history/summaries.html
http://apps.who.int/iris/bitstream/10665/155082/1/roadmapsitrep_11March2015_eng.pdf?ua=1
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<th>Cases/Deaths</th>
<th>% Fatality</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002/03</td>
<td>EBOV</td>
<td>Republic of Congo</td>
<td>143/128</td>
<td>89.5%</td>
<td>Occurred in Mbomo and Kéllé</td>
</tr>
<tr>
<td>2003</td>
<td>EBOV</td>
<td>Republic of Congo</td>
<td>35/29</td>
<td>82.8%</td>
<td>Occurred in Mbomo and Mbandza</td>
</tr>
<tr>
<td>2004</td>
<td>SUDV</td>
<td>South Sudan</td>
<td>17/7</td>
<td>41%</td>
<td>Occurred in Yambio, South Sudan</td>
</tr>
<tr>
<td>2004</td>
<td>EBOV</td>
<td>Russia</td>
<td>1/1</td>
<td>100%</td>
<td>Result of laboratory contamination</td>
</tr>
<tr>
<td>2007</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>264/187</td>
<td>70.8%</td>
<td>Occurred in Kasai</td>
</tr>
<tr>
<td>2007/08</td>
<td>BDBV</td>
<td>Uganda</td>
<td>149/37</td>
<td>24.8%</td>
<td>Occurred in Bundibugyo, first BDBV case</td>
</tr>
<tr>
<td>2008</td>
<td>RESTV</td>
<td>Philippines</td>
<td>6/0</td>
<td>0%</td>
<td>Observed in pig slaughter house and farm, first case in pigs</td>
</tr>
<tr>
<td>2008/09</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>32/15</td>
<td>46.8%</td>
<td>Occurred in Mweka and Luebo health zones, Kasai</td>
</tr>
<tr>
<td>2011</td>
<td>SUDV</td>
<td>Uganda</td>
<td>1/1</td>
<td>100%</td>
<td>Occurred in Luwero district</td>
</tr>
<tr>
<td>2012</td>
<td>SUDV</td>
<td>Uganda</td>
<td>24/17</td>
<td>70.8%</td>
<td>Occurred in Kibaale</td>
</tr>
<tr>
<td>2012</td>
<td>BDBV</td>
<td>Democratic Republic of Congo</td>
<td>77/36</td>
<td>46.7%</td>
<td>Occurred in Orientale province</td>
</tr>
</tbody>
</table>

Phylogeny of the family Filoviridae

- Phylogenetic analysis showed separate clade for the Guinean EBOV strain in a sister relationship with other known EBOV strains.
- Suggests Guinean EBOV strain evolved in parallel with strains from the Democratic Republic of Congo and Gabon from a recent ancestor.
- Not introduced from the above countries into Guinea.
- Potential reservoirs of EBOV, fruit bats species Hypsignathus monstrosus, Epomops franqueti, and Myonycteris torquata, present in large parts of West Africa.
- EBOV might have circulated undetected in this region.
- Guinea outbreak highlights outbreaks in the West African subregion.

Transmission Chains in the Outbreak of Ebola Virus Disease in Guinea
Ebola Virus Outbreaks - Lessons Learned

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<tr>
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<th>Country</th>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>Guinea</td>
<td>3285/2170</td>
<td>66%</td>
<td>Widespread</td>
</tr>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>Liberia</td>
<td>9343/4162</td>
<td>44.5%</td>
<td>Widespread</td>
</tr>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>Mali</td>
<td>8/6</td>
<td>75%</td>
<td>Bamako, via Guinea</td>
</tr>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>Nigeria</td>
<td>2018/101</td>
<td>50%</td>
<td>Lagos, via Liberia</td>
</tr>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>Senegal</td>
<td>393/164</td>
<td>40%</td>
<td>Dakar, via Guinea</td>
</tr>
<tr>
<td>2014-2015</td>
<td>EBOV</td>
<td>Sierra Leone</td>
<td>11619/3629</td>
<td>31.2%</td>
<td>Widespread</td>
</tr>
<tr>
<td>2014-2015</td>
<td>EBOV</td>
<td>Spain</td>
<td>1/0</td>
<td>0%</td>
<td>Patient came from Sierra Leone,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>via Liberia</td>
</tr>
<tr>
<td>2014-2015</td>
<td>EBOV</td>
<td>Spain</td>
<td>1/0</td>
<td>0%</td>
<td>Patient came from Sierra Leone,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>via Liberia</td>
</tr>
</tbody>
</table>

2014 Ebola Virus – United Sates (N=11)

[Image]
2014 Ebola Virus – United States (N=11)

- October 23, 2014, patient 4 (physician), diagnosed in New York City; he had just returned from working with Doctors Without Borders in Guinea
- Total number of deaths: 2
- Patient 1: Physician evacuated from Sierra Leone – died in Nebraska
- Cases first diagnosed in US: 4
- Cases evacuated to US from other countries: 7
- Recoveries from Ebola: 9

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<tr>
<th>Year</th>
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<th>Country</th>
<th>Cases/Deaths</th>
<th>% Fatality</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>United States</td>
<td>4/1</td>
<td>25%</td>
<td>Dallas, via Liberia, and New York via Guinea</td>
</tr>
<tr>
<td>2013-2014</td>
<td>EBOV</td>
<td>United Kingdom</td>
<td>1/0</td>
<td></td>
<td>Volunteer of an Ebola center in Sierra Leone, flew from Freetown to London via Casablanca, Morocco, then to Leeds, England</td>
</tr>
<tr>
<td>2014</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>70/42</td>
<td>60%</td>
<td>Boende district</td>
</tr>
<tr>
<td>2017</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>8/4</td>
<td>50%</td>
<td>Likati</td>
</tr>
<tr>
<td>2018</td>
<td>EBOV</td>
<td>Democratic Republic of Congo</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>Bikoro</td>
</tr>
</tbody>
</table>

Map of Guinea Showing Initial Locations of the Outbreak of Ebola Virus Disease - 2014

First known case of Ebola in Nigeria was a traveler exposed in Liberia.

*July 17, 2014, under observation in Monrovia, Liberia, hospital for possible Ebola.*

*Left the hospital against medical advice.*

*Despite advice against travel, on July 20, 2014, flew by from Monrovia via Accra, Ghana, to Lagos, Nigeria.*

*Hospitalized, was confirmed positive for acute Ebola in Lagos and died on July 25.*
Ebola Virus Outbreaks - Lessons Learned (Nigeria)

The Role of the Polio Program Infrastructure in Response to Ebola Virus Disease Outbreak in Nigeria 2014


Ebola Virus Outbreaks - Lessons Learned (Nigeria)

- The Role of the Polio Program Infrastructure in Response to Ebola Virus Disease Outbreak in Nigeria 2014

Ebola - family of viruses contain single, linear, negative sense ssRNA genomes, known as Filoviridae.

Filoviruses - thread-like appearance under the electron microscope
- Divided into two genera
  - The Ebola-like species: Sudan, Reston, and Zaire
  - Marburg virus, with a single species: The Lake Victoria Marburg virus
- 14,000 nm long, and 80 nm in diameter
- Within the center of the Ebola virus is the nucleocapsid, consisting of the helical ssRNA genome, wrapped around NP, VP35, VP30, and L proteins
- This assembly is encased in a viral envelope derived from the host cell membrane, with 10 nm long viral glycoprotein (GP) spikes attached
- Viral protein VP24, and VP40 are located between the capsid and viral envelope
- The length of each Ebola virus is approximately 19kb and codes for one non-structural and seven structural proteins as follows:
  - 3'-leader-NP-VP35-VP40-GP/sGP-VP30-VP24-L-trailer-5'
Ebola Structure

Ebola Virus Life Cycle

http://www.oxfordhumanists.org/?page_id=1863
Ebola Virus Life Cycle

1. Ebola virus (EBOV) binds to attachment factors and receptors on the cell surface via glycoprotein (GP)
2. Virus is then placed into and transferred to various compartments in the cell - endosomes, macropinosomes, and lysosomes - to keep the virus in a "trash can" and digest it. Virus "tricks" its way out in late endosome
3. Viral nucleocapsid (four proteins — NP, VP35, VP30 and L (RNA Polymerase) and the viral genome) is released into the cytoplasm
4. Viral genome is transcribed into mRNA with through viral proteins VP35, VP30 and L (RNA Polymerase)
5. Viral mRNAs translated into respective proteins via cell machinery.
6. mRNAs encoding GP brought to endoplasmic reticulum (ER), GP is synthesized and modified
7. GP is further modified in the Golgi and delivered to the plasma membrane in secretory vesicles
8. At plasma membrane, ribonucleoprotein complex (RNA plus nucleoprotein (NP)) and associated viral proteins assemble with membrane-associated proteins (matrix proteins VP24 and VP40, and GP), resultant virions bud from the cell surface
9. Other forms of GP, soluble GP (sGP) — secreted

http://www.oxfordhumanists.org/?page_id=1863
Ebola Virus Outbreaks - Lessons Learned

- Ebola virus encodes two types of its glycoprotein gene:
  - sGP (secreted glycoprotein) and GP (glycoprotein)
  - sGP is a small, non-structural dimeric form, which is transcribed directly from the viral mRNA.
  - It is not found in viral particles, but is secreted into the bloodstream, where it is believed to serve as decoy that absorbs Ebola-directed antibodies, during Ebola cell invasion.
  - The second glycoprotein stems from transcriptional editing of the glycoprotein origin of replication, which encodes a transmembrane-bound form.
  - This Ebola virus envelope glycoprotein spike appears at the cell surface, inserted into the host membrane, thereby triggering viral cell attachment and cell fusion.
  - Post-translation, GP is cleaved into two subunits, GP1, and GP2.
  - GP1 serves as an attachment to host cells, GP-2 functions to mediate fusion of viral and host membranes.

- The function of other Ebola virus genes is as follows:
  - VP24 is essential in the correct assembly of a functional nucleocapsid.
  - Lack of VP24 results in reduced transcription/translation of VP30.
  - VP30 is essential for RNA synthesis.
  - VP35 / VP30 / VP40 suppress interferon production.
  - These proteins respond to cell infection, via sequestration of cellular antiviral, cell growth inhibition, as well as immunoregulatory functions.
  - VP40, which is located underneath the viral envelope, serves to maintain structural integrity of the virus.
  - Mediates budding because of its innate ability to eject itself from cells.
  - Other proteins:
    - The leader and trailer sections carry critical information.
    - Regulate transcription, replication, as well as packaging of the genome into new virions.

Methods of Transmission

Ebola Virus Outbreaks - Lessons Learned

Methods of Transmission

- Hemorrhagic fever
- Contact with infected body fluids

Pathophysiology

- Proinflammatory cytokines, chemokines and growth factors primarily synthesized by monocytes and dendritic cells that represent the basis of innate immune reaction to pathogens.
- At moderately high levels, these soluble mediators act as first-line defense while recruiting circulating mononuclear cells to sites of infection.
- They also increase endothelial permeability, activate macrophage and dendritic cell cytolytic functions, as well as induce adaptive immune responses by affording co-stimulatory signals to naïve T cells.
- Dendritic cells are known to initiate adaptive immune response by presenting antigens to T lymphocytes as well as stimulating T and B cell differentiation.
- Early productive replication of the Ebola and Marburg viruses in macrophages as well as dendritic cells apparently impair both innate and adaptive immune responses to these viruses.

- Ebola virus spreads from the initial infection site (small lesions) to regional lymph nodes, liver, and spleen.
- Monocytes and macrophages within the lymphoid tissues are frequently the initial targets.
- The early part of the immune system, aids the Ebola virus to practically spread throughout other cells without adequate control.
- Dendritic cells, hepatocytes, fibroblasts, endothelial cells, adrenal cortical cells, are sanctuaries for Ebola virus replication.
- Ebola virus does not infect lymphocytes, their rapid loss appears to be via apoptosis.
- Prominent feature of disease.
Ebola Virus Outbreaks - Lessons Learned

Pathophysiology

- Substantial loss of lymphocytes results
- Infection-mediated impairment of dendritic cells
- Release of soluble factors from monocytes and macrophages

Soluble factors released from target cells

- Contribute to the impairment of the vascular system leading to vascular leakage
- Infected macrophages release large amounts of nitric oxide (NO), a gaseous hormone
- Which normally functions in cell communication
- High NO concentrations serve as a depressant to mitochondrial membrane potential
- Resulting in apoptosis of proximal natural killer cells
- Tissue damage
- Loss vascular integrity
- Shock and NO-induced hypotension.

Ebola Virus Outbreaks - Lessons Learned

Pathophysiology

- Shock and multiorgan failure
  - Systemic virus spread and replication
  - General dysregulation of the host immune response
  - Coagulation abnormalities
  - Impairment of the vascular system
  - Hypotension
Ebola Virus Outbreaks - Lessons Learned

Pathophysiology
- Fatal Ebola virus outcomes - characterized by hypersecretion of proinflammatory agents
- Cytokines - IL-1β, IL-1RA, IL-6, IL-8, IL-15 and IL-16
- Chemokines
- Growth factors
- Coagulation and fibrinolysis defects during Ebola virus infections are manifested
- Petechiae, ecchymoses, mucosal hemorrhage, bleeding at venipuncture sites, gastrointestinal tract
- Other indicators of coagulopathy characteristic of Ebola virus infections
- Disseminated intravascular coagulation, hypotension, vascular dysfunction
- Hallmark of human fatal Ebola viral infection

Diagnosis
- One of the reasons for this high rate of mortality with Ebola virus infections relates to its diagnosis
- Most cases of Ebola virus infection outbreaks have occurred in equatorial Africa and West Africa
- Diagnoses has been difficult here - lack of advanced diagnostic equipment and medical care
- While portable real-time thermocyclers and various simple serological assays for field use have been developed
- Implementation in Sub-Saharan Africa have been difficult, if not impossible
- Diagnoses can be simple
- Initial Ebola symptoms such as fever, headache and weakness
- Recent history of travel to an endemic area.
Ebola Virus Outbreaks - Lessons Learned

Diagnosis
- Unfortunately in Africa, the diagnosis may be difficult, and complicated
- Myriad ailments such as typhoid fever, malaria, yellow fever, viral hepatitis, meningitis, sepsis, cholera, leptospirosis, Chikungunya fever, hemorrhagic fever, scabies, typhus, visceral leishmaniasis, and trypanosomiasis, have similar presentations
- Must be differentiated from Ebola virus
- IgG-specific antibodies appear within 6 to 18 days post onset and may persist in the system for several years
- Presence of IgM antibodies or rising IgG titer is strong presumptive diagnosis
- Decreasing IgM or increase in IgG titer, especially a four-fold increase, or both, in successive paired serum samples are indicative of recent Ebola viral infection
- Thus later in the course of the disease, or post recovery, direct IgM and IgG antibody testing are indicated

Therapy - Blood Products
- Several positive case reports have been observed in the management of these patients with blood transfusion from convalescent patients
- To date this appears to be the most positive lead to managing these patients
- The use of blood transfusion from convalescent patients was first employed by Democratic Republic of Congo physicians during the 1995 Ebola outbreak
- Passive immunotherapy with convalescent-phase human blood in Kikwit
- Initially in a nurse who contracted Ebola during the care of Italian nuns with Ebola virus infection who died
- The nurse did well with blood transfusion, then these physicians later replicated passive immunotherapy with convalescent phase human blood in 8 additional patients
- 7 of the 8 patients survived resulting in approximately 90% positive outcome
- Results of these case reports were later published in the American Journal of Infectious Diseases in 1999

Table 1. Laboratory tests used in diagnosis and laboratory tests available for diagnosis

<table>
<thead>
<tr>
<th>Timeline of Infection</th>
<th>Diagnostic tests available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within a few days after symptoms begin</td>
<td>Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgG ELISA, Polymerase chain reaction (PCR), Visceral leishmaniasis.</td>
</tr>
<tr>
<td>Later in disease course or after recovery</td>
<td>IgG and IgM antibodies.</td>
</tr>
<tr>
<td>Retrospectively in deceased patients</td>
<td>Immunohistochemistry testing, PCR, Visceral leishmaniasis.</td>
</tr>
</tbody>
</table>
Ebola Virus Outbreaks - Lessons Learned

Therapy - Blood Products
- Following the 2013-2014 Ebola outbreak, several patients in Africa and the United States survived with the use of blood transfusions.
- In July 2014, a Texas physician Dr. Kent Brantly caring for Ebola patients in Liberia via an international relief group - Samaritan’s Purse, was diagnosed with Ebola.
- A 14-year-old boy who had treated and survived, donated his blood to Brantly.
- Dr. Brantly survived, and went on to donate blood to other patients in the United States diagnosed with Ebola.
- The World Health Organization has since endorsed this approach of blood transfusion in the management of Ebola patients.


Ebola virus outbreaks in Africa: Past and present, Onderstepoort Journal of Veterinary Research 79(2), Art. #451, 8 pages

Therapy - Monoclonal Antibodies

ZMapp
- Consists of three monoclonal antibodies, initially obtained from mice exposed to Ebola virus proteins, chimerized with human constant regions.
- Components are chimeric monoclonal antibody c13C6 derived from an earlier antibody cocktail, "MB-003" and two chimeric monoclonal antibodies from another antibody cocktail, "ZMab", c2G4 and c4G7.
- MB-003 is the product which consists of humanized chimeric antibodies, c13C6, h13F6 and c6D8.
- MB-003 tested in rhesus macaque monkeys infected with Ebola virus, at 1 hour, 24 and 48 hours post infection:
  - All animals treated one hour post infection survived, while 4 of the 6 animals treated 24 to 48 hours post infection survived.
- ZMab, on the other hand, is a compound consisting of three monoclonal antibodies, namely, m1H3, m2G4, and m4G7.
- Ebola virus infected macaque monkeys treated with ZMab within 24 hours of infection, survived.

ZMapp large scale production has been challenging.
- Genes encoding for the chimeric antibodies, are inserted into viral vectors, subsequently, tobacco plants are infected with the viral vector encoding the antibodies with Agrobacterium cultures.
- Antibodies are extracted, and purified from the plants, a process which takes months to achieve.

Development of ZMapp: consortium of scientists
- Public Health Agency of Canada
- Defyrus
- US Army Medical Research Institute of Infectious Diseases (USAMRIID)
- Kentucky Bioprocessing
- Mapp Biopharmaceutical (San Diego)
Ebola Virus Outbreaks - Lessons Learned
Therapy - Antiviral Agents

Brincidofovir
- Broad spectrum antiviral agent developed by Chimerix, USA
- Prodrug to cidofovir
- Effective against cytomegalovirus, adenovirus, smallpox, and ebolavirus
- Clinical trials for the treatment of ebolavirus in Liberia
- Used during the 2014 ebolavirus outbreak in the United States

Favipiravir (T705, Avigan)
- Broad-spectrum RNA polymerase inhibitor derivative antiviral agent developed by Toyama Chemical, Japan
- The drug is known to be active against West Nile virus, influenza virus, yellow fever virus, arenaviruses, bunyaviruses, and coronaviruses
- Favorably has also shown efficacy in mouse ebolavirus disease models

Lamivudine
- (2',3'-dideoxy-3'-thiacytidine, also known as 3TC), is nucleoside analog reverse transcriptase inhibitor
- Has activity against HIV/AIDS, and HBV
- Successful use in 13 of 15 patients during the 2014 Ebola outbreak in Liberia
- Scientists unable to validate such results in in vitro models

BCX4430 (Immucillin-A)
- Antiviral adenosine analog, originally developed for hepatitis C
- Demonstrated efficacy for Ebola virus, and Marburg virus
- Efficacy against other RNA viruses; bunyaviruses, arenaviruses, paramyxoviruses, coronaviruses
- Efficacy observed in animal models only

JK-05
- Inhibitor of RNA polymerase, with broad spectrum antiviral activity
- Shown to be efficacious against Ebola in mouse models

Antisense Therapeutics
- TKM–Ebola
- Antisense therapeutics is derived from antisense technology
- A gene is selected
- Oligos of fluorescein and JO4M (RNA or chemical entity) are synthesized
- Oligo is hybridized and the messenger RNA is removed by the gene
- Antisense, silencing all the gene
- Concept applied in Ebola virus therapeutics
- Small interfering RNA (siRNA)
- Phosphorodiamidate morpholino oligomers (PMOs)
- Targets ebolavirus L protein
- TKM–Ebola, is a small interfering RNA agent
- Targets three of the seven ebolavirus proteins, namely L polymerase, VP24, and VP35
- Clinical trials are being conducted with these agents
Ebola Virus Outbreaks - Lessons Learned

**Therapy - Ion Channel Blockers**

Amodarone, dronedarone, and verapamil

- There is some evidence from in vitro studies these agents may prevent entry of Ebola virus into cells.

**Exogenous**

- There is some evidence from in vitro studies these agents may prevent entry of Ebola virus into cells.

**Estrogens**

- Indications for the treatment of metastatic breast cancer in postmenopausal women with estrogen receptor positive or null breast tumors.
- Demonstrated to prevent Ebola virus progression in vitro models.

**Therapy - Nonsteroidal, ovulatory stimulant**

Clomiphene

- Indicated for the treatment of ovulatory dysfunction in women desiring pregnancy.
- Observed to inhibit Ebola virus progression in vitro models.

**Vaccines**

- cAd3-EBO Z
  - Attenuated bivalent vector vaccine derived from the chimpanzee adenovirus type 3 (chAd3)
  - Genetically engineered, to express glycoproteins from both the Zaire, and Sudan ebolavirus strains.
  - Aims at providing immune response these strains.
  - Clinical trials being carried out through the efforts of the National Institute of Allergy and Infectious Disease, and Oxford University.

- VSV-EBOV
  - Developed, using vesicular stomatitis virus, genetically engineered to express Ebola virus glycoprotein.
  - Aims at providing immune response against Ebola virus.
  - Variants have protective response against Marburg Virus.
  - Developed at the Canadian National Microbiology Laboratory.

- EBOV GP
  - Nanoparticle recombinant vaccine.
  - Based on the 2014 Guinea Ebola virus strain.
  - Demonstrated efficacy in preclinical trials.
  - Nasovet

- Currently in development by the Russian Health Ministry.
- Effective against Ebola and Marburg Virus, set for clinical trials in West Africa in 2015.
Ebola Virus Outbreaks - Lessons Learned

**Infection Control**
- Infection prevention and control associated with Ebola virus is multi-facitorial
- Infection prevention should aim at controlling the risk of infection in the healthcare environment
- Reducing the risk of human-to-human transmission
- Reducing the risk of wild-life to human transmission
- Implementation of outbreak containment measures
- Control measures
  - Use of proper personal protective equipment
  - Proper hand washing
  - Proper burial
  - Identification of those who might have been in contact with Ebola patients, and monitoring them for 21 days
  - Animals such as fruit bats, monkeys or apes known to be infected with Ebola virus should be handled with care
  - If they absolutely have to be eaten, should be properly cooked

**Conclusions**
- Ebola remains a formidable threat to mankind in recent times
- Almost one-half century since the initial presentation of the filovirus associated ailments, in Germany, there appears to no end in sight for the outbreaks of this disease
- What is alarming is not the number of outbreaks, since 1967, but rather the lack of developing cure
- While scientists are out in full force more than ever before in search of new compounds, clinical trials are also underway
- Eradication and decrease in mortality will come with substantial education, in areas such as contact precautions, bush meat handling, processing and consumption
- A panel of experts from the World Health Organization states blood plasma and whole blood transfusion should be the treatment priority and approach for now
- The major test will occur sooner or later with another outbreak
- Rapid response, as well as mortality data will indicate whether or not we learned to manage any lessons from previous outbreaks