Before the Ebola epidemic in West Africa from 2013 to 2016, doctors did not realize how much damage the disease could leave in its wake, because previous outbreaks were small and survivors few. Eye disease, with the specter of blindness, has become a dreaded complication.

There are about 17,000 Ebola survivors in West Africa, and researchers estimate that 20 percent of them have had a severe inflammation inside the eye, uveitis. It can cause blindness, but even if it resolves and sight returns, cataracts can quickly follow. …One goal has been to look for the virus in the eyes of survivors with cataracts, to let local surgeons know whether it is safe to operate.

Dr. Ian Crozier, an infectious-disease specialist who contracted Ebola while treating patients in Sierra Leone in 2014 … was blinded in one eye by uveitis and recovered — but then lost his sight a second time, to a cataract. Nearly two months after he had seemingly recovered from Ebola, and after his blood was free of it, severe uveitis suddenly developed — and fluid inside Crozier’s eye was teeming with active virus. At that time, uveitis was also emerging in West Africa.

Sierra Leone’s Ministry of Health and Sanitation was eager for Emory’s help, “Ian’s story was the turning point for survivors.”
Family: Filoviruses

• Latin: *filo* = ’filament’

A virus that is unusually pathogenic in humans

• 1967: Marburg, Germany. Lab workers preparing primary cell cultures from African green monkeys imported from Uganda resulted in an outbreak of a previously unrecognized disease
  – 31 cases, 7 deaths

• 1976: Outbreak of hemorrhagic fever in Zaire (now DRC) & Sudan (Ebola River)
  – 500 diagnosed cases, 460 deaths!

• From these two outbreaks, 2 novel viruses (Marburg & Ebola) were isolated - placed in a new family, the *Filoviridae*
Filoviruses

- Genus = Marburg Viruses
- Genus = Ebola Viruses

Species =
- Zaire Ebola Virus (ZEBOV)
- Sudan Ebola Virus (SEBOV)
- Tai Forest Ebola Virus (ICEBOV)
- Bundibugyo Ebola Virus
- Reston Ebola Virus (REBOV)

Filoviruses are biosafety level 4 pathogens. So, much less is known about their replication since few labs can study them.

Replication cycle is similar to paramyxoviruses; gradient of transcription, and genomic organization.

<table>
<thead>
<tr>
<th>Nucleoprotein (NP)</th>
<th>RNA encapsidation; mRNA versus genome transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP35 (like P)</td>
<td>polymerase cofactor/IFN antagonist</td>
</tr>
<tr>
<td>VP40 (like M)</td>
<td>Major Matrix protein; virion assembly and budding</td>
</tr>
<tr>
<td>GP = Glycoprotein</td>
<td>Virus entry</td>
</tr>
<tr>
<td>sGP, made from unedited message</td>
<td>?</td>
</tr>
<tr>
<td>VP30 (unique to filo)</td>
<td>transcription activation</td>
</tr>
<tr>
<td>VP24 (unique to filo)</td>
<td>(minor matrix protein) IFN antagonist</td>
</tr>
<tr>
<td>Polymerase (L)</td>
<td>Transcription and replication</td>
</tr>
</tbody>
</table>
Filoviruses are non-segmented negative stranded RNA viruses

Niemann-Pick C1 (NPC1), a lysosomal cholesterol transporter is an intracellular receptor for Ebola virus
1. GP cleaved by host CatL/B protease to uncover receptor binding domains

2. Attachment to intracellular receptor

3. L, NP, VP35, VP30 for transcription

4. Loads at 3' end and starts and stops at each gene like paramyxoviruses

5. Transition to replication on membranes. VP30 goes off replication complex

6. VP40 (matrix) drives assembly at the plasma membrane

7. Budding as long filopodia that reach out to the next cell

A GFP-VP40 fusion protein expressed in HEK293T cells shows robust virus-like particle assembly and egress at the plasma membrane (A). (B) corresponds to inset indicated in (A).
Filoviruses- vs. Paramyxoviruses

- **Similarities**
  - single stranded, non-segmented negative sense RNA
  - mRNA synthesis by starting at intergenic regions, stuttering, and restarting. Same polar expression of genes
  - switch from transcription to replication via N binding (called NP in Ebola), but Ebola also uses VP30 for transcription only
  - replicate in cytoplasm

- **Differences**
  - some genes are overlapping
  - fusion is initiated by cleavage of gp within the infected cell
  - additional genes that act on innate immune system
  - assembly and shape of virion is much different
  - envelope gene has both attachment and fusion

Transmission of Ebola Virus

- **Human to human:** direct contact with the blood, secretions, organs, or other bodily fluids of infected persons (often health care workers)
- **Human to human:** Burial ceremonies where mourners have direct contact with the body of the deceased person
- **Zoonosis:** handling of infected chimpanzees, gorillas, and forest antelopes
- **Human to human:** sexual contact after symptom recovery (rare)
Human Ebola outbreaks in Africa 1976-2004

No known cases between 1979 and 1994, but frequently since 2004

Distinct viral species co-circulate in Africa

What might explain this?

Sequence divergence as a function of time of isolation

1.5% divergence but isolated 20 yrs and 1000 km apart

Gp sequences only
Distinct viral species co-
circulate in Africa.

Lack of geographical clustering with deep phylogenies of Ebola genera

What could cause this?


Site of largest recorded Ebola outbreak before 2014, 425 people

Characteristics of an animal reservoir

• Reservoir host contains a higher genetic diversity of virus than the target host.

• Harbor the virus continuously at the group level (with or without disease)

• Naturally infected beyond the geographical area of the target host

• Why are each of these important?
EBOV must be present in a reservoir species

EBOV is also lethal to chimps, gorillas, and duikers

reservoir host(s) → intermediate host(s)? → amplification?

humans

Animal to human transmission in filovirus outbreaks
Are fruit bats the EBOV reservoir?
Investigation of 2001-2005 outbreaks in Gabon

- Three trapping expeditions in areas close to chimp and gorilla carcasses
- Tested 679 bats, 222 birds, 129 small terrestrial vertebrates
- 16 Ab+ bats, 13 PCR+ bats (no overlap)
- Previous attempts focused on insects and small mammals had consistently been negative

Luebo DRC; 186 deaths. Index case linked to contact with bats

Mechanisms involved in EBOV pathogenesis

- Virus enters body through skin abrasions or through mucosa of the eye
- Primary replication site is monocytes/macrophages
- Virus can reach titers of $1 \times 10^9$ per ml of blood within 2 days after infection
- Infected monocytes release proinflammatory cytokines TNFα, IL-6, IL-8, others
- Cytokines cause death/loss of function of endothelial cells which compromises the vascular system (microvascular endothelial cells also directly infected late in disease)
- Severe bleeding, coagulation abnormalities, multiple organ failures
Mechanisms involved in EBOV pathogenesis

1. Primary targets
   - NK cells
   - EBOV
   - Lymphocytes

2. Cytokine storm
   - Macrophages
   - Dendritic cells
   - Tissue factor expression
   - Disseminated intravascular coagulation

3. Pathological effects on vascular system

But, very active and sustained CD4 and CD8 cell responses in surviving patients treated in the US

Historical and geographical distribution of filovirus outbreaks

Filovirus species represented by the different colors and the number of cases by the size of the circles.

West African outbreak is Ebola Zaire, but probably not an imported case.

What data would support this conclusion?
2014/2015 Ebolavirus outbreak in West Africa

Cases and deaths
Data up to 27 March 2015

Guinea 3811 10 675
Liberia 609 4
Sierra Leone 14 124
Italy 14 124
Mali 4
Nigeria 20 8
Senegal 14 124
Spain 14 124
United Kingdom 14 124
United States of America 14 124
Total 11 323 28 646

Additional contained outbreak in March 2016 from a woman with sexual exposure to a survivor.
http://apps.who.int/gho/data/node.ebola-sitrep

The west African outbreak of 2014/15 from a single source

Lineage A was the initial outbreak in March 2014, expanded around this area and then declined. From lineage A a second lineage (B) emerged in May/June 2014 and expanded into Sierra Leone and Liberia.

Lineage B continued to spread into Sierra Leone, Liberia, and further into Guinea. EBOV entered Mali from Guinea via two separate routes.

R0 estimated at 1.4-2.0

This outbreak was different because it reached urban population centers.
Antivirals and vaccines

• Treatment
  current is hydration and other symptom based
  – Monoclonal antibodies (ZMapp)
  – Convalescent sera
  – siRNA
  – Broad acting nucleosides, other drugs from existing approved drugs

• Vaccines
  – ChAd3 or VSV vectors expressing EBOV GP

Problems associated with testing these

Date: Fri 20 Oct 2017
Source: WHO Media centre, news release [edited]

WHO supports containment of rare virus on Uganda-Kenya border

WHO is working to contain an outbreak of Marburg virus disease (MVD) that has appeared in eastern Uganda on the border with Kenya.

At least one person is confirmed to have died of MVD and several hundred people may have been exposed to the virus at health facilities and at traditional burial ceremonies in Kween District, a mountainous area 300 km [approx. 86 mi] northeast of Kampala.

The 1st case was detected by the Ministry of Health on [17 Oct 2017]; a 50-year-old woman who died at a health centre of fever, bleeding, vomiting and diarrhoea on [11 Oct 2017]. Laboratory testing at the Uganda Virus Research Institute (UVRI) confirmed the cause of death as MVD.

The woman's brother had also died of similar symptoms 3 weeks earlier and was buried at a traditional funeral. He worked as a game hunter and lived near a cave inhabited by _Rousettus_ bats, which are natural hosts of the Marburg virus.

One suspected and one probable case are being investigated and provided with medical care. An active search for people who may have been exposed to or infected by the virus is underway.
**Ebolaviruses: Not just in Africa**

**Discovery of an Ebolavirus-Like Filovirus in Europe**

Ana Negredo¹, Gustavo Palacios³, Sonia Vázquez-Morón¹, Félix González³, Hernán Dopazo⁶, Francisca Molerö¹, Javier Juste⁴, Juan Quetglas⁴, Nazir Savji⁴, María de la Cruz Martínez¹, Jesús Enrique Herrera¹, Manuel Pizarro⁴, Stephen K. Hutchison⁷, Juan E. Echevarría¹, W. Ian Lipkin⁷, Antonio Tenorio⁷

Massive die-off of bats in caves in Spain, Portugal, and France
Found Ebola-like virus = Lloviu Virus (LLOV) not present in caves with healthy bats

**Discovery of Swine as a Host for the Reston ebolavirus**


Outbreak of respiratory and abortion syndrome in pigs in the Phillipines
Isolated new virus related to Reston. Probably not pathogenic in pigs. 6/141 humans in contact with pigs were positive for antibody to the virus

**Things you should know about Filoviruses**

- Similarities and differences in genome and replication between filoviruses and other negative strand RNA viruses
- Unusually rapid pathogenesis/high incidence of death
- Indications of a host reservoir/what are the transmission patterns?
- 2014 Epidemic in Western Africa
- **No Discussion paper: midterm due next Tuesday by class time.**