Emerging Viruses

Part III

Zoonoses and why they occur

The Influenza, Ebola, and Zika Paradigms
Convergent forces of disease emergence

- Globalization
- Rapid Air Travel
- Expanding Populations
- "Mega-Cities"
- Poverty
- Deforestation
- Microbial Evolution
- Altered Ecosystems
- Environmental Changes
Expanding viral niches

- Successful encounters require access to susceptible and permissive cells
- Population density and health are important factors
- Virus populations will endure in nature only because of serial infections (a chain of transmission)
Emerging viruses

- *Emerging virus* - causative agent of a new or previously unrecognized infection
- The term became popular in 1990s, but emerging viruses are not new.
- Since the rise of agriculture - 11,000 years ago - new infectious agents have invaded human populations because they can be sustained by numbers that were unknown before agriculture and commerce
- Only recently have we become good at detecting emerging viruses
Emerging viruses

- Expanded host range with an increase in disease not previously obvious
- Transmission of a virus from a wild or domesticated animal to humans - *zoonosis*
- Cross-species infection may establish a new virus in the population (SIV moving from chimps to humans) to become HIV
- Often cross-species infection cannot be sustained (e.g. Ebola and Marburg from bats to humans)
The general interactions of hosts and viruses

- Stable: maintains virus in ecosystem
- Evolving: passage of virus to naive population (same or different host)
- Dead-end: one way passage to different species
- Resistant host: infection blocked
# Some Zoonoses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Drivers of Emergence</th>
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</thead>
<tbody>
<tr>
<td>Dengue virus</td>
<td><em>Flaviviridae</em></td>
<td>Urban population density, mosquito breeding</td>
</tr>
<tr>
<td>Ebolavirus</td>
<td><em>Filoviridae</em></td>
<td>Human contact with natural host; bushmeat</td>
</tr>
<tr>
<td>Hantaan virus</td>
<td><em>Bunyaviridae</em></td>
<td>Agriculture: human/rodent contact</td>
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<tr>
<td>Hendra virus</td>
<td><em>Paramyxoviridae</em></td>
<td>Bats to horses to stable workers</td>
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<tr>
<td>HIV</td>
<td><em>Retroviridae</em></td>
<td>Bushmeat trade</td>
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<tr>
<td>Influenza virus</td>
<td><em>Orthomyxoviridae</em></td>
<td>Pig/blrd agriculture</td>
</tr>
<tr>
<td>Junin virus</td>
<td><em>Arenaviridae</em></td>
<td>Agriculture: human/rodent contact</td>
</tr>
<tr>
<td>Nipah virus</td>
<td><em>Paramyxoviridae</em></td>
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<tr>
<td>Machupo virus</td>
<td><em>Arenaviridae</em></td>
<td>Agriculture: human/rodent contact</td>
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<tr>
<td>Rift Valley virus</td>
<td><em>Bunyaviridae</em></td>
<td>Dams, Irrigation</td>
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<tr>
<td>Sin Nombre virus</td>
<td><em>Bunyaviridae</em></td>
<td>Weather, human/rodent contact</td>
</tr>
<tr>
<td>West Nile virus</td>
<td><em>Flaviviridae</em></td>
<td>Mosquito</td>
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</tbody>
</table>
Mechanisms of Zoonoses

- RNA viruses: error prone RNA polymerase, no correction mechanism
- One misincorporation in $10^4 - 10^5$ nucleotides polymerized ($10^6$ greater than host DNA genome)
- In RNA viral genome of 10 kb, this frequency leads to one mutation in 1-10 genomes
Influenza Virus

A respiratory Virus
Effect of 1918 Influenza Outbreak on Life Expectancy

Spread of Respiratory Viruses

- Respiratory secretions - aerosols produced by coughing, sneezing, speaking
- Nasal secretions contaminating hands, tissues

http://www.virology.ws/2013/01/23/slow-motion-sneezing/
Influenza Infection Characteristics

• Common respiratory pathogens in humans
• Can lead to pneumonia
• Continual source of novel influenza viruses
  – Extensive host-range diversity
  – Extensive genetic diversity
  – ability to reassort genetically
  – Emergence of periodic pandemics
• Pandemics cause higher morbidity and mortality than annual epidemics
• Infection of both upper and lower respiratory tract
• Influenza infects entire respiratory tract
• Infection severely damages lining of the airway passages
• Secondary bacterial infections often ensue
• Immune responses contribute to pathogenesis
Influenza virus
An orthomyxovirus

SEGMENTED RNA genome (genome in eight pieces)

Enveloped

Two major surface proteins
HA – Hemagglutinin (fusion protein)
NA – Neuraminidase (detaches virus from host cell)
If infected with $\geq 2$ strains, both HA & NA + other RNA segments can swap = new serotype
The Influenza Hemagglutinin

green = HA (trimer)
yellow = NA (tetramer)
red = fusion peptide
Receptor binding weak
Easy to mutate so can bind alternative sialic acid isomer
Antibodies to one strain not bind well to another
Sialic acid: receptor for influenza viruses

- Sialic acids: N-acetyllneuraminic acid (A,B); 9-O-acetyl-N-neuraminic acid (C)
- $\alpha(2,6)$ preferentially bound by human strains, $\alpha(2,3)$ by avian
Sialic Acid Interaction with HA

Human

Avian
Antigenic drift: Influenza virus
Schematic showing the known emergence events of influenza A viruses in mammals that led to extended outbreaks, including epidemics and pandemics.

Selecting an influenza virus vaccine

WHO GISN* 
WHO CC† 
WHO CC-CDC‡/FDA§ 
FDA 
FDA 
FDA 
Manufacturers 
Clinic 

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec 

Surveillance 
Select strains 
Prepare reassortants 
Standardize antigen 
Assign potency 
Review/license 
Formulate/test/package 
Vaccinate 

*World Health Organization Global Influenza Surveillance Network 
†WHO Collaborating Centres 
‡US Centers for Disease Control and Prevention 
§US Food and Drug Administration

http://www.microbe.tv/twiv/twiv-413/ on how strains are selected
Inactivated influenza vaccine

- 3000-49000 deaths/yr in US due to influenza virus
- Vaccine: virus grown in embryonated chicken eggs, formalin-inactivated or detergent or chemically disrupted virions
- 75-100 million doses manufactured each year US
- 60% effective in healthy children and adults <65 yr
- Protection correlates with serum antibodies to HA, NA
- Vaccine produced in cell culture avoids egg allergies (Flucelvax)
Problems with the Current Influenza Vaccine

• Major circulating virus (H3N2) is most virulent
• Vaccine virus currently propagated in eggs
• Virus wants to adapt to optimal growth in eggs
• Appropriate mutants outgrow input virus
• This year’s mutants are not as antigenic as input virus (fewer neutralizing antibodies made)
• H3N2 virus only 65% of currently circulating virus
• Vaccine still effective against 35% of the circulating viruses
Antigenic drift: Influenza virus
Mutations in egg derived H2N2 viruses affect antigenic structure
Inhibitors for when Vaccines do not work

**Symmetrel (Amantadine)**

- Interacts with influenza viral M2 protein (ion channel)
- Blocks entry of protons into virion, prevents uncoating
Amantadine Binding Sites
Inhibitors for when Vaccines do not work

Influenza virus NA inhibitors
Influenza virus NA inhibitors

- Designed to mimic natural ligand, sialic acid
- Closer inhibitor to natural compound, less likely target can change to avoid binding drug while maintaining viable function
Neuraminidase Inhibitor Resistance Testing Results on Samples Collected Since October 1, 2016

<table>
<thead>
<tr>
<th></th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
<th>Peramivir</th>
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<tbody>
<tr>
<td></td>
<td>Virus Samples tested (n)</td>
<td>Resistant Viruses, Number (%)</td>
<td>Virus Samples tested (n)</td>
</tr>
<tr>
<td>Influenza A (H1N1)pdm09</td>
<td>240</td>
<td>0 (0.0)</td>
<td>234</td>
</tr>
<tr>
<td>Influenza A (H3N2)</td>
<td>1,525</td>
<td>0 (0.0)</td>
<td>1,525</td>
</tr>
<tr>
<td>Influenza B</td>
<td>566</td>
<td>0 (0.0)</td>
<td>566</td>
</tr>
</tbody>
</table>

Circulating H1N1 and H3N2 viruses are largely resistant to adamantanes, not recommended for use

http://www.cdc.gov/flu/weekly/index.htm
Thoughts for New Vaccine Approaches

**Problem**
- Many HA epitopes nonneutralizing
- Current vaccines grown in eggs
- Ferrets = test animal
- No consideration of previous exposure history
- Virus inhibits natural immune responses

**Recommended solution**
- Develop HA without these epitopes but retaining native structure
- Express HA on cells w/o other viral proteins
- Genetic comparisons
- Understand effects of immunological legacy
- Use viruses debilitated in immune inhibition
Future influenza vaccines?

- Virus-like particles: synthesis of HA alone in cells leads to production of immunogenic particles
- Has also been done in plants
- 1 square meter of plants produces 20,000 doses at under $0.20/dose
To Optimize Production of Neutralizing Antibodies: Consider Conformational Changes During Fusion

Native: metastable structure

First change: Head-group separation

Activated: prehairpin

Post-fusion: 6-helix bundle
To Optimize Production of Neutralizing Antibodies

- Broadly neutralizing human mAbs
- Prime-boost
- HA stem antigen

- Stem region invariant among strains
- Antibodies to this region inhibit conformational changes required for fusion
Modulation of Viral Attack on Immune System
Interfering with Interferon Inhibition

Generating live attenuated HIS virus vaccines
HIS viruses confer improved vaccination against influenza virus strains. This approach could be used to generate more effective live attenuated vaccines against other viruses.

Libraries of mutant influenza virus strains are screened for IFN-I sensitivity. IFN-I-sensitive strains are selected and sent for RNA sequencing.

IFN-sensitive strains are sequenced and mutations incorporated into a single virus, creating a HIS virus that is attenuated in vitro and in vivo.

Antigenic determinants are retained.

1 Naïve mice or ferrets are vaccinated with the HIS influenza virus. 2 Mice or ferrets are challenged with homologous or heterologous virus strains.

Animals challenged with HIS virus exhibited superior protection compared to mock challenged animals with respect to both reduced mortality and viral loads.

(HIS = high interferon sensitivity)

J. Teijaro & D. Burton, Science, 359: 277-278, 2018
Ebola Virus

A Blood-Borne Virus
Filamentous, enveloped, nonsegmented negative-strand RNA virus; family Filoviridae (Marburg = sister virus)

Hemorrhagic fever virus on the NIAID list of category A priority pathogens (up to 90% fatal)

Requires proteolytic processing and binding to intracellular receptor for activation.

Fusion mechanism still unknown
Ebola virus displays a carpet of Glycoprotein (GP) proteins on its surface. GP is the viral fusion protein that associates with multiple cell surface proteins for internalization. It binds to the target cell receptor (internal) for fusion, and the exact fusion trigger is still unknown.
How are humans infected?

- A classic zoonosis
- Index case: contact with animal carcass* (bushmeat)
- Transmitted to other humans by close contact with infected fluids
- Chains of human infections short
- $R_0 = 2$

*not always identified
Filovirus ecology

- Marburg virus has been isolated from cave-dwelling fruit bat (*Rousettus aegyptiacus*)
- Zaire Ebolavirus RNA, antibodies found in three tree-roosting bats (but not infectious virus)
- Humans, gorillas, chimpanzees are dead-end hosts
Ebola virus outbreak examples

- Gabon, 1996 (Zaire ebolavirus, 37 cases) A chimpanzee found dead in the forest was eaten by people hunting for food. Eighteen people who were involved in butchering the animal became ill. Ten other cases occurred in their family members.

- Gabon, 1996-97 (Zaire ebolavirus, 60 cases) The index case was a hunter who lived in a forest camp. A dead chimpanzee found in the forest at the time was infected with Ebola virus.
Ebola virus emergence in Guinea

Virology Lectures 2017 - Prof. Vincent Racaniello - Columbia University
Host entry

- Mucosal surfaces
- Breaks or abrasions in skin
- Parenteral (e.g. contaminated needles)
- Virus detected in skin, body fluids, nasal secretions, blood, semen
Human-human transmission

- Contact with infected blood or body fluids (urine, saliva, sweat, feces, vomit, breast milk, semen) from someone who is sick or has died
- Contact with contaminated objects (needles, syringes)
- Not by insects, water, food, or aerosol
Ebola virus disease: Clinical features

- Incubation period 2-21 days (not contagious)
- Early symptoms: fever, headache, muscle pain, diarrhea, vomiting, stomach pain
- Peak illness: rash, hemorrhage, convulsions, severe metabolic disturbances, diffuse coagulopathy
- 30-90% case fatality ratio in Africa
Immunopathogenesis

- Many inflammatory mediators produced, especially by infection/activation of monocytes/macrophages
- Imbalanced cytokine production => disease
- Impairment of vascular and coagulation systems
- These dysregulation activities contribute to high rate of viral replication and pathogenesis
Clinical features: Multisystem involvement

- Systemic (prostration)
- Gastrointestinal (anorexia, nausea, vomiting, abdominal pain, diarrhea)
- Respiratory (chest pain, shortness of breath, cough)
- Vascular (conjunctival injection, postural hypotension, edema)
- Neurological (headache, confusion, coma)
Pathogenesis

- Extensive necrosis in parenchymal cells of many organs (liver, spleen, kidney, gonads)
- Broad cell tropism: Monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, epithelial cells
- Elevation of liver enzymes, shock (adrenal)
- Massive lymphocyte death but not infected

- Death usually from massive organ failure
Other ongoing issues now being evidenced by survivors of 2013-2016 epidemic
Filovirus Life Cycle: A Primary Target of Pharmacological Research

- Antibodies to inhibit external Virus (Vaccines)
- Antivirals to inhibition of fusion
- Antivirals and siRNA to inhibit replication
Biosafety level 4 (BSL-4)

- High mortality
- Person to person transmission
- No approved vaccine or antiviral

Threading the NEIDL https://youtu.be/tqAjkjGq8Ug
Pseudotyped Virus Production for Study Under BSL-2 Conditions

1. Transfect GP cDNA into BHK-21 cells
2. Infect with VSVΔG*-G helper virus
3. Harvest virus, purify through sucrose gradient

Use GFP virus to infect target cells
Score # green (infected) cells
Verify in animals (BSL-4)
Ebola Virus Glycoprotein (GP)

**Internal Fusion Loop (IFL):**
Wraps around Protein hugging adjacent monomer of the trimer

- All small molecule fusion inhibitors bind the interface between the IFL and the rest of the protein
- Inhibitors destabilize GP, triggering fusion relevant conformational changes prematurely
Zmapp, the best known passive vaccine

- Raised in mice immunized with virus-like particles
- Chimerized into human IgG1 scaffold
- Produced in tobacco plants
A More Protective Pair of Antibodies

Fully protective in limited NHP tests ≥ 5 days post infection
**Pseudotyped Virus for Vaccine Production**

1. **Transfect GP cDNA**
   - BHK-21 cells

2. **Infect with VSVΔG*-G helper virus**
   - GP expressed at cell surface and on virions

3. **Harvest virus, purify through sucrose gradient**

**Use GP virus to infect target cells**

GP expressed at cell surface and on virions

Antibodies made to this GP
Strategies for Ebola Virus Vaccines

- Have shown protection for 1 yr.
What have we learned?

- Every infectious disease is a global problem
  - 4 cases in US: 2 imported (Dallas, NYC), 2 locally acquired
- Ebolavirus vaccines have been ready for clinical trials for some time
- What other viruses should we be preparing for?
Zika Virus (ZIKV)

A Mosquito-Transmitted Virus
ZIKA Virus (ZIKV)

an arthropod-borne virus (arbovirus) in the genus *Flavivirus* and the family *Flaviridae*
ZIKV and its Closest Relatives

- ZIKV
- Spondweni
- Dengue
- Yellow Fever
- West Nile
- Japanese Encephalitis
The complex life cycle of an arbovirus

Stable host-virus interactions

Not for ZIKV
ZIKV Departures from the Arbovirus Paradigm

- Human-to-human contact (escape the mosquito portion of the cycle)
- Humans are amplifying hosts (contribute to mosquito-animal cycle)
- Blood-borne transmission
- Crosses the Placenta
- Transmitted sexually
- Persists in semen
All ZIKV in US to date introduced from Caribbean, not Brazil.
Mosquito Ranges in US
The ZIKV Genome
The Flavivirus Life Cycle

A. Virus attachment and entry
B. Virus fusion and disassembly in the endosome
C. Protein translation, polyprotein processing on membranes
D. Viral RNA replication on membranes
E. Immature virus assembly and budding into ER
F. Virus maturation and Furin cleavage of prM
G. Mature virus release

ER, Golgi, TGN

Nucleus

doi: 10.1016/j.antiviral.2008.05.004
Zika virus

- Disease: rash, fever, joint pain, conjunctivitis, headache (similar to dengue, chikungunya)
- Incubation period 2-10 days
- 1 in 5 develop symptoms; 5 day course
- Fatalities rare
Mutations That Increase Pathogenicity

• A mutation in prM is associated with microcephaly

• Epidemic strains isolated after 2012 have a mutation in the NS1
  – enables the protein to suppress IFN-β induction
  – enhances virus production in mosquitos

• Additional mutations exist; significance not yet known
Central nervous system complications associated with Zika virus infection

**Adults**

- Guillain-Barré Syndrome (post-infection autoimmune neuropathy; weakness, paralysis, death)
- Acute myelitis
- Encephalopathy
- Meningoencephalitis

**Infants**

- Microcephaly
- Lissencephaly
- Macular atrophy
ZIKV Kills Neuronal Embryonic Stem Cells

https://doi.org/10.1016/j.celrep.2016.08.038
ZIKV Damages the Placenta

Fig. 9 Schematic summary of placental damage following maternal ZIKV-infection. Cross section diagram of placental vasculature demonstrating maternal spiral arteries that perfuse the intervillus space. Notations A–D summarize the placental tissue damage, alterations in perfusion, and immune response to ZIKV infection during pregnancy.
Zika virus shedding

- Semen
- Urine (PCR) - up to 30 days after symptom onset
- Saliva (more frequently than in blood)
- Blood
- Breast milk
- Except for blood, unknown how virus reaches these sites
The DENV Problem

- Initial infection causes mild infection
- Second infection much more serious
- Antibody response is actually helping the virus infect immune cells (Antibody Dependent Enhancement = ADE)
- Antibodies to DENV and ZIKV cross-react with opposite viruses
- Prior exposure to DENV makes ZIKV infection worse
- DENV endemic in S. America and Caribbean
- These are the areas where ZIKV pathogenesis was worst
The ZIKV and DENV Surface Proteins Have Nearly Identical Structures

Zika Virus Vaccines In Development

- Attenuation: Replication competent natural virus vaccine
- Cloning: Bacterial cell
- Expression: Protein
- Cloning: Virus-like particle vaccine
- Inactivation: Inactivated virus vaccine

Being most actively pursued
Substitute prM & E Proteins from all strains of ZIKV and DENV
Include NS T-cell stimulating epitopes
Zika virus DNA vaccine

Advantage of DNA Vaccine: Can include multiple antigens
Include DNA for E Proteins from all strains of ZIKV and DENV
Include NS T-cell stimulating epitopes
Zikv E DIII contains ZIKV-specific sequences
3 Zikv specific epitope sites on E DIII identified
No cross reaction with other flaviviruses

DIII movement critical to fusion function
Antibodies to DIII inhibit fusion
To Make a ZIKV DIII Vaccine

- Fuse ZIKV E DIII to HBcAg (the core protein of HBV) = HBcAg-zDIII
- HBcAg stimulates T cells to improve antibody production
- Express in tobacco plants
- Purify VLPS
- DIII retains native structure
- Immunize mice
- HBcAg-zDIII elicits potent neutralizing antibodies
- HBcAg-zDIII elicits potent cellular immune response
- HBcAg-zDIII induced antibodies do not cross-react with other flaviviruses (no ADE ressponse)

These results warrant follow-up clinical trials
Questions?
How common are host range jumps?

- Dead end: Very common
- Those that produce sustaining transmission: Rare
- Can we predict them? No
- But we can know what is out there, and react (preparedness)
Summary

- Virus life cycle requirements
- Viral survival strategies
- Host immune response
- The virus-host “tug-of-war”
- Vaccine and Antiviral strategies to aid host survival
- Virus modes of transmission require relevant protection measures
- Improving our understanding of virus-host interactions can aid design of improved antivirus defenses
Other Benefits of Studying Viruses

• Viral oncogenes helped establish the hallmarks of cancer development
• Most tissue culture cells transformed using viral oncogenes
• Viruses have taught us most of what we know about cell biology
• Viruses have helped define cells and functions of the immune system
• Genetic code worked out using bacteriophages
Current uses of viruses

• Cancer community learning strategy from virology field (and vice-versa)

• Drug delivery strategies
  – Use packaging and targeting abilities of viruses
  – Substitute surface proteins and/or receptor binding sites onto benign viruses
  – Use VLPs with desired protein-targeting motifs on surface
  – Use lipid nanoparticles with surface proteins or receptor binding motifs displayed on surface
  – Use chimeric proteins with drug attached to viral receptor binding motif