Biosafety from the Field to the Lab

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Vertebrate-borne Zoonoses

**Marburg Virus Disease**
- Marburg virus
- Egyptian fruit bat

**Ebola Virus Disease**
- Ebola Zaire virus
- Ebola Ivory Coast virus
- Ebola Sudan virus
- Hypsignathus, Epomops, and Myonycteris spp. bats

**Bolivian Hemorrhagic Fever**
- Machupio virus
- Vesper mouse
- Chapare virus
- Unknown - presumably rodent

**Brazilian Hemorrhagic Fever**
- Sabia virus
- Unknown - presumably rodent

**Hepatitis E**
- Hepatitis E virus
- Swine, wild deer, and boars

**Rabies**
- Rabies virus
- Bats of many species

**Venezuelan Hemorrhagic Fever**
- Guanarito virus
- Cane mouse

**Lassa Fever**
- Lassa virus
- Multimammate mouse

**Hantavirus Disease**
- > dozen viruses
- Various rodent species

**Nipah Encephalitis**
- Nipah virus
- Pteropus spp. flying foxes (bats)

**Hendra Encephalitis**
- Hendra virus
- Pteropus spp. flying foxes (bats)

**SARS/MERS**
- Coronaviruses
  - Chinese horseshoe bat
  - Egyptian tomb bat?

**Monkeypox**
- Monypox virus
  - Squirrels, Gambian rats

**Avian Influenza**
- Avian influenza viruses
  - Waterfowl

**Unknown**
- Various rodents and reservoir hosts
Vertebrate Reservoirs of Zoonotic Agents

- To perpetuate, viruses must:
- Persistently infect their reservoirs \textit{without}
  - substantial pathology
  - eliciting a sterilizing immune response
- Infect another susceptible host \textit{before}
  - the immune response controls the virus
  - the host dies

Reservoir hosts rarely suffer disease from the viruses

If you want to understand the \textit{biology and ecology} of an infectious, you have to study it in the context of its \textit{reservoir host}
## Model Systems for the Study of Zoonotic Viruses

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Value for zoonosis research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal model, conventional</td>
<td>Easy to maintain and breed</td>
<td>Heterologous pathogen-host relationship</td>
<td>Limited</td>
</tr>
<tr>
<td>(e.g., laboratory mouse, rat)</td>
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<td>Species-specific reagents available</td>
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<tr>
<td>Animal model, reservoir host</td>
<td>Homologous pathogen-host relationship</td>
<td>Husbandry and breeding limited to few species</td>
<td>Limited to few species</td>
</tr>
<tr>
<td>(natural reservoir host)</td>
<td></td>
<td>Species-specific reagents rarely available</td>
<td></td>
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<tr>
<td>Cell culture, conventional</td>
<td>Easy to culture</td>
<td>Heterologous pathogen-host relationship</td>
<td>Suitable as a basic model, but less useful for more complex questions on pathogen-host interaction</td>
</tr>
<tr>
<td>(e.g., Vero E6, tumor cell lines, HUVECs, monocytes, dendritic cells)</td>
<td></td>
<td>Accumulation of mutations/deregulation of important cellular pathways due to high passage numbers possible</td>
<td></td>
</tr>
<tr>
<td>Cell culture, reservoir host-derived</td>
<td>Homologous pathogen-host relationship</td>
<td>Very few reservoir-derived cell lines available so far</td>
<td>High</td>
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<tr>
<td></td>
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<td>Thorough characterization necessary</td>
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<tr>
<td></td>
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<td>Species-specific reagents rarely available</td>
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</tr>
</tbody>
</table>

**Studying immunity to zoonotic diseases in the natural host — keeping it real**

*Andrew G. D. Bean, Michelle L. Baker, Cameron R. Stewart, Christopher Cowled, Celine Deffrasnes, Lin-Fa Wang, and John W. Lowenthal*  
*NATURE REVIEWS | IMMUNOLOGY*  
*Volume 13 | December 2013 | 851*

CSIRO Biosecurity Flagship, Australian Animal Health Laboratory, Geelong, Victoria, Australia

**Reservoir Host Immune Responses to Emerging Zoonotic Viruses**

*Judith N. Mandl, Rafi Ahmed, Luis B. Barreiro, Peter Daszak, Jonathan H. Epstein, Herbert W. Virgin, and Mark B. Feinberg*  
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*5Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA*  
*6Merck Vaccines, Merck & Co. Inc., West Point, PA 19486, USA*  

Eckerle et al., *Viruses*, 2014
Vertebrate Reservoirs of Zoonotic Agents

- Field research
  - Many variables that cannot be controlled
  - Many risks other than the agent are associated with the work
  - Tailored occupational health oversight
  - Careful thought to mitigate those risks
  - Plans if things go wrong
- Lab research
  - Bring the animal species into the lab
    - Capture and end-use
    - Establishment of a colony
  - Quarantine period
  - What else might you bring in with the animal?
    - "Specific pathogen free" animals
    - Deep sequencing to ID other infectious agents may not be reliable
Difficulties in Zoonotic Disease Research

- Work in remote and/or undeveloped areas, often in difficult surroundings
- Cultural barriers
- Electricity is often unavailable
- Laboratory facilities are often unavailable
- Must work with personal protective equipment
- Equipment must be transported to destination
- Animal reservoirs are not model organisms
- Lack of reagents and methods
- Governmental issues
  - Work visas
  - Permits
  - Export
- One new virus/2 years
- Human as sentinels
Projects: Rodent and Bat-borne Viruses

- Hantaviruses
- Arenaviruses
- MERS-CoV
- Nipah virus
- Ebolaviruses
- Zika virus
- Deer mice
- Artibeus bats
- Carollia bats
- Syrian hamsters
Field Work with Reservoirs
New World Hantavirus Biosafety

*Hantavirus Cardiopulmonary Syndrome*

- **BSL-2**: Laboratory manipulation of viruses not known to cause human disease
- **BSL-2 with BSL-3 precautions**: Manipulating tissues from euthanized animals infected with HCPS-causing hantaviruses
- **BSL-3**
  - Laboratory manipulation and propagation of viruses that cause HCPS
- **Animal infections with viruses not known to cause human disease**
  - Can transition live cells to BSL-2
- **BSL-4**: Animal infections with viruses that cause HCPS
  - Cannot transition live cells to BSL-3 or BSL-2

MMWR 43:RR-7 1994
Establishment of a Deer Mouse Colony

- Deer mice (*Peromyscus maniculatus*) are the principal reservoir host of Sin Nombre hantavirus (SNV)
- Found throughout western North America
- Other species of *Peromyscus* found in other parts of North America
- Founders live-trapped in western Colorado, June 2000
- Bled at capture for serology (one positive)
- Individually quarantined for 42 days (outdoors)
- Bled again for serology
  - All were negative and moved into animal facility
  - Breeding within a month
  - Subsequent serology was negative

We did not find what we were looking for (hantavirus)

But we did not find what we *weren’t* looking for, either!
Establishment of a Deer Mouse Colony
Establishment of a Deer Mouse Colony
Hantavirus Disease

- Two clinical diseases with many similarities
  - Hemorrhagic Fever with Renal Syndrome (Eurasia) - BSL-3
  - Hantavirus Cardiopulmonary Syndrome (Americas) - BSL-3/ABSL-4
- Both are thought to have immunopathologic components
- No virus damage to the endothelium
- Pronounced inflammatory immune response 10 to 35 days post exposure
- About 200,000 cases and about 10,000 deaths per year
Hantaviruses

- Negative stranded RNA viruses
- Global distribution
- Enveloped
- Trisegmented
  - S - nucleocapsid (NSs?)
  - M - Gn and Gc glycoproteins
  - L - RNA-dependent RNA polymerase
- Zoonotic reservoirs (no pathology)
  - Rodents
  - Shrews
  - Moles
  - Bats
- Reservoirs remain infected, perhaps for life, despite an immune response
  (Easterbrook et al., *PNAS*, 2007; Schountz et al., *PNAS*, 2007)
- In rodent reservoirs a regulatory T cell response occurs
Hantaviruses and Their Rodent Reservoirs

Why don’t reservoir rodents have pathology when infected with their hantaviruses?

Why are they unable to clear the virus?
Rodent-Borne New World Hantaviruses

Deer mice are susceptible to Andes virus, but clear infection (Spengler et al., PLoS One, 2013)

How do deer mouse immune responses differ to SNV and ANDV? (ABSL-4, RML)
Gene Expression Cluster Analysis

Deer mouse. S=SNV infected (5); A=ANDV infected (8)

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<th>100</th>
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<th>200</th>
<th>200</th>
<th>100</th>
<th>-</th>
<th>800</th>
<th>1600</th>
</tr>
</thead>
</table>

- Th1/Th2 (low) 5/5 SNV
- Th1/Th2 (high) 6/8 ANDV
- Th2 2/8 ANDV

Fold

Th1
- IFNg
- TNF
- LTA
- Il18rap
- Ifi1
- Runx3
- Socs1
- Socs5
- Stat1
- Stat4
- Tbx21
- Traf6
- Il4
- Il4ra
- Il13
- Il5
- Il6
- Ccl5
- Ccl2
- Cxcl10
- Gata4
- Gfi1
- Gpr144
- Icos
- Irf4
- Irf8
- Jak1
- Nfatc2ip
- TGFb
- IL10
- Ccr6
- Pou2f2
- Foxp3
- TLR7
- Casp3
- Ccl7
- Myb
- Mx1
- Lrnc32
- Pkd2
- Tnfrsf9
- Zbtb7b
- Cxcl2

Th2

Treg

Antiviral
Gene Expression Profiles of Deer Mice Infected with SNV (blue) or ANDV (red) for 14 Days

T Follicular Helper B Cell Maturation

T Cell-B Cell Activation
Model for Hantavirus Infection of Reservoirs

SNV
- Virus
- Th1/Th2
- Treg

ANDV
- Virus
- Th1/Th2
- Th2/Tfh

Time
Maporal Hantavirus

- Isolated from rice rats in Venezuela
- Phylogenetic cluster with Andes virus
- Not known to cause disease in humans (ABSL-3)
- Causes disease in Syrian hamsters similar to HCPS
Maporal Virus Infects Deer Mice
MAPV Induces a Modest Host Response in Deer Mice

MAPV infection of deer mice is more similar to SNV than to ANDV infection. A surrogate ABSL-3 model for SNV?
Bats As Reservoir Hosts of High Impact Viruses

- Rabies virus and other lyssaviruses
- Parmyxoviruses
- Henipaviruses
- Sosuga virus?
- Filoviruses
- Coronaviruses

Are bats “special” and, therefore, well-suited as reservoir hosts?
Bats

- Order Chiroptera - “hand-wing”
- About 25% of mammalian species (~1200)
- Essential ecological roles
- Pollination
- Insect control
- Diet (species-specific)
- Fruits
- Insects
- Nectar
- Blood
- Vertebrates
Evolution of Placental Mammals

Nishihara et al., 2009, PNAS
Bats in Trinidad and Tobago
Bat Models for Infectious Disease Research
Jamaican Fruit Bat Colony at Colorado State University

- Species: *Artibeus jamaicensis*
- Most common bats in the Americas
- Fruit bats
- Genome and transcriptomes available
- Currently producing antibody reagents
- Adults - 40-45 grams
- Two offspring per year
- Currently ~60 bats in the colony
- One of two bat colonies in USA for infectious disease research
- Experimental infections
  - MERS-CoV
  - Tacaribe virus
  - Zika virus
  - Bat H17 and H18 influenza viruses
Procedures
Middle East Respiratory Syndrome Coronavirus Appears to be a Bat Virus

Sequence from Egyptian tomb bat *(Taphozous perforatus)*

*Memish et al., 2013*
Experimental Infection

- 12 bats
- Animal Biosafety Level 3, Colorado State University
- Two doses per bat (10 infected, 2 uninfected)
  - $10^6$ TCID$_{50}$ IP
  - $2.5 \times 10^5$ TCID$_{50}$ IN
- Daily oral and rectal swabs for real-time PCR
- Euthanize two bats on days 2, 4, 7, 14 and 28
Temperatures

Two bats euthanized at each of these time points.

None of the bats exhibited signs of clinical disease.
Detection of MERS CoV RNA in Tissues

TaqMan PCR

No detectable vRNA in tissues from day 28 (bats 9 and 10)
Lung Histopathology/IHC
# Bat Infection Summary

<table>
<thead>
<tr>
<th></th>
<th>swabs (PCR)</th>
<th>tissue (PCR)</th>
<th>blood (PCR)</th>
<th>tissue (virus isolation)</th>
<th>histology (IHC)</th>
<th>Seroconversion (ELISA &amp; VN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoV/B1</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CoV/B2</td>
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<tr>
<td>CoV/B3</td>
<td>+</td>
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<tr>
<td>CoV/B4</td>
<td>+</td>
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<tr>
<td>CoV/B5</td>
<td>+</td>
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<tr>
<td>CoV/B6</td>
<td>+</td>
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<tr>
<td>CoV/B7</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>CoV/B8</td>
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<tr>
<td>CoV/B9</td>
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<tr>
<td>CoV/B10</td>
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<td>+</td>
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</table>
Acknowledgments

- Colorado State University
  - Richard Bowen, DVM, PhD
  - Sandy Quackenbush, PhD
  - Joel Rovnak, PhD
  - Ken Olson, PhD
  - Bill Black, PhD
  - Charles H. Calisher, PhD
  - Barry Beaty, PhD

- NIAID RML
  - Heinz Feldmann, MD
  - Vincent Munster, PhD
  - Joe Prescott, PhD

- University of New Mexico
  - Brian Hjelle, MD

- University of the West Indies, Trinidad and Tobago
  - Christine Carrington, PhD
  - Janine Seetahal, DVM
  - Vernie Ramkisson

- Pontifical Catholic University of Valparaiso, Chile
  - Fernando Torres-Perez, PhD
  - Mariana Acuna-Retamar, DVM

- Funding
  - NIH R15 AI054461
  - NIH R15 AI089419
  - NIH Contract AI25489
  - NIH R56 AI110535