Emerging Vector Borne Pathogens
Mammalian Reservoirs of Zoonotic Agents:
From the Field to the Lab
There are more than 4,600 species of mammals

About 1,800 of those are rodents (Rodentia)

About 1,200 of those are bats (Chiroptera)

65% of mammalian species
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?

Sin Nombre virus and the deer mouse (*Peromyscus maniculatus*)
## Sin Nombre Virus Infection: Human vs. Deer Mouse

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Human (HCPS)</th>
<th>Deer Mice (no disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus in lungs</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Infected cells</td>
<td>Capillary endothelial cells</td>
<td>Capillary endothelial cells</td>
</tr>
<tr>
<td>Endothelial damage</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cardiopulmonary disease</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pulmonary inflammation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pulmonary T cell infiltrates</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Pro-inflammatory</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>T cells</td>
<td>CD4$^+$ and CD8$^+$ CTL (PBL)</td>
<td>CD4$^+$ Treg</td>
</tr>
<tr>
<td>Neutralizing antibody</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mortality</td>
<td>36% (immunopathology)</td>
<td>None</td>
</tr>
<tr>
<td>Fate of virus</td>
<td>Clearance in survivors</td>
<td>Lifelong infection</td>
</tr>
</tbody>
</table>

And rats infected with Seoul hantavirus
Schountz et al., PNAS 2007
Easterbrook et al., PNAS 2007
Schountz et al., JVI 2012
New World Hantaviruses

Peromyscus maniculatus

Sin Nombre
Muleshoe
Isla Vista
El Moro Canyon
Choclo
Caño Delgado
Maporal
Oligoryzomys delicatus
Oligoryzomys longiseta
Oligoryzomys longicaudatus
Oligoryzomys flavescens

New York
Peromyscus leucopus
Prospect Hill
Microtus pennsylvanicus
Bloodland Lake
Microtus ochrogaster
Monongahela
Peromyscus maniculatus
Bayou
Oryzomys palustris
Black Creek Canal
Sigmodon hispidus
Rio Segundo
Reithrodontomys mexicanus
Laguna Negra
Calomys laucha
Araraquara
Bolomys lasiurus
Maciel
Necromys benefactus
Lechiguanas
Oligoryzomys flavescens

Andes
Oligoryzomys longicaudatus
Pergamino
Akodon azarae
Andes Virus Infects Deer Mice

But they appear to clear it without disease

Seroconversion

Viral RNA Copy Number (Taq-Man)

Spengler et al., PLOS One 2013

72 hour lymph node cultures from a deer mouse infected with ANDV 14 days before

Schountz et al., submitted
Experimental Infection

16 Deer Mice
5 infected with SNV < Reservoir
8 infected with ANDV < Nonreservoir
3 uninfected controls

Harvest lymph nodes
14 days post infection

72 hour lymph node cultures from a deer mouse infected with ANDV 14 days before

94 Gene PCR Immunoarray (GAPDH normalized ΔΔCt)

No Antigen
10 ug/ml ANDV N

BSL-4, Rocky Mountain Laboratories Hamilton, MT, USA
Antibody Titers to Nucleocapsid
ELISA day 14

SNV: 0/5 (<100)
ANDV: 7/8 (titers of 100 or 200)
Uninfected: 0/3 (<100)
Th2/B Cell Gene Expression
94-gene real-time PCR array

Fold Increased Expression

* 95% CI

Class Switching/
Affinity Maturation

SNV ANDV
Conclusions

• Deer mice infected with ANDV appear healthy
  • No behavioral or hematological differences
• Most of the 94 immune genes on the array were not modulated
• Of expressed genes, most were at similar levels
• However, Th2 expression levels higher in ANDV-infected deer mice
  • Class switching and affinity maturation
Hantaviruses and Their Rodent Reservoirs

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

Subtle immune response?

Why are they unable to clear the virus?

Failure to seroconvert quickly?
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
• BSL-4: Animal infections with viruses that cause HCPS

Can transition live cells to BSL-2

Cannot transition live cells to BSL-3 or -2
Maporal Virus

• Hantavirus isolated from fulvous pygmy rice rat (*Oligoryzomys fulvescens*) in western Venezuela

• Principal reservoir host is delicate pygmy rice rat (*Oligoryzomys delicatus*)

• No known cases of human disease

• Causes an HCPS-like disease in Syrian golden hamsters (*Mesocricetus auratus*)

• Animal BSL-3
  
  Deer mouse susceptibility?
Two species of Caribbean fruit bat: *Artibeus jamaicensis* and *Carollia perspicillata*. 
Field Work with Reservoirs
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