Biosafety from the Field to the Lab

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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

Venezuelan Hemorrhagic Fever

Guanarito virus

Cane mouse

Lassa Fever

Lassa virus

Multimammate mouse

Hantavirus Disease

> dozen viruses

Various rodent species

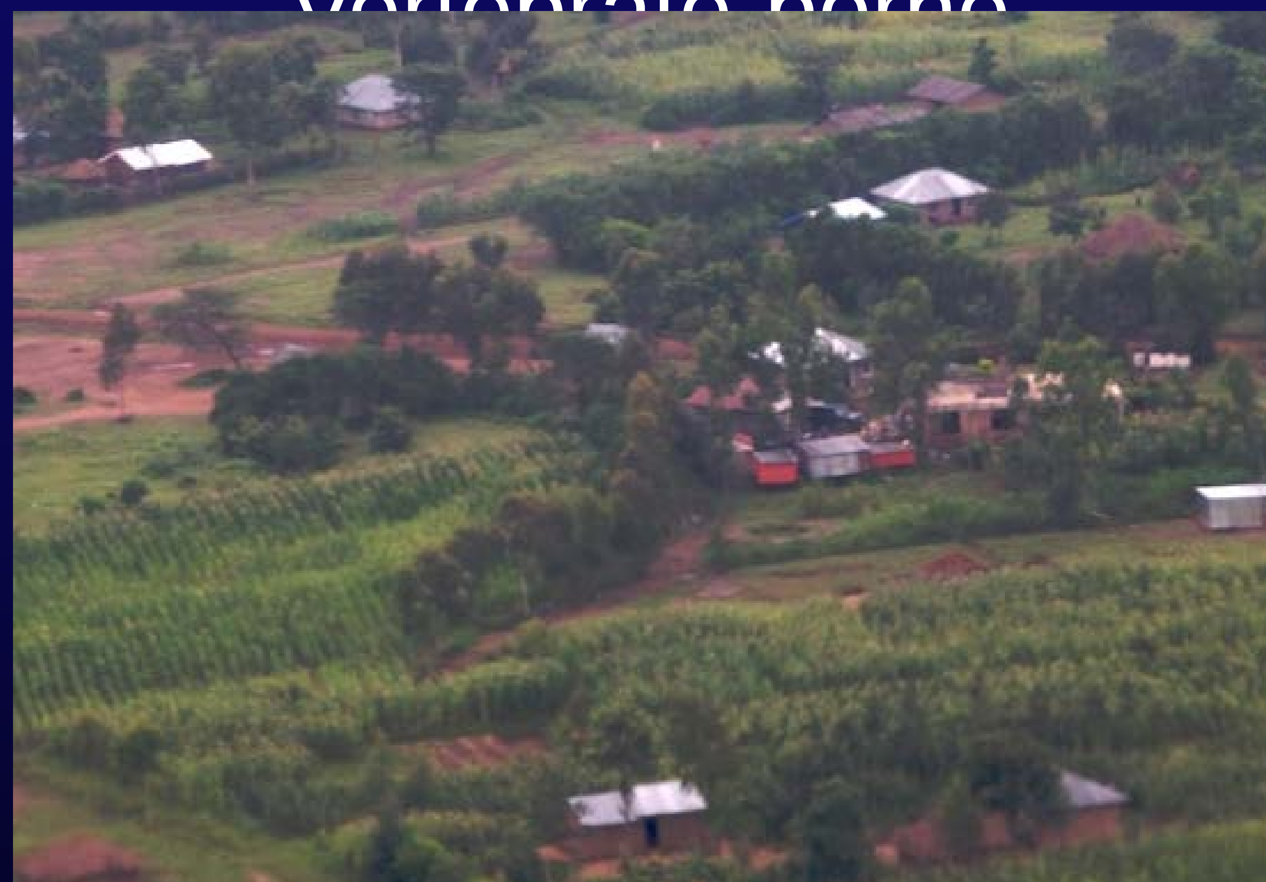
Bolivian Hemorrhagic Fever

Machupo virus

Vesper mouse

Chapare virus

Unknown - presumably rodent



Nipah Encephalitis

Nipah virus

Pteropus spp. flying foxes (bats)

Hendra Encephalitis

Hendra virus

Pteropus spp. flying foxes (bats)

Brazilian Hemorrhagic Fever

Sabia virus

Unknown - presumably rodent

SARS/MERS

Coronaviruses

Chinese horseshoe bat
Egyptian tomb bat?

Monkeypox

Monkeypox virus

squirrels, Gambian rats

Hepatitis E

Hepatitis E virus

swine, wild deer, and boars

Argentine Hemorrhagic Fever

Junin virus

Grass field mouse
Dark field mouse
Corn mouse

Rabies

Rabies virus

Bats of many species

Avian Influenza

Avian influenza viruses
waterfowl

Vertebrate Reservoirs of Zoonotic Agents

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

Reservoir hosts rarely suffer disease from the viruses

If you want to understand the biology and ecology of an infectious, you have to study it in the context of its reservoir host

Model Systems for the Study of Zoonotic Viruses

Mode … zoonosis research

Animal model, c…
(e.g., laboratory …

Limited

Animal model, re…
(natural reservoir …

…imited to few species

Species-specific reagents
rarely available

Cell cultu…
Vero E…
HUVECs…

…odel, but
…complex
…en-host

Cell cul…

necessary

Species-specific reagents
rarely available

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

Andrew G. D. Bean[1], Michelle L. Baker[1], Cameron R. Stewart[1], Christopher Cowled[1], Celine Deffrasnes[1], Lin-Fa Wang[1,2] and John W. Lowenthal[1,3]

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

Cell

Leading Edge
Review

Reservoir Host Immune Responses to Emerging Zoonotic Viruses

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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

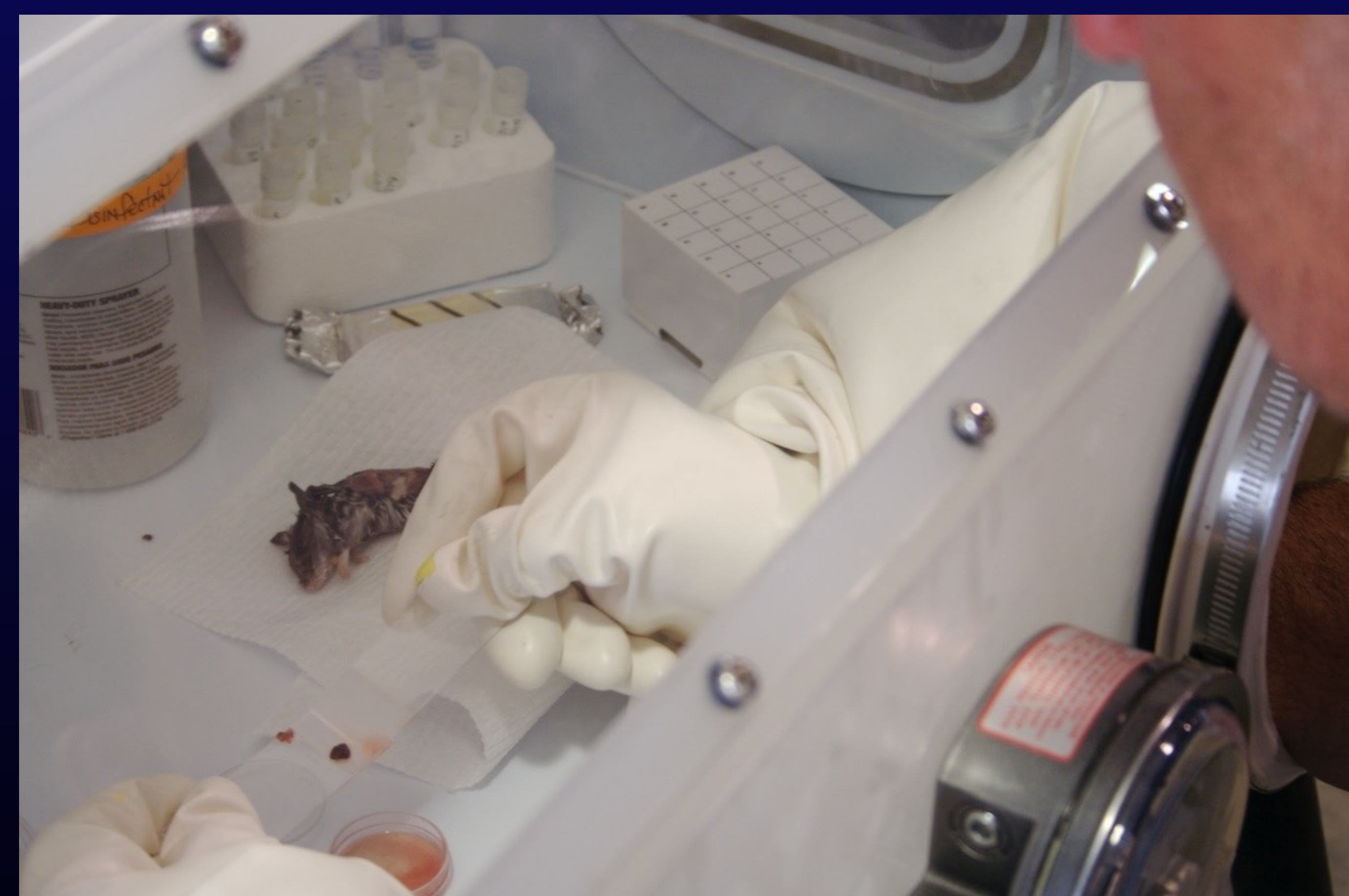
- Work in remote and/or undeveloped area
  often in difficult surroundings
- Cultural barriers
- Electricity is often unavailable
- Laboratory facilities are often unavailable
- Must work with personal protective equipment
- Equipment must be transported to destination
- Animal reservoirs are not model 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 -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 facilit
  - 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

Schmaljohn and Hjelle, EID, 1997

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
- In rodent reservoirs a regulatory T cell response occurs
  (Easterbrook et al., *PNAS*, 2007; Schountz et al., *PNAS*, 2007)

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

New World Hantaviruses

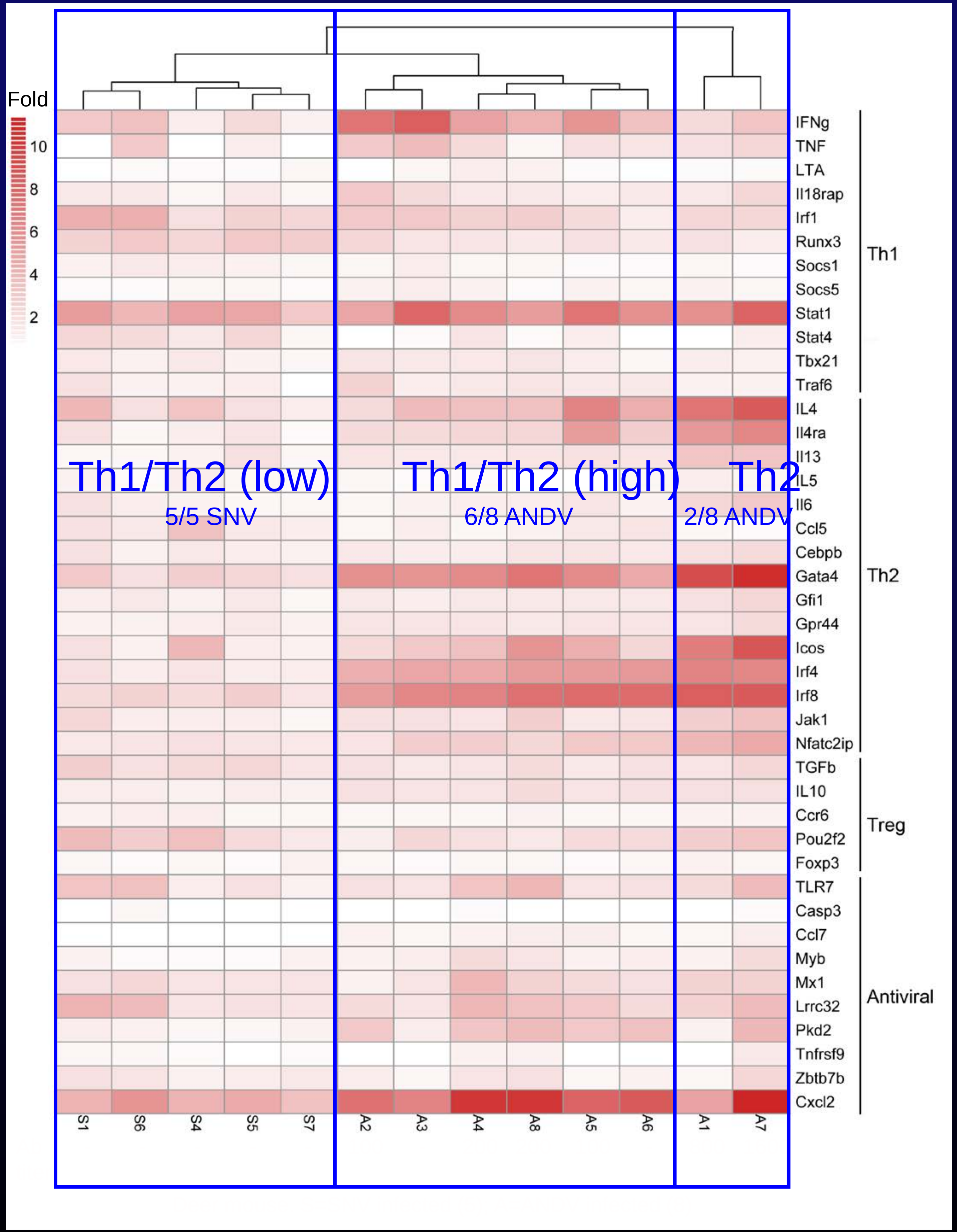
Deer mice are susceptible to Andes virus, but clear infection

(Spengler et al., PLoS One, 2013)

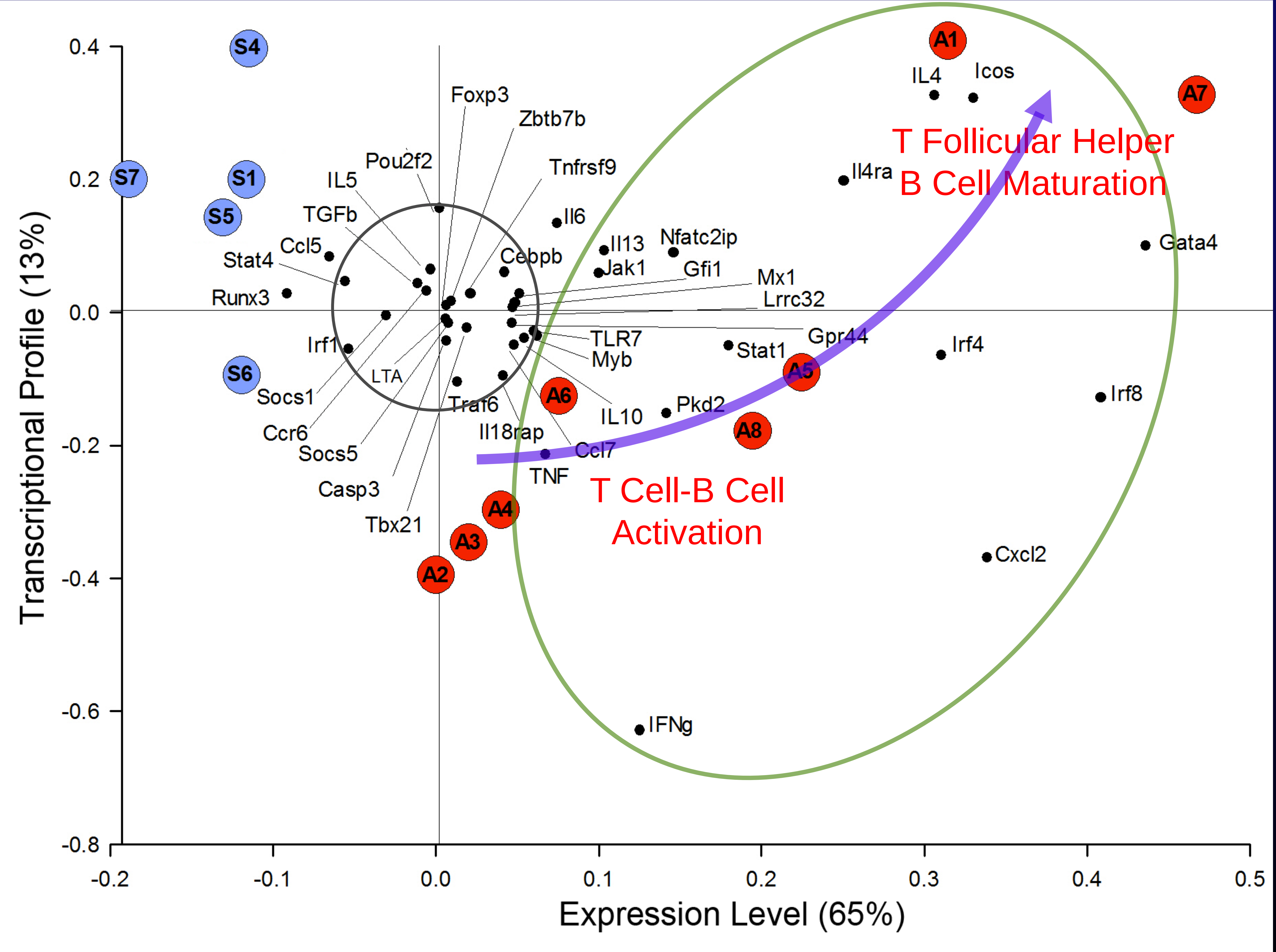
How do deer mouse immune responses differ to SNV and ANDV?

(ABSL-4, RML)

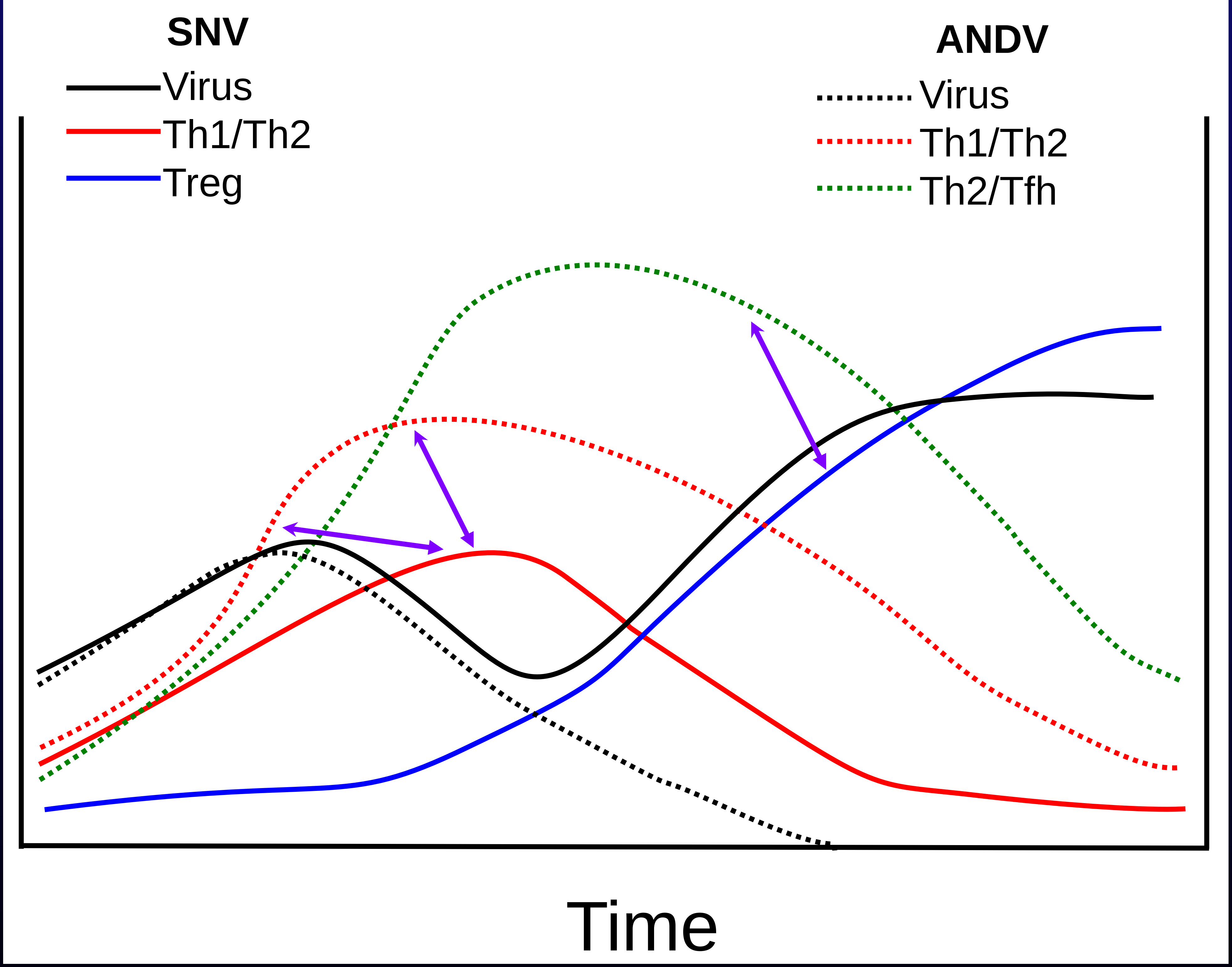
Gene Expression Cluster Analysis



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



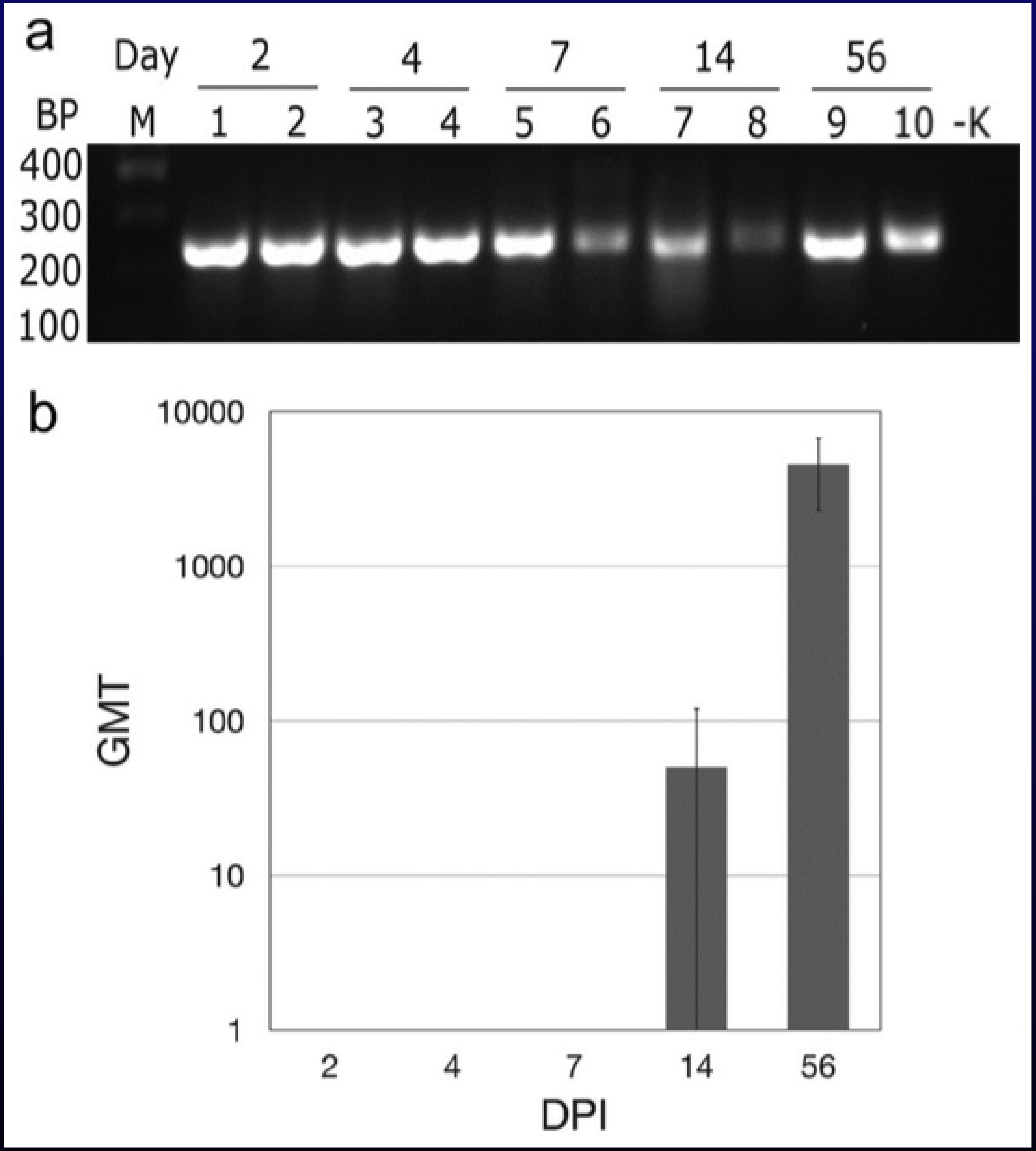
Model for Hantavirus Infection of Reservoirs



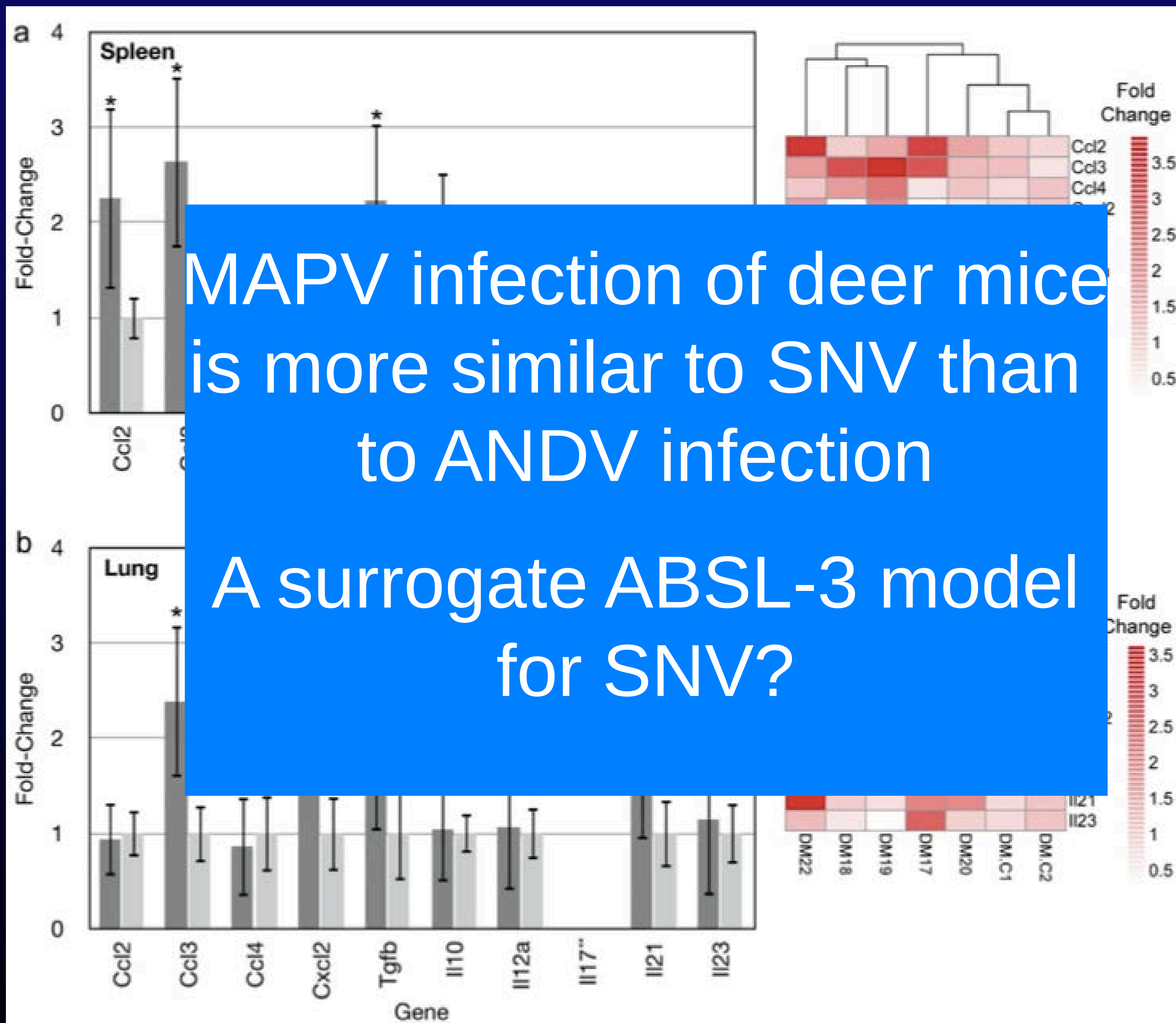
Maporal Hantavirus

- 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

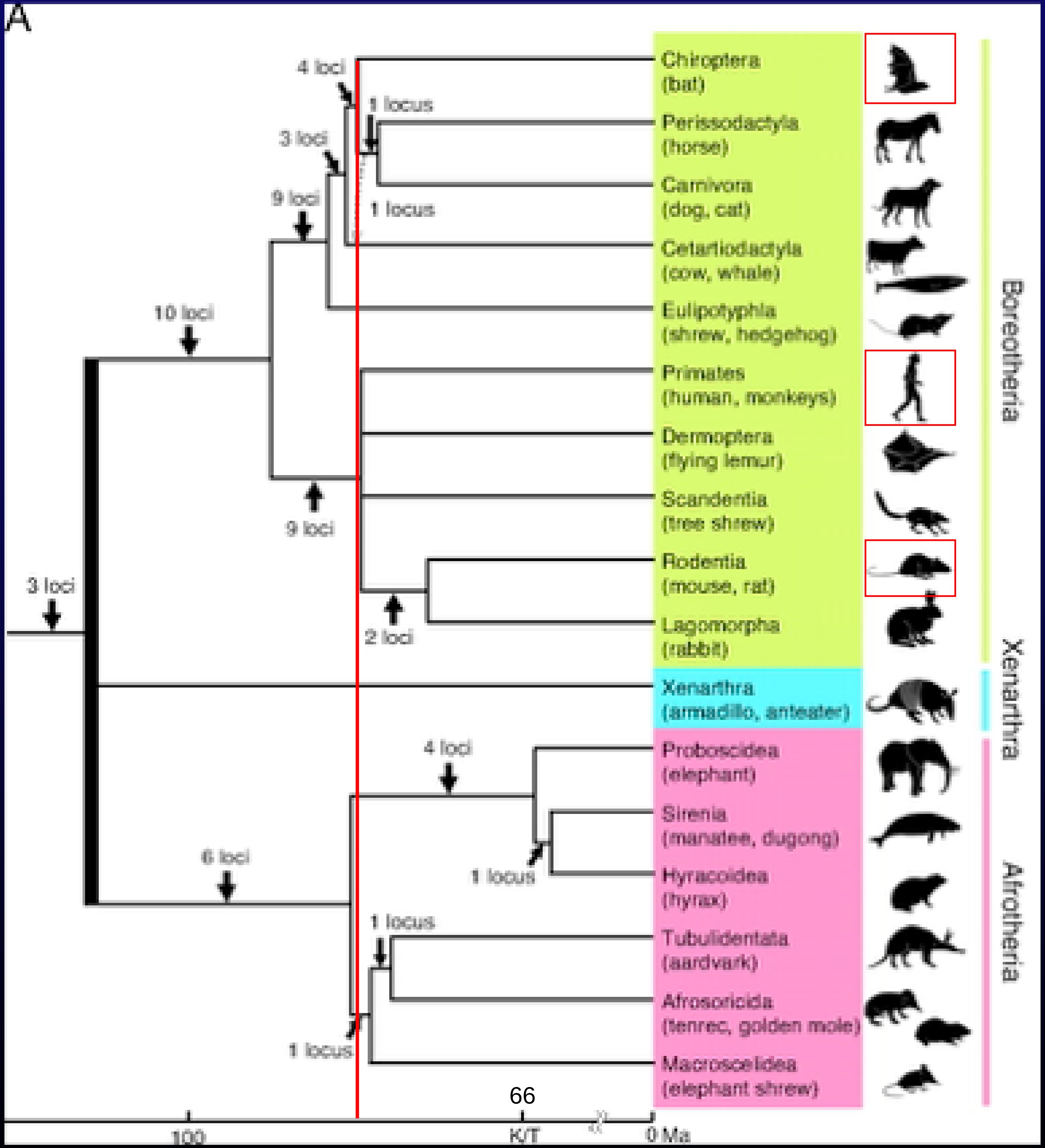
- Rabies virus and other lyssaviruses
- Parmyxoviruses
- Henipaviruses
- Sosuga virus?
- Filoviruses
- Coronaviruses

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

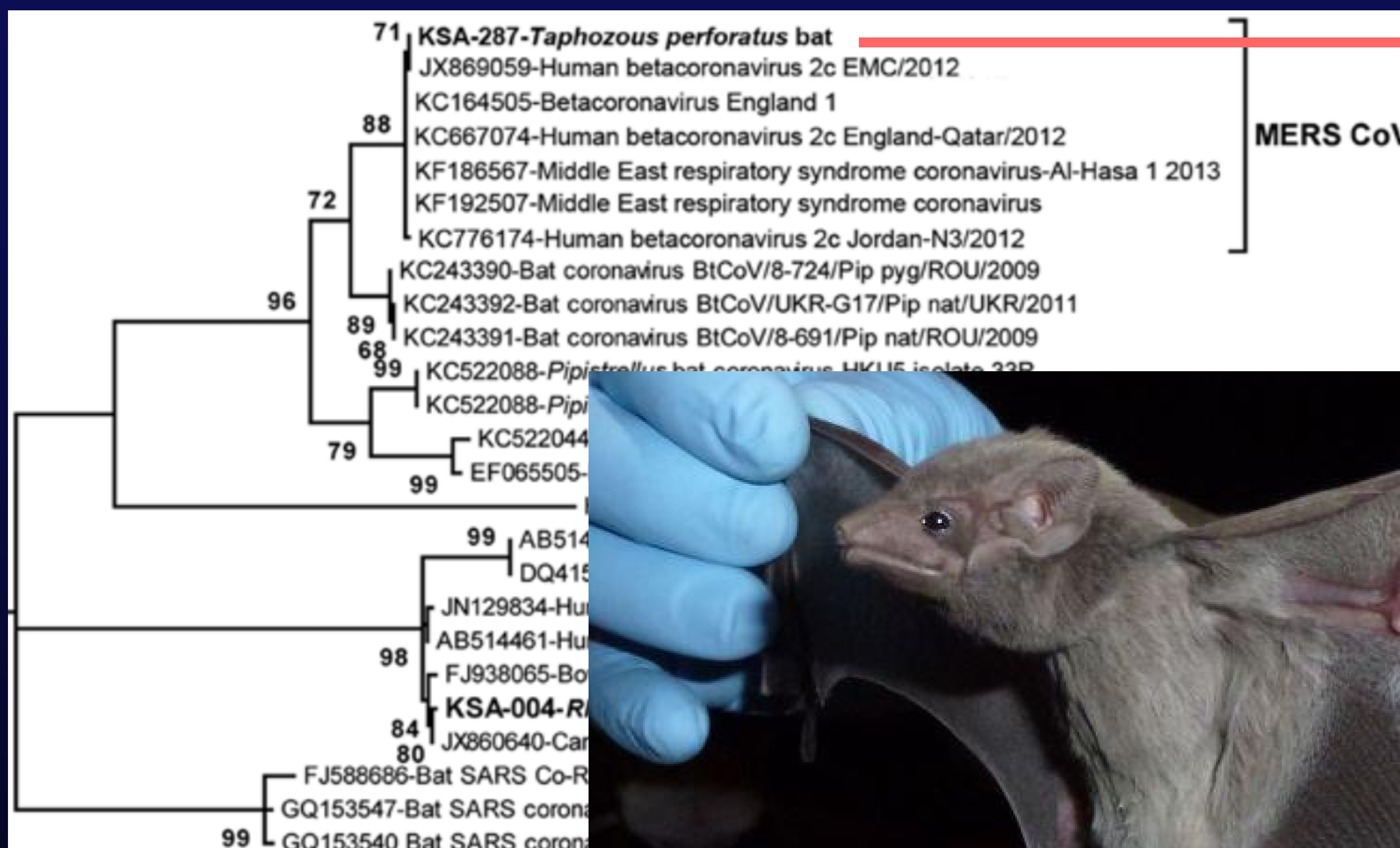
Bat Models for Infectious Disease Research

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



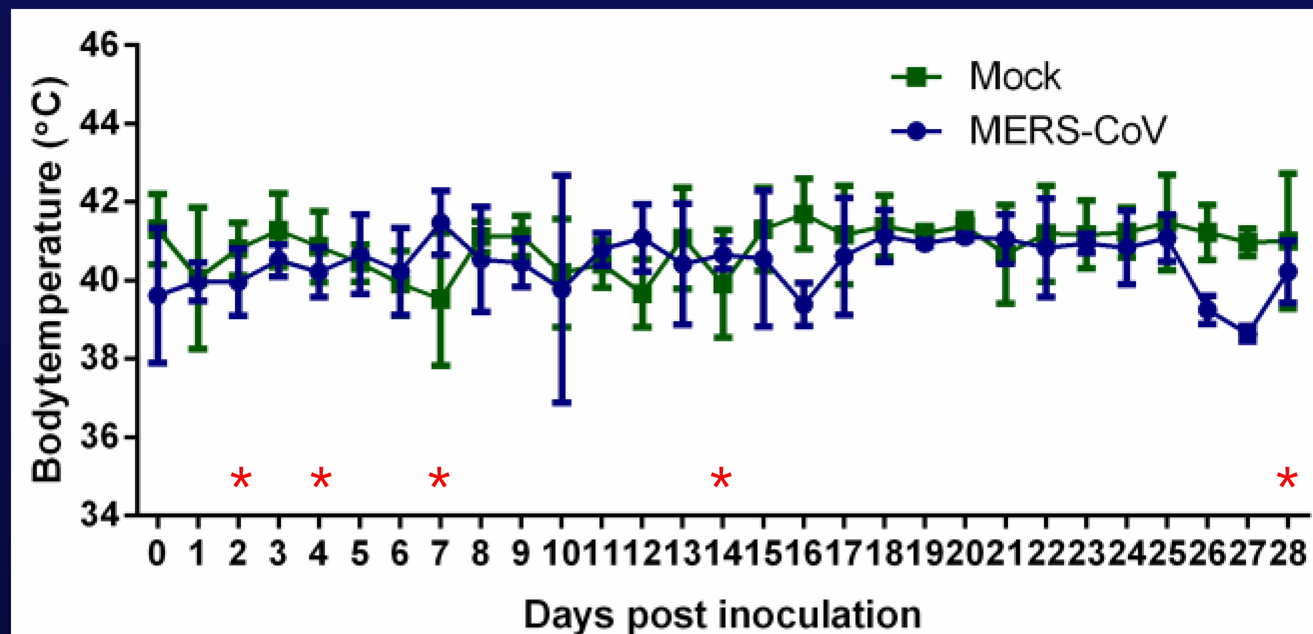
Sequence from Egyptian tomb bat (*Taphozous perforatus)*

Memish et al., 2013

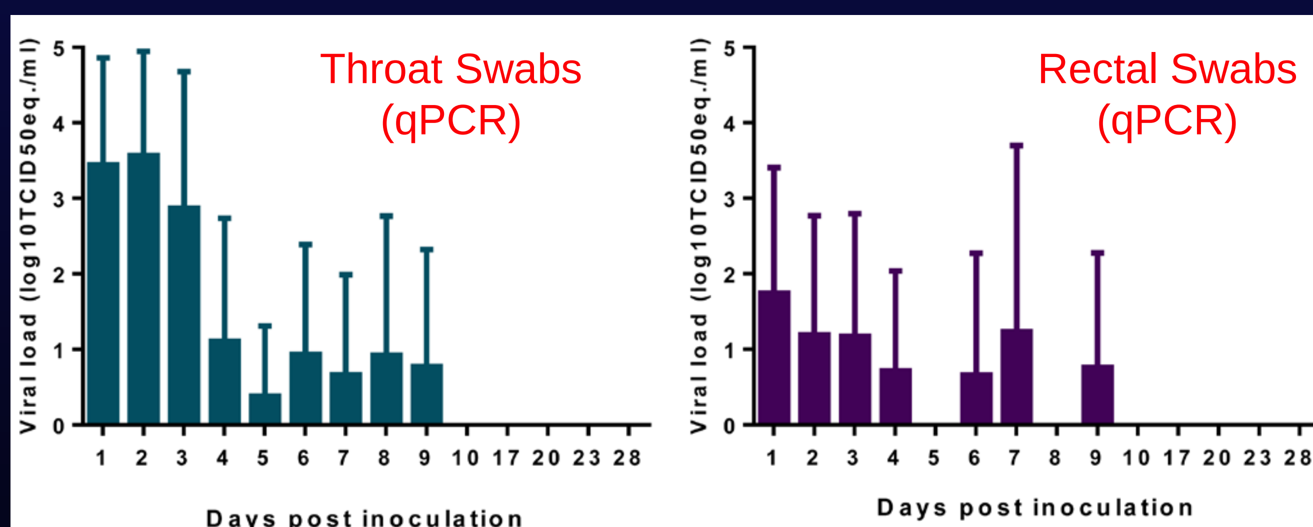
Experimental Infection

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

Temperatures



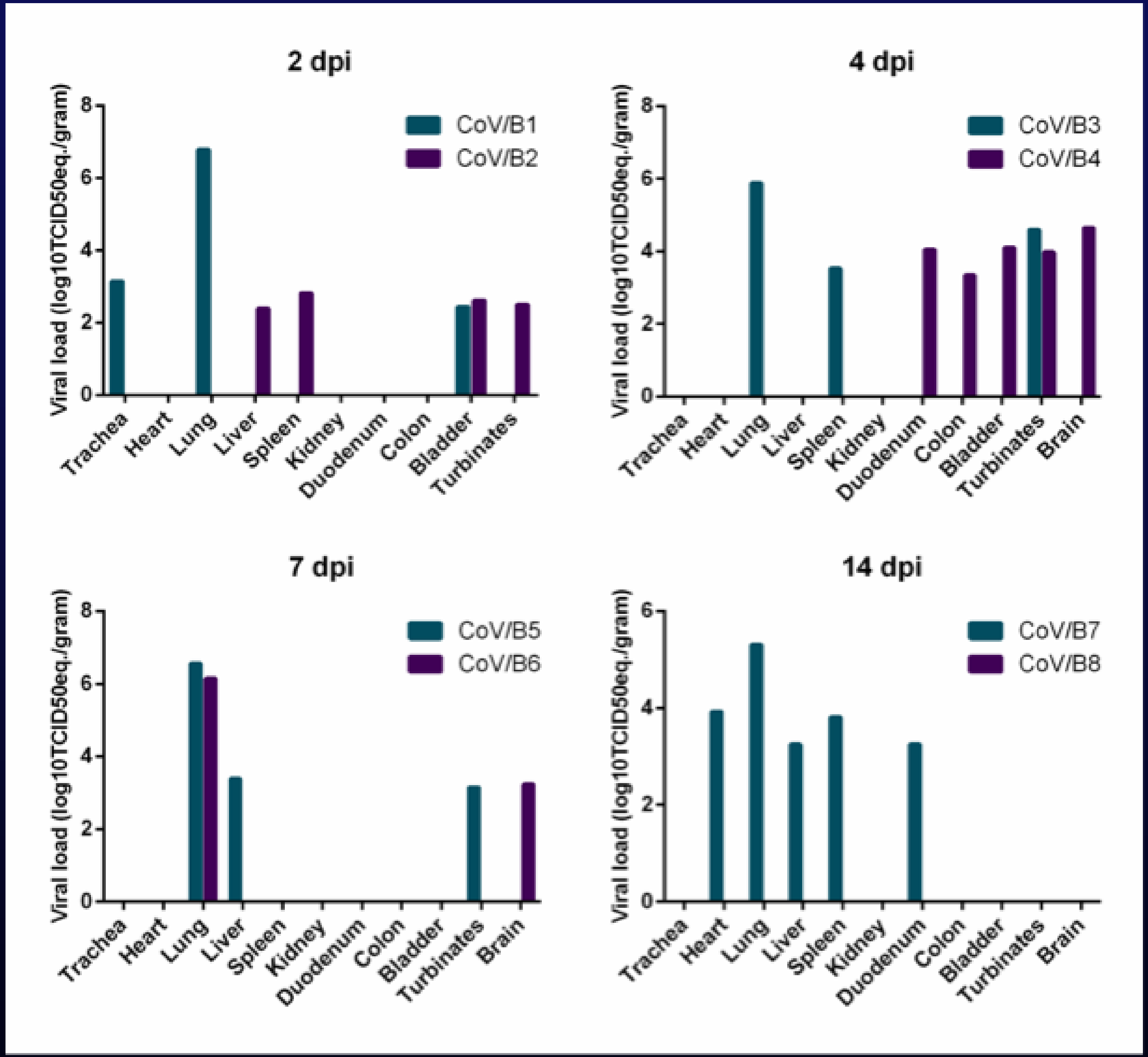
Two bats euthanized at each of these time points



None of the bats exhibited signs of clinical disease

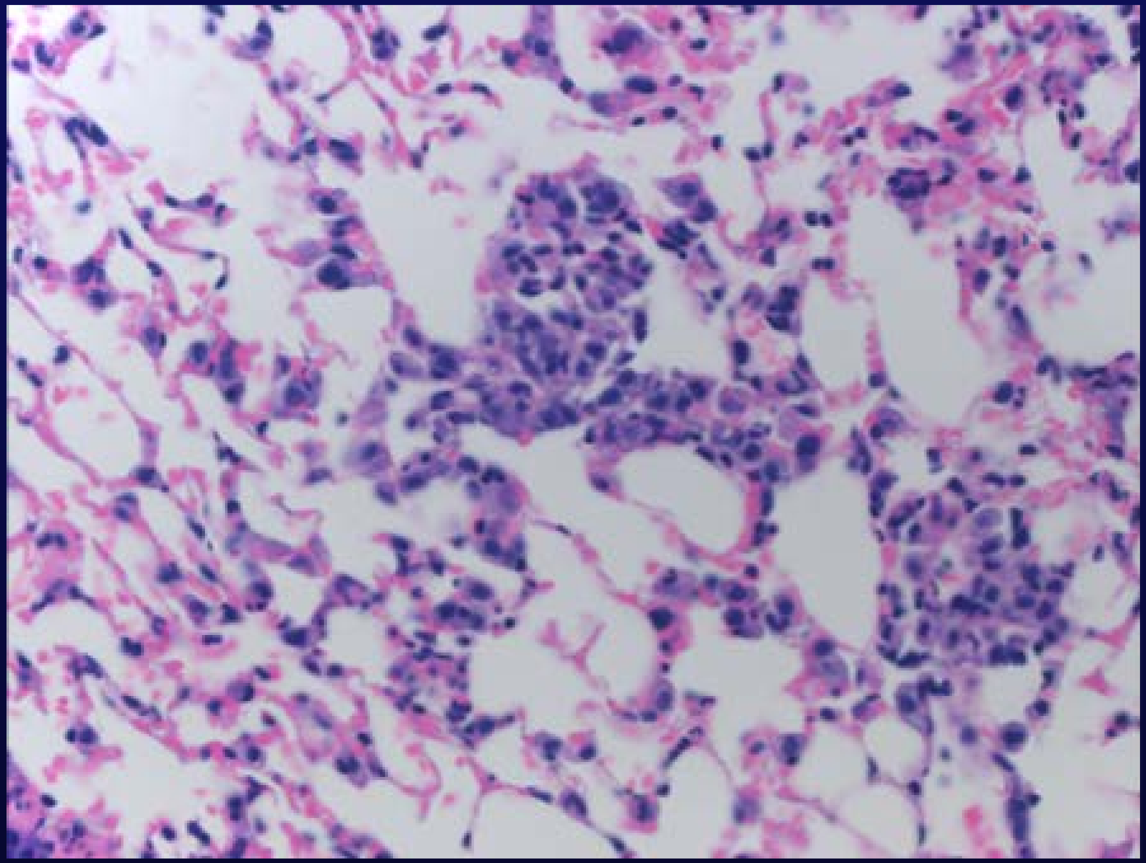
Detection of MERS CoV RNA in Tissues

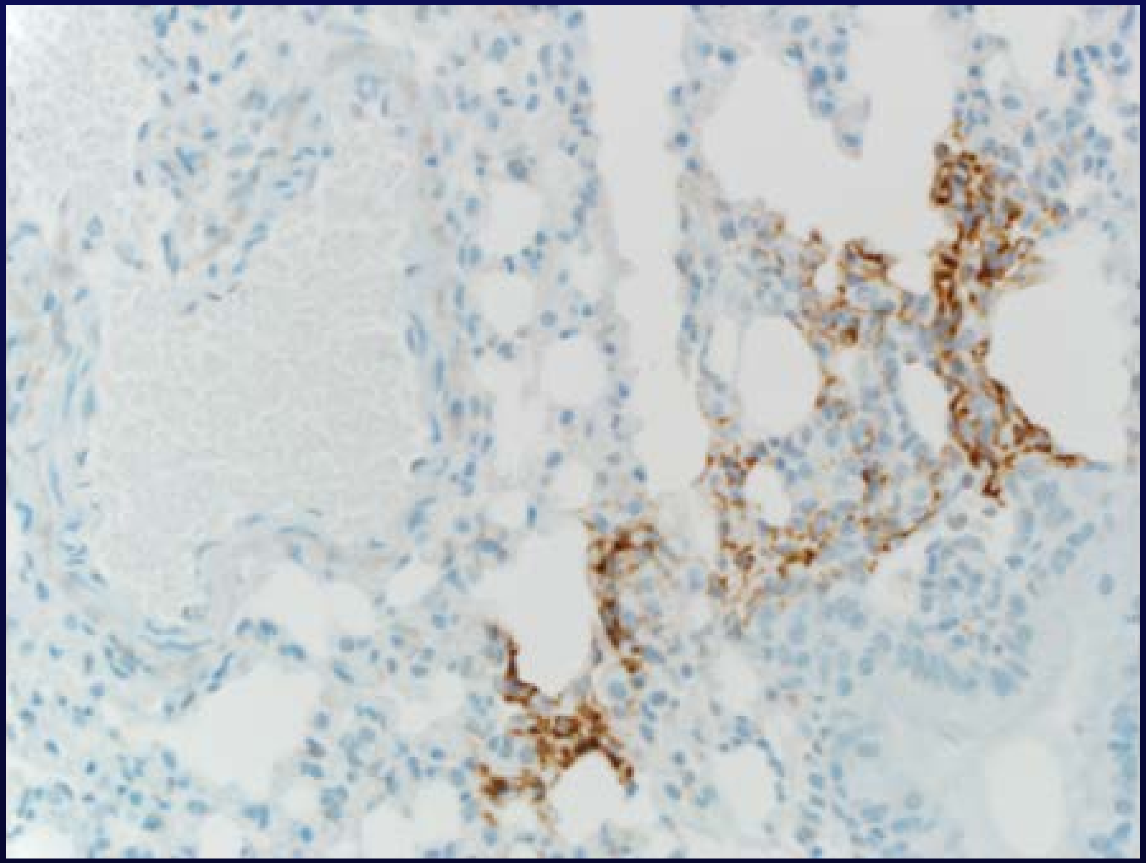
TaqMan PCR



No detectable vRNA in tissues from day 28 (bats 9 and 10)

Lung Histopathology/IHC





Bat Infection Summary

	Evidence for MERS-CoV replication in Jamaican fruit bats					
	swabs (PCR)	tissue (PCR)	blood (PCR)	tissue (virus isolation)	histology (IHC)	Seroconversion (ELISA & VN)
CoV/B1	+	+	+	+	+	
CoV/B2	+	+	+			
CoV/B3	+	+	+		+	
CoV/B4	+	+				
CoV/B5	+	+			+	
CoV/B6	+	+		+		
CoV/B7	+	+		+		+
CoV/B8	+					
CoV/B9	+					
CoV/B10				+		

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