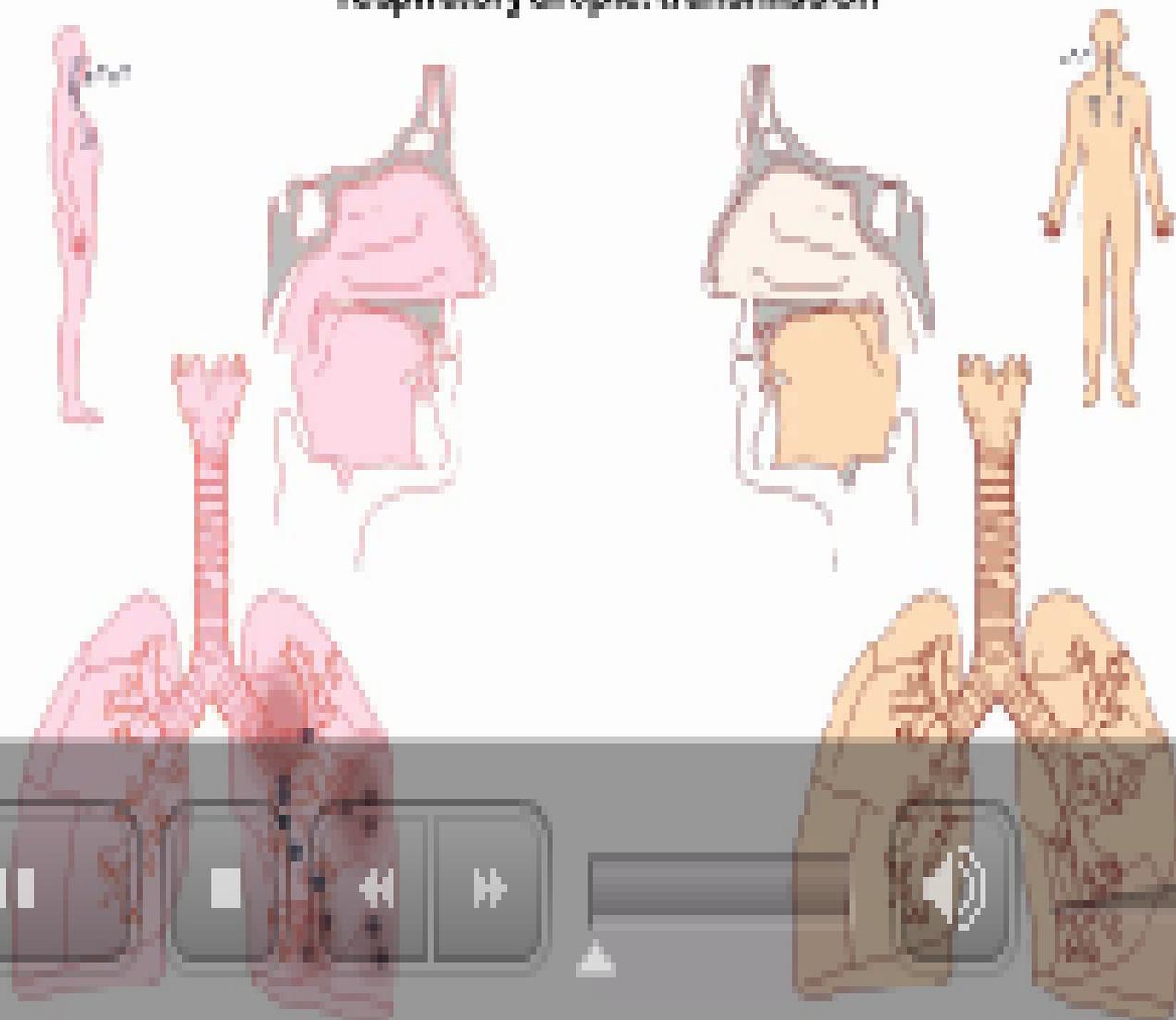
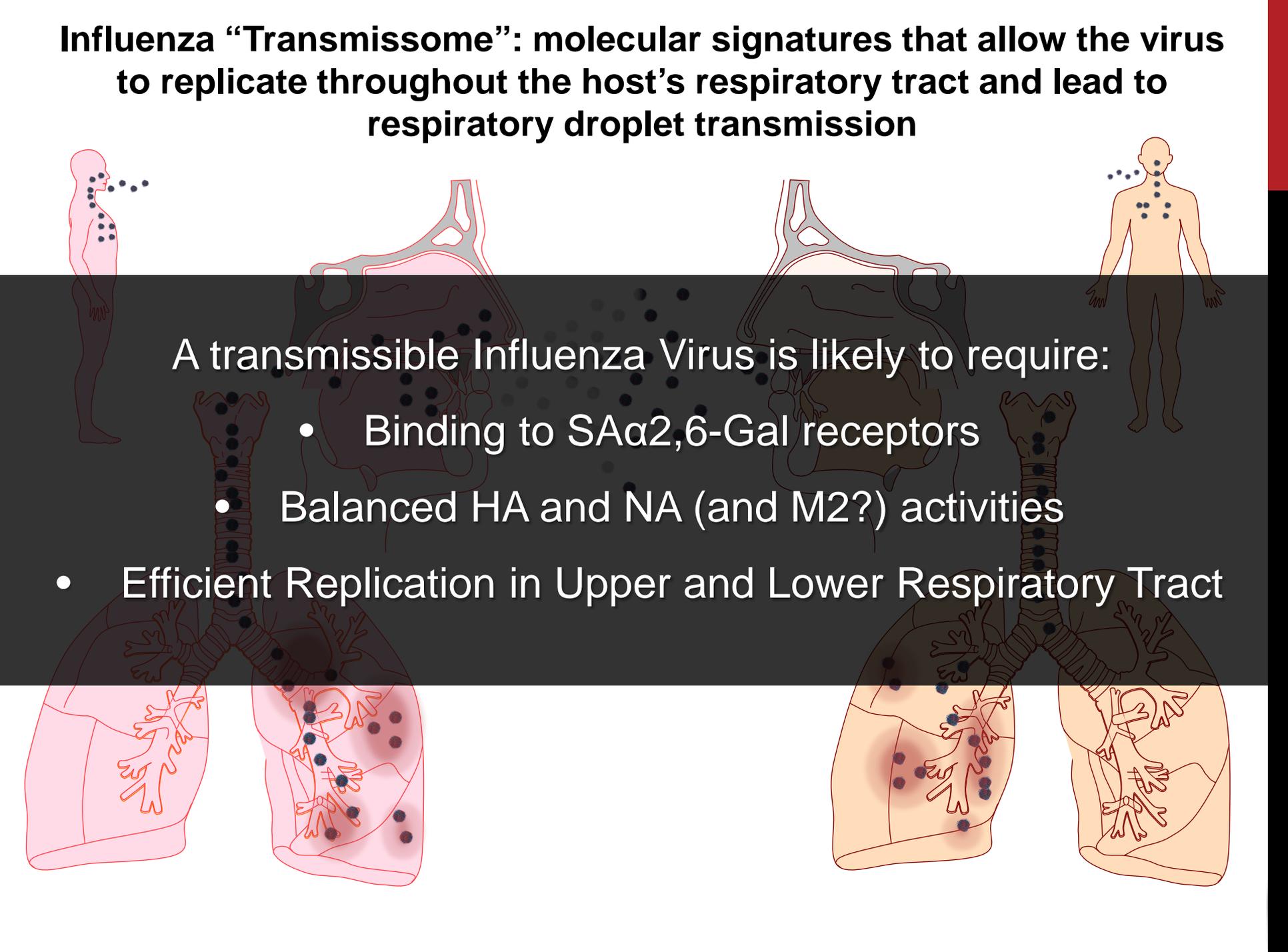


Influenza "Tiger disease": molecular signatures that allow the virus to replicate throughout the host's respiratory tract and lead to respiratory droplet transmission



Influenza “Transmissible”:

molecular signatures that allow the virus to replicate throughout the host’s respiratory tract and lead to respiratory droplet transmission



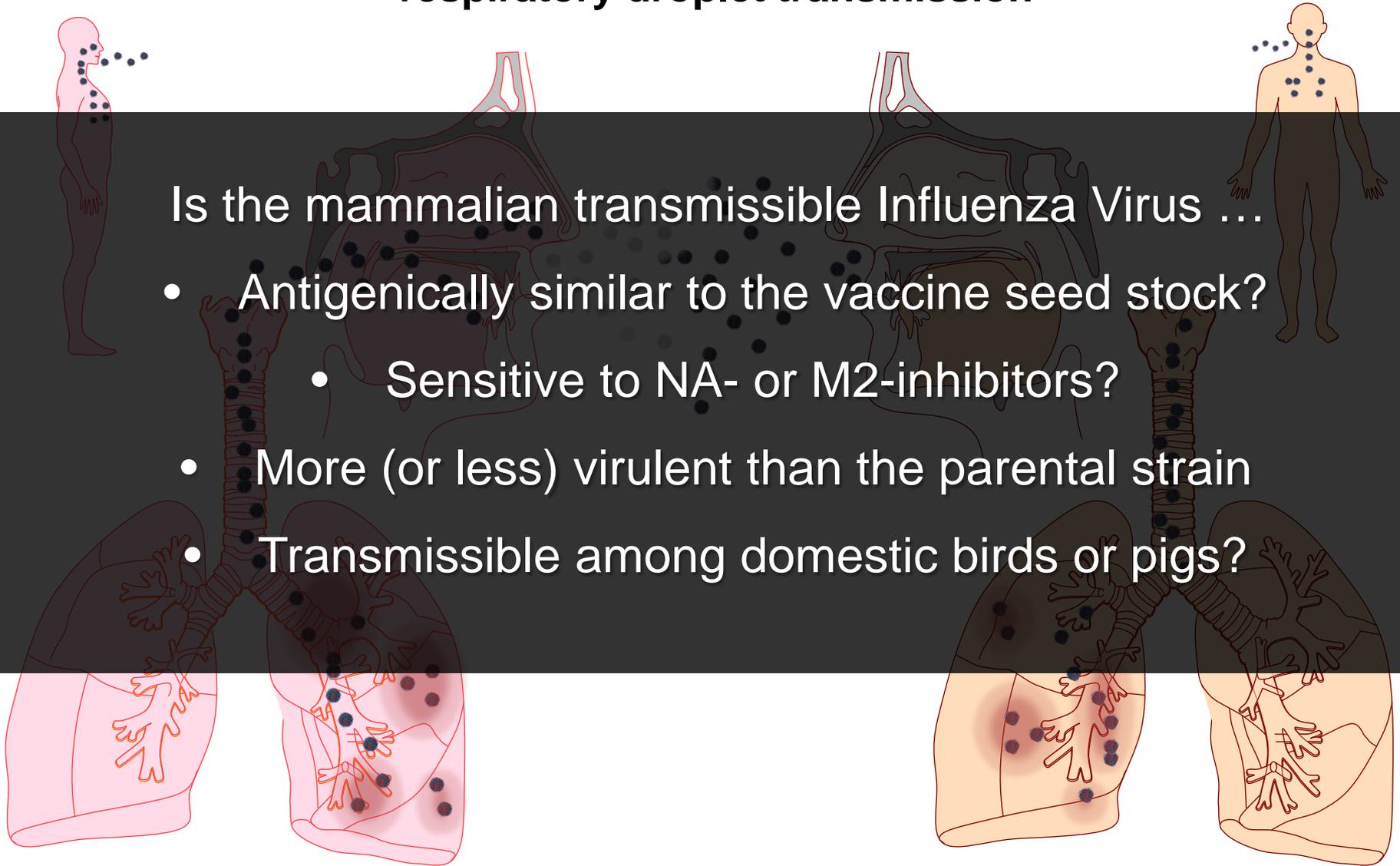
A transmissible Influenza Virus is likely to require:

- Binding to SA α 2,6-Gal receptors
- Balanced HA and NA (and M2?) activities
- Efficient Replication in Upper and Lower Respiratory Tract

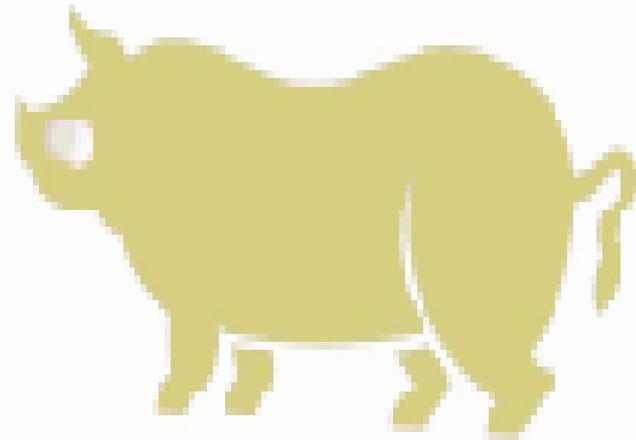
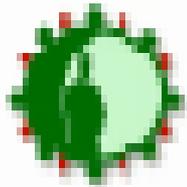
Influenza “Transmissible”: molecular signatures that allow the virus to replicate throughout the host’s respiratory tract and lead to respiratory droplet transmission

Is the mammalian transmissible Influenza Virus ...

- Antigenically similar to the vaccine seed stock?
 - Sensitive to NA- or M2-inhibitors?
- More (or less) virulent than the parental strain
- Transmissible among domestic birds or pigs?

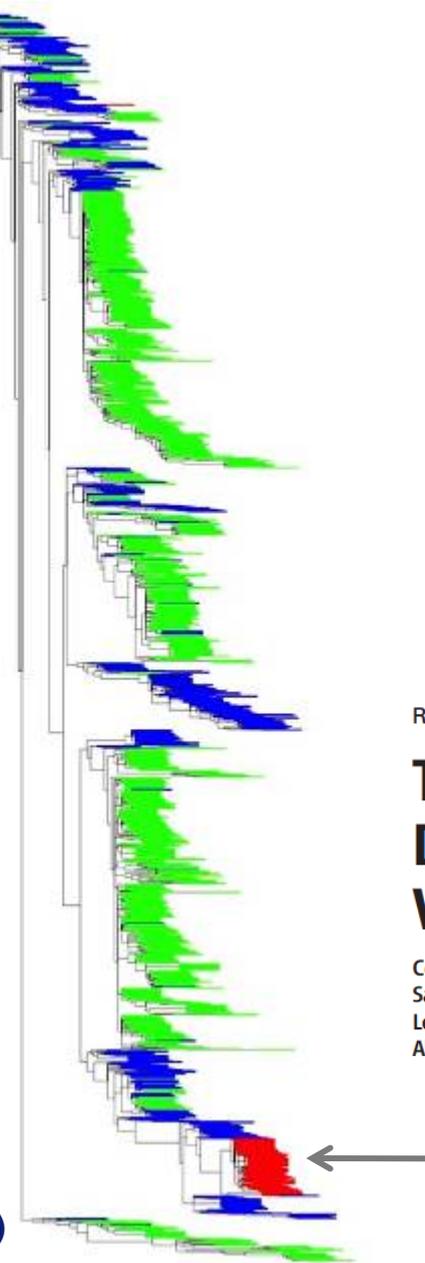


Reassortment is how Flu has SEX!



Asia - avian

Herfst *et al* set
Number of
nucleotide mutations
in HA necessary for
aerosol transmission

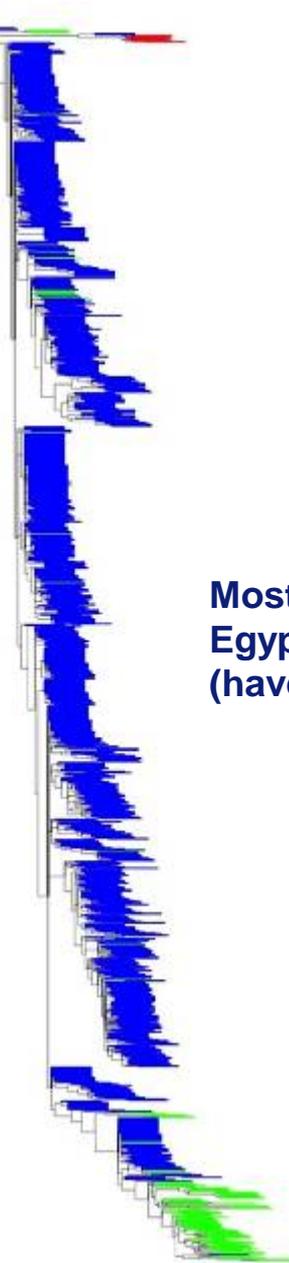


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Mostly from
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(have E627K)

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auwen,⁷
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mith^{1,2,3,7*}



Russell et al., Science (2012)

Asia - avian

Africa and Middle East - avian

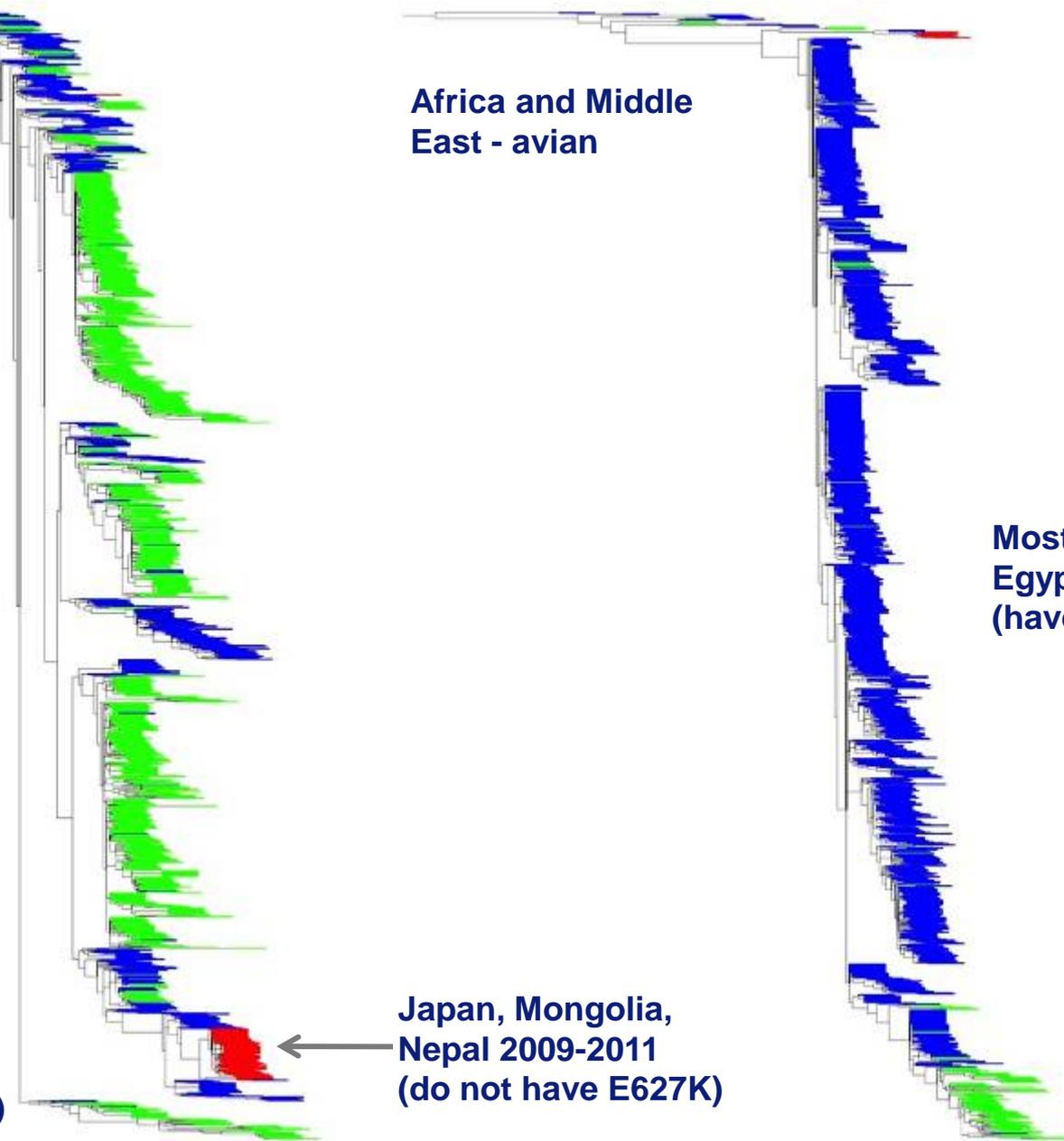
Herfst *et al* set
Number of nucleotide mutations in HA necessary for aerosol transmission

3 mutations
4 mutations
5 mutations

Mostly from Egypt (have E627K)

Japan, Mongolia, Nepal 2009-2011 (do not have E627K)

Russell *et al.*, Science (2012)



Position T189A: RBS and antigenic profile

2



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Proc Natl Acad Sci U S A. 2009 May 5; 106(18): 7565–7570. PMID: PMC2670882
Published online 2009 April 20. doi: [10.1073/pnas.0900877106](https://doi.org/10.1073/pnas.0900877106).

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Microbiology

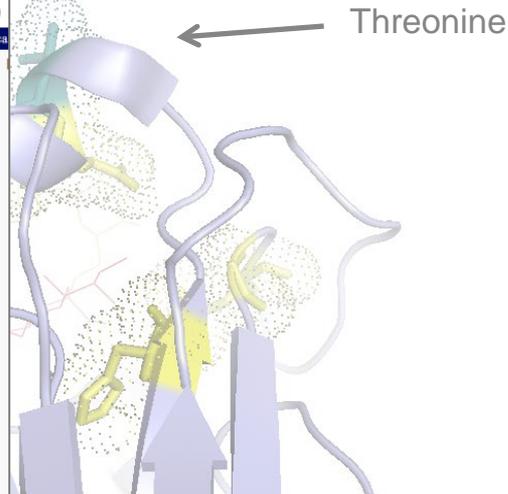
Minimal molecular constraints for respiratory droplet transmission of an avian–human H9N2 influenza A virus

Erin M. Sorrell,¹ Hongquan Wan,^{1,2} Yonas Araya, Haichen Song,³ and Daniel R. Perez⁴

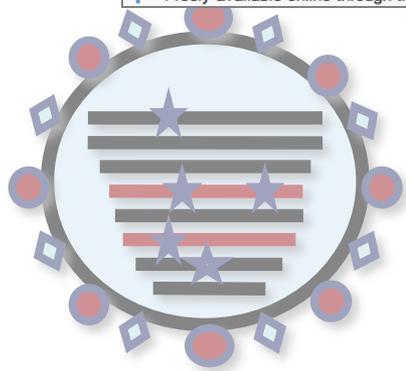
Department of Veterinary Medicine, University of Maryland, College Park, and Virginia–Maryland Regional College of Veterinary Medicine, 8075 Greenmead Drive, College Park, MD 20742
⁴To whom correspondence should be addressed. E-mail: dperez1@umd.edu
Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved March 17, 2009
Contributed by
Author contributions: D.R.P. designed research; E.M.S., H.W., Y.A., and H.S. performed research; E.M.S., H.W., H.S., and D.R.P. analyzed data; and E.M.S., H.W., and D.R.P. wrote the paper.
¹E.M.S. and H.W. contributed equally to this work.
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³Present address: Synbiotics Corporation, 8075 Greenmead Drive, College Park, MD 20742.

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P10



Alanine



PNAS

Compatibility of H9N2 avian influenza surface genes and 2009 pandemic H1N1 internal genes for transmission in the ferret model

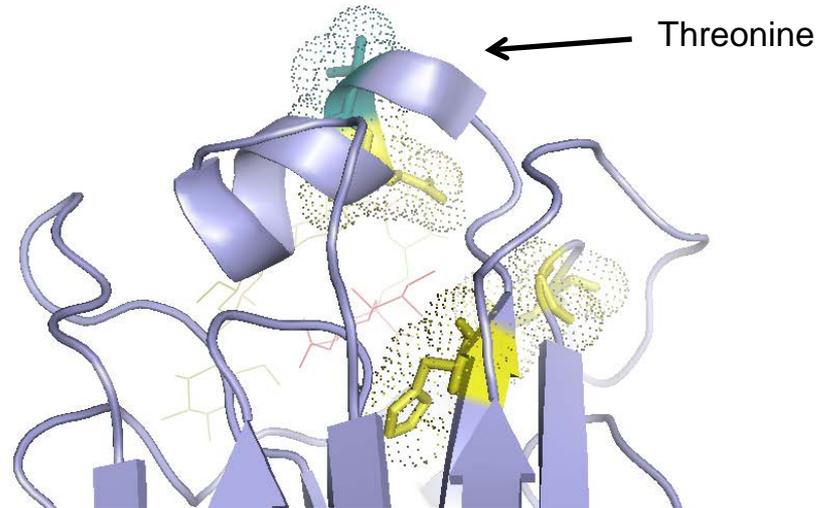
J. Brian Kimble^a, Erin Sorrell^a, Hongxia Shao^a, Philip L. Martin^b, and Daniel Roberto Perez^{a,1}

^aDepartment of Veterinary Medicine, University of Maryland, College Park and Virginia–Maryland Regional College of Veterinary Medicine, College Park, MD 20742; and ^bCenter for Advanced Preclinical Research, Science Applications International Corporation/National Cancer Institute, Frederick, MD 21702
Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved June 10, 2011 (received for review May 19, 2011)

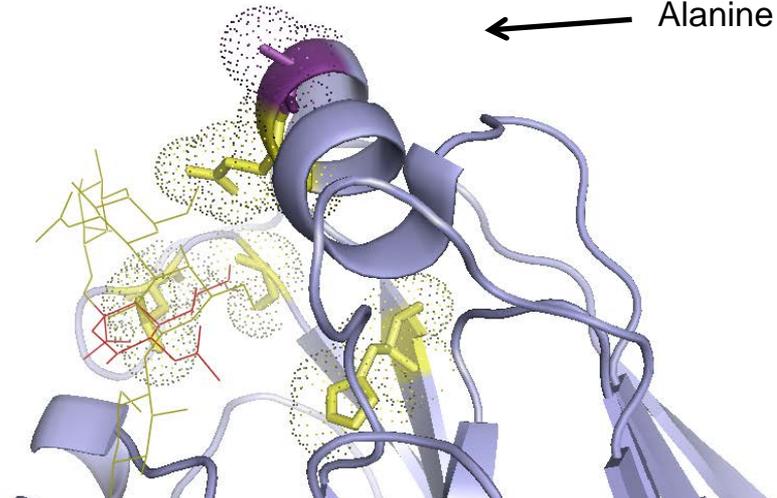
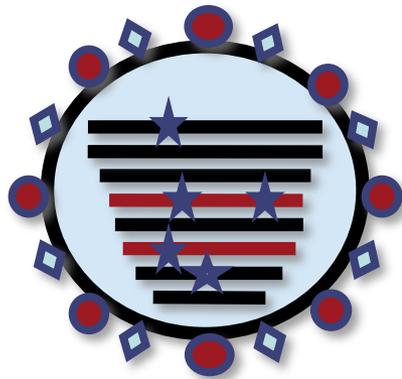


Position T189A: RBS and antigenic profile

2WF10:6M98

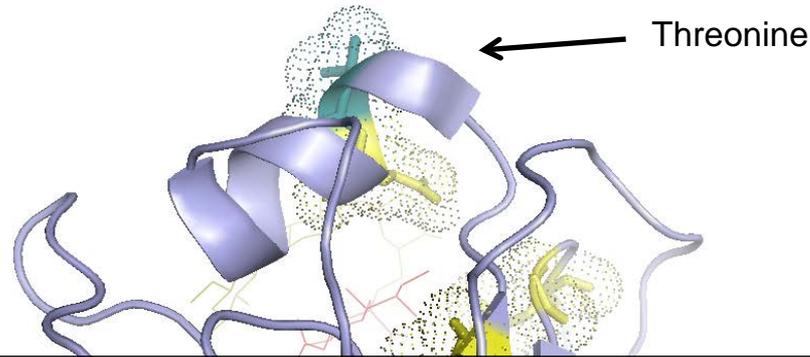


P10-2WF10:6M98



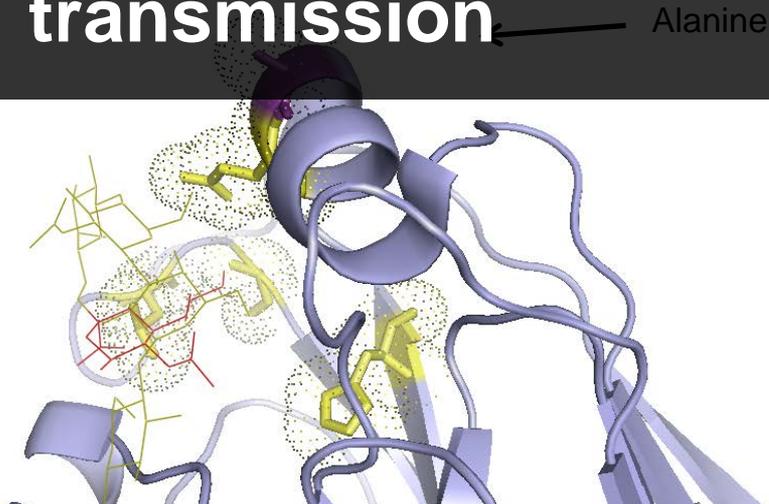
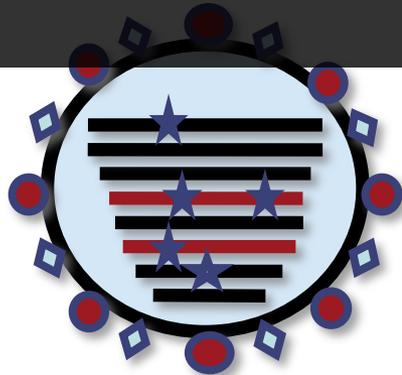
Position T189A: RBS and antigenic profile

2WF10:6M98

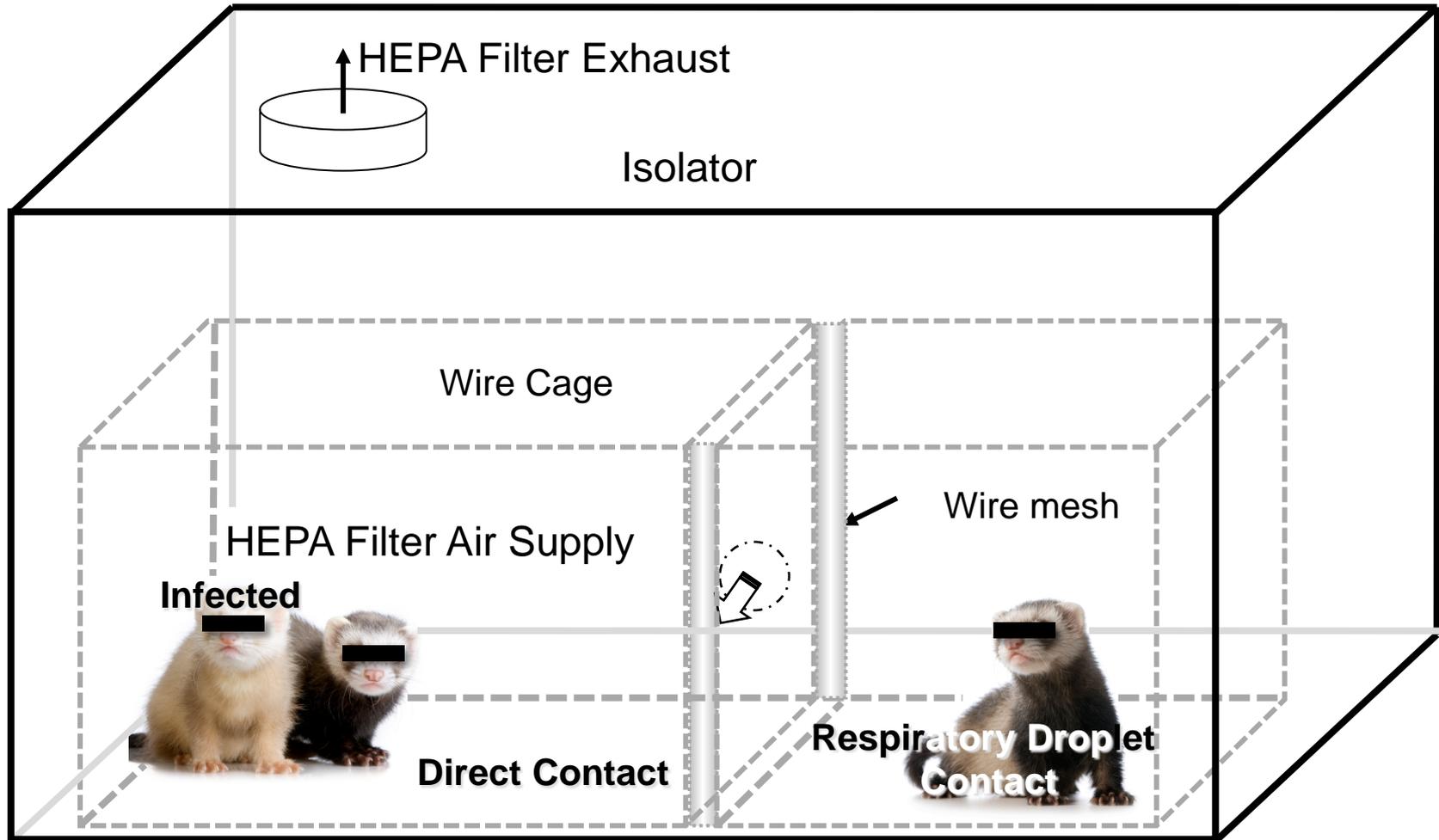


An amino acid change on the tip of the HA protein, near the RBS, is crucial for respiratory transmission

P10-2WF10:6M98



Ferrets recapitulate human infection with Influenza A viruses



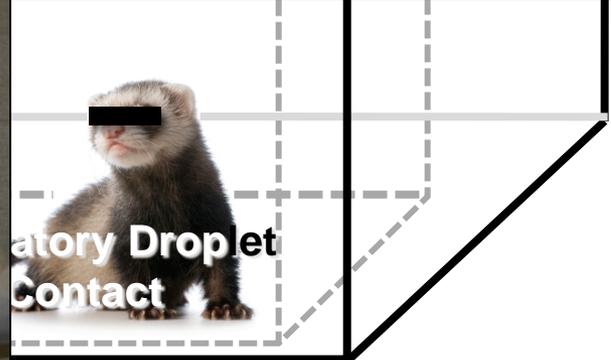
Ferrets recapitulate human infection with Influenza A viruses



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Laboratory Droplet
Contact



Biosecurity BSL3 enhanced conditions:

- Multiple security check points
 - Access to BSL2+ area
 - Access to BSL3 enhanced ante-room
 - Access to BSL3 enhanced shower area
 - Access to Lab/Animal Rooms
- Video monitoring
- Double lock freezer with combination of key and combination locks
- Virus stocks accessible to two individuals (PI and designated personnel)
- “Buddy” system while working with HPAIVs



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosecurity BSL3 enhanced conditions:

- Multiple security check points

- Access to BSL2+ area

- Access to BSL3 enhanced ante-room

- Access to BSL3 enhanced animal room

- Access to Lab/Animal Rooms

In countries where H5N1 is endemic, these viruses circulate freely, out of control

- Video monitoring

- Double lock freezer with combination of key and combination locks

- Virus stocks accessible to two individuals (PI and designated personnel)

- “Buddy” system while working with HPAIVs



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosafety BSL3 enhanced conditions:

- Personnel – Clearance and Training:
 - FBI clearance
 - Training
 - Intensive (at least three months before being allowed to work independently)
 - Proficiency on SOPs (Annual and/or Unannounced)
 - Facility Operation
 - Emergency Response
 - Annual Table Top exercises



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosafety BSL3 enhanced conditions:

- Personnel – Clearance and Training:
 - FBI clearance
 - Training



In countries where H5N1 is endemic, people have little

understanding of infectious diseases or proper hygiene conditions.

Children are usually responsible for backyard domestic animals

- Intensive (at least three months before being allowed to work independently)
- Proficiency on SOPs (Annual and/or Unannounced)
- Facility Operation
- Emergency Response
- Annual Table Top exercises



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosafety BSL3 enhanced conditions:

- Personnel – Layers of protective clothing/equipment:
 - Scrubs
 - Full body Tyvek suits
 - PAPR – Whole head hood
 - Three pairs of disposable shoe covers – one pair is removed while exiting lab or animal room – a new pair is donned
 - Three pairs of disposable gloves – changes as necessary, and while exiting lab or animal room – a new pair is donned
 - Disposable lab coats – don in animal rooms or lab, change as necessary



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosafety BSL3 enhanced conditions:

- Personnel – Layers of protective clothing/equipment:
 - Scrubs
 - Full body Tyvek suits



In countries where H5N1 is endemic, people wear no protective clothing. During eradication efforts, it is not uncommon to observe personnel wearing inappropriate protective clothing or equipment

- Three pairs of disposable gloves – changes as necessary, and while exiting lab or animal room – a new pair is donned
- Disposable lab coats – don in animal rooms or lab, change as necessary



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosafety BSL3 enhanced conditions:

- Personnel – Exit Protocol:
 - Remove disposable lab coat before entering autoclave/shower area
 - Wipe hood cover/PAPR with clorox wipes
 - Spray hood cover with 70% ethanol
 - Remove shoe covers (one last pair of shoe covers should remain on)
 - Remove disposable gloves (one last pair of disposable gloves should remain on)
 - Enter autoclave/shower area, remove hood cover and PAPR, inspect for damage
 - Dispose (in biohazard bags) Tyvek suit and last pair of shoe covers and gloves.
 - Shower out (shampoo and soap)



Biosecurity/Biosafety measures while working with mammalian transmissible avian influenza viruses

Biosafety BSL3 enhanced conditions:

- Personnel – Exit Protocol:
 - Remove disposable lab coat before entering autoclave/shower area
 - Wipe hood cover/PAPR with clorox wipes
 - Spray hood cover with 70% ethanol

During eradication efforts, it is not uncommon to observe inappropriate (and inhumane) disposal of live or dead animals

- Remove disposable gloves (one last pair of disposable gloves should remain on)
- Enter autoclave/shower area, remove hood cover and PAPR, inspect for damage
- Dispose (in biohazard bags) Tyvek suit and last pair of shoe covers and gloves.
- Shower out (shampoo and soap)



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