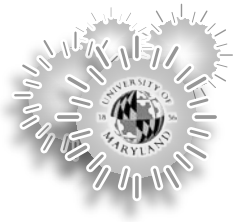


ABSA Annual Meeting
Orlando, FL
October 22, 2012

Of Men, Birds, Pigs and…Flu

Daniel R. Perez, PhD

Department of Veterinary Medicine – University of Maryland, College Park

VIRGINIA - MARYLAND
REGIONAL COLLEGE OF VETERINARY MEDICINE

aicaps

CEIRS

Influenza types

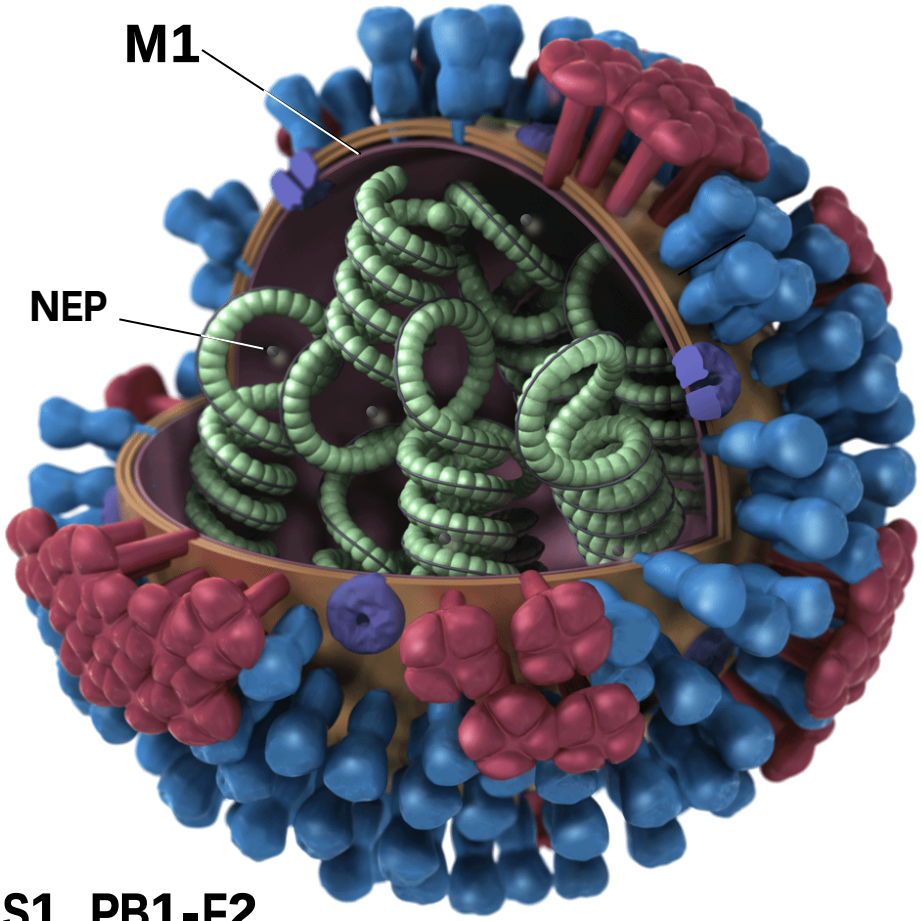
Type A	**Potentially severe illness**
	Epidemics and pandemics
	Rapidly changing
Type B	**Usually less severe illness**
	Epidemics
	More uniform
Type C	**Usually mild or asymptomatic illness**
	Minimal public health impact

Centers for Disease Control and Prevention. Influenza Prevention and Control. Influenza. Available at: http://www.cdc.gov/ncidod/diseases/flu/fluinfo.htm.

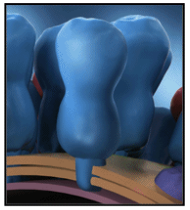
Influenza A virus

- Family: *Orthomyxoviridae*

8 Segments of negative sense single stranded RNA

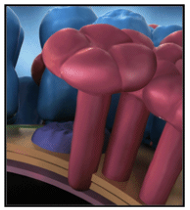


www.cdc.gov



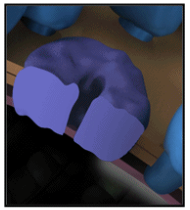
HA — α2-6Gal, α2-3Gal

Hemagglutinin



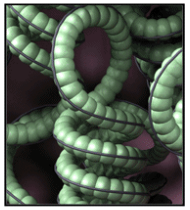
NA

Neuraminidase



M2

M2 Ion Channel



PB1, PB2, PA

NP

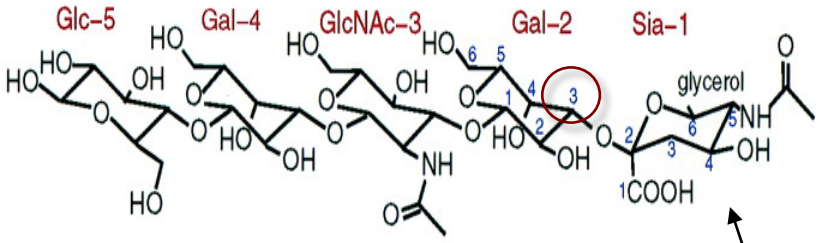
vRNA

vRNP

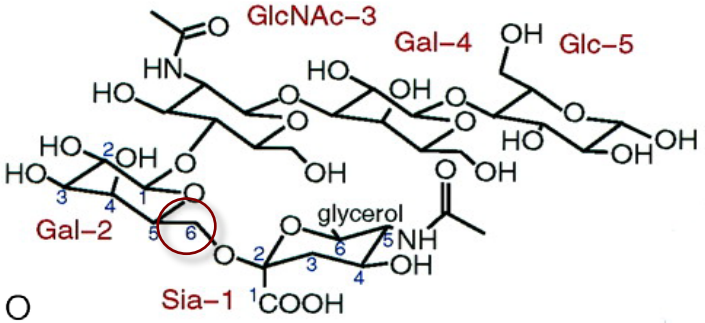
RNP

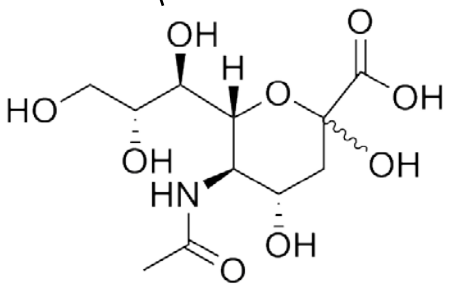
A change in receptor specificity is thought to favor host switching of influenza A viruses

SAα2,3-Gal



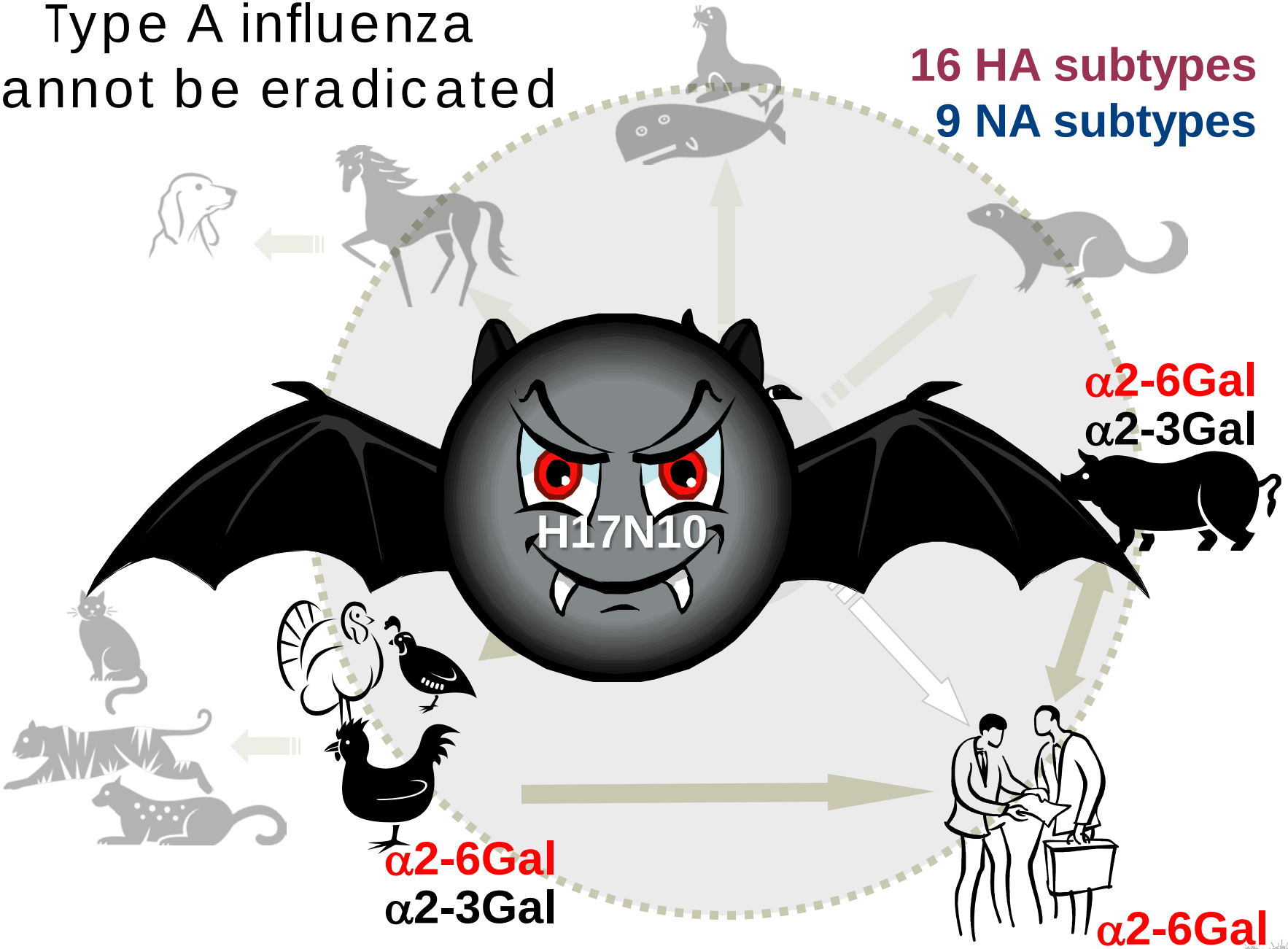
SAα2,6-Gal



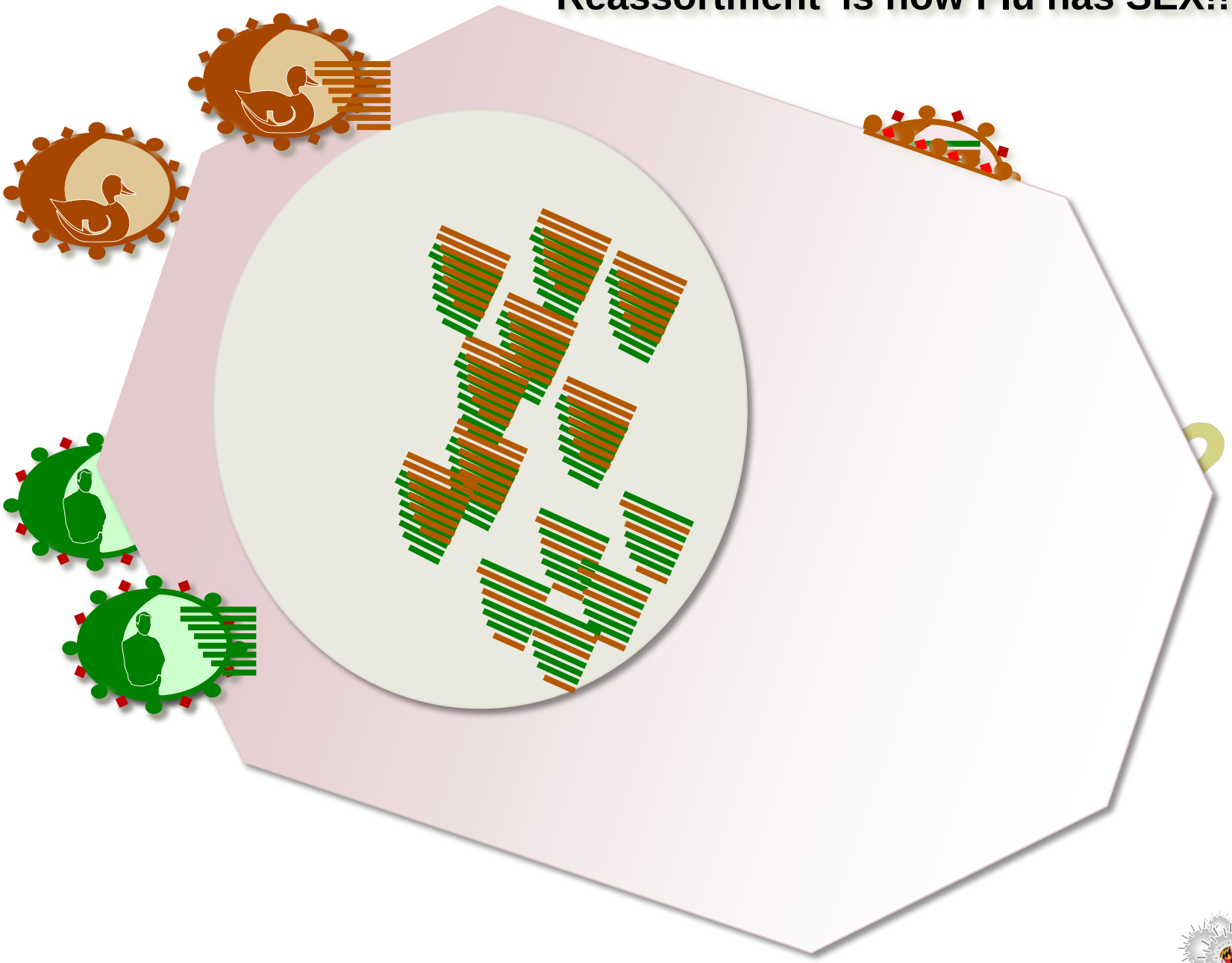


Sialic acid
(*N*-acetyl neuraminic acid)

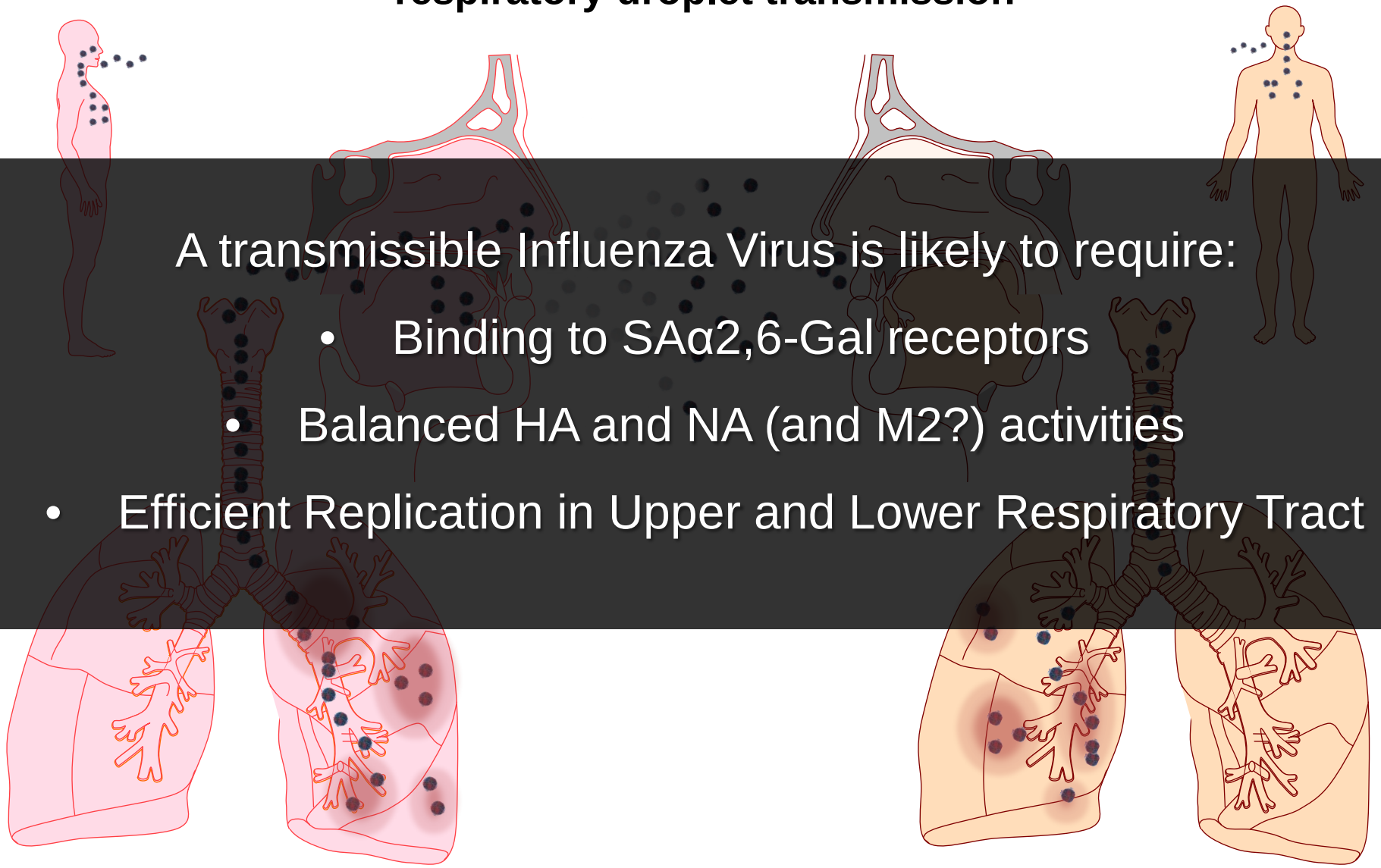
Type A influenza cannot be eradicated



Reassortment is how Flu has SEX!!



Influenza “Transmissome”: 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 SAα2,6-Gal receptors
- Balanced HA and NA (and M2?) activities
- Efficient Replication in Upper and Lower Respiratory Tract

Influenza in domestic birds

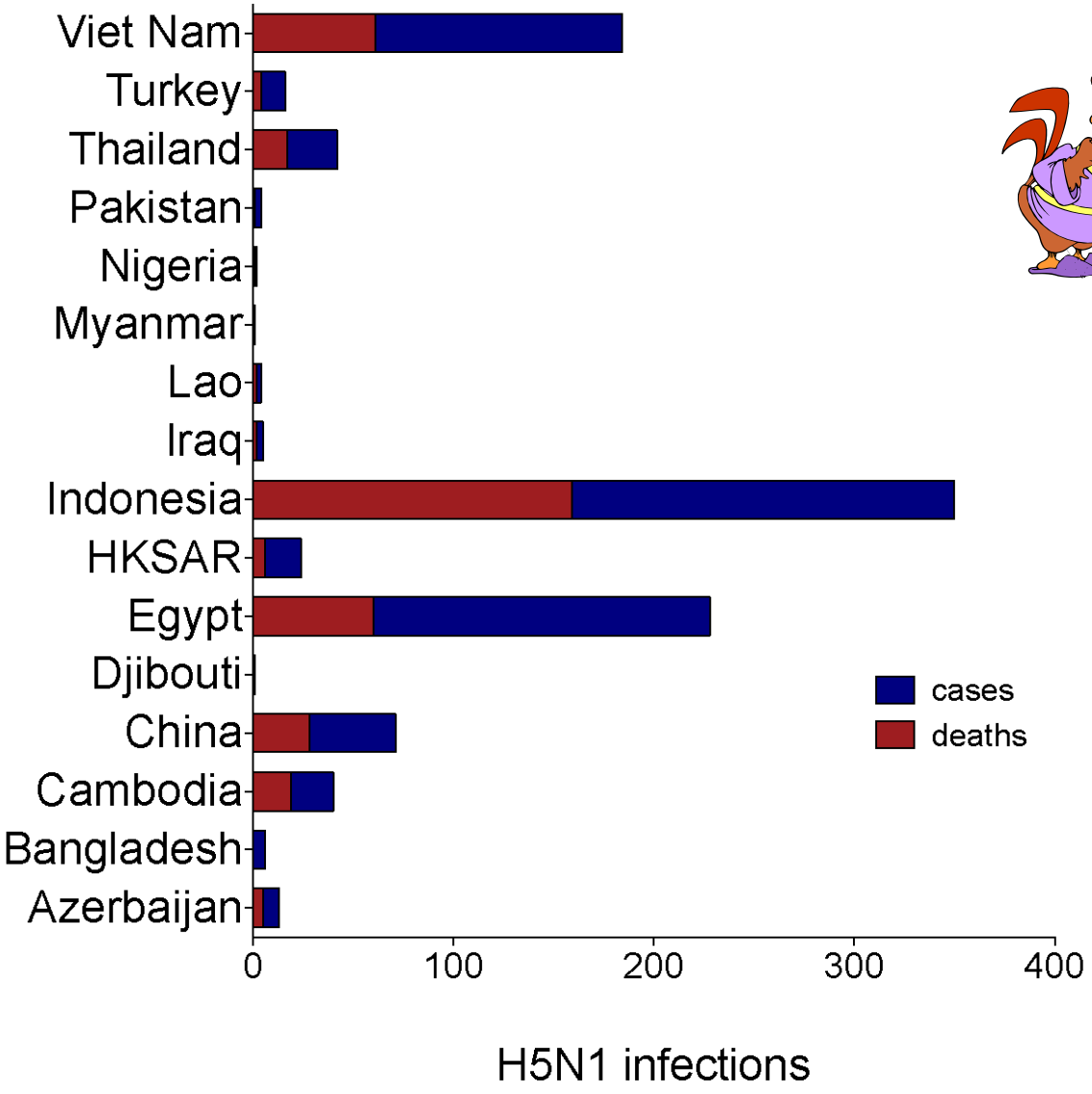
Defined as fowl plague in 1878.

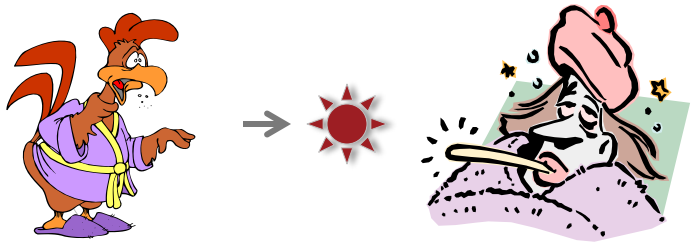
Significant economic losses.

Low Pathogenic Avian Influenza Viruses – LPAIV

- Associated with outbreaks of varying intensity in domestic birds.
- Progenitors of …
  - **Highly Pathogenic Avian Influenza Viruses - HPAIV**
  - H5 and H7 subtypes.
  - Acummulation of basic amino acids at the HA cleavage site.
  - ~26 outbreaks since 1959, 13 since 1990
  - ~13 H7 subtype, ~13 H5 subtype

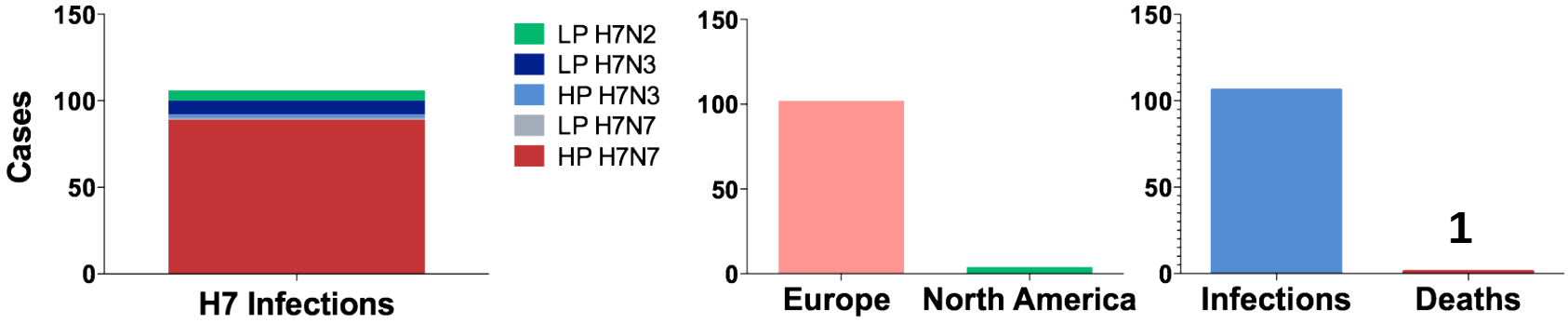
Number of human cases and deaths due to H5N1 infections/country since 1997



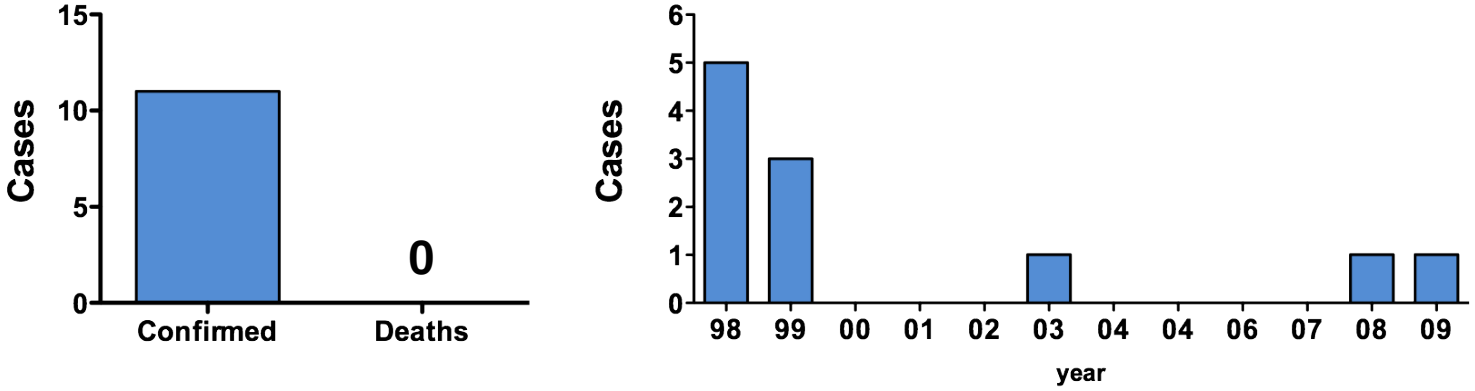


608 cases /
359 deaths

Number of cases and deaths due to H7 infections



Number of cases and deaths due to H9 infections



Seroprevalence of H9N2 in humans

Region	Population	% Sero- prevalence	Citation
Xinjiang, CH	General (64% poultry exposure)	1.7	Jia et al. 2009, *J Clin Vir*
Liaoning, CH	General (67% poultry exposure)	1	Jia et al. 2009, *J of Clin Vir*
Guangzhou, CH	Poultry workers	3-15	Wang et al. 2009, *NEJM*
Iran	General	2.5	Hadipour and Pazira 2011, *J An Vet Adv.*

- 1-4% seroprevalence in the general public in China
  - Only used single antigenic strain, may be higher
- 9-15% in poultry workers in Guangdong Province
- **Cross-reaction with H2N2 antibodies or other human influenza viruses??**

Overview of H9N2 avian influenza viruses

A/guinea fowl/Hong Kong/WF10/99 (H9N2) - RGWF10

Nature

Aquatic birds

Land-based poultry

Before 1988

After 1988-1994

Laboratory

Early isolates do not or replicate poorly (1997 and before)

Recent field isolates replicate well (1998 and later)

Bind preferentially to human-like sialic acid receptors

1999 and later

1998 and later

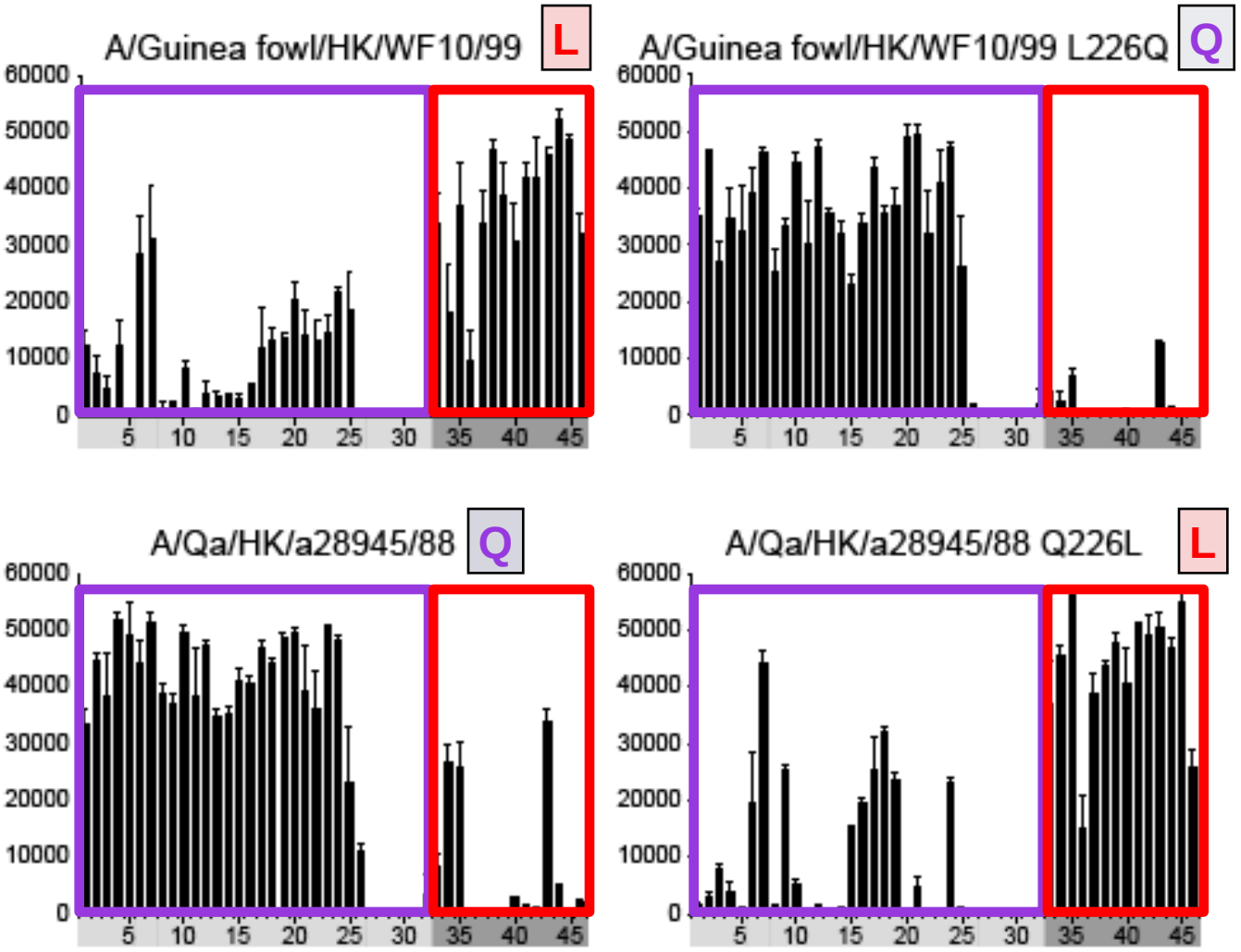
H9N2 geographic range

H9N2 avian influenza viruses of the G1-like lineage show the largest geographic spread

H9 influenza viruses go where H5N1 viruses go!

Previous pandemic influenza viruses have not been associated with major disease outbreaks in birds

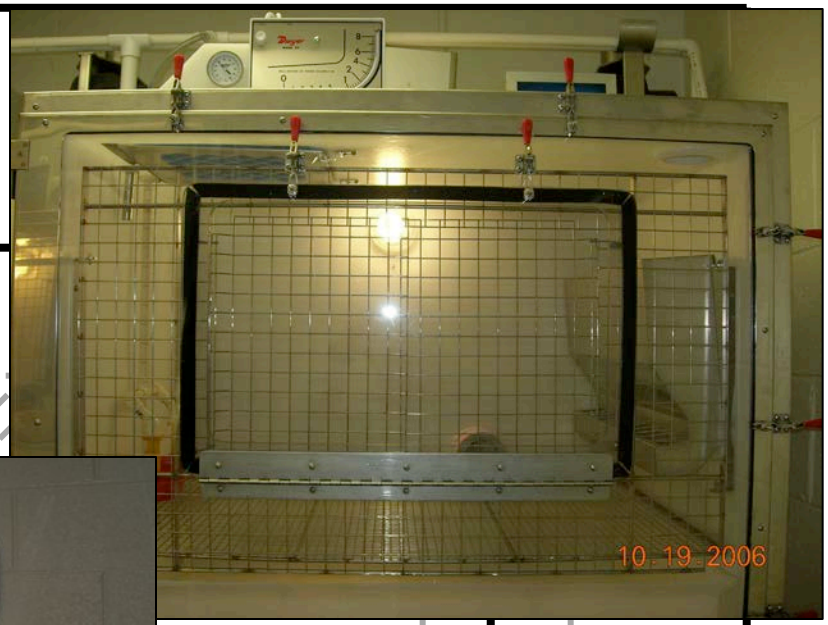
Contemporary H9N2 avian influenza viruses with human-like receptor specificity (L226) infect mostly nonciliated cells in HAE cultures.

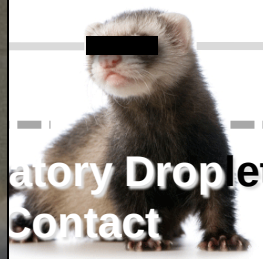


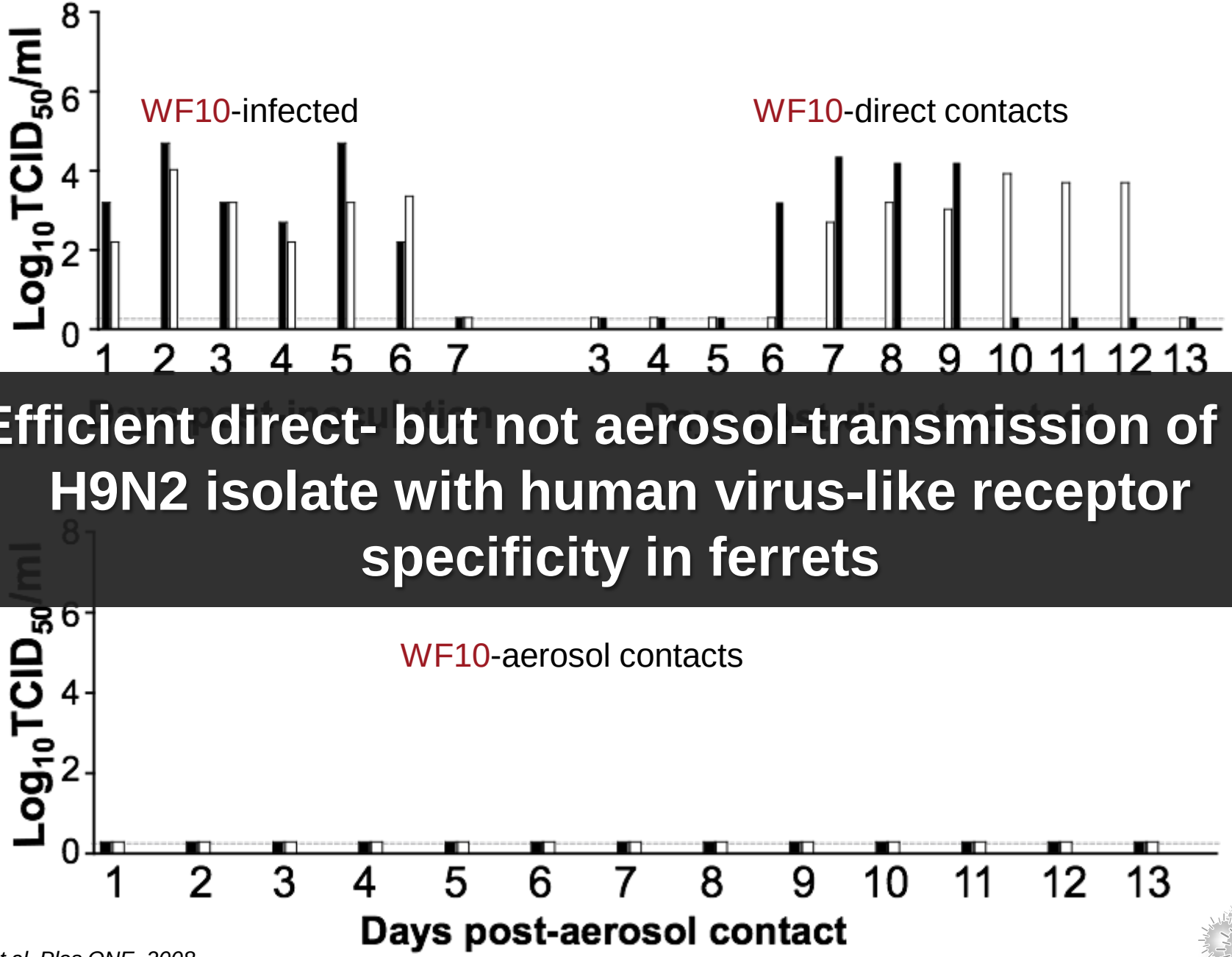
Collaborator: James Stevens, CDC

Wan & Perez, JVI 2007

Ferrets recapitulate human infection with Influenza A viruses







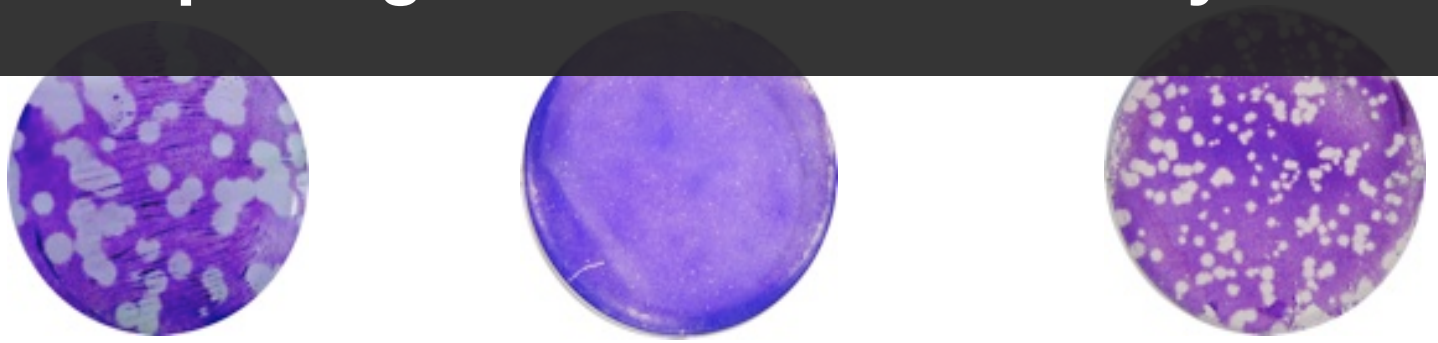
Efficient direct- but not aerosol-transmission of a H9N2 isolate with human virus-like receptor specificity in ferrets

Wan et al. Plos ONE, 2008

Would an avian/human H9N2 reassortant virus show improved replication and transmission in ferrets?

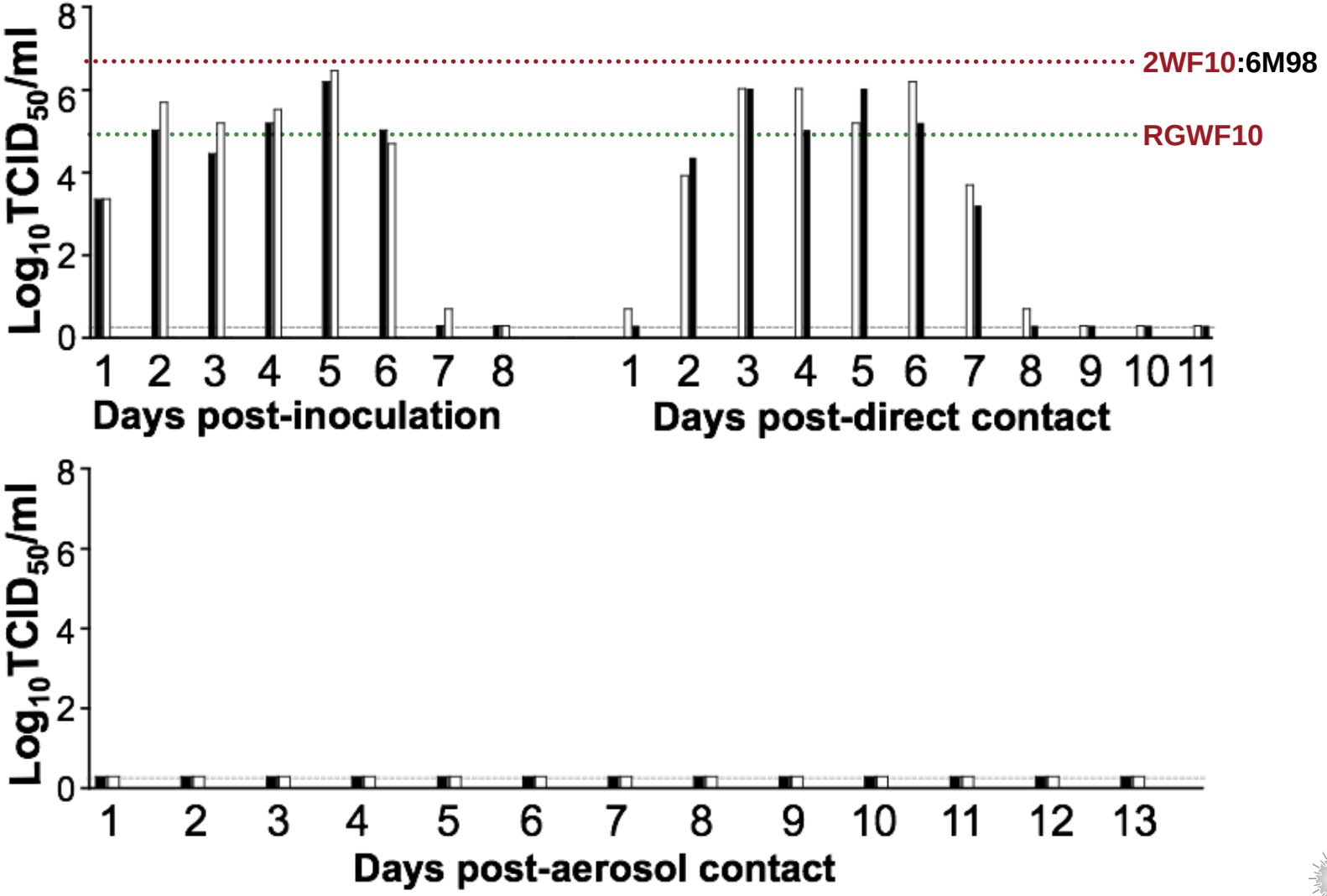
RGMemphis98 × RGWF10 (L226) → 2WF10:6M98

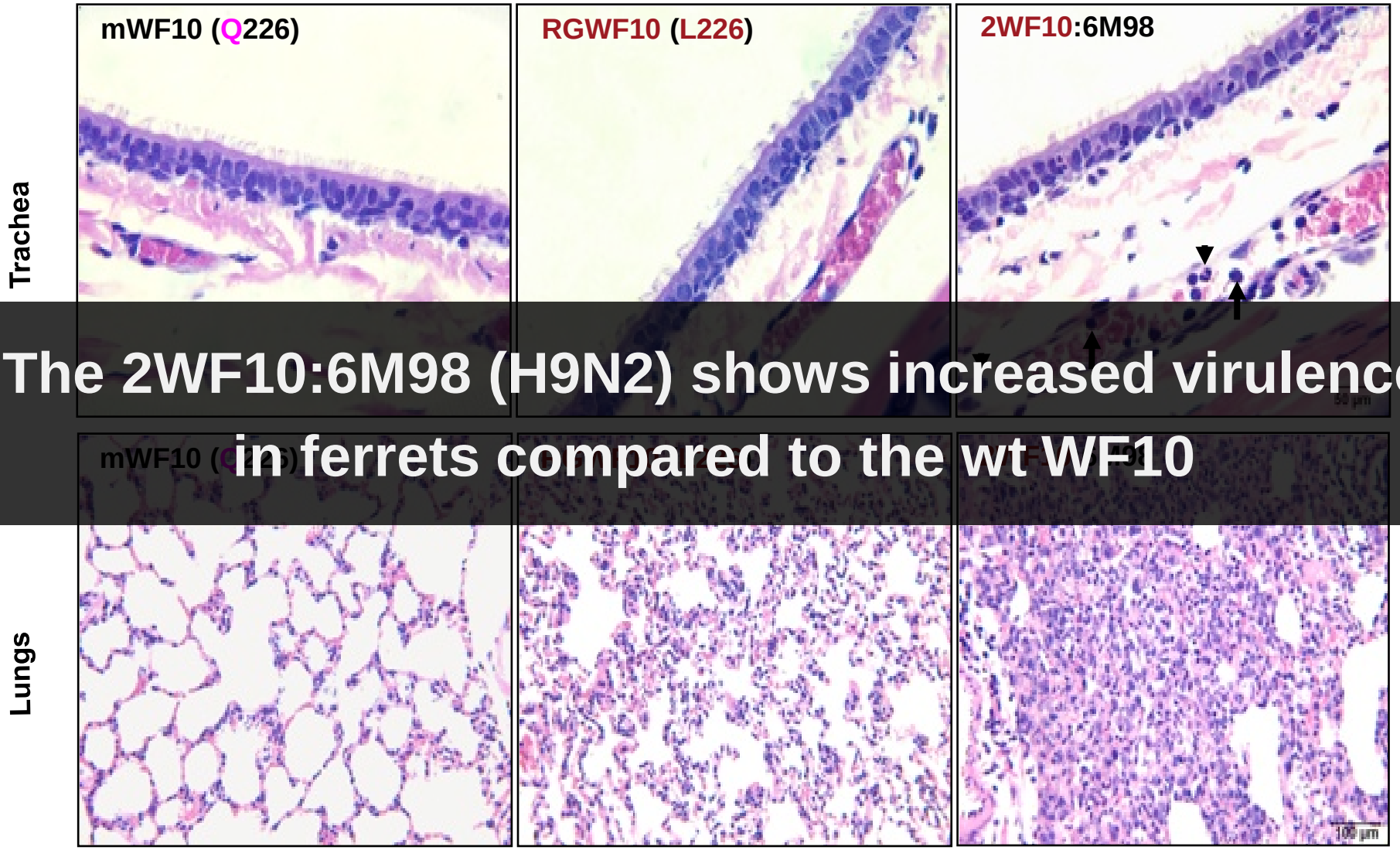
Plaque morphology cannot be used as a marker of pathogenesis/transmissibility



Wan et al. Plos ONE, 2008

Efficient direct- but not aerosol-transmission of the avian/human 2WF10:6M98 (H9N2) virus in ferrets



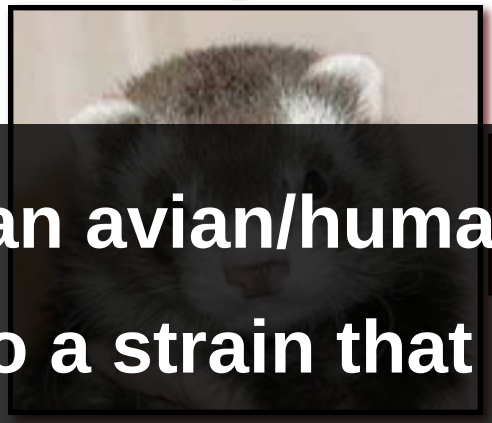


The 2WF10:6M98 (H9N2) shows increased virulence in ferrets compared to the wt WF10

Is it possible to generate a H9N2 virus that transmits by aerosol in ferrets? - Implications for pandemic preparedness

2WF10:6M98

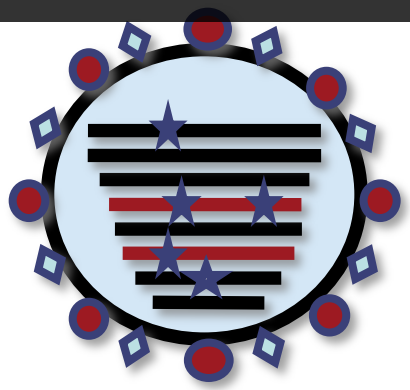
Passage 1



Adaptation of an avian/human H9N2 virus in ferrets leads to a strain that can transmit by respiratory droplets

Nasal washes used to infect new set of ferrets

P10-2WF10:6M98



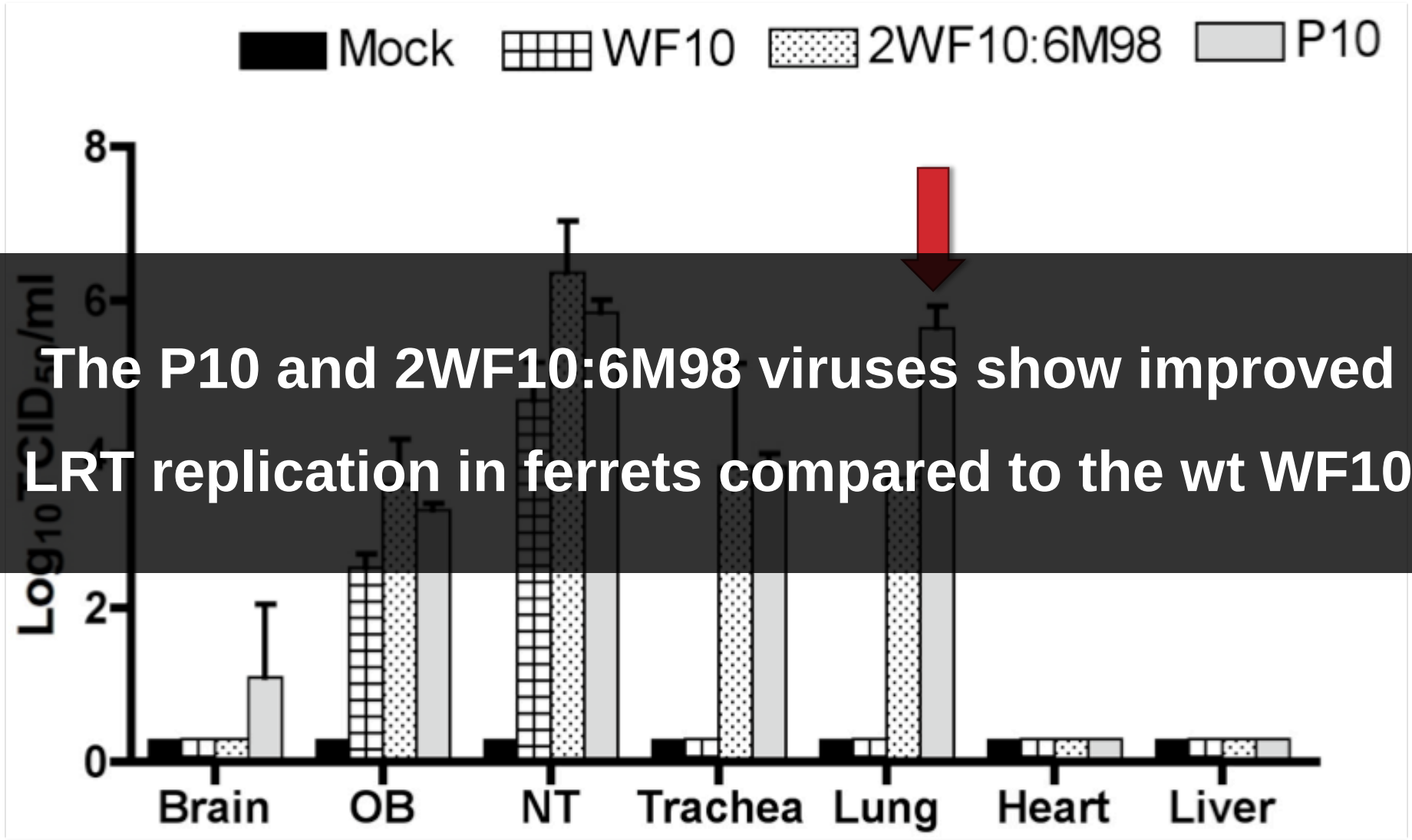
Passage 10

Sorrell et al. PNAS, 2009

Gene	Origin	Amino Acid Position	Parent	P8	P9	P10	RCP10	RCP10$_2$
PB2	Human	374	L	I	I	I	I	I
PB1	Human	No changes[a]		nd[b]	nd			
PA	Human	No changes		nd	nd			
HA	Avian	HA1 189	T	T	T	T	A	A
		HA2 192	G	G	*G*/R[c]	*G*/R[c]	R	R
NP	Human	No changes		nd	nd			
NA	Avian	28	I	V	V	V	V	V
M1	Human	110	H	H/***Y***[c]	Y	Y	Y	Y
M2	Human	No changes		nd	nd			
NS1	Human	No changes		nd	nd			
NEP	Human	No changes		nd	nd			

[a] No amino acid changes detected between the parent and either the RCP10 or the RCP10$_2$ viruses. [b] nd, sequencing not done. [c] Bold and italicized letter denotes more prominent residue at particular amino acid position based on electropherograms of sequencing profiles.

Five amino acid changes occurred during adaptation of the 2WF10:6M98 virus in ferrets

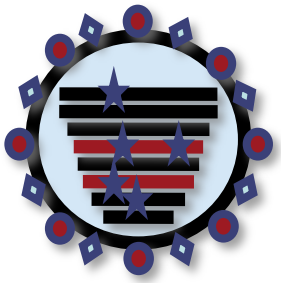


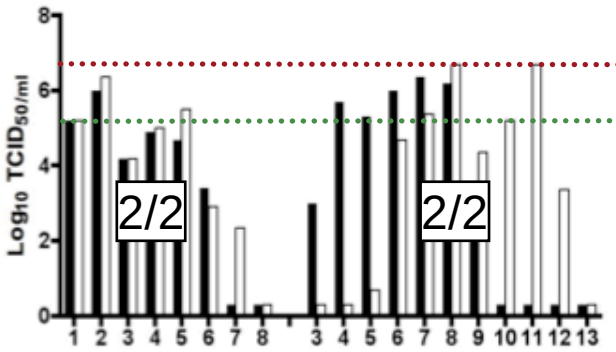
The P10 and 2WF10:6M98 viruses show improved LRT replication in ferrets compared to the wt WF10

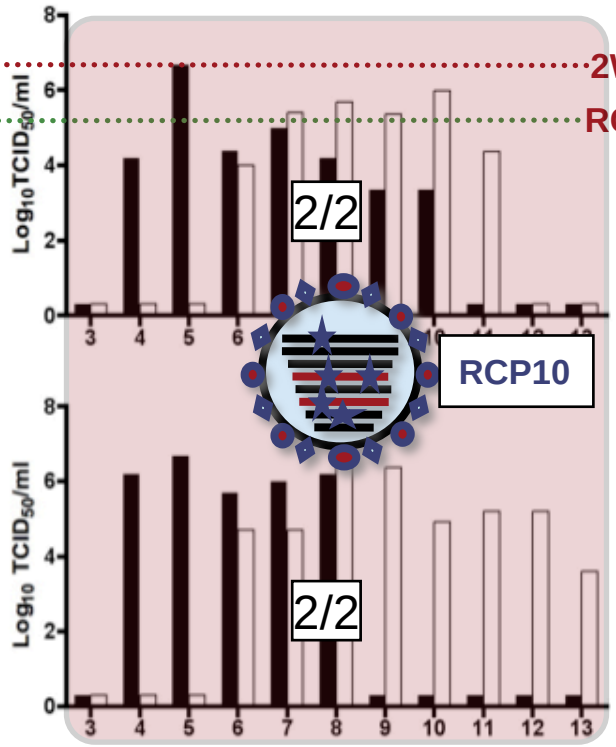
The P10-2WF10:6M98 (H9N2) virus shows consistent aerosol transmission in ferrets

Round 1

P10-2WF10:6M98

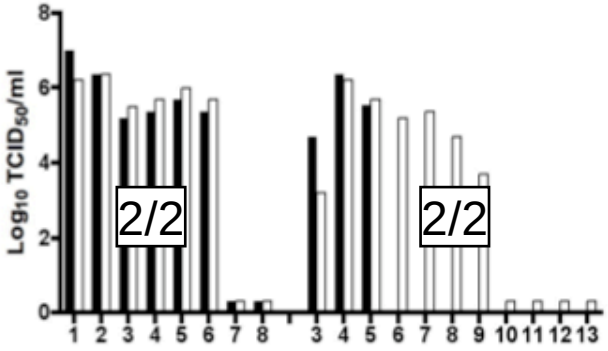






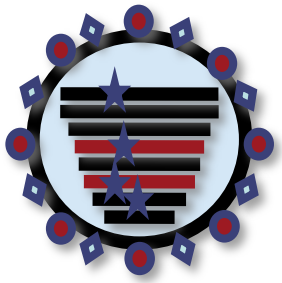
Round 2

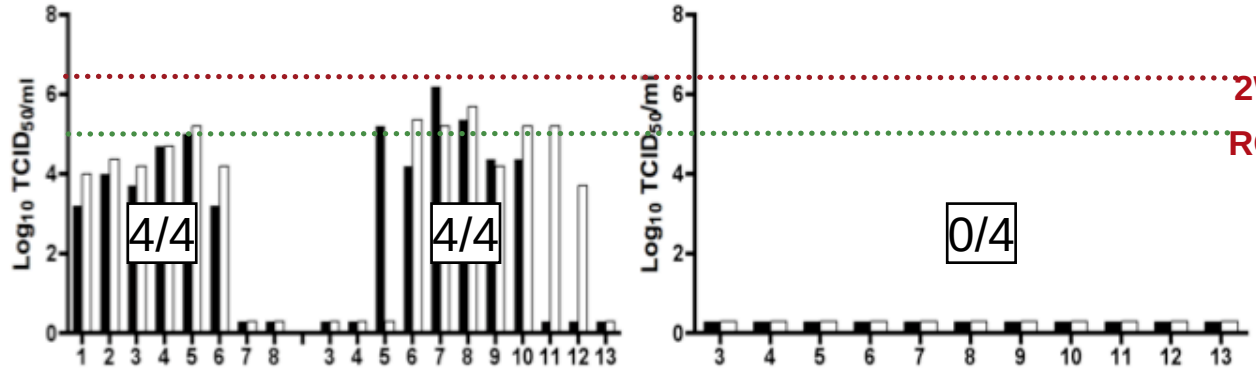
P10-2WF10:6M98



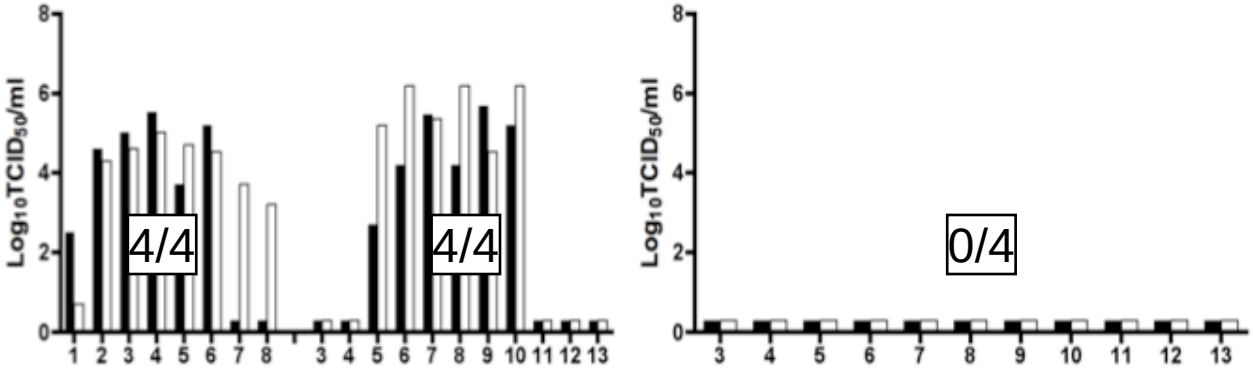
The P10-2WF10:6M98 (H9N2) virus shows consistent aerosol transmission in ferrets

RCP10 (A189,G192)

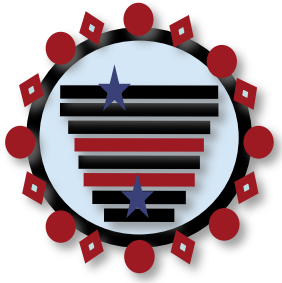


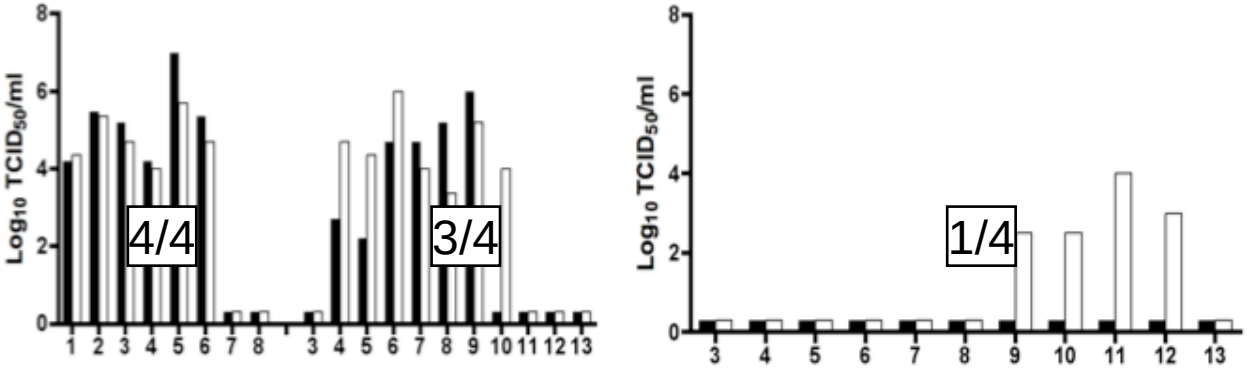


RCP10 (T189,R192)

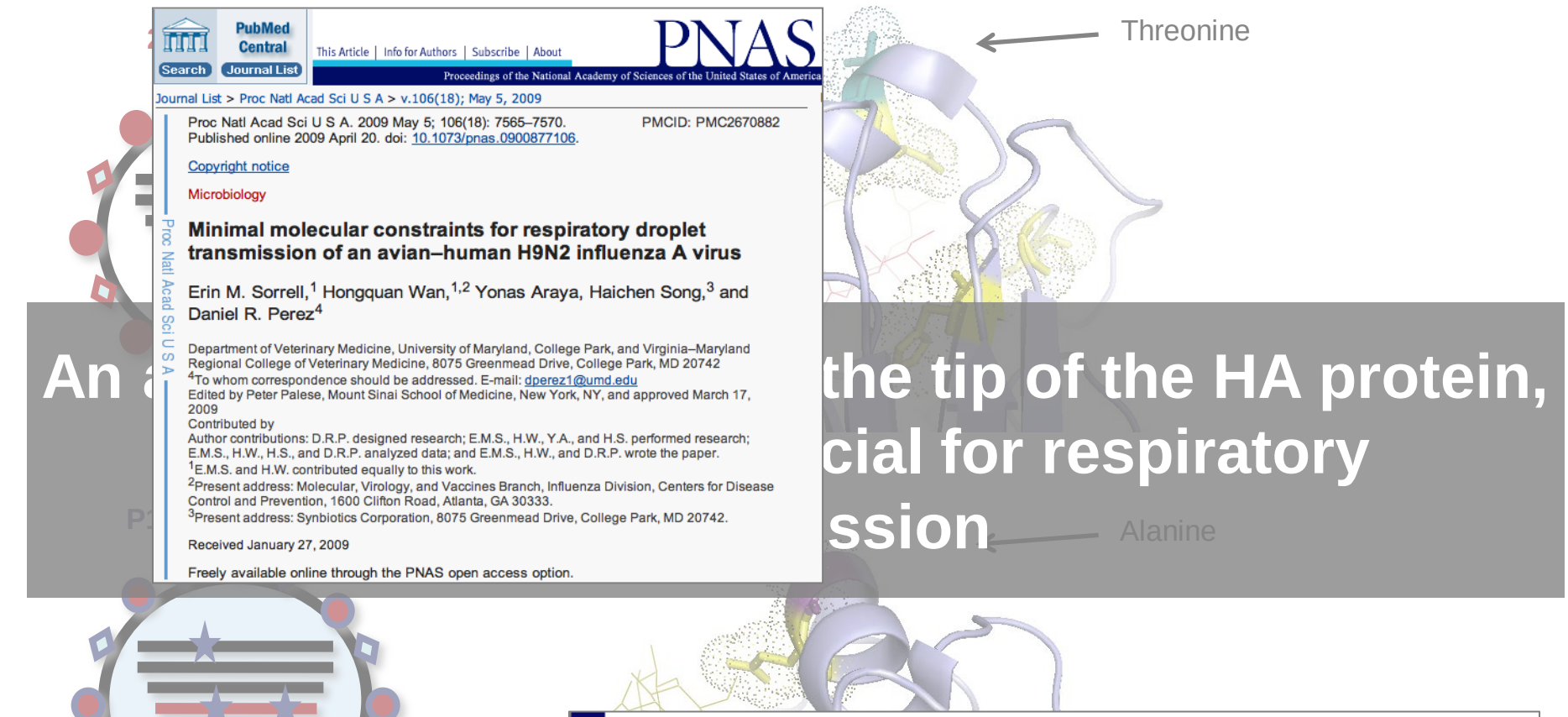


2WF10:6RCP10





Position T189A: RBS and antigenic profile



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PNAS
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Journal List > Proc Natl Acad Sci U S A > v.106(18); May 5, 2009

Proc Natl Acad Sci U S A. 2009 May 5; 106(18): 7565–7570. PMCID: PMC2670882
Published online 2009 April 20. doi: 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. Perez[4]

Department of Veterinary Medicine, University of Maryland, College Park, and Virginia–Maryland Regional College of Veterinary Medicine, 8075 Greenmead Drive, College Park, MD 20742
[4]To whom correspondence should be addressed. E-mail: dperez1@umd.edu
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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.
[1]E.M.S. and H.W. contributed equally to this work.
[2]Present address: Molecular, Virology, and Vaccines Branch, Influenza Division, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333.
[3]Present address: Synbiotics Corporation, 8075 Greenmead Drive, College Park, MD 20742.

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Proc Natl Acad Sci U S A

An a… the tip of the HA protein, …cial for respiratory …ssion

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]

[a]Department of Veterinary Medicine, University of Maryland, College Park and Virginia-Maryland Regional College of Veterinary Medicine, College Park, MD 20742; and [b]Center for Advanced Preclinical Research, Science Applications International Corporation/National Cancer Institute, Frederick, MD 21702

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Epidemiology of SIVs in North America since 1918

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Published online 2010 October 14. doi: 10.1371/journal.ppat.1001145.

Variations in the Hemagglutinin of the 2009 H1N1 Pandemic Virus: Potential for Strains with Altered Virulence Phenotype?

Jianqiang Ye,[1,2] Erin M. Sorrell,[1,2] Yibin Cai,[1,2] Hongxia Shao,[1,2] Kemin Xu,[1,2] Lindomar Pena,[1,2] Danielle Hickman,[1,2] Haichen Song,[3] Matthew Angel,[1,2] Rafael A. Medina,[4,5,6] Balaji Manicassamy,[4,5,6] Adolfo Garcia-Sastre,[4,5,6] and Daniel R. Perez[1,2*]

[1]Virginia-Maryland Regional College of Veterinary Medicine, College Park, Maryland, United Sta
[2]Department of Veterinary Medicine, University of Maryland, College Park, Maryland, United St
[3]Synbiotics Corporation, College Park, Maryland, United States of America
[4]Department of Microbiology, Mount Sinai School of Medicine, New York, New York, United Sta
[5]Institute of Global Health and Emerging Pathogens, Mount Sinai School of Medicine, New York
States of America
[6]Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, New
United States of America
Raul Andino, *Editor*
University of California San Francisco, United States of America
* E-mail: dperez1@umd.edu

Conceived and designed the experiments: J. Ye, E. Sorrell, D. Perez. Performed the experimer
Sorrell, Y. Cai, H. Shao, K. Xu, L. Pena, D. Hickman, M. Angel. Analyzed the data: J. Ye, E. Sor
Pena, D. Hickman, R. Medoma. B. Manicassamy, A. Garcia-Sastre, D. Perez. Contributed
reagents/materials/analysis tools: Y. Cai, H. Shao, K. Xu, L. Pena, D. Hickman, H. Song, R. Me
Manicassamy, A. Garcia-Sastre. Wrote the paper: J. Ye, E. Sorrell, M. Angel, D. Perez.

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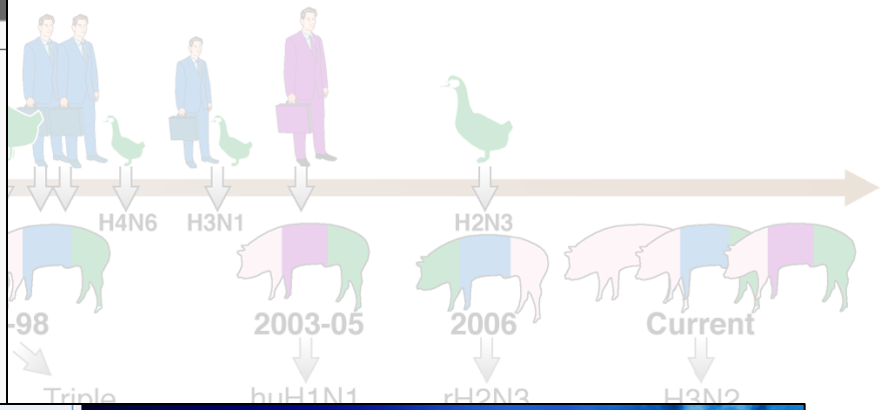


Journal List > PLoS Curr > v.1;

Version 2. PLoS Curr. 2009 August 25 [revised 2009 August 27]; 1: RRN1011. PMCID: PMC2762341
doi: 10.1371/currents.RRN1011. Other versions

Influenza

Fitness of Pandemic H1N1 and Seasonal influenza A viruses during Co-infection

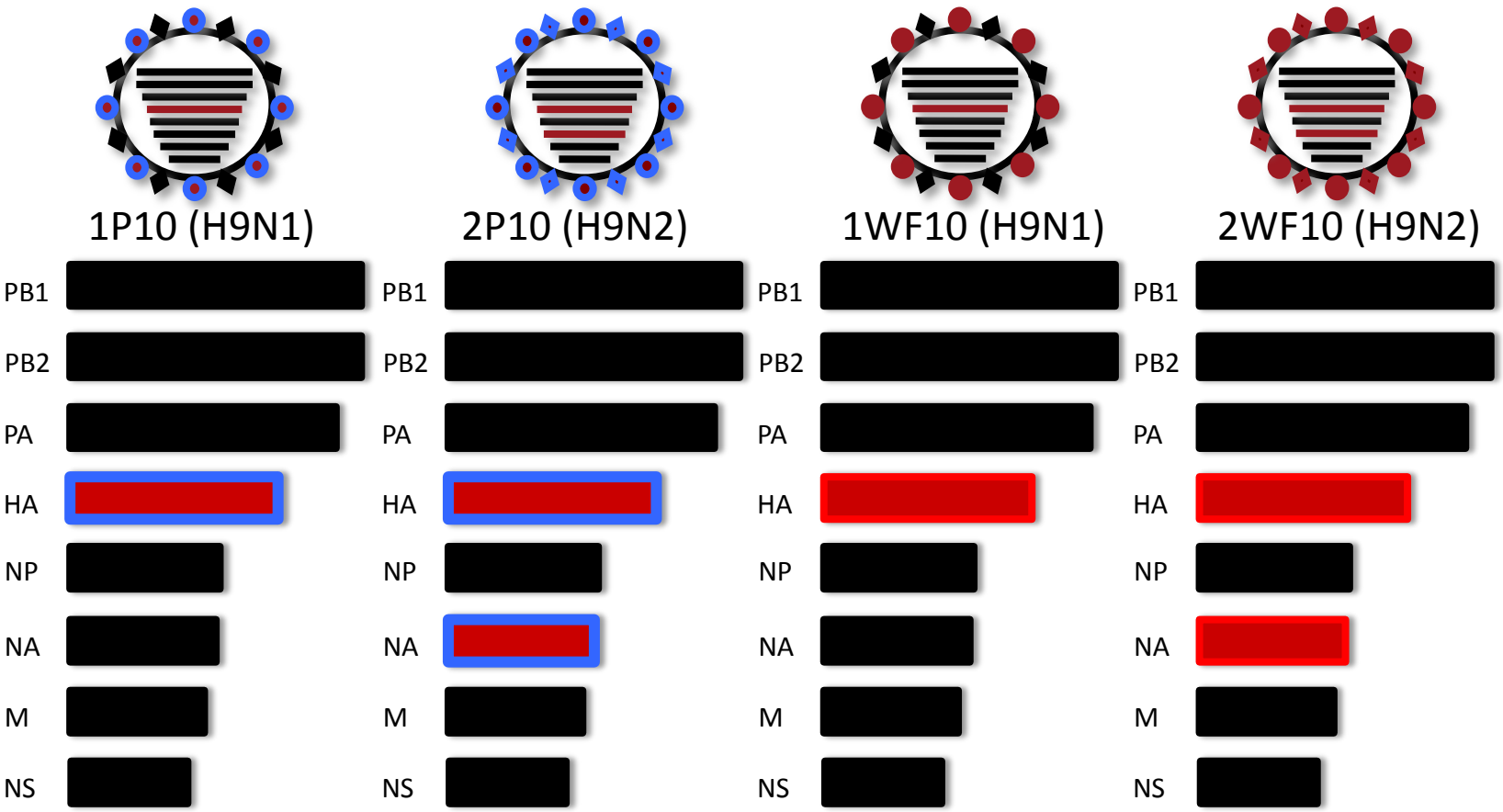
Evidence of competitive advantage of pandemic H1N1 influenza versus seasonal influenza

Daniel Roberto Perez,[*] Erin Sorrell,[†] Matthew Angel,[‡] Jianqiang Ye, Danielle Hickman,[*] Lindomar Pena,[¶] Gloria Ramirez-Nieto,[#] Brian Kimble,[†] and Yonas Araya[†]

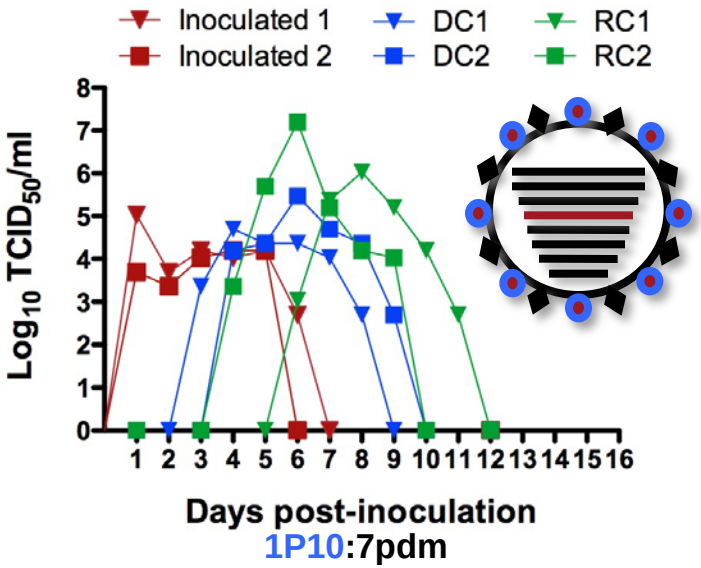
[*]University of Maryland, College Park; [†]University of Maryland; [‡]Virginia-Maryland Regional College of Veterinary Medicine; [¶]Virginia-Maryland Regional College of Veterinary Medicine-Maryland Campus and [#]Universidad Nacional De Colombia

Accepted August 25, 2009.

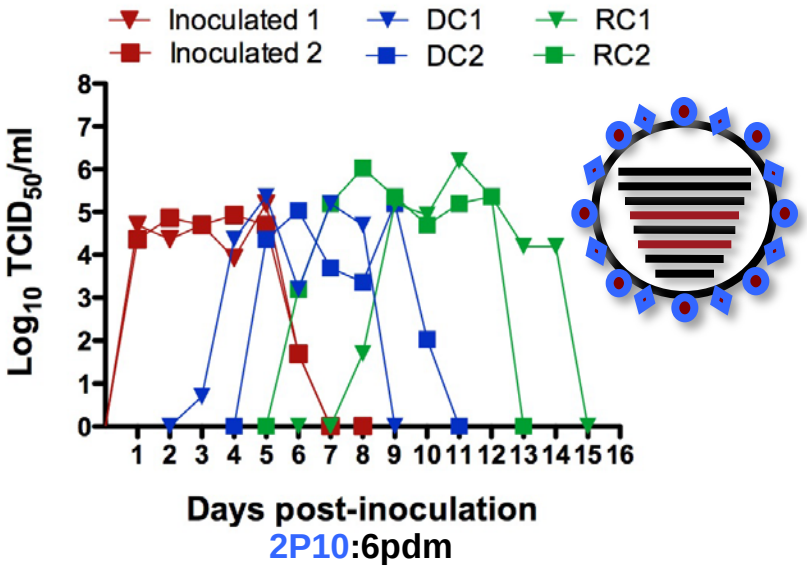
How does the 2009 Pandemic change how we view H9s?



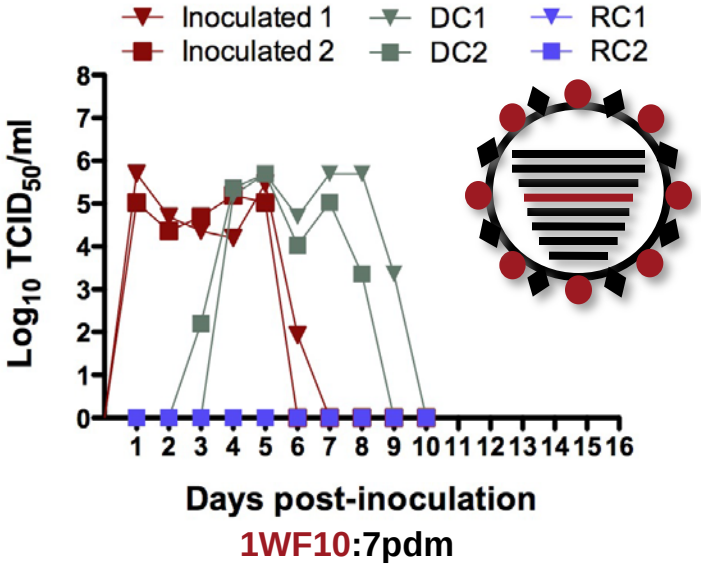
H9N2:H1N1pdm reassortants can be transmitted by respiratory droplets in ferrets



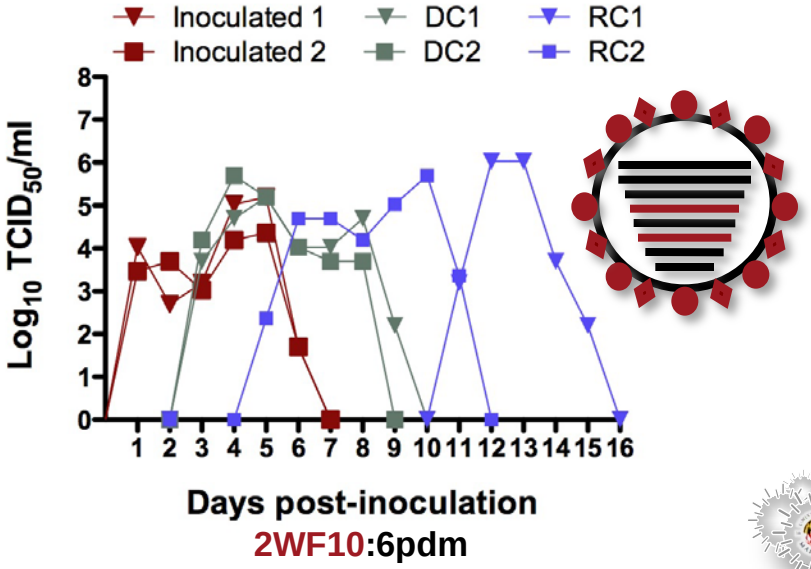
1P10:7pdm



2P10:6pdm



1WF10:7pdm



2WF10:6pdm

Summary H9N2 viruses – Risk Assessment

Contemporary H9N2 avian influenza viruses with human-like receptor specificity infect mostly nonciliated cells in cultures of human airway epithelium (HAE).

L226 containing H9N2 viruses show efficient direct - but not aerosol - transmission in ferrets. No signs of disease.

An avian/human H9N2 reassortant shows improved replication in ferrets with no detectable respiratory droplet transmission.

Respiratory droplet transmission in ferrets of an avian/human H9N2 virus requires few amino acid changes with major implications in replication, virulence and antibody recognition profiling.

H9 HA in the context of N2 or N1 NA is compatible with respiratory transmission when the internal genes are derived from the H1N1pdm virus.

LETTER

doi:10.1038/nature10831

Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai[1], Tokiko Watanabe[1,2], Masato Hatta[1], Subash C. Das[1], Makoto Ozawa[1,3], Kyoko Shinya[4], Gongxun Zhong[1], Anthony Hanson[1], Hiroaki Katsura[5], Shinji Watanabe[1,2], Chengjun Li[1], Eiryo Kawakami[2], Shinya Yamada[5], Maki Kiso[5], Yasuo Suzuki[6], Eileen A. Maher[1], Gabriele Neumann[1] & Yoshihiro Kawaoka[1,2,3,5]

H5N1

Science **336**, 1534 (2012);
DOI: 10.1126/science.1213362

REPORT

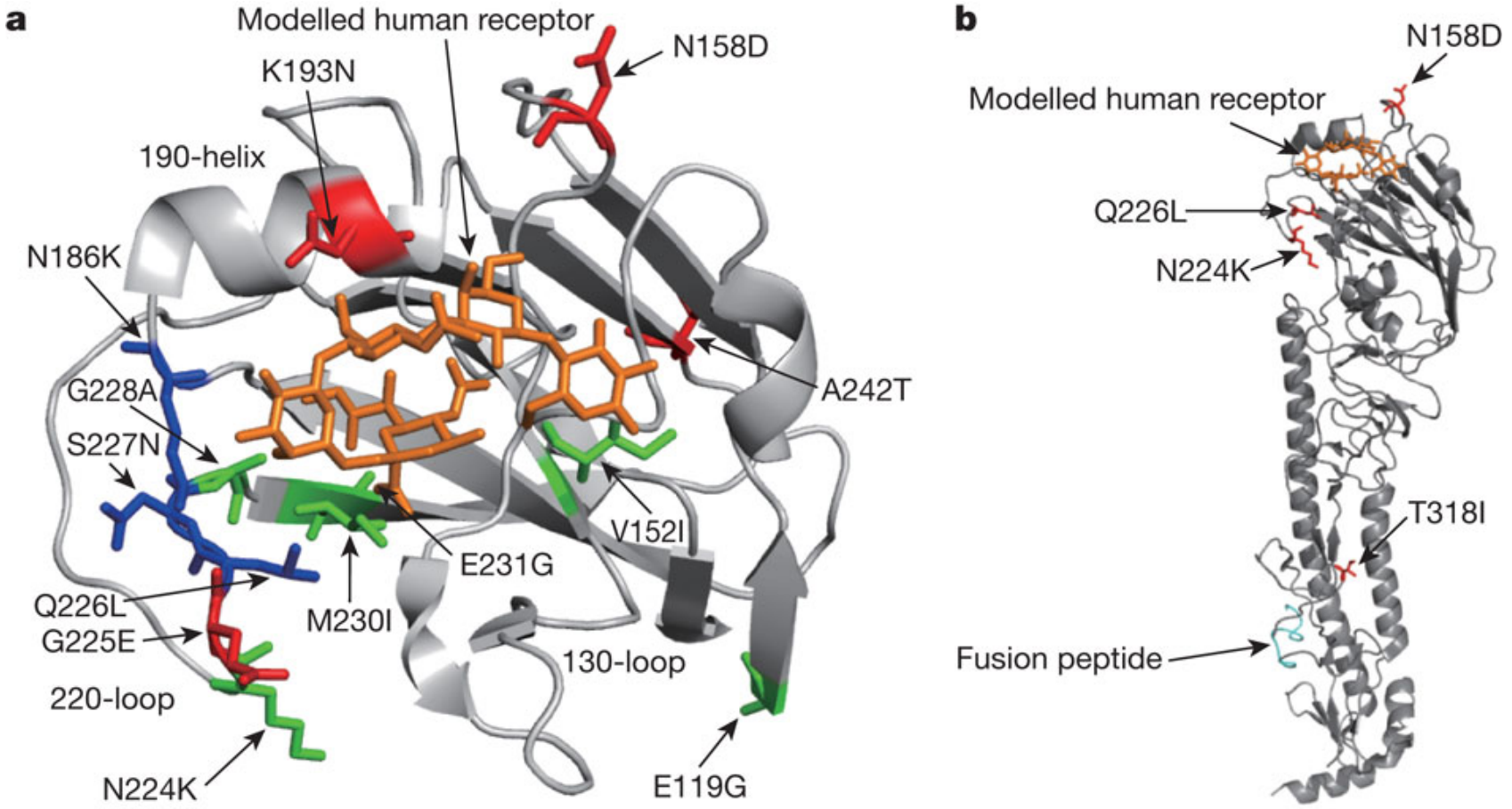
Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

Sander Herfst,[1] Eefje J. A. Schrauwen,[1] Martin Linster,[1] Salin Chutinimitkul,[1] Emmie de Wit,[1]* Vincent J. Munster,[1]* Erin M. Sorrell,[1] Theo M. Bestebroer,[1] David F. Burke,[2] Derek J. Smith,[1,2,3] Guus F. Rimmelzwaan,[1] Albert D. M. E. Osterhaus,[1] Ron A. M. Fouchier[1]†

Transmissible H5N1 viruses: Chronology of events

- September 2011, Prof. Ron Fouchier of the Erasmus Medical Center (Rotterdam, Netherlands) reported at a public scientific meeting the results of genetically modifying influenza A (H5N1) viruses transmissible through the air between ferrets.
- Similar research at the University of Wisconsin-Madison, USA under Dr. Yoshihiro Kawaoka except that the transmissible viruses are reportedly not lethal to ferrets.
- Manuscripts submitted to the journals Science and Nature, respectively…
  - Both sets of studies funded by the US National Institutes of Health (NIH) and came under review by the U.S. National Science Advisory Board for Biosecurity (NSABB) prior to publication.

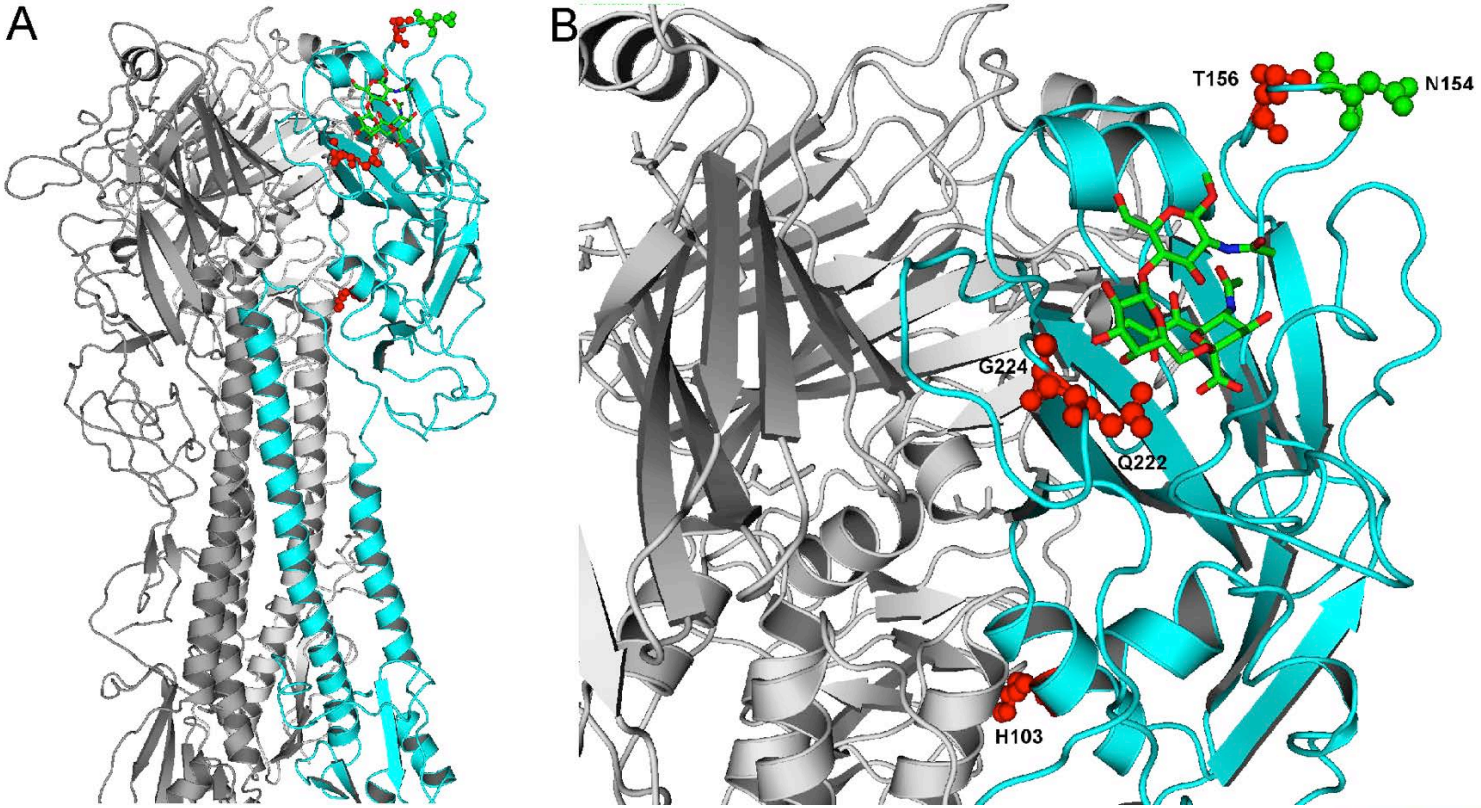
Localization of amino acid changes identified in this study on the three-dimensional structure of the monomer of VN1203 HA (Protein Data Bank accession 2FK0)[15].



M Imai *et al*. *Nature* **000**, 1-9 (2012) doi:10.1038/nature10831

nature

Serial passaging in ferrets
- Sequence analysis -



H103: forms part of the trimer interface

T156: part of a N-linked glycosylation sequon

Herfst et al., Science (2012)

Transmissible H5N1 viruses: Interlocking issues

- *A voluntary moratorium on the research has been declared by the groups capable of undertaking such research. (Still in effect)*
- Public concern that laboratory-modified H5N1 viruses could accidentally cause an influenza pandemic;
- The need for assessment of the balance of public health risk and benefit in research;
- Appropriate laboratory biosafety requirements of research on the evolution of pathogens to become more virulent or transmissible;
- The need and ability to revise risk assessments concerning H5N1 viruses;
- A potential threat to the new pandemic influenza preparedness framework for influenza virus and benefit sharing;
- and academic freedom to publish.

Federal Register /Vol. 77, No. 201 /Wednesday, October 17, 2012 / Proposed Rules – Tier 1

- *“Current epidemiologic evidence indicates that, once transmitted into a human host, H5N1 viruses may result in more severe disease in humans than other subtypes of influenza.”*

- *“Listing influenza viruses that contain an HA from the goose/Guangdong/1/96 lineage as an HHS select agent will ensure that the focus of regulation will also be on the potential impact of these viruses on human health as well as agriculture.”*

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- *“Designating HPAI containing an HA from the Goose/Guangdong/1/96 lineage an HHS select agent, in addition to its status as a USDA select agent, may help to ensure that HPAI strains that have the greatest potential for major direct effects on human health will be regulated with a focus on protection of human health.”*

Federal Register /Vol. 77, No. 201 /Wednesday, October 17, 2012 / Proposed Rules – Tier 1

- The HHS/CDC's Intragovernmental Select Agents and Toxins Technical Advisory Committee (ISATTAC) recognized …
  - **That study of the Goose/Guangdong/1/96 lineage-derived viruses could lead to significant public health benefits for understanding pandemic influenza, improved diagnostics, and the development of more effective countermeasures.**
  - **Therefore, the risks posed by these viruses need to be weighed against any adverse impact that a regulation will have on legitimate research.**

Federal Register /Vol. 77, No. 201 /Wednesday, October 17, 2012 / Proposed Rules – Tier 1

- Establishment of a Docket and Request for Specific Input on Certain Topics
  - **Should special precautions (i.e., safety and containment measures) be considered when working with strains of HPAI containing the HA from the Goose/Guangdong/1/96 lineage that have been shown to be transmissible between mammals beyond those recommended for non-mammalian transmissible HPAI?**

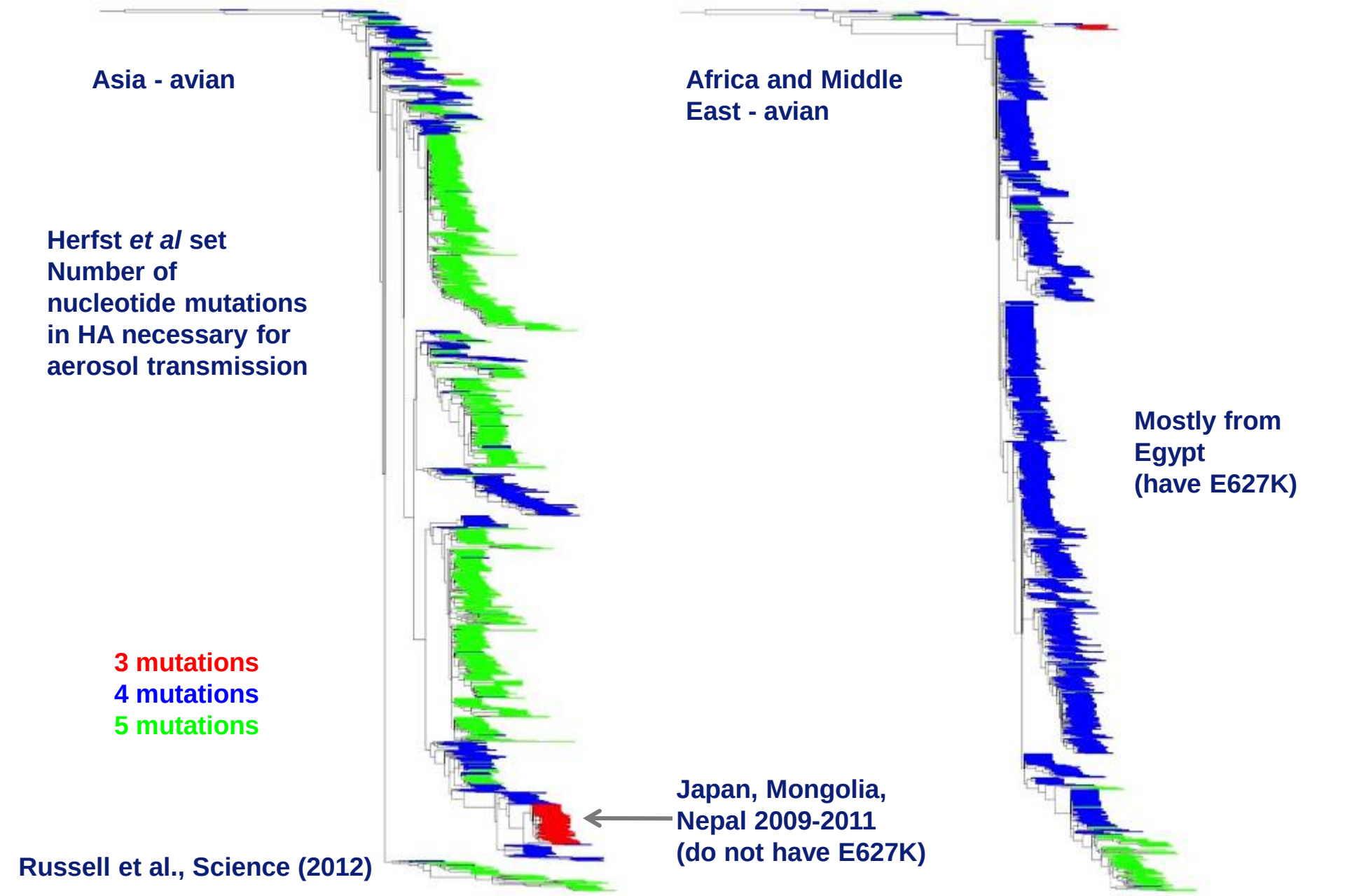
HPAI H5N1 transmission - Will such virus emerge in nature?

www.sciencemag.org **SCIENCE** VOL 336 22 JUNE 2012

REPORT

The Potential for Respiratory Droplet–Transmissible A/H5N1 Influenza Virus to Evolve in a Mammalian Host

Colin A. Russell,[1,2,3] Judith M. Fonville,[1,2] André E. X. Brown,[4] David F. Burke,[1,2] David L. Smith,[3,5,6] Sarah L. James,[1,2] Sander Herfst,[7] Sander van Boheemen,[7] Martin Linster,[7] Eefje J. Schrauwen,[7] Leah Katzelnick,[1,2] Ana Mosterín,[1,2,8] Thijs Kuiken,[7] Eileen Maher,[9] Gabriele Neumann,[9] Albert D. M. E. Osterhaus,[7] Yoshihiro Kawaoka,[9,10,11,12] Ron A. M. Fouchier,[7] Derek J. Smith[1,2,3,7]*



UNIVERSITY OF CAMBRIDGE
Center for Pathogen Evolution

WHO Collaborating Center
Modeling, Evolution, and Control of Emerging Infectious Diseases

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

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Ilaria Capua, Giovanni Cattoli, Isabella Monne

NIH NIAID
CDC-HHS
NIFA-USDA