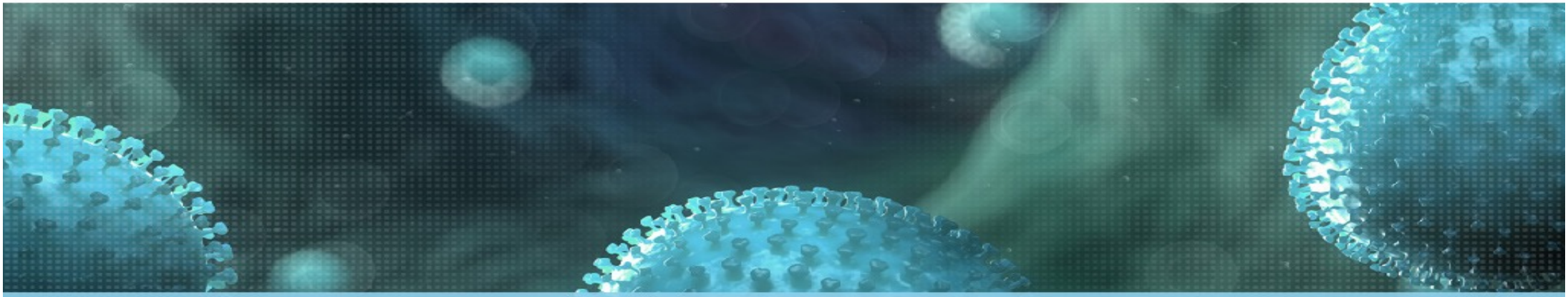
Erasmus MC

Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT



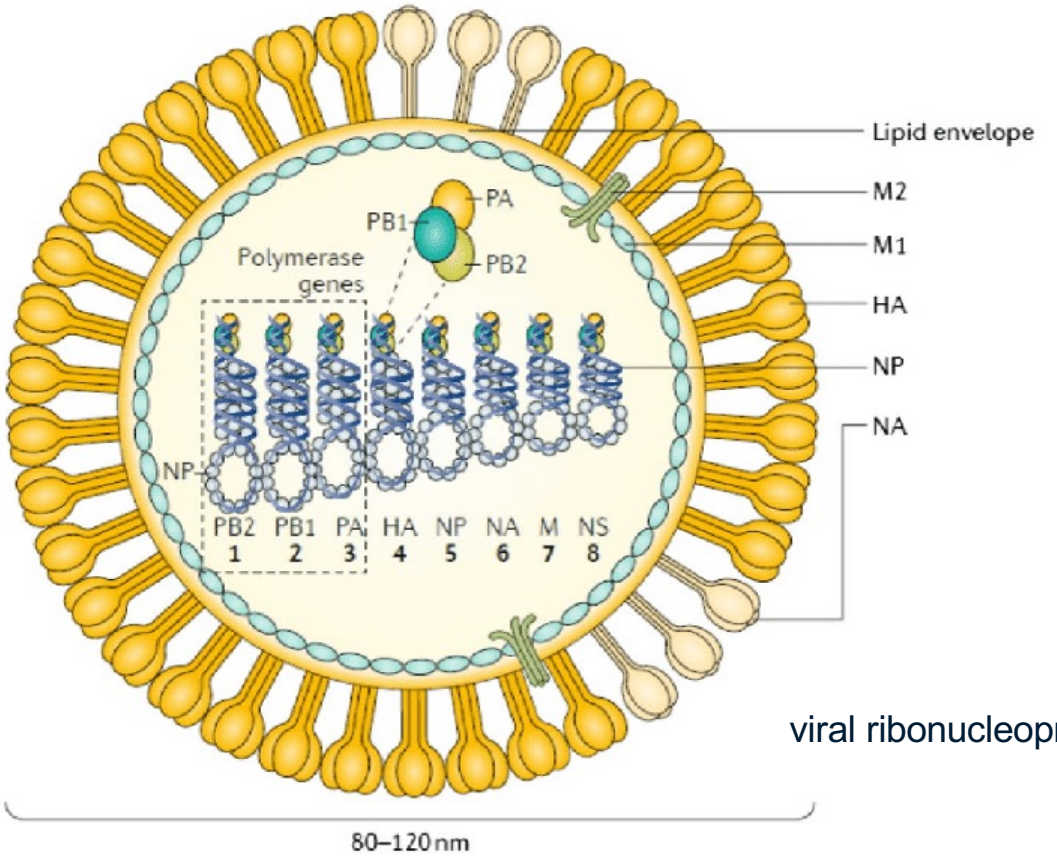
Mammalian adaptation of influenza A viruses

Croucher Course 2025, Hong Kong, July 15

Sander Herfst, PhD
Professor, Respiratory Virus Transmission

Influenza A virus

Structure



- Negative-sense ssRNA
- Segmented genome (8)

- **Different subtypes**
  - Nomenclature based on HA and NA
  - H1 - H19
  - N1 - N11

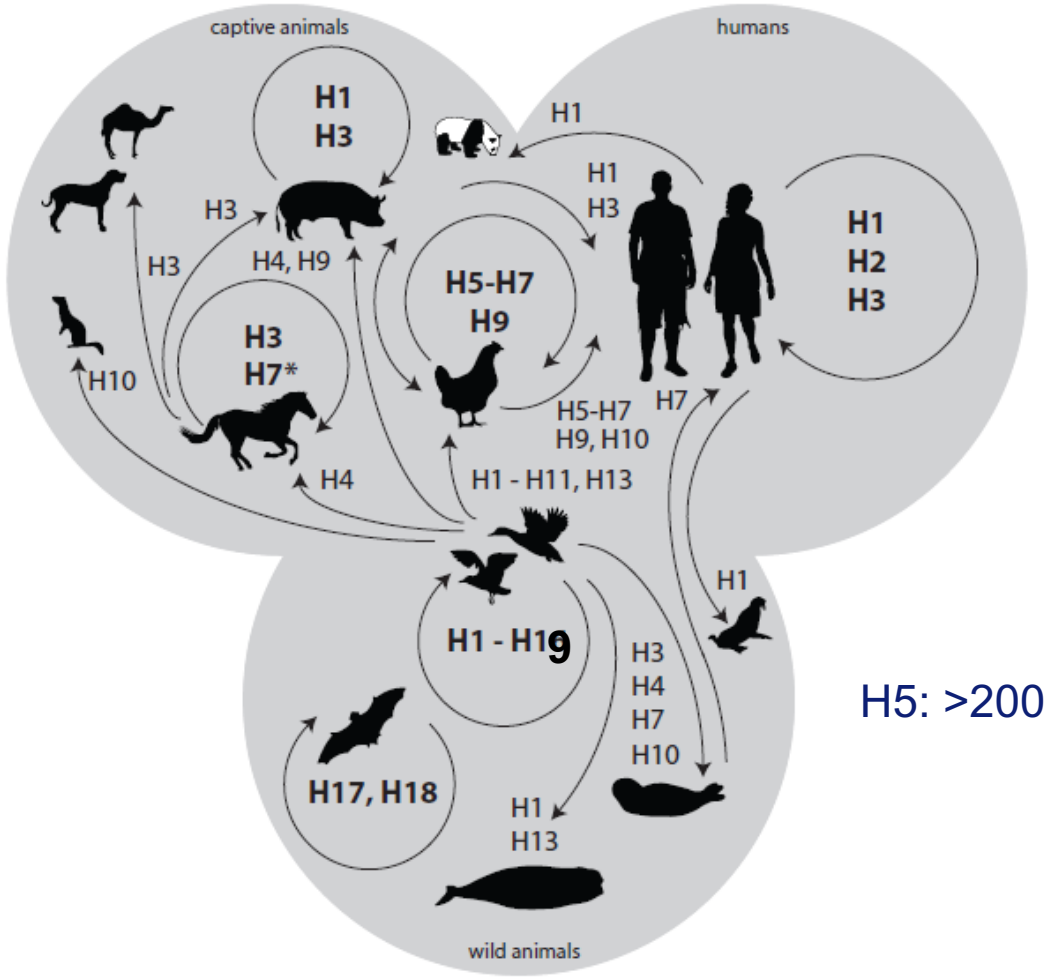
    e.g. H5N1

viral ribonucleoproteins (vRNPs)

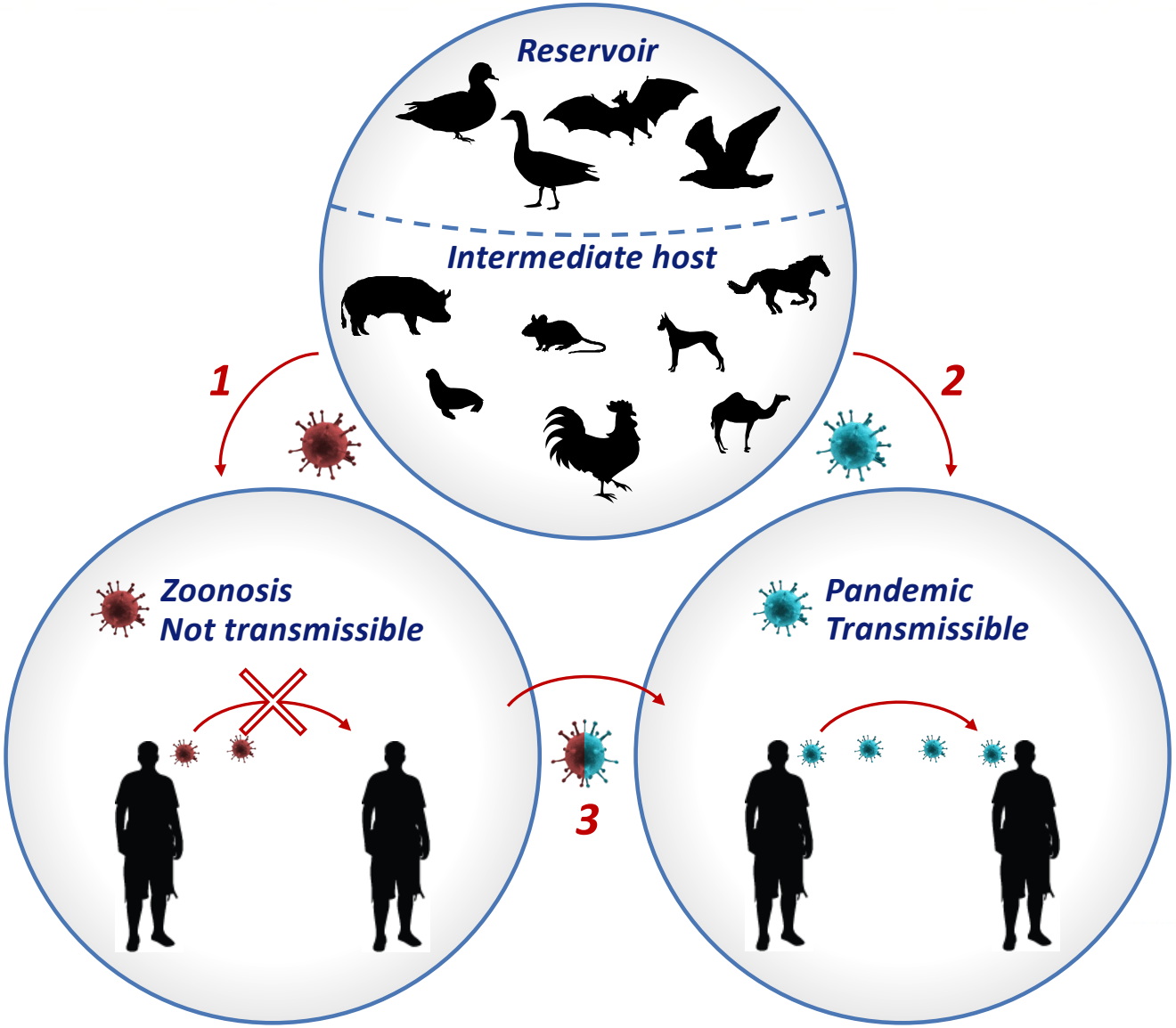
A DEPARTMENT OF Erasmus MC

Influenza A virus

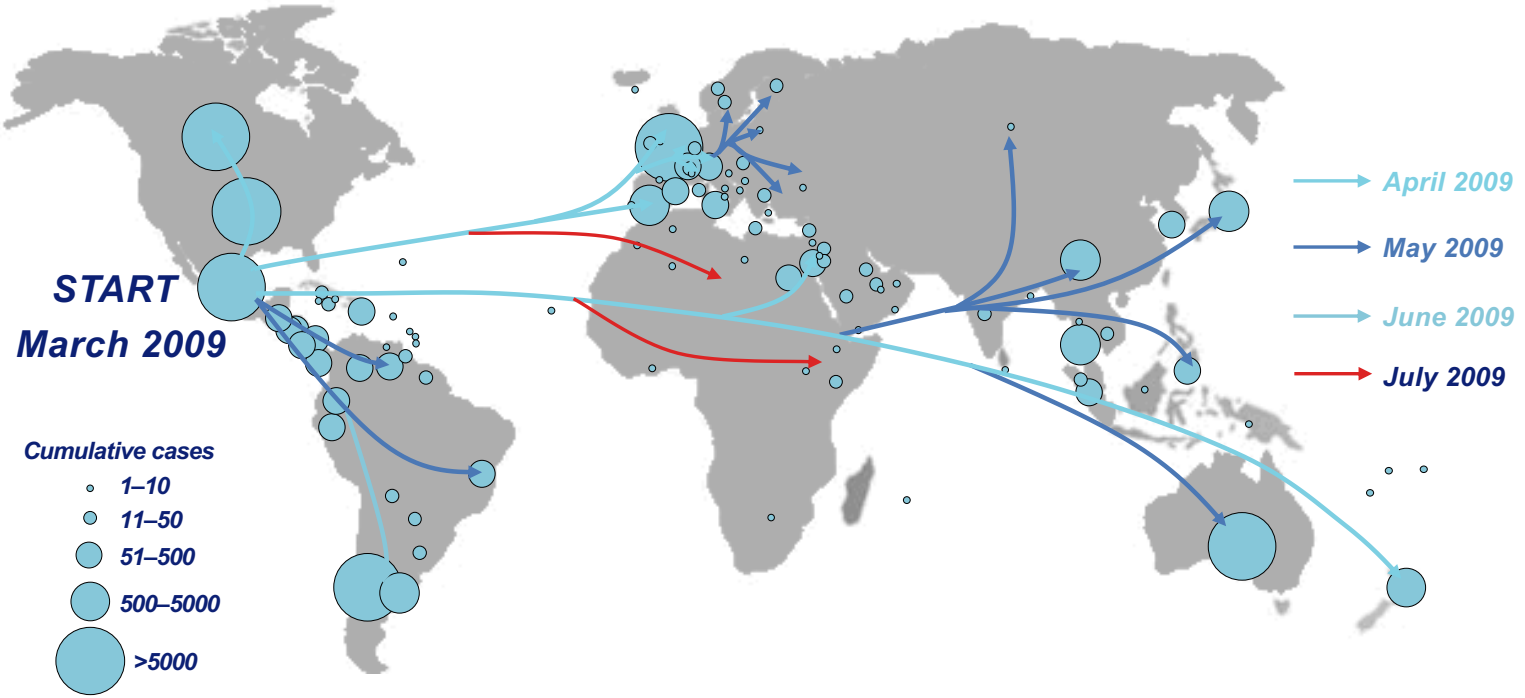
- Reservoir & zoonosis -



H5: >200 mammalian species

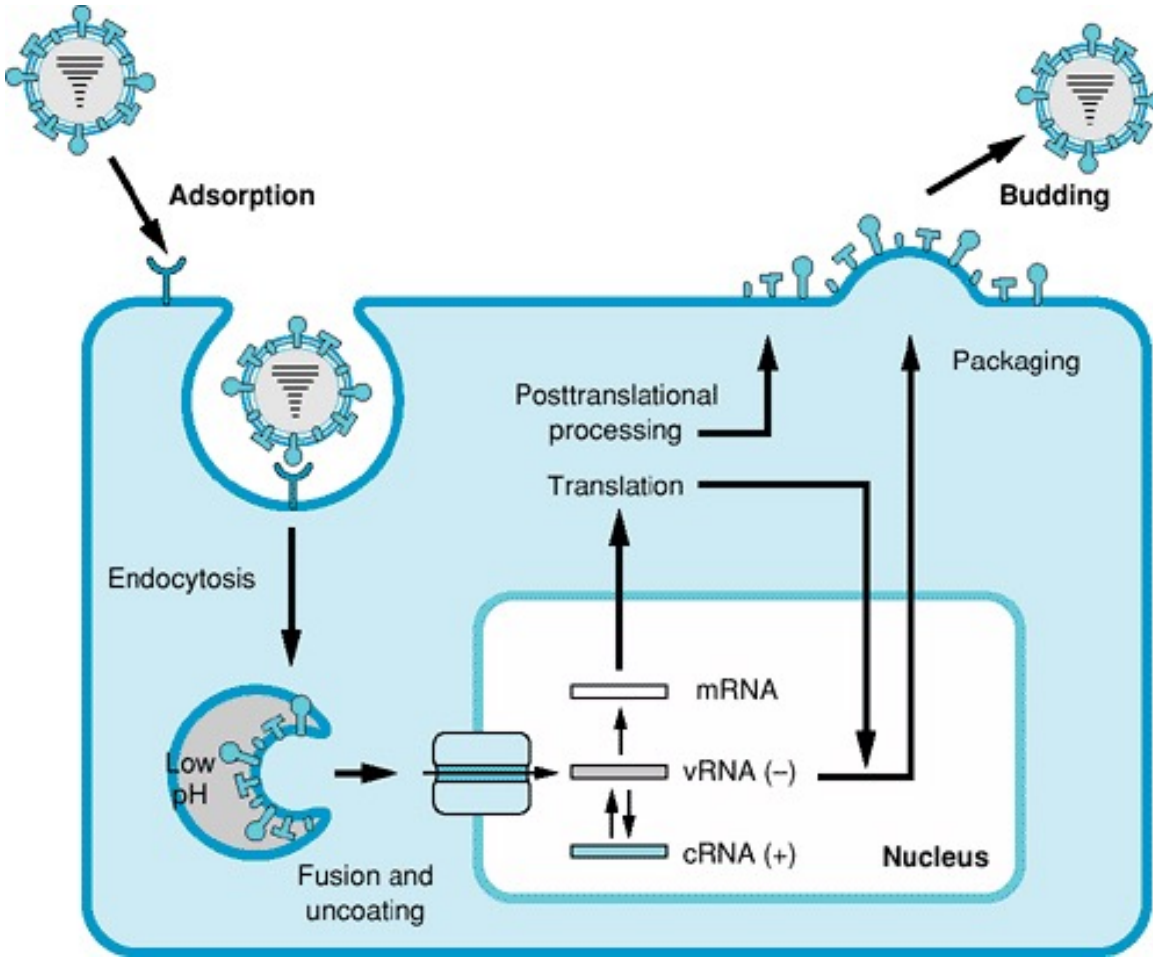

Reservoir
Intermediate host
1
2
3
Zoonosis
Not transmissible
Pandemic
Transmissible

Spread of pandemic H1N1 in 2009



A DEPARTMENT OF Erasmus MC

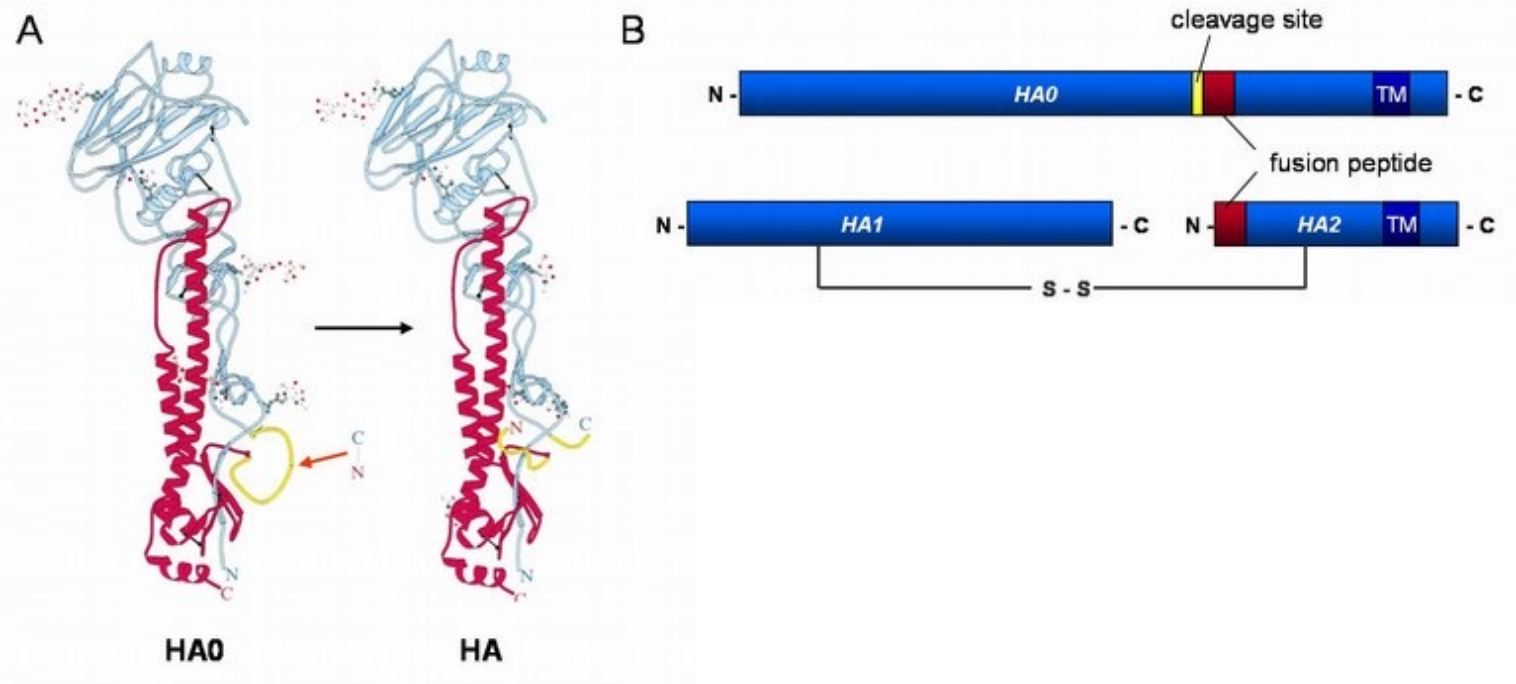
Influenza A virus
- Replication -



VIROSCIENCE LAB
WHERE SKILLS MEET TO STUDY & PROTECT
PARTMENT OF Erasmus MC

Hemagglutinin
- Fusion / Cleavage -

HA0 → HA1 + HA2 (trypsin-like proteins in respiratory tract)



Hemagglutinin
- Differences in host range -



Avian flu

Target:
Intestinal tract

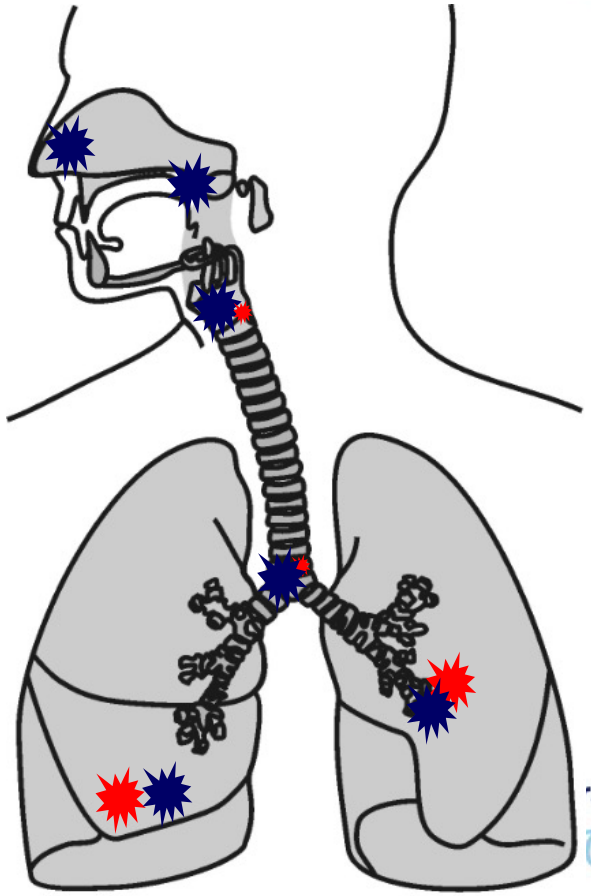
Receptor:
Neu5Ac-**α2,3**-Gal



Human flu

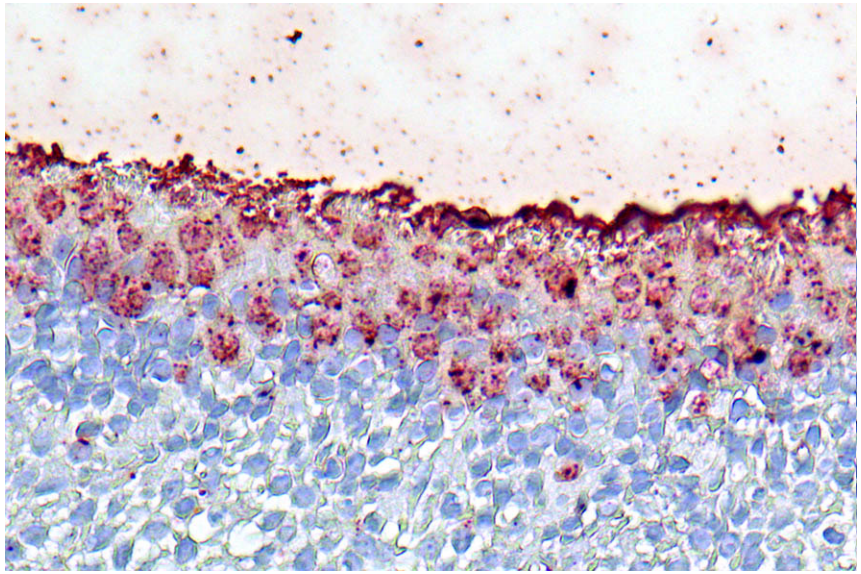
Target:
Upper airways

Receptor:
Neu5Ac-**α2,6**-Gal



asmus MC

Virus histochemistry



= FITC-labeled influenza A virus

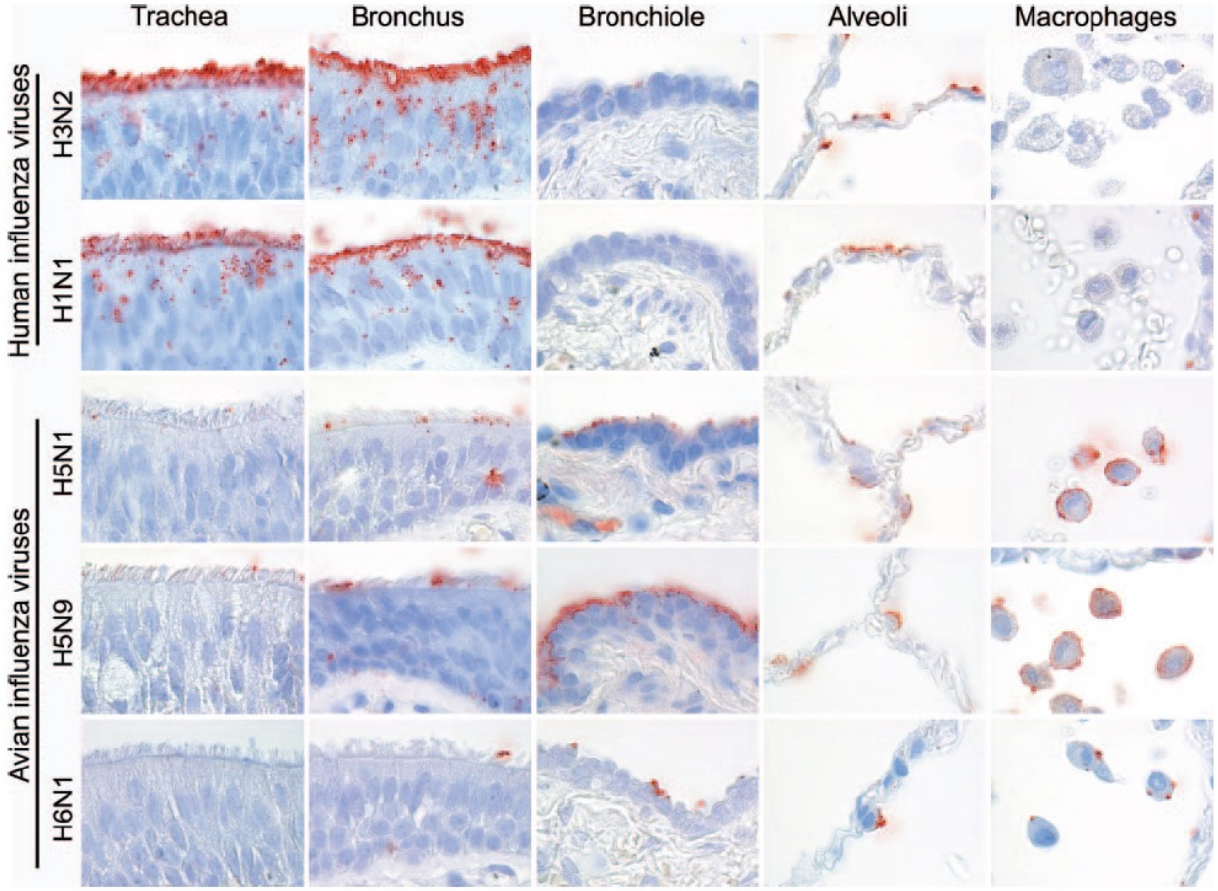
= Rabbit anti FITC-HRP

Van Riel, Science (2006)

A DEPARTMENT OF Erasmus MC

Influenza A virus

- Host differences in sialic acid receptors -

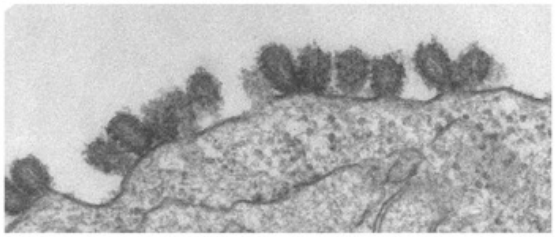


URT: **α2,6** SA
LRT: **α2,3** SA

Van Riel et al., Science (2006)

A DEPARTMENT OF Erasmus MC

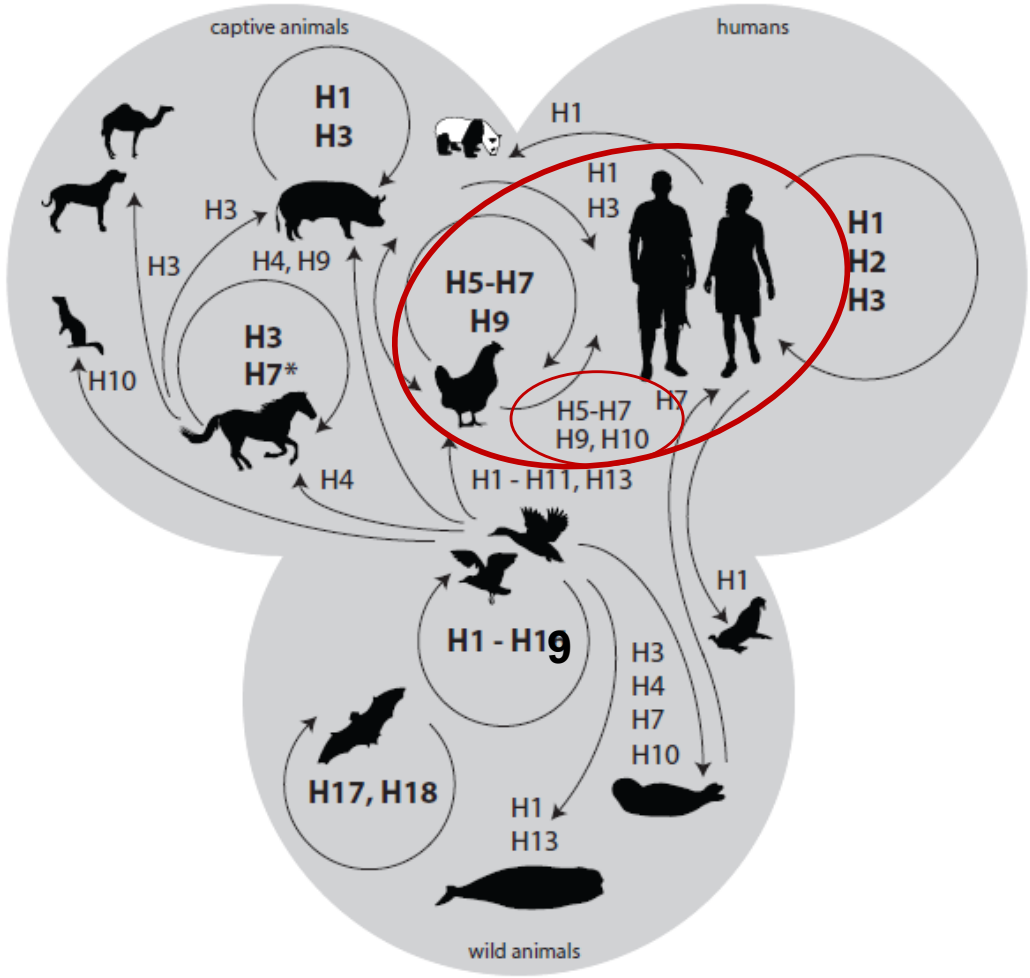
Influenza A virus
- Replication -



VIROSCIENCE LAB
WHERE SKILLS MEET TO STUDY & PROTECT
DEPARTMENT OF Erasmus MC

Influenza A virus

- Reservoir & zoonosis -



DEPARTMENT OF Erasmus MC

Avian influenza
-Pathogenicity -

LPAI:

- Low pathogenic avian influenza
- Common in wild birds (and poultry)
- Decreased egg production in poultry
- Mild respiratory symptoms in poultry

HPAI:

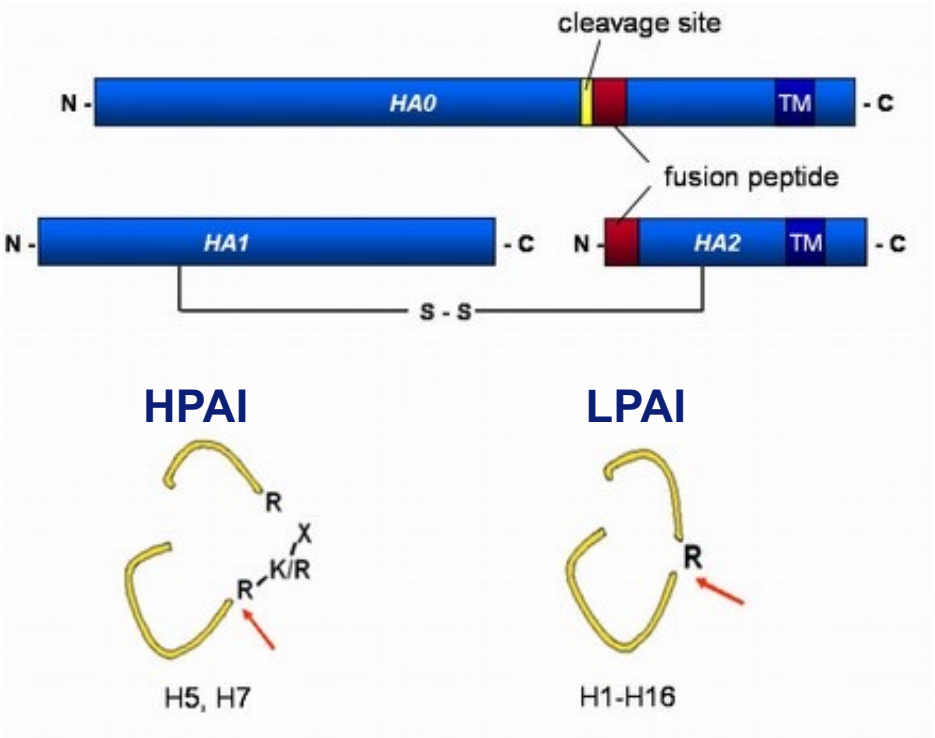
- Highly pathogenic avian influenza
- H5 and H7 subtypes
- Arises in poultry
- Mortality up to 100% in poultry
- Multi basic cleavage site

Highly pathogenic avian influenza in poultry

- HA cleavage site is main determinant of virulence -

LPAI (wild birds and poultry)
- Trypsin-like proteases
- Replication in resp/intestinal tracts

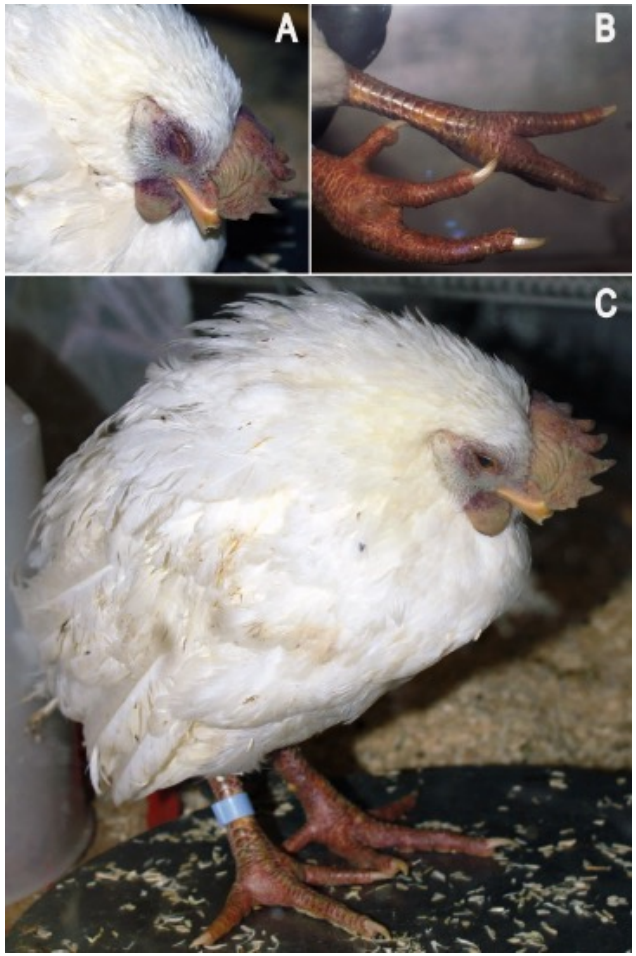
HPAI (poultry)
- HA cleavage by multiple proteases
- Replication systemic
- Restricted to H5 and H7 subtypes



A DEPARTMENT OF Erasmus MC

Basic cleavage site

- Not specific to H5 and H7 (in the lab): H6 -



In nature only H5 and H7 viruses.

Introduction of MBCS in H6 in the lab:

-HA cleaved in absence of trypsin
-Virus replicated in absence of trypsin
-Cyanosis: discoloration of the skin due to poor circulation or inadequate oxygenation of the blood.

H6 IVPI:
0 → 1.4 (>1.2)

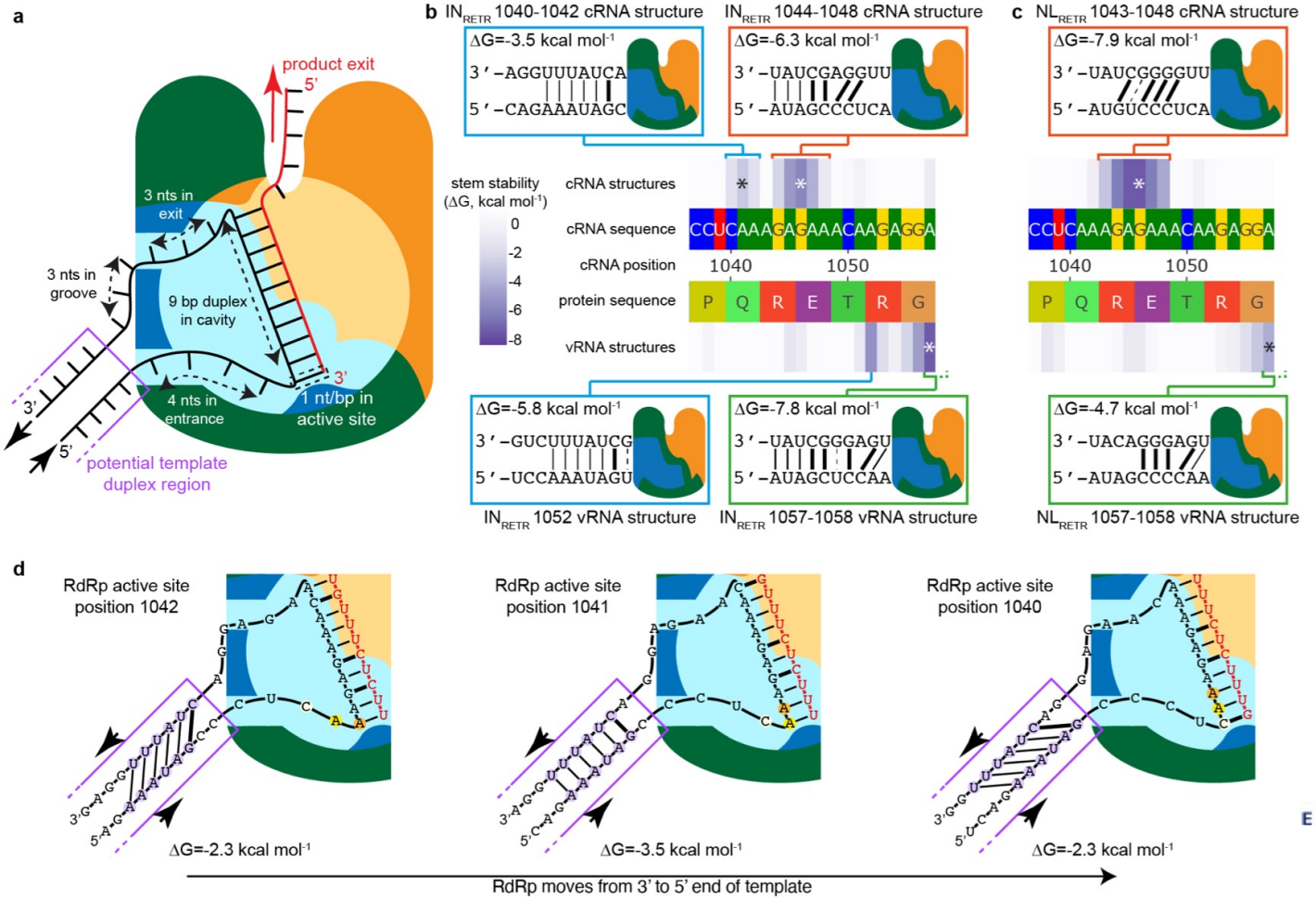
Not in human H3N2 in ferrets!

Schrauwen et al., J Gen Virol, 2011

Munster et al., J Virol, 2010

A DEPARTMENT OF Erasmus MC

Transient RNA structures underlie highly pathogenic avian influenza virus genesis



Funk et al., 2025

bioRχiv
THE PREPRINT SERVER FOR BIOLOGY

ENT OF Erasmus MC

Birdflu in Asia, Africa, Middle-East, Europe
- H5N1 -



-May 1997;1 child infected, died

-Virus identified as H5N1

(J.C. De Jong et al., Nature 389: 554 (1997))

-December 1997, 17 infected, 5 died

-Birds in markets infected with highly pathogenic avian influenza

-Birds in markets culled

>2003; Numerous countries, 3 continents

-Culling not always first option

-100s millions poultry deaths

-Transmission to mammals (incl. humans)

HPAI H5N1 virus

- A continuing threat -

Cumulative number of confirmed human cases† for avian influenza A(H5N1) reported to WHO, 2003-2025

Country	2003-2009*		2010-2014*		2015-2019*		2020-2024*		2025		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Australia	0	0	0	0	0	0	1	0	0	0	1	0
Azerbaijan	8	5	0	0	0	0	0	0	0	0	8	5
Bangladesh	1	0	6	1	1	0	0	0	2	0	10	1
Cambodia	9	7	47	30	0	0	16	6	3	3	75	46
Canada	0	0	1	1	0	0	1	0	0	0	2	1
Chile	0	0	0	0	0	0	1	0	0	0	1	0
China	38	25	9	5	6	1	3	1	1	0	57	32
Djibouti	1	0	0	0	0	0	0	0	0	0	1	0
Ecuador	0	0	0	0	0	0	1	0	0	0	1	0
Egypt	90	27	120	50	149	43	0	0	0	0	359	120
India	0	0	0	0	0	0	1	1	1	1	2	2
Indonesia	162	134	35	31	3	3	0	0	0	0	200	168
Iraq	3	2	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	2	2	0	0	0	0	1	0		0	3	2
Mexico	0	0	0	0	0	0	0	0	1	1	1	1
Myanmar	1	0	0	0	0	0	0	0	0	0	1	0
Nepal	0	0	0	0	1	1	0	0	0	0	1	1
Nigeria	1	1	0	0	0	0	0	0	0	0	1	1
Pakistan	3	1	0	0	0	0	0	0	0	0	3	1
Spain	0	0	0	0	0	0	2	0	0	0	2	0
Thailand	25	17	0	0	0	0	0	0	0	0	25	17
Turkey	12	4	0	0	0	0	0	0	0	0	12	4
United Kingdom of Great Britain and Northern Ireland	0	0	0	0	0	0	5	0	1	0	6	0
United States of America**	0	0	0	0	0	0	68	1	3	0	71	1
Viet Nam	112	57	15	7	0	0	2	1	1	0	130	65
Total	**468**	**282**	**233**	**125**	**160**	**48**	**102**	**10**	**13**	**5**	976	470

*2003-2009, 2010-2014, 2015-2019 and 2020-2024 total figures. Breakdowns by year available on subsequent tables.
** For the United States of America, in 2024, cases reported as A(H5) are included here.
†This count includes reported detections in asymptomatic individuals. In some cases, the confirmation of infection versus transient contamination of the nasopharynx/oropharynx with virus particles after exposure to infected birds or contaminated environment remains inconclusive. Total number of cases includes number of deaths. Counts are according to reporting country which may differ from country where exposure may have occurred.
WHO reports only laboratory-confirmed cases. All dates refer to onset of illness.
Source: WHO/GIP, data in HQ as of 27 May 2025.

World Health Organization

Lethal in ~50%?
Probably not…

TMENT OF Erasmus MC

Birdflu in Asia, Africa, Middle-East, Europe
- H5N1 -



Why does this virus infect mammals?

Why does this virus kill mammals?

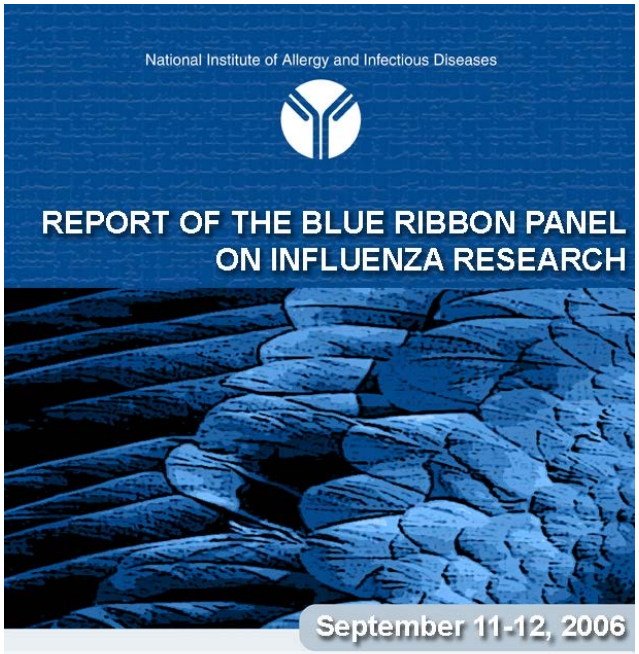
Do antiviral drugs work, can the virus become resistant?

Do current vaccines provide protection, can the virus escape?

How does this virus spread in animals (and humans)

Can this virus cause the next pandemic?

Relevance of understanding virus properties that favor transmission

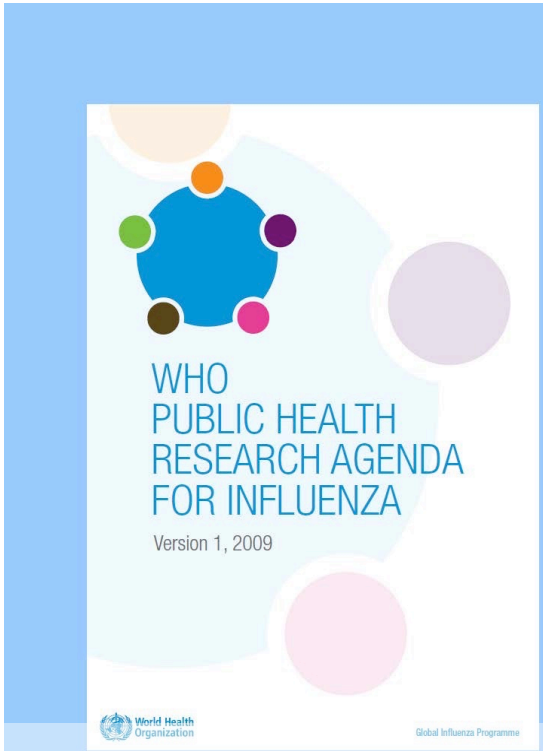


NIH recommended research on influenza virus transmission.

"Learning more about how influenza viruses circulate between animal reservoirs and about the evolutionary pressures that lead to the emergence and spread of new viral sub-types - especially the factors that favor transmission from animals to humans - are urgent research priorities."

A DEPARTMENT OF Erasmus MC

Relevance of understanding virus properties that favor transmission



WHO recommended research on the factors related to pandemics including transmissibility

1.1.1 Investigate virus-specific factors associated with zoonotic and pandemic potential (e.g., infectivity, transmissibility and pathogenicity)

1.1.2 Assess the animal host-specific factors associated with zoonotic and pandemic potential (e.g. infectivity, transmissibility and pathogenicity)

1.1.3 Study the environmental and animal management/husbandry-specific factors associated with zoonotic and pandemic potential (e.g. infectivity, transmissibility)

A DEPARTMENT OF Erasmus MC

Transmissible H5N1

- One million dollar question -

What does it take for an avian (H5N1) virus to cause a pandemic?

Reassortment with human flu?

Influenza
- Pandemics of the last century -



1918

"Spanish flu"

~50 million deaths

A(H1N1)



1957

"Asian flu"

~2 million deaths

A(H2N2)



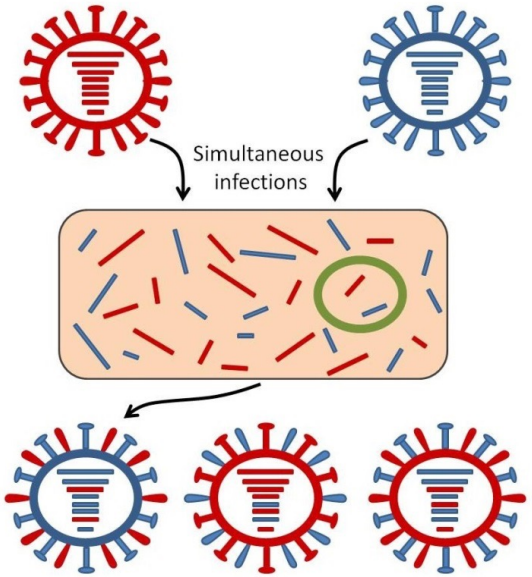
1968

"Hong Kong flu"

~1 million deaths

A(H3N2)

Reassortment



A DEPARTMENT OF Erasmus MC

Transmissible H5N1

- One million dollar question -

What does it take for an avian (H5N1) virus to cause a pandemic?

Reassortment with human flu?

How much does the virus need to mutate (and where)?

Airborne transmission
- Experimental approach -

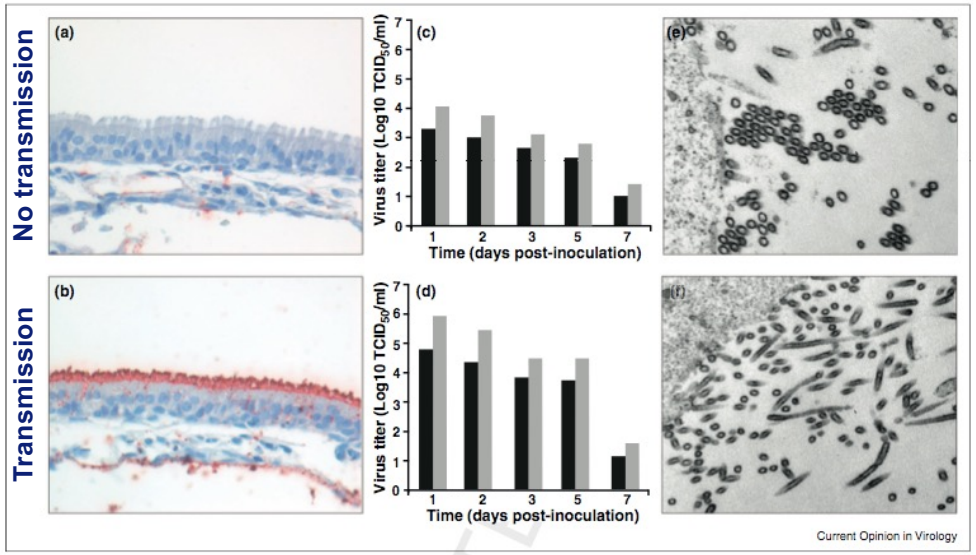
Hypothesis minimal requirements

Sorrell et al., Curr Opinion Virol (2011)

Airborne transmission
- Experimental approach -

Hypothesis minimal requirements

- **Better attachment to and infection of cells in the URT**
- **Increased replication in the URT**
- **Efficient release of virus particles**



Sorrell et al., Curr Opinion Virol (2011)

A DEPARTMENT OF Erasmus MC

Infectious disease research
- Two approaches -

Loss-of-function
Take virulent/transmissible/resistant virus and test by e.g. mutational analyses

Gain-of function
Take non-virulent/non-transmissible/sensitive virus and test by e.g. mutational analyses





Erasmus MC

HPAI A/H5N1 virus

- Requirements for H2H transmission -

Hypothesis minimal requirements

- **Better attachment to and infection of cells in the** URT ✓ HA-Q222L,G224S
- **Increased replication in the URT** ✓ PB2-E627K
- **Efficient release of virus particles**

Avian virus polymerase cannot replicate in mammalian cells because PB2 is incompatible

Nature. 1977 Dec 15;270(5638):617-8. doi: 10.1038/270617a0.

A single gene determines the host range of influenza virus

J W Almond

But E627K fixes the problem…

JOURNAL OF VIROLOGY, Apr. 1993, p. 1761–1764 Vol. 67, No. 4
0022-538X/93/041761-04$02.00/0
Copyright © 1993, American Society for Microbiology

A Single Amino Acid in the PB2 Gene of Influenza A Virus Is a Determinant of Host Range

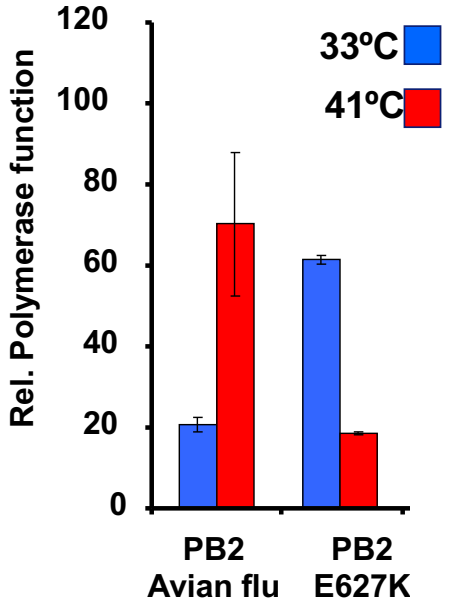
E. KANTA SUBBARAO,[1]* WILLIAM LONDON,[2] AND BRIAN R. MURPHY[1]

HPAI A/H5N1 virus

- Requirements for H2H transmission -

Hypothesis minimal requirements

- **Better attachment to and infection of cells in the** URT ✓ HA-Q222L,G224S
- **Increased replication in the URT** ✓ PB2-E627K
- **Efficient release of virus particles**



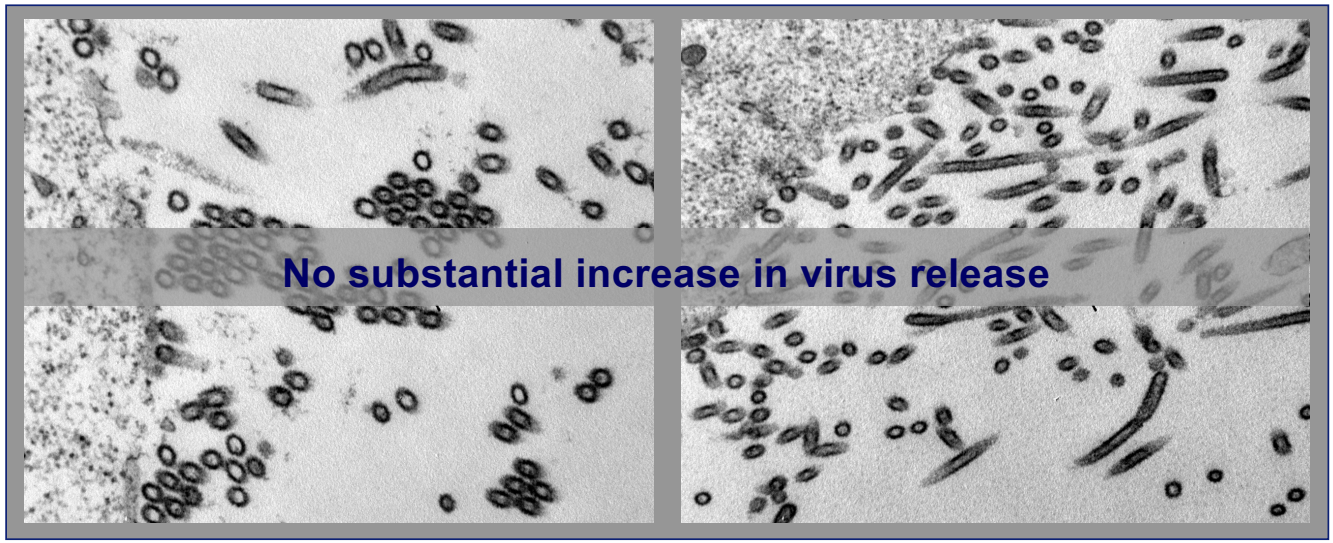
MENT OF Erasmus MC

HPAI A/H5N1 virus

- Requirements for H2H transmission -

Hypothesis minimal requirements

- **Better attachment to and infection of cells in the** URT ✓ HA-Q222L,G224S
- **Increased replication in the URT** ✓ PB2-E627K
- **Efficient release of virus particles** X



The ferret model for influenza

- Risk assessment, pathogenesis, transmission, vaccination/challenge, serology -



Susceptible to natural infection
Anatomy of airways similar to humans
Respiratory disease and lung pathology similar to humans
Patterns of virus attachment similar to humans

Airborne transmission

- Ferret model -



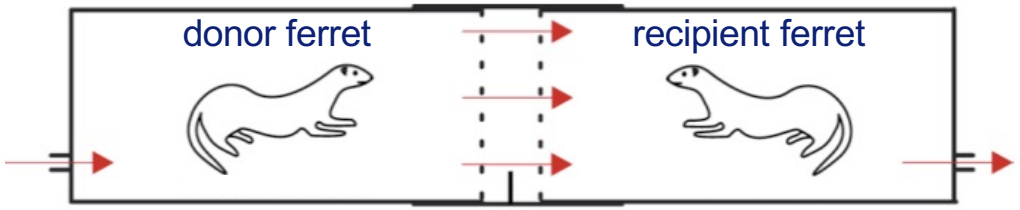
= α 2,3 SA = α 2,6 SA

A DEPARTMENT OF Erasmus MC

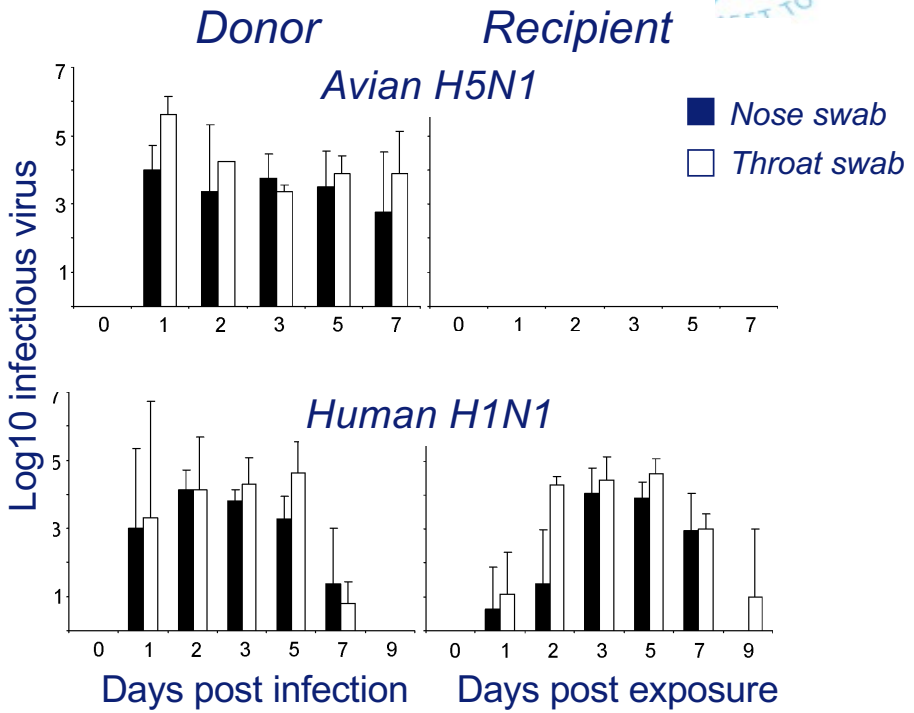
Airborne transmission

- H5N1 HA Q222L,G224S PB2 E627K -

ABSL3+





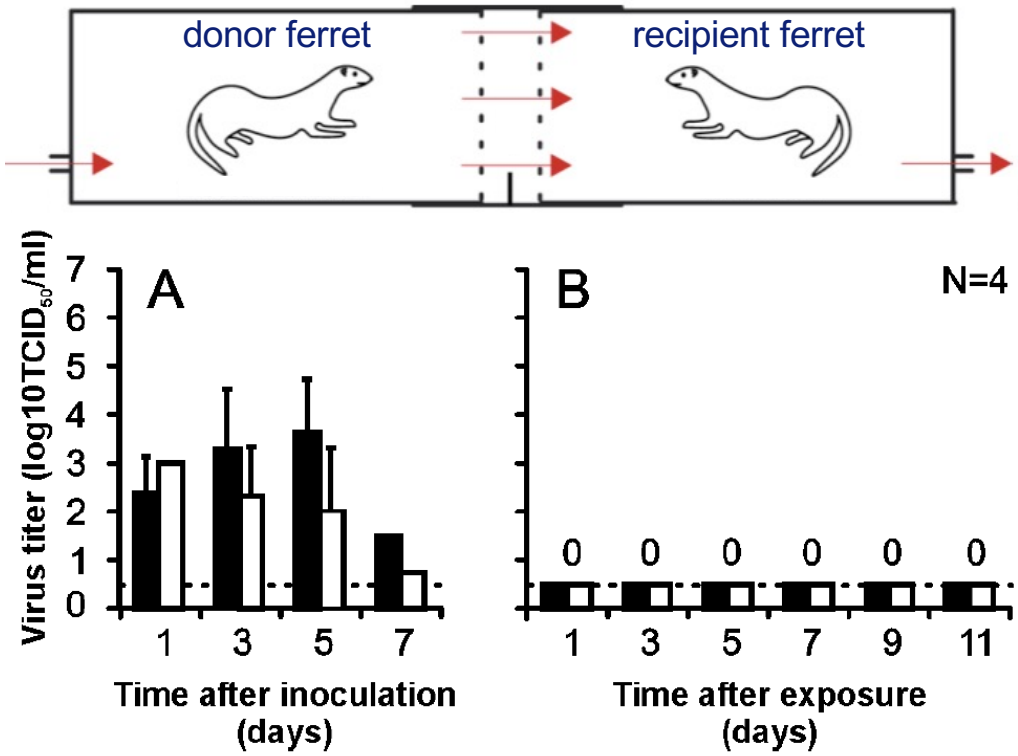


Richard et al., Nat Commun, 2020 – SARS-2
Kutter et al., Nat Commun, 2021 – SARS-2
De Vries et al., Science, 2021 – SARS-2

Munster et al., Science, 2009 – pH1N1
Herfst et al., Science, 2012 – H5N1
Richard et al., Nature, 2013 – H7N9
Linster et al., Cell, 2014 – H5N1

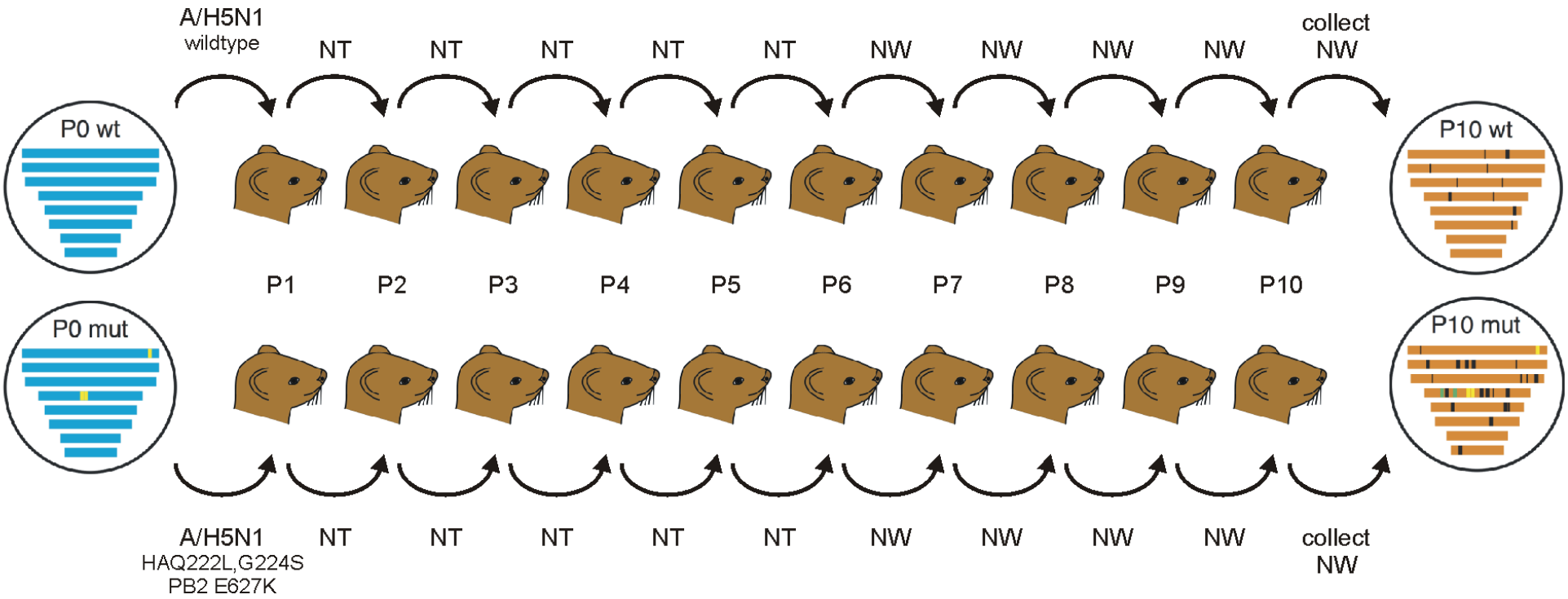
Airborne transmission

- H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ -



Herfst et al., Science (2012)

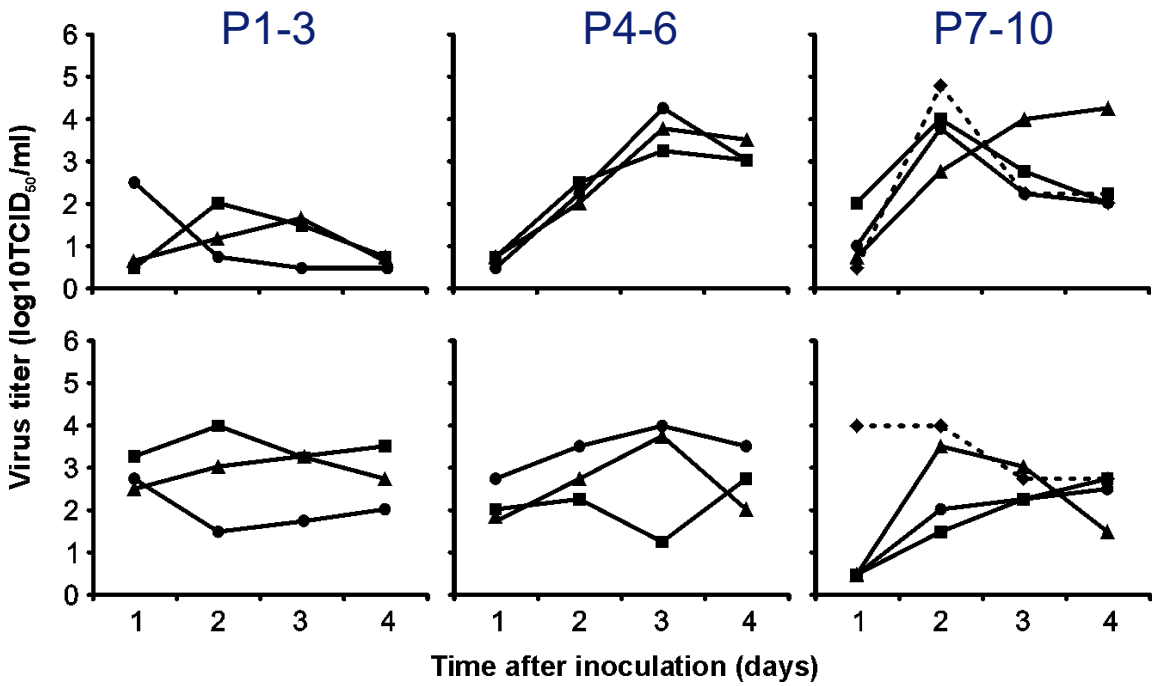
Serial passaging in ferrets
- A/Indonesia/5/05 -



A DEPARTMENT OF Erasmus MC

Serial passaging in ferrets

- Virus shedding from the nose -



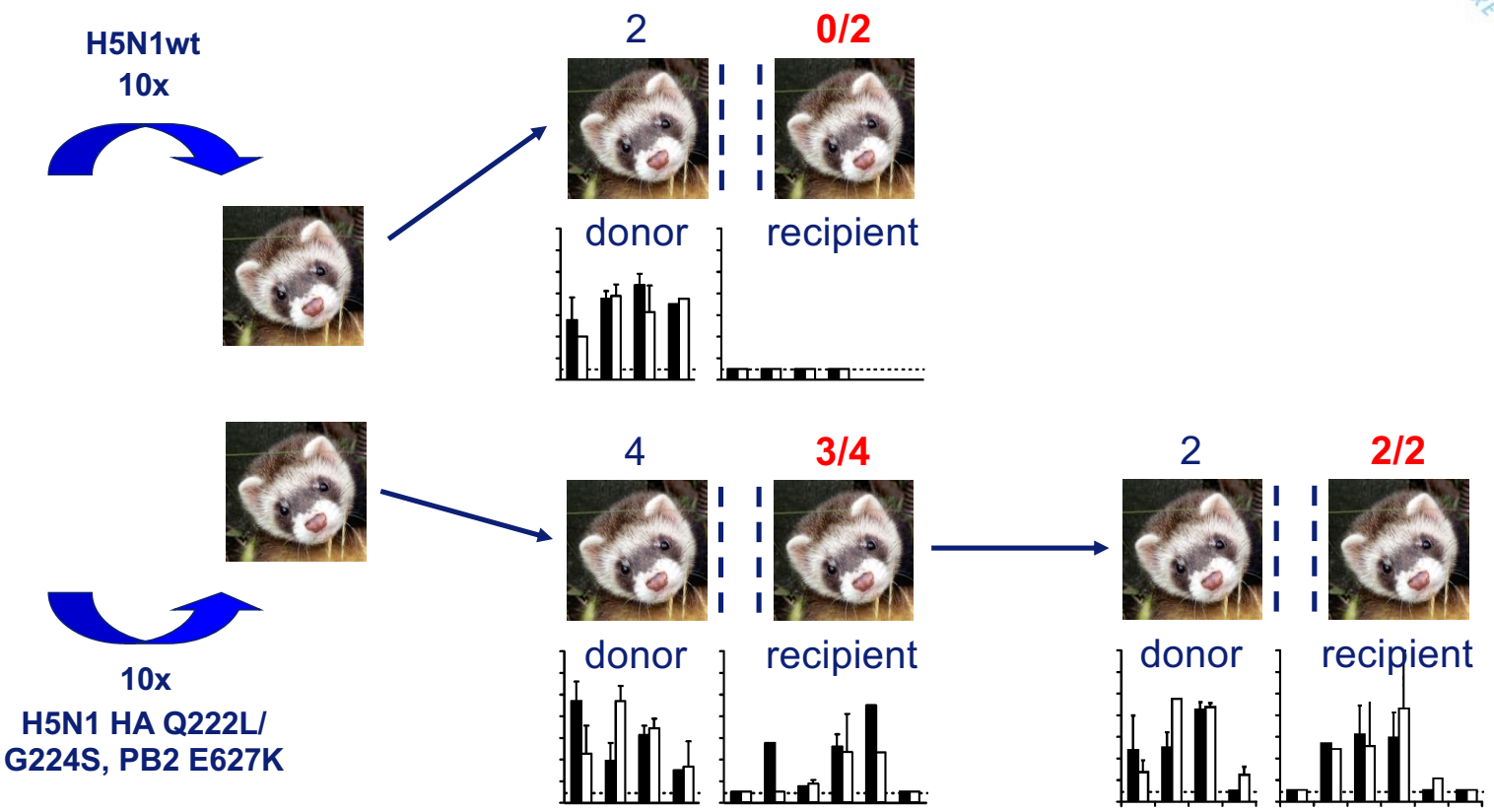
H5N1
HA Q222L/
G224S
PB2 E627K

H5N1wt

A DEPARTMENT OF Erasmus MC

Airborne transmission

- Passaged viruses -



Herfst et al., Science (2012)

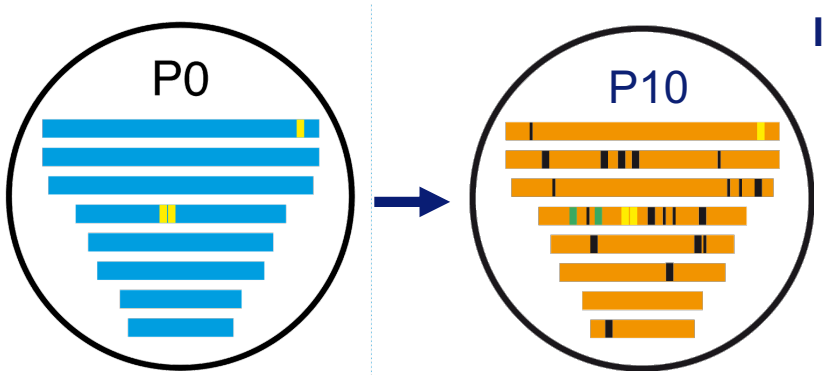
A DEPARTMENT OF Erasmus MC

A/H5N1 virus transmission

- Sequence analysis of airborne viruses -



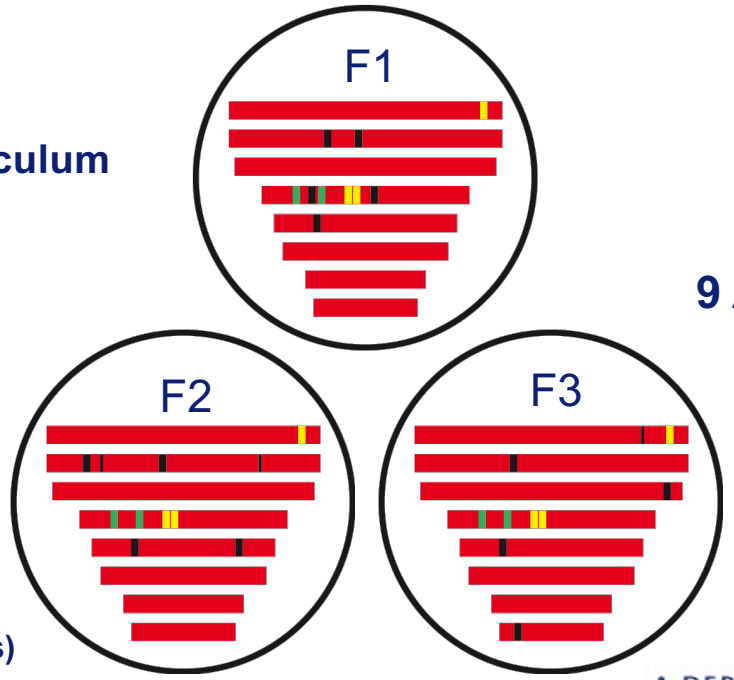
A/H5N1 HA
Q222L,G224S
PB2 E627K



454 deep sequencing:
Numerous substitutions
associated with adaptation

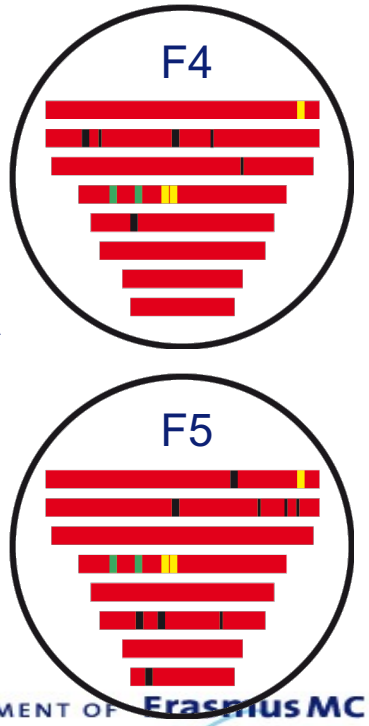
Inoculum

Transmission 1



Transmission 2

9 AA



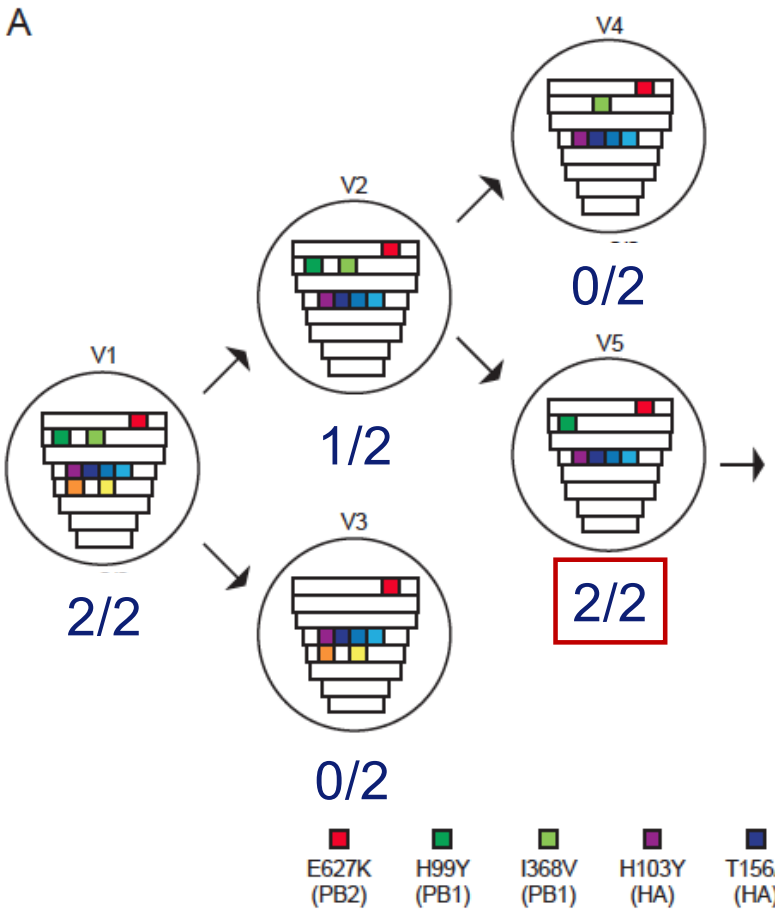
- **Silent substitution**
- **Amino acid change**
- **Introduced (mutagenesis)**
- **Consistent in airborne viruses**

Linster et al. Cell, 2014

A DEPARTMENT OF Erasmus MC

H5N1 virus transmission

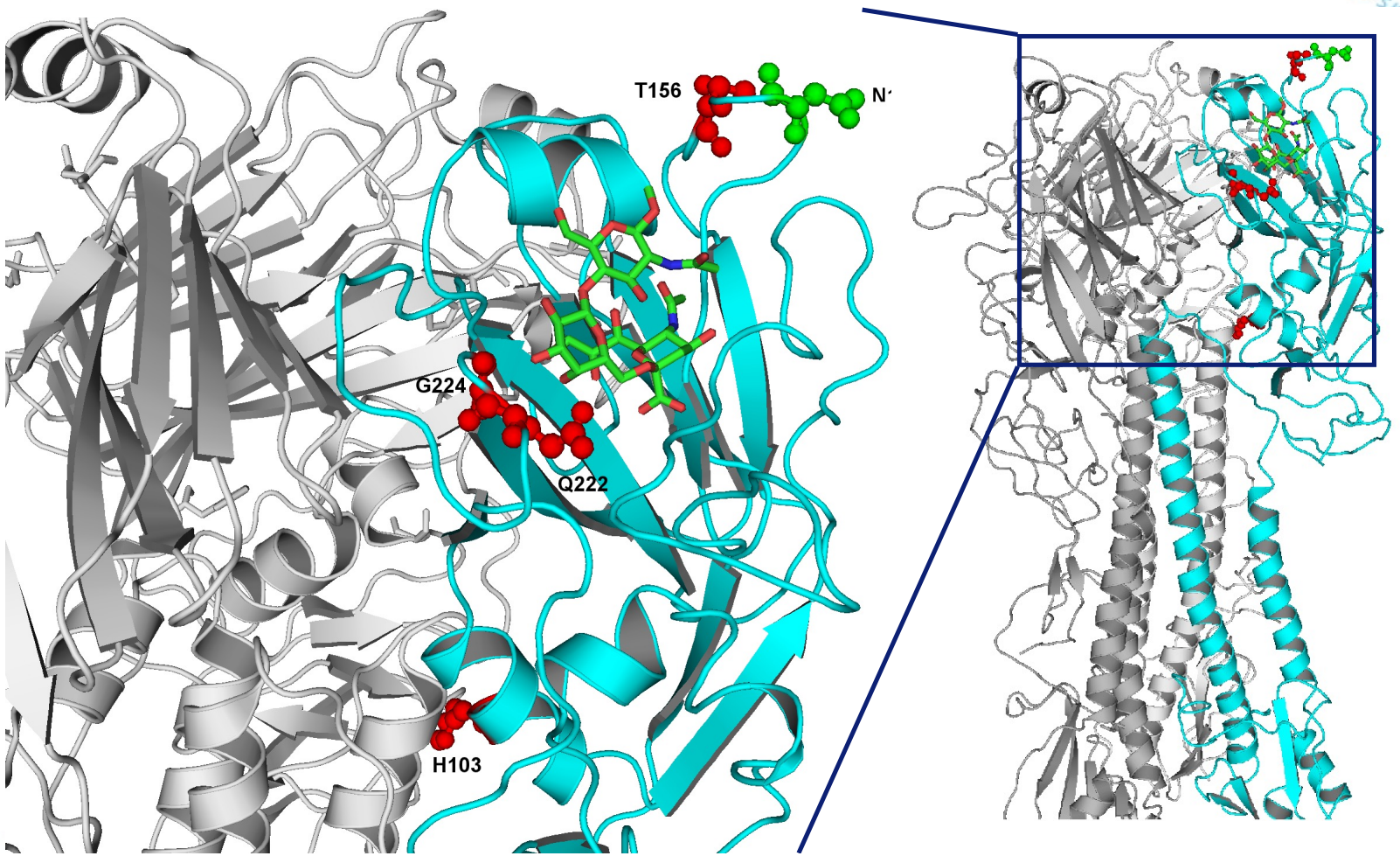
- A minimal set of mutations defined -



*PB2 E627K**
PB1 H99Y
*HA Q222L**
*HA G224S**
HA H103Y
HA T156A

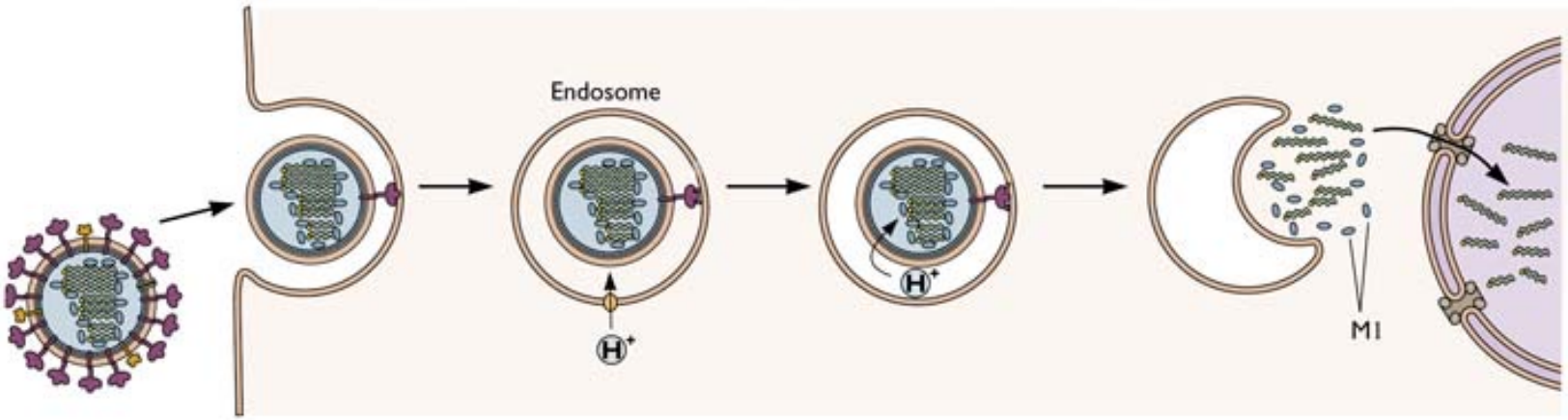
Linster et al. Cell, 2014

Airborne-transmissible A/H5N1
- Mutations in HA -



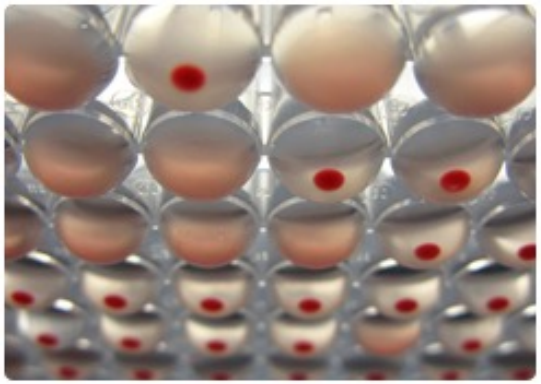
Airborne-transmissible A/H5N1

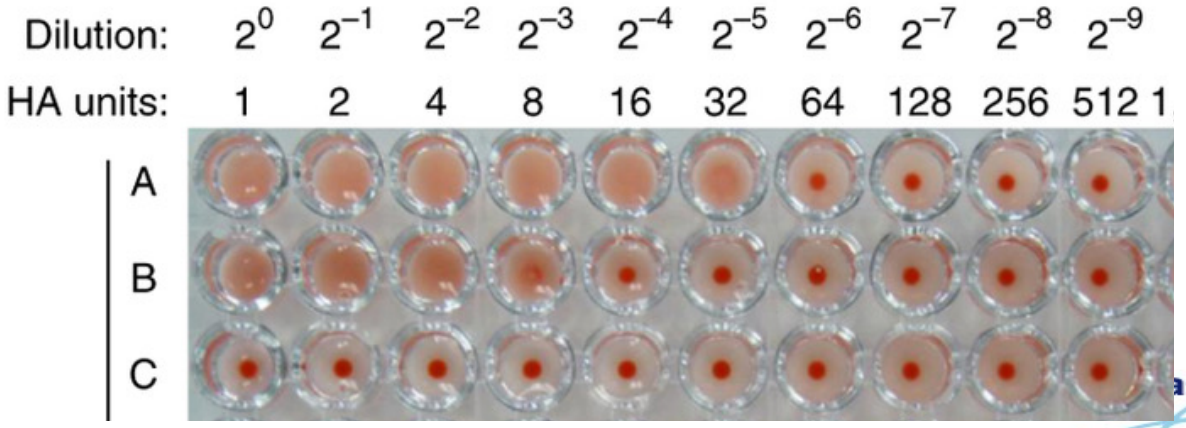
- Phenotypic analysis, binding & fusion -



Airborne A/H5N1 virus

- Receptor binding to red blood cells -





Airborne A/H5N1 virus

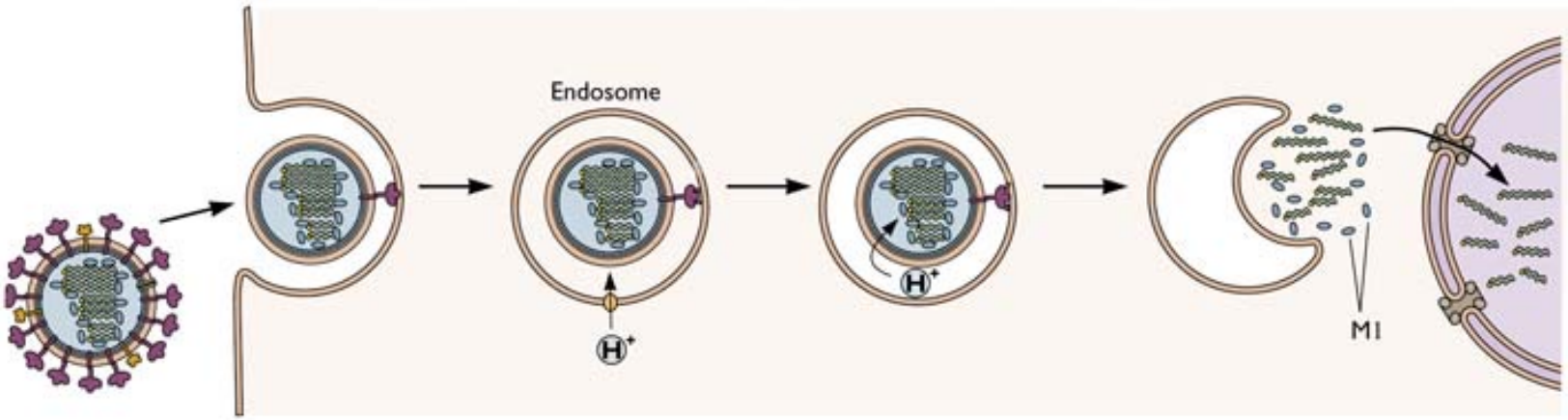
- Receptor binding to red blood cells -

VIRUS	SUBTYPE	TRBC	2,3 SA (avian)	2,6 SA (human)
A/Netherlands/213/03	H3N2	64	0	64
A/H5N1	H5N1	64	16	0
A/H5N1$_{\text{HA Q222L,G224S}}$	H5N1	64	0	24
A/H5N1$_{\text{HA H103Y, T156A}}$	H5N1	64	48	0
A/H5N1$_{\text{HA H103Y, T156A, Q222L,G224S}}$	H5N1	64	4	32

Linster et al. Cell, 2014

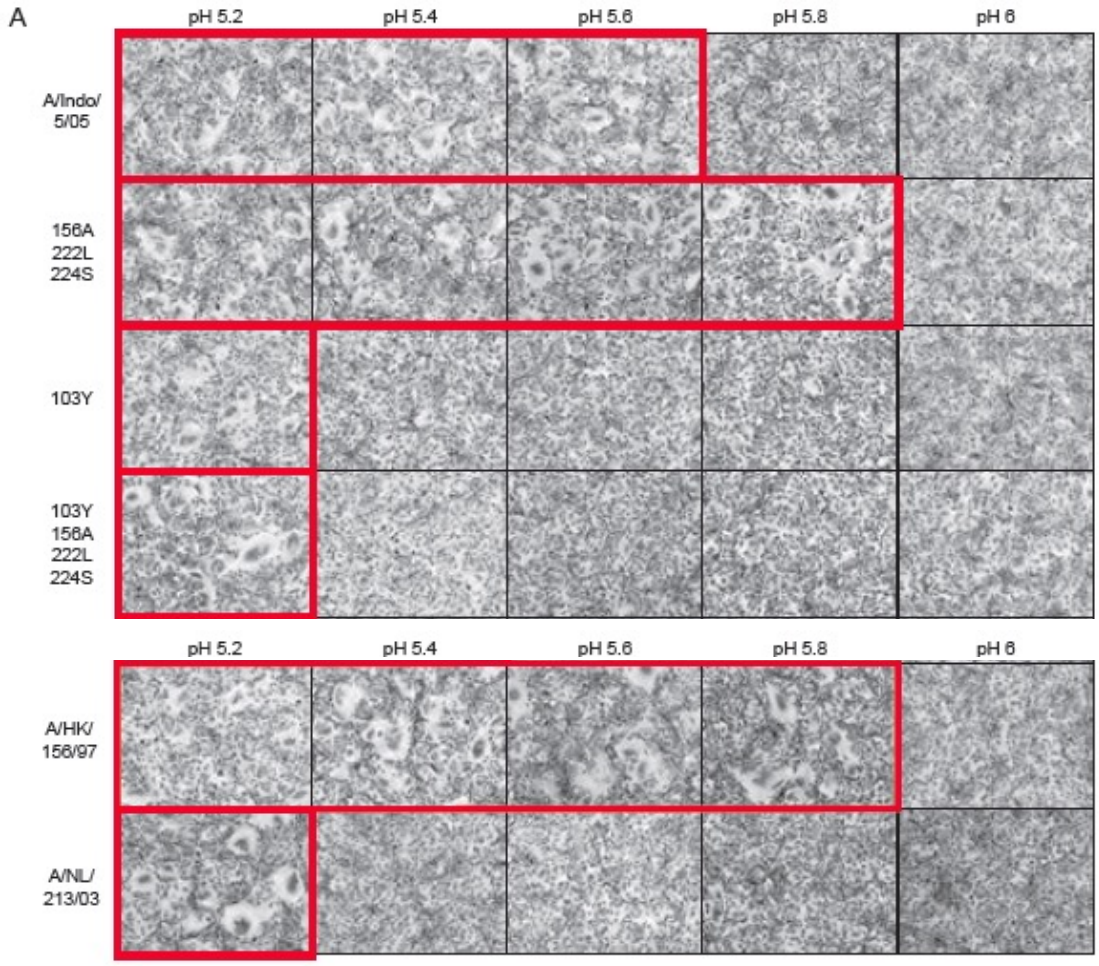
Airborne-transmissible A/H5N1

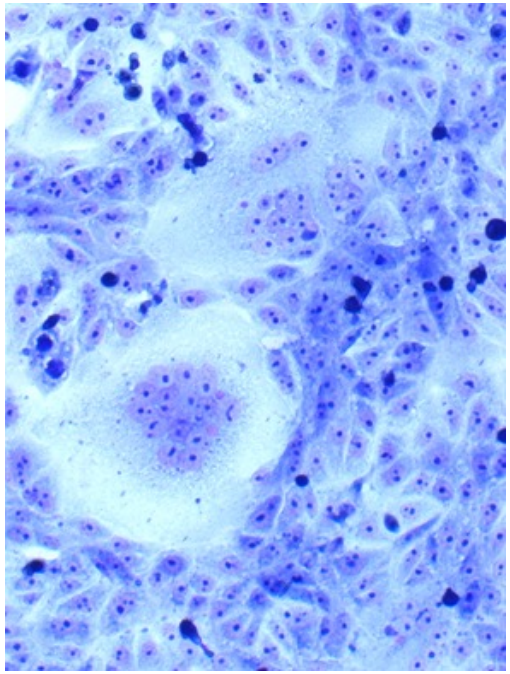
- Phenotypic analysis, binding & fusion -



Airborne-transmissible A/H5N1

- HA: fusion and stability -

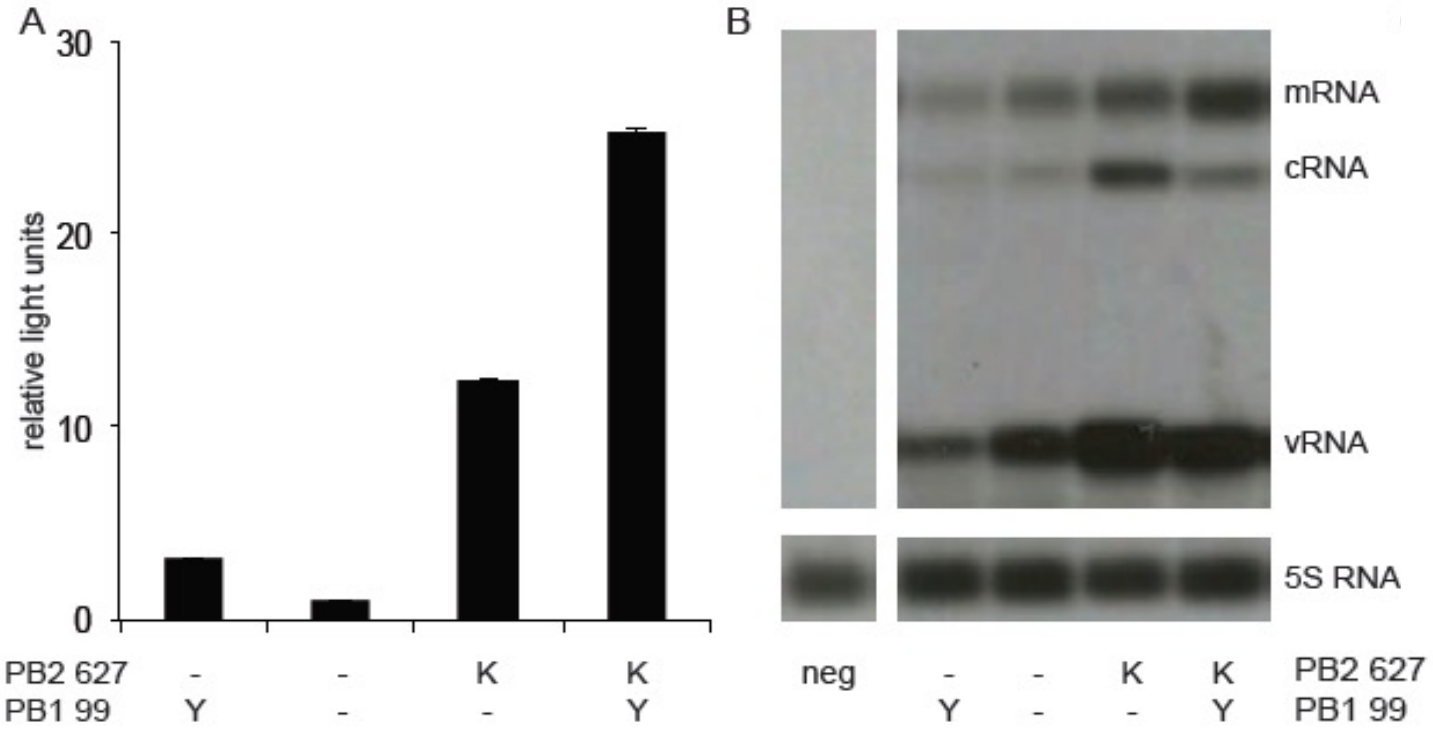


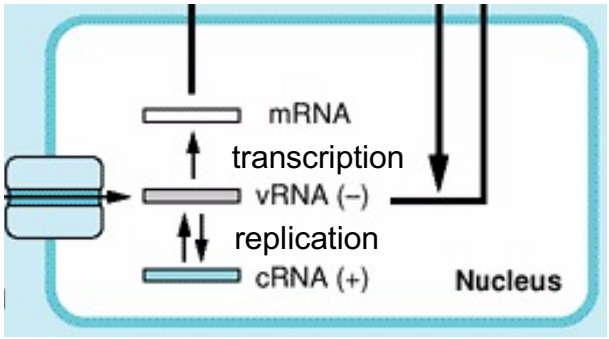


Linster et al. Cell, 2014

A/H5N1 virus transmission

- Polymerase adaptation -



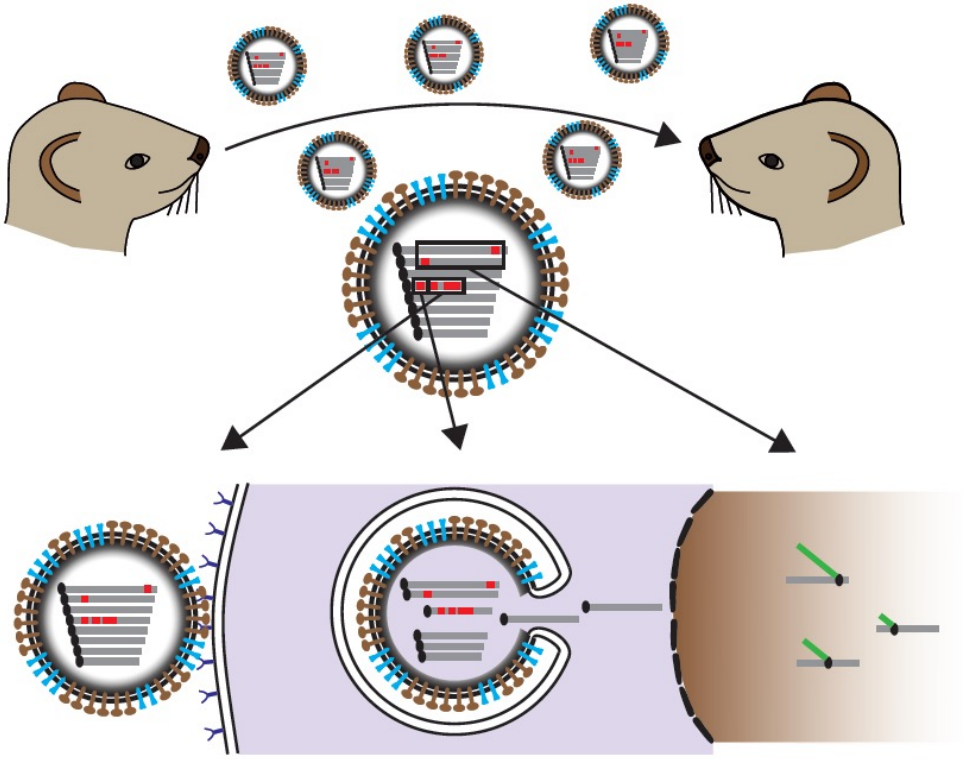


Linster et al. Cell, 2014

A DEPARTMENT OF Erasmus MC

Influenza A/H5N1 virus transmission

- 5 or 6 mutations -



Binding Fusion / stability replication

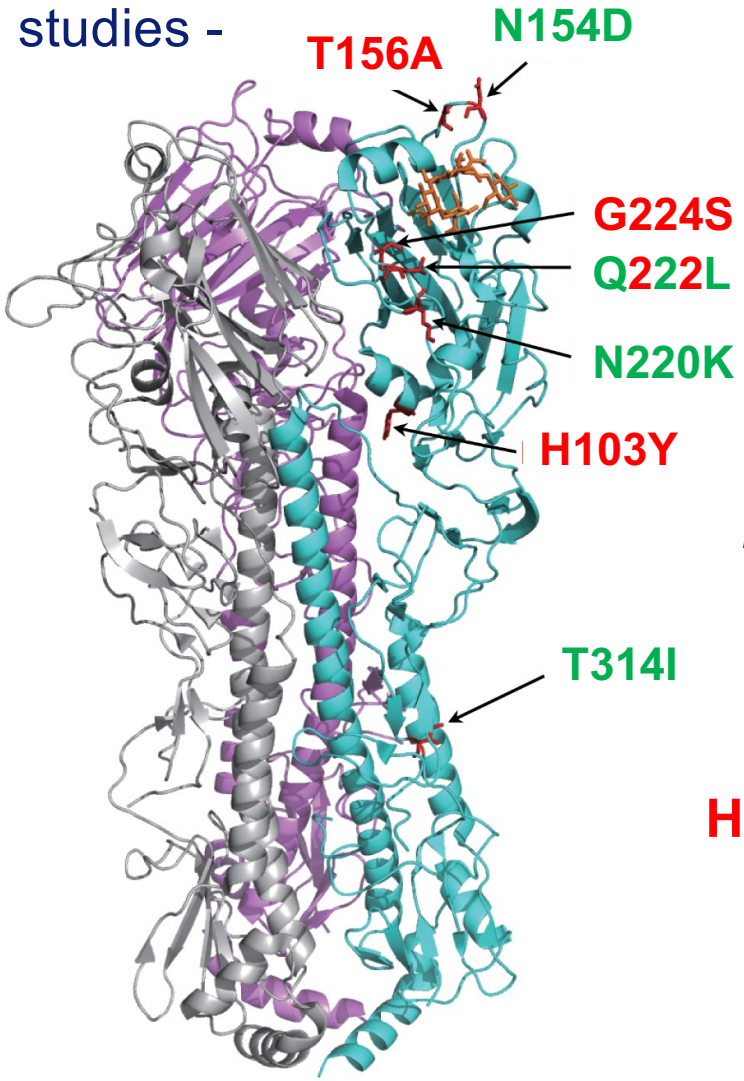
Phenotypes > genotypes!!

Linster et al. Cell, 2014

A DEPARTMENT OF Erasmus MC

Influenza A/H5N1 virus transmission

- Similarities between studies -



Phenotypes > genotypes!!

Imai, Nature, 2012

Herfst, Science, 2012

Influenza A/H5N1 virus transmission
- Similarities between studies -



THE NEW YORK TIMES, SUNDAY, JANUARY 8, 2012

The New York Times

An Engineered Doomsday

A lethal virus needs to be destroyed, or better contained, and future research closely reviewed

Scientists have long worried that an influenza virus that has ravaged poultry and wild birds in Asia might evolve to pose a threat to humans. Now scientists financed by the National Institutes of Health have shown in a laboratory how that could happen. In he process they created a virus that could kill tens or hundreds of millions of people if it escaped confinement or was stolen by terrorists.

We nearly always champion unfettered scientific research and open publication of the results. In this case it looks like the research should never have been undertaken because the potential harm is so catastrophic and the potential benefits from studying the virus so speculative.

Unless the scientific community and health officials can provide more persuasive justifications than they have so far, the new virus, which is in the Netherlands, ought to be destroyed. Barring that, it should be put in a few government-controlled laboratories with the highest containment and the two scientific journals that plan to publish the studies omit any details that might help terrorists figure out how to unleash a devastating pandemic. That presumably includes details on how the engineered virus was made and details on the precise mutations that allowed it to go airborne.

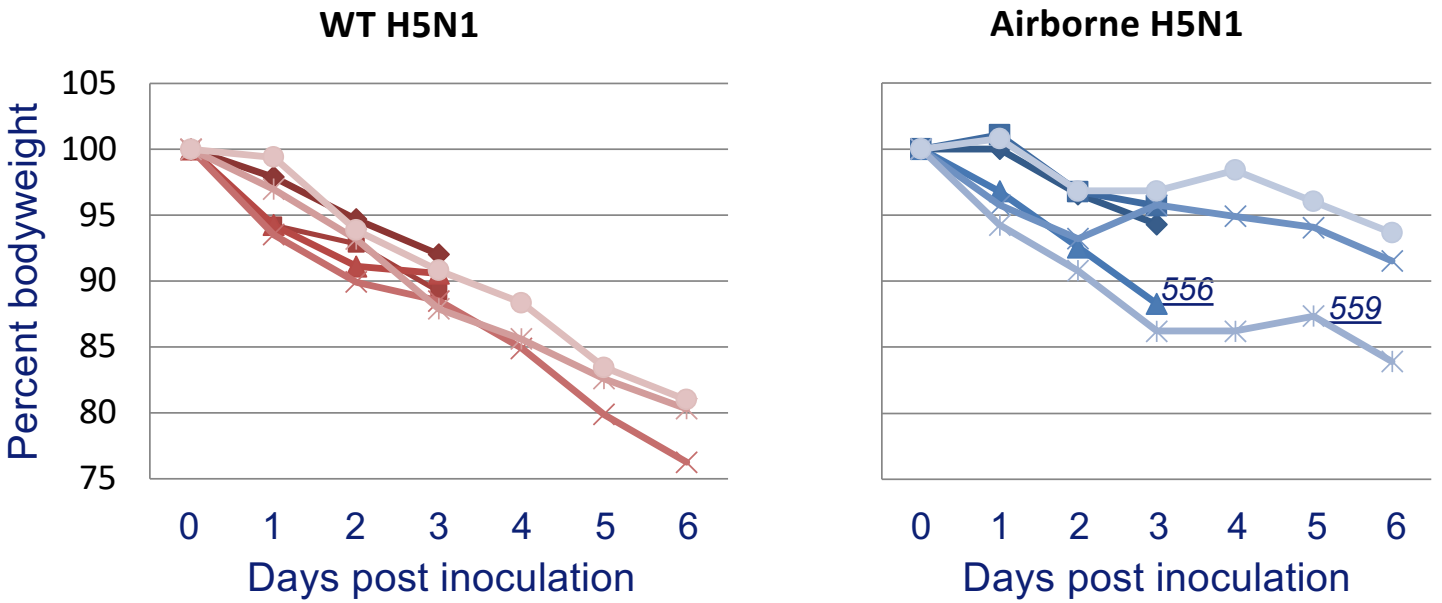
We doubt that anything at all should be published, but it seems clear that something will be.

The two journals reviewing the papers seem inclined to follow the advisory board's recommendations that the research be published in a redacted form, provided there is some way for researchers who need the information to gain access to the full details. The Erasmus team believes that more than 100 laboratories and perhaps 1,000 scientists around the world need to know the precise mutations to look for. That would spread the information far too widely. It should suffice to have a few of the most sophisticated lab-

rasmus MC

Airborne A/H5N1

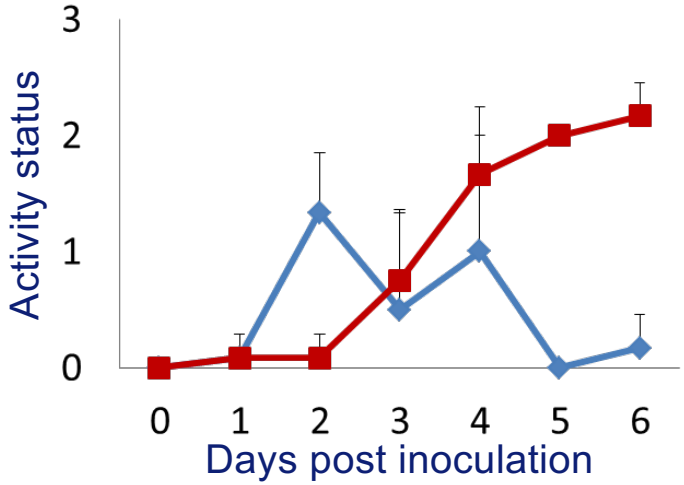
- Pathogenicity in ferrets -



A DEPARTMENT OF Erasmus MC

Airborne A/H5N1

- Pathogenicity in ferrets -

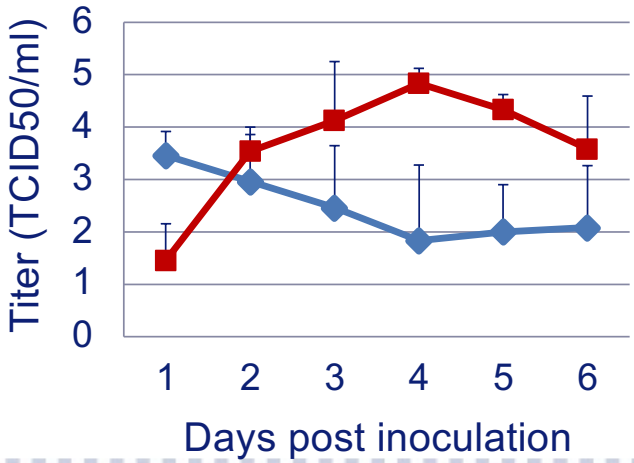




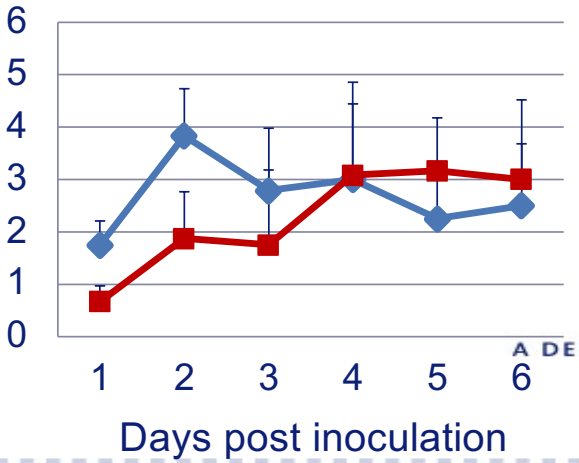
Activity status

0 - Alert and playful
1 - Alert and playful only when stimulated
2 - Alert but not playful when stimulated
3 - Neither alert nor playful when stimulated

Throat



Nose





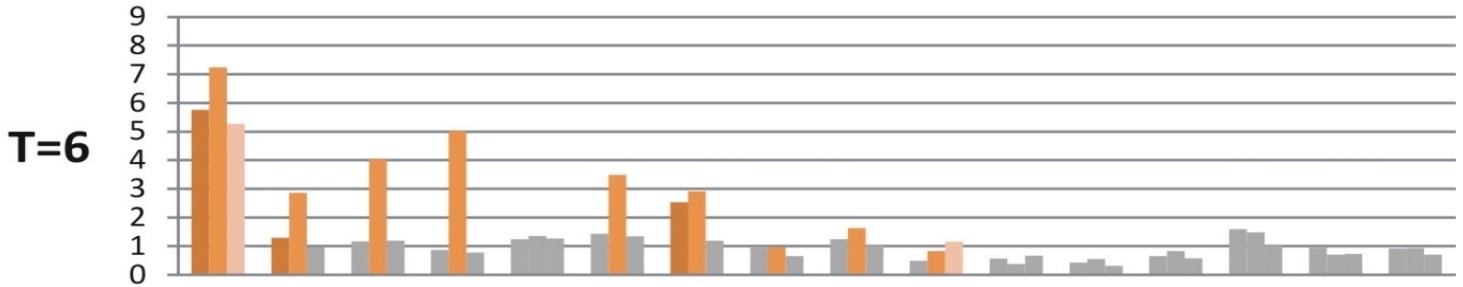
A DEPARTMENT OF Erasmus MC

Airborne A/H5N1

- Pathogenicity in ferrets -

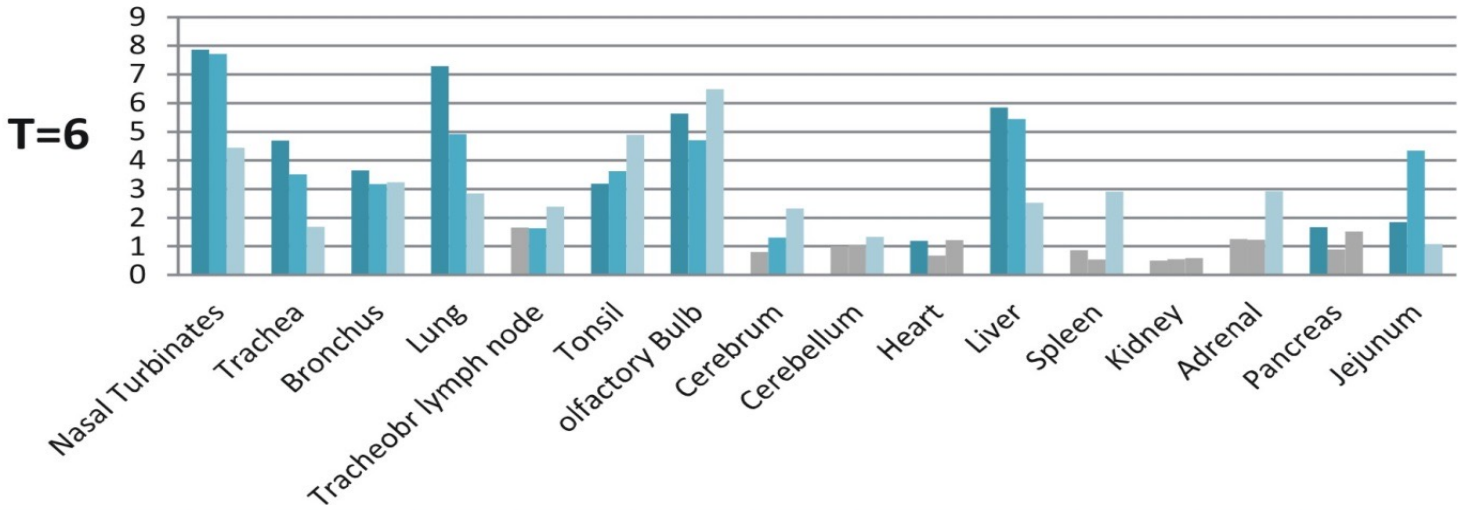
ID (% lung lesions)

Airborne H5N1



F557 (5%)
F559 (80%)
F560 (1%)

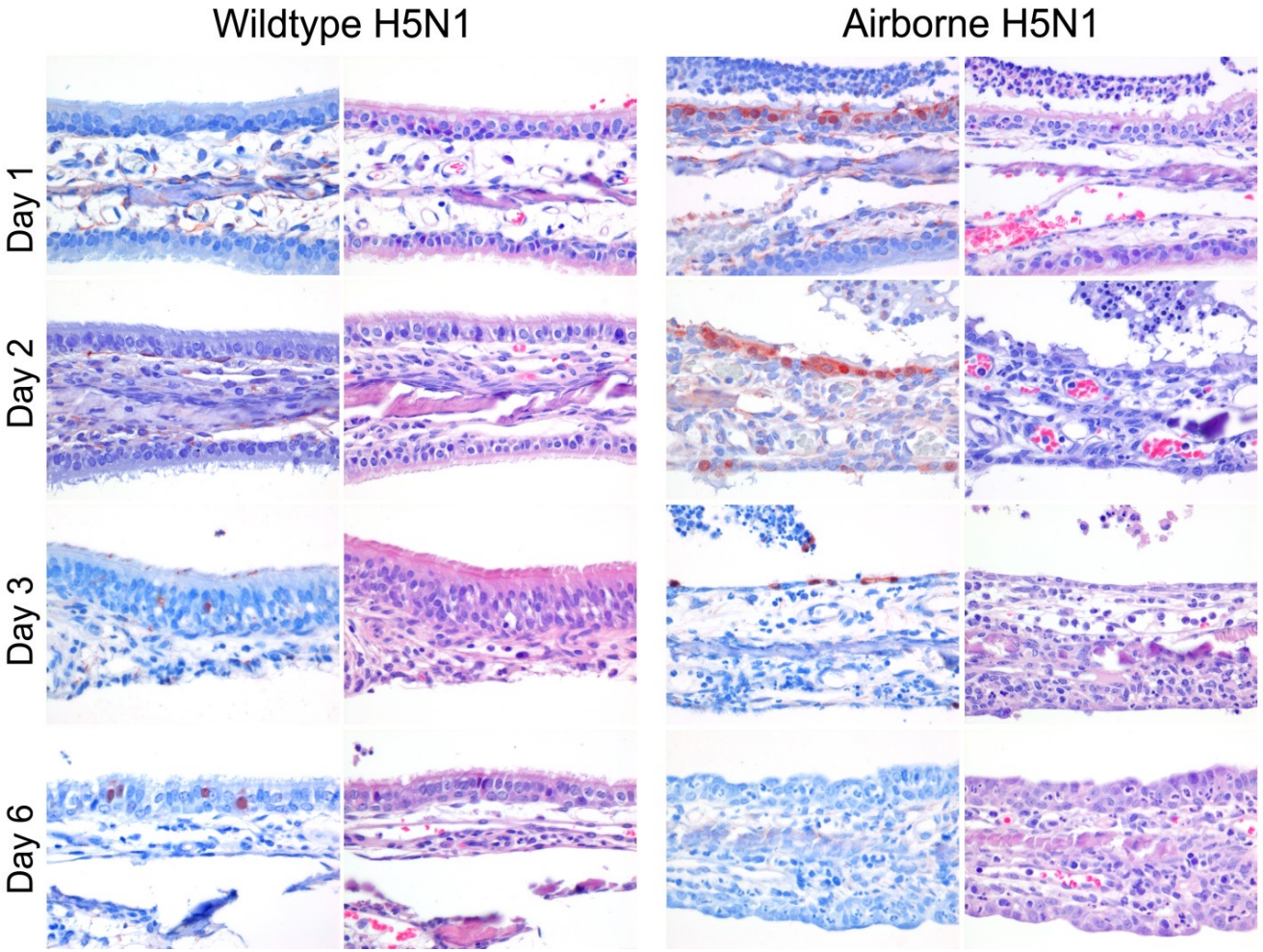
WT H5N1



F570 (70%)
F571 (50%)
F572 (0%)

Airborne A/H5N1

- Pathogenicity in ferrets -



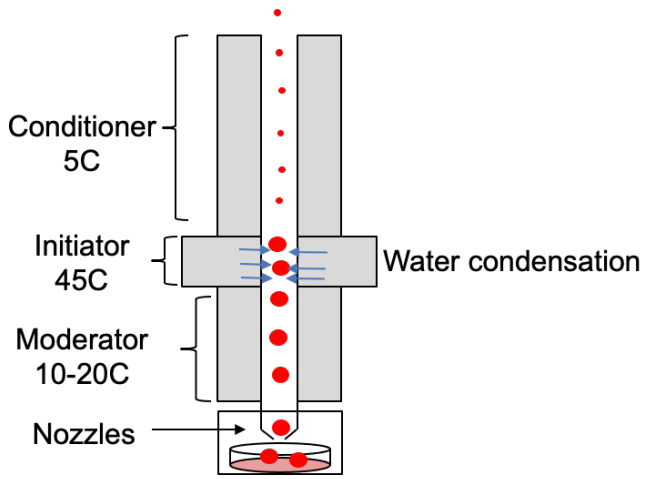
VIROSCIENCE LAB
WHERE SKILLS MEET TO STUDY & PROTECT

A DEPARTMENT OF Erasmus MC

Influenza A virus shedding kinetics

- BioSpot characteristics -

- Sampling principle
  - Aerosol particles are exposed to a supersaturated water environment in the initiator phase of the sampling (45° C)
  - Condensation growth → larger droplets are gently deposited onto liquid surface in a petri dish (contains 2mL of liquid medium)
  - Flow rate BioSpot: 15L/min



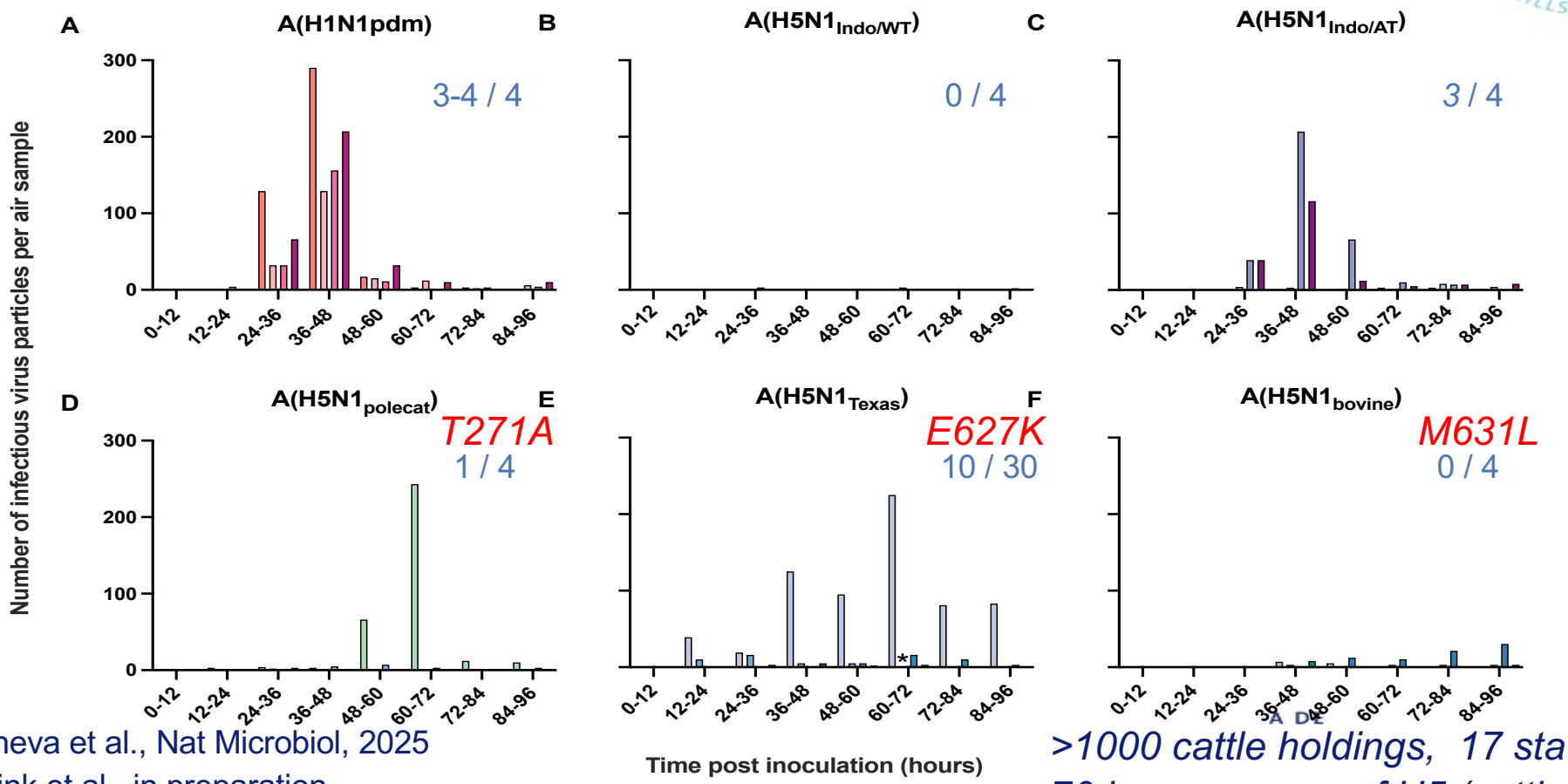


Aerosol Devices Inc

A DEPARTMENT OF Erasmus MC

Influenza A virus shedding kinetics

- Infectious virus -



Tosheva et al., Nat Microbiol, 2025

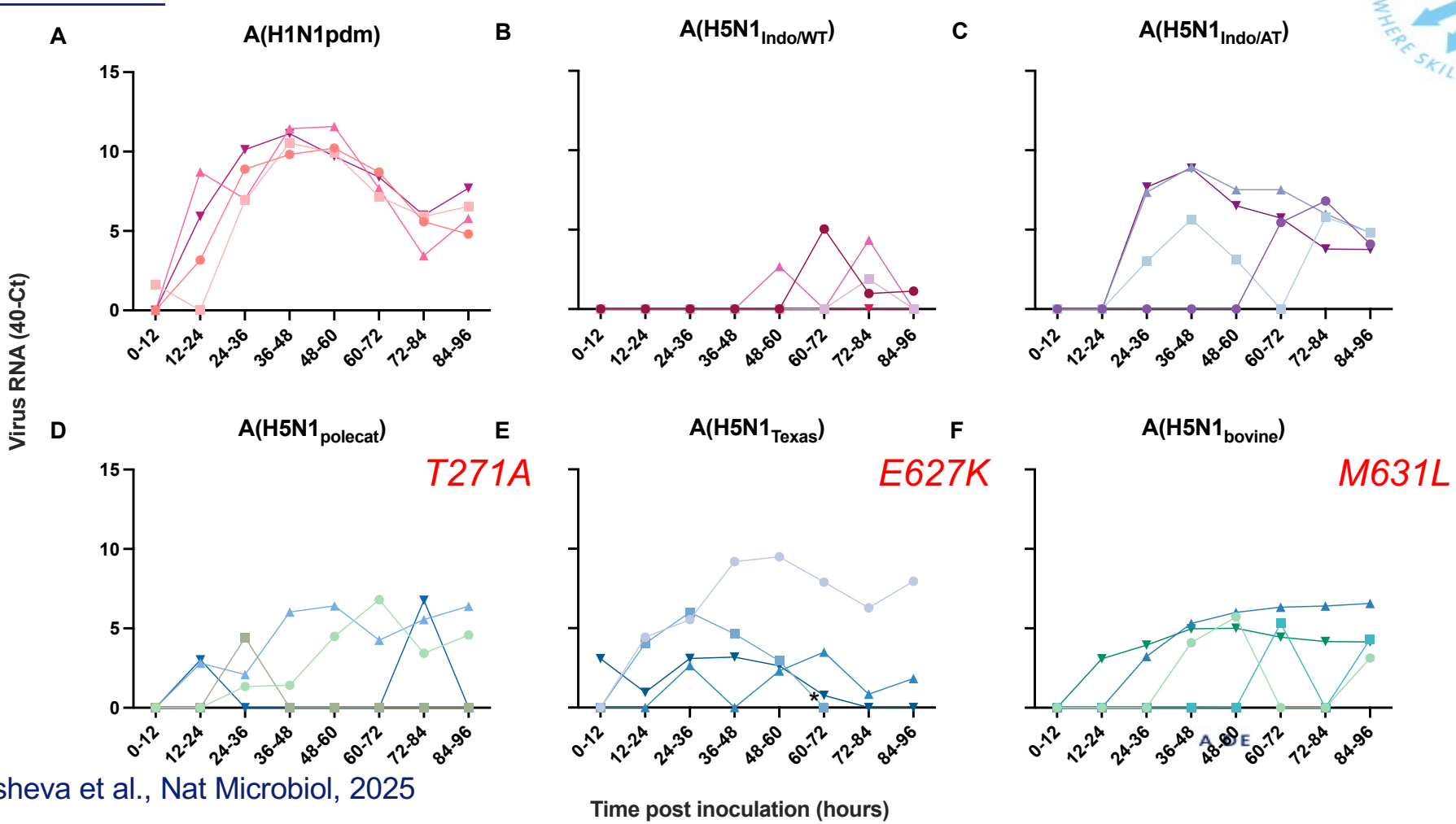
Rijnink et al., in preparation

>1000 cattle holdings, 17 states (USDA)

70 human cases of H5 (cattle / poultry)

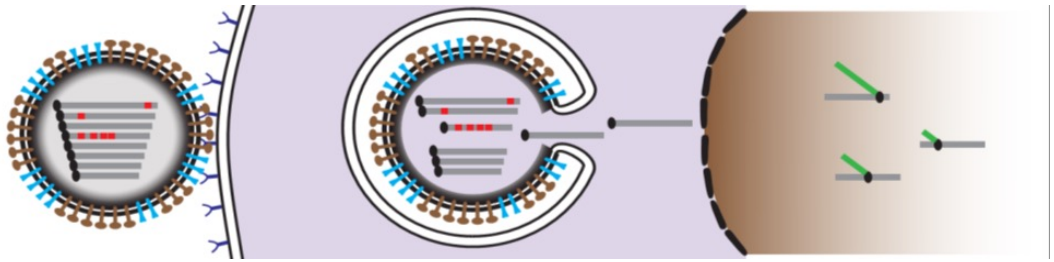
Influenza A virus shedding kinetics

- Viral RNA -



Tosheva et al., Nat Microbiol, 2025

Airborne transmission phenotypes



	Binding	Stability	Replication
H5N1 wt	✗	✗	✗
H5N1 Airborne	✓	✓	✓

Avian influenza A(H7N9) virus

- Limited airborne transmission between ferrets -



nature

Pathogenesis and transmission of avian influenza A (H7N9) virus in ferrets and mice

Jessica A. Belser[1], Kortney M. Gustin[1], Melissa B. Pearce[1], Taronna R. Maines[1], Hui Zeng[1], Claudia Pappas[1], Xiangjie Sun[1], Paul J. Carney[1], Julie M. Villanueva[1], James Stevens[1], Jacqueline M. Katz[1] & Terrence M. Tumpey[1]

Characterization of H7N9 influenza A viruses isolated from humans

Tokiko Watanabe[1]*, Maki Kiso[2]*, Satoshi Fukuyama[1]*, Noriko Nakajima[3]*, Masaki Imai[4]*, Shinya Yamada[2], Shin Murakami[5], Seiya Yamayoshi[2], Kiyoko Iwatsuki-Horimoto[2], Yoshihiro Sakoda[6], Emi Takashita[4], Ryan McBride[7], Takeshi Noda[2], Masato Hatta[8], Hirotaka Imai[8], Dongming Zhao[1], Noriko Kishida[4], Masayuki Shirakura[4], Robert P. de Vries[7], Shintaro Shichinohe[6], Masatoshi Okamatsu[6], Tomokazu Tamura[6], Yuriko Tomita[1], Naomi Fujimoto[1], Kazue Goto[1], Hiroaki Katsura[2], Eiryo Kawakami[1], Izumi Ishikawa[1], Shinji Watanabe[1,9], Mutsumi Ito[2], Yuko Sakai-Tagawa[2], Yukihiko Sugita[2], Ryuta Uraki[2], Reina Yamaji[2], Amie J. Eisfeld[8], Gongxun Zhong[8], Shufang Fan[8], Jihui Ping[8], Eileen A. Maher[8], Anthony Hanson[8], Yuko Uchida[10], Takehiko Saito[10], Makoto Ozawa[11,12], Gabriele Neumann[8], Hiroshi Kida[6,13], Takato Odagiri[4], James C. Paulson[7], Hideki Hasegawa[3], Masato Tashiro[4] & Yoshihiro Kawaoka[1,2,5,8,14]

Limited airborne transmission of H7N9 influenza A virus between ferrets

Mathilde Richard[1]*, Eefje J. A. Schrauwen[1]*, Miranda de Graaf[1], Theo M. Bestebroer[1], Monique I. J. Spronken[1], Sander van Boheemen[1], Dennis de Meulder[1], Pascal Lexmond[1], Martin Linster[1], Sander Herfst[1], Derek J. Smith[2], Judith M. van den Brand[1], David F. Burke[2], Thijs Kuiken[1], Guus F. Rimmelzwaan[1], Albert D. M. E. Osterhaus[1] & Ron A. M. Fouchier[1]

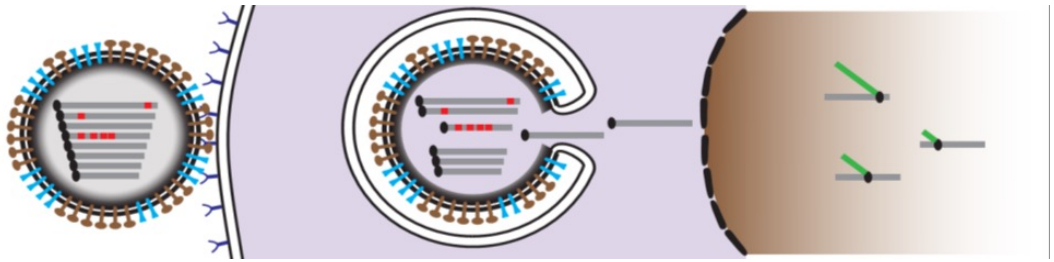
H7N9 Influenza Viruses Are Transmissible in Ferrets by Respiratory Droplet

Qianyi Zhang,[1,2]* Jianzhong Shi,[1]* Guohua Deng,[1]* Jing Guo,[1]* Xianying Zeng,[1]* Xijun He,[1] Huihui Kong,[1] Chunyang Gu,[1,2] Xuyong Li,[1] Jinxiong Liu,[1] Guojun Wang,[1,2] Yan Chen,[1] Liling Liu,[1] Libin Liang,[1] Yuanyuan Li,[1] Jun Fan,[1] Jinliang Wang,[1] Wenhui Li,[1] Lizheng Guan,[1] Qimeng Li,[1,2] Huanliang Yang,[1] Pucheng Chen,[1] Li Jiang,[1] Yuntao Guan,[1] Xiaoguang Xin,[1] Yongping Jiang,[1] Guobin Tian,[1] Xiurong Wang,[1] Chuanling Qiao,[1] Chengjun Li,[1] Zhigao Bu,[1] Hualan Chen[1,2]†

Science

A DEPARTMENT OF Erasmus MC

Airborne transmission phenotypes



	Binding	Stability	Replication
H5N1 wt	✗	✗	✗
H5N1 Airborne	✓	✓	✓
H7N9 Asia	✓	✗	✓

Flu-associated mortality among harbor seals in 2014



425 deaths
March - October 2014

152 deaths (Anholt)
June-August 2014

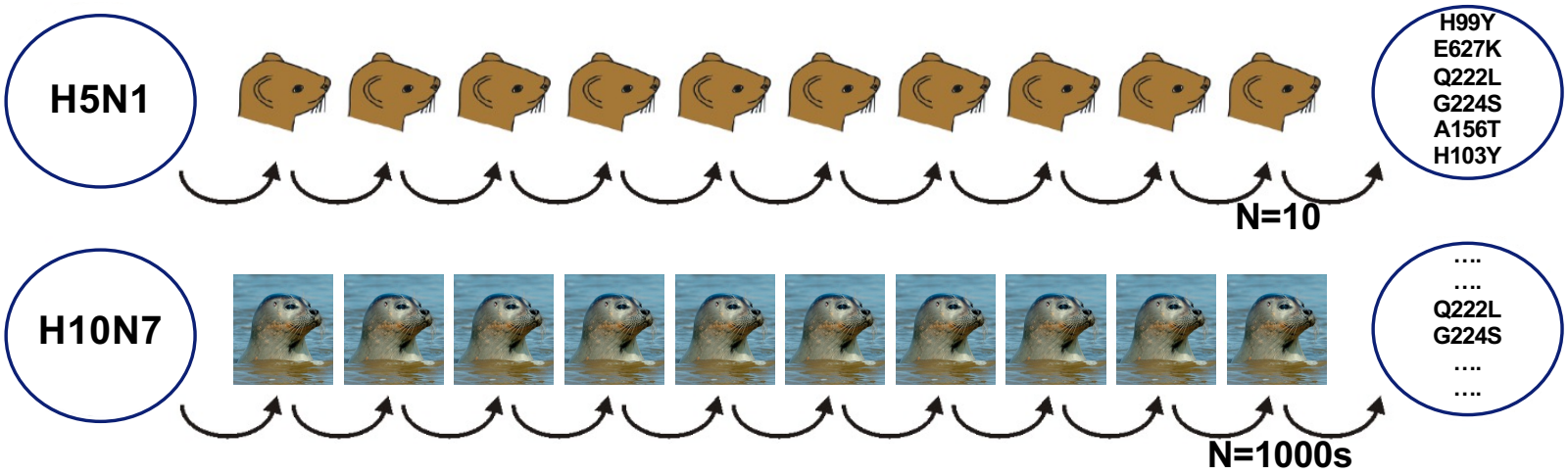
Approx. 1400 deaths
September- November 2014

First cases in the Netherlands
November 2014

Zohari et al Eurosurveillance 2014, Krog et al EID 2015, Bodewes et al EID 2015

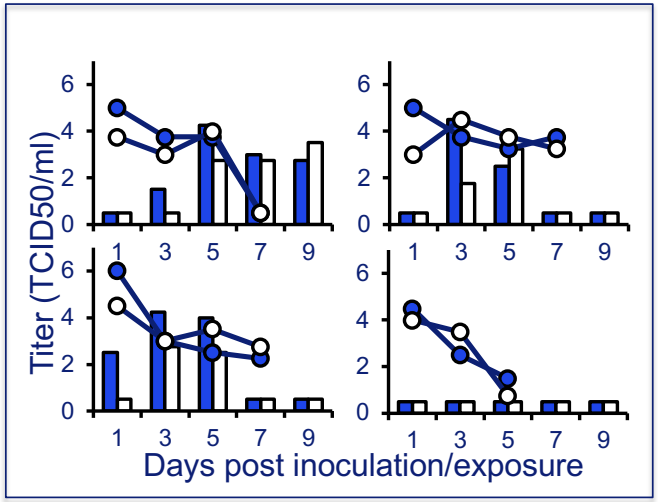
- **Research on influenza transmission**
- **- BLS3+ labs and *in-vivo* -**

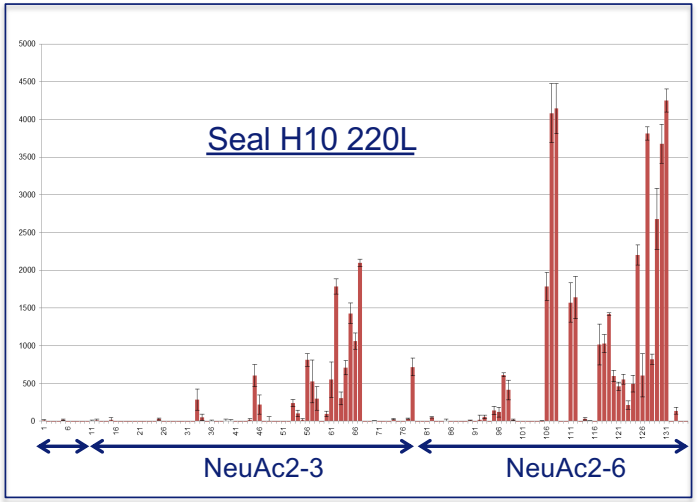
Herfst et al. 2012

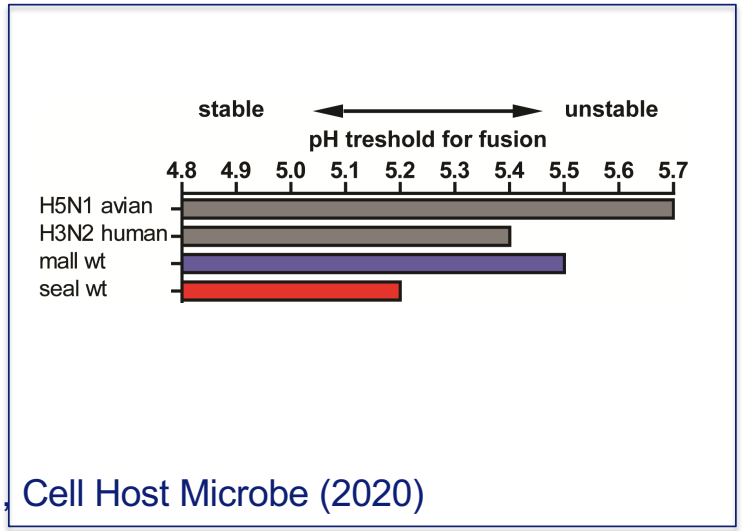


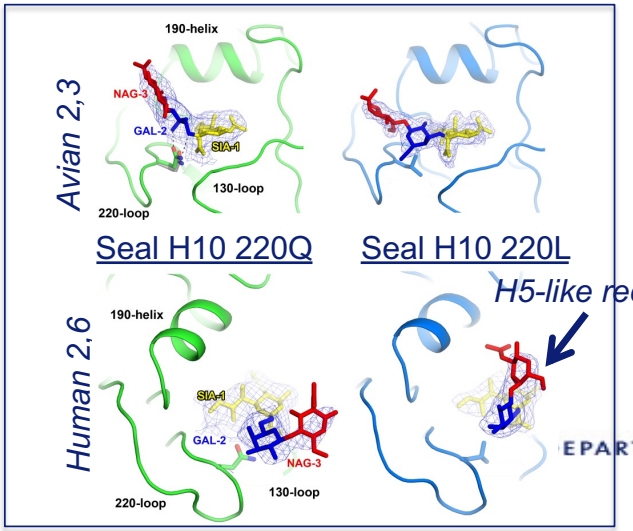
Mother Nature et al. 2014

Efficient airborne transmission of seal H10N7



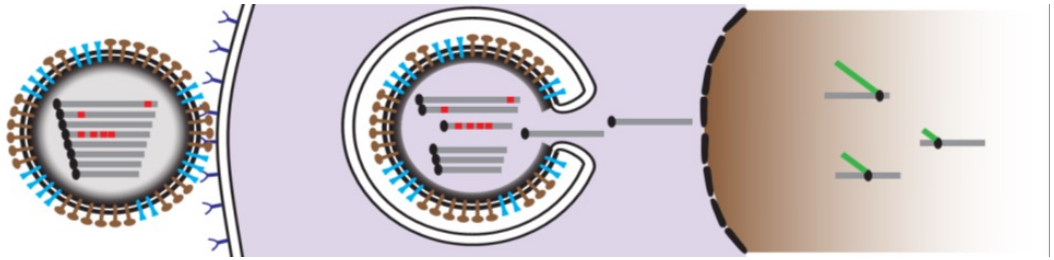






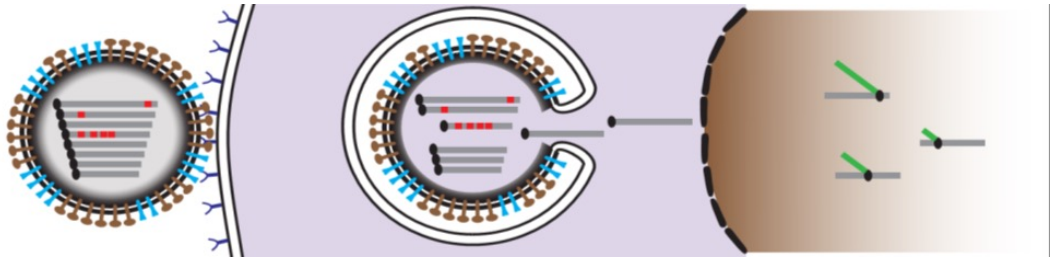
Herfst et al., Cell Host Microbe (2020)

Airborne transmission phenotypes



	Binding	Stability	Replication
H5N1 wt	✗	✗	✗
H5N1 Airborne	✓	✓	✓
H7N9 Asia	✓	✗	✓
H10N7 seal	✓	✓	✓

Airborne transmission phenotypes



	Binding	Stability	Replication	
H5N1 wt	✗	✗	✗	
H5N1 Airborne	✓	✓	✓	
H7N9 Asia	✓	✗	✓	
H10N7 seal	✓	✓	✓	
H5N6 China ‘14 (human)	✗	✗	✓	*Herfst et al, 2018*
H5N8 Europe ‘14 (chicken)	✗	✗	✗	*Richard et al, 2015*
H5N6 Europe ‘17 (gull)	✗	✗	✗	*Herfst et al, 2023*

Making airborne transmissible H5N1:

- H5N1 can **adapt** to mammals and gain airborne transmission (mammals)
- Adaptation : receptor binding, HA stability, polymerase adaptation (host restriction facors)
- For A(H5N1) a failure to transmit corresponds to a **lack of viral expulsion into the air** by donor animals (as opposed to mechanisms acting in the environment or at the recipient)
- Increased **knowledge** and continuous **surveillance** is required to assess whether viruses have pandemic potential, and prevent future pandemics
- **Gain-of-function** studies are essential in studying viral transmission
- **Risks** of gain of function studies can be mitigated

Acknowledgements

Herfst Lab:
Ilona Tosheva
Suzanne Mijnhardt
Kain Saygan
Bea Bellido Martin
Maarten Wilbrink
Dennis de Meulder
Bianca van Kekem
Theo Bestebroer
Abilash Ravi
Fabien Filaire

Former Members
Pascal Lexmond
Jasmin Kutter
Bob Boogaard
Elwin Verveer
Martin Linster
Miruna Rosu

Dept. Viroscience:
Mathilde Richard
Pieter Fraaij
Marion Koopmans
Massimo Palmarini
Ron Fouchier

Richard Webby (St. Jude Children's hospital)
Andy Bowman (Ohio State Univ)
Yoshi Kawaoka (Univ of Wisconsin-Madison)
Anice Lowen (Emory University)