Lessons Learnt from Emerging Viruses: (Coronavirus and Influenza A Virus)

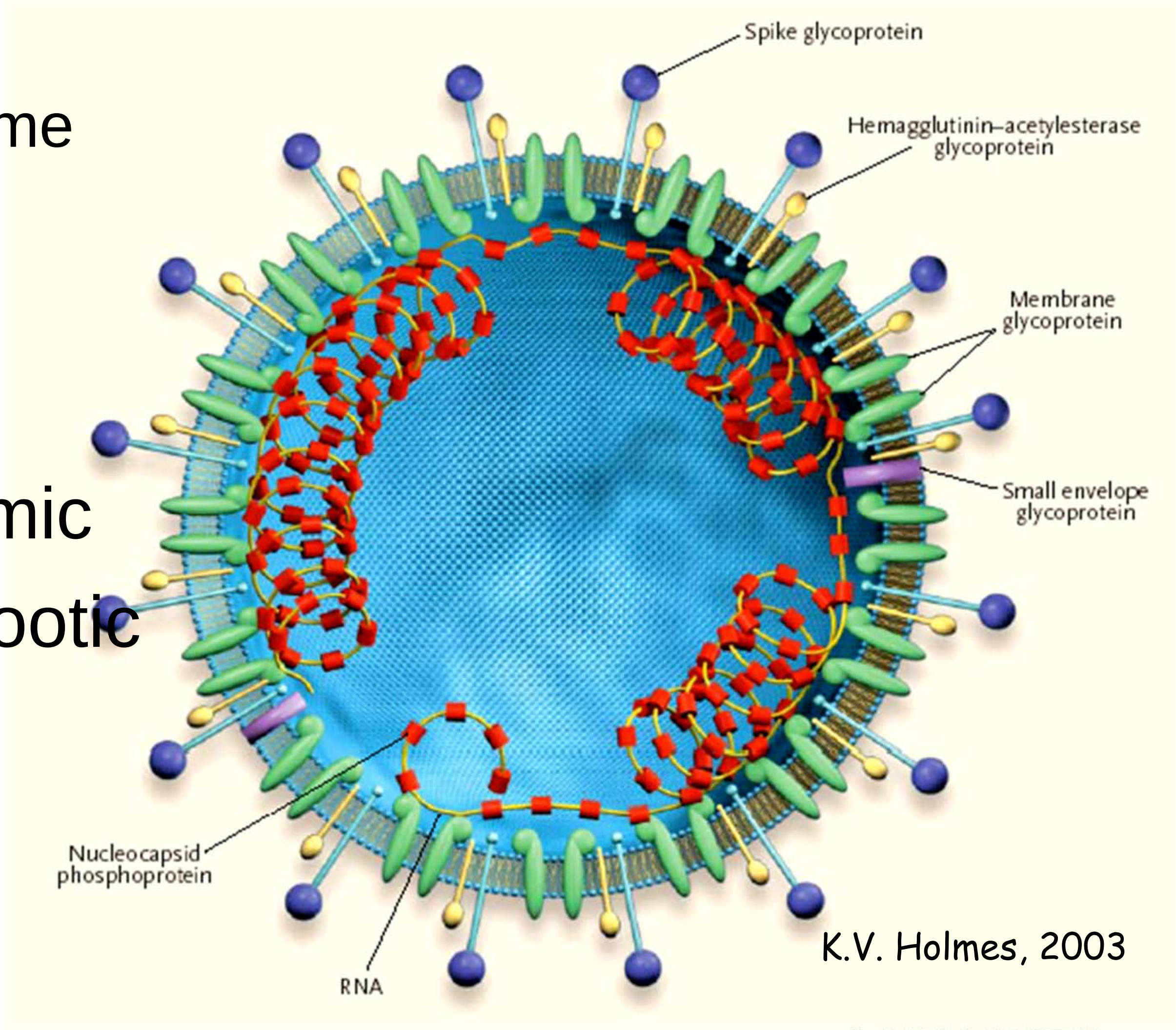
Outline

- Coronavirus Background
- SARS Outbreak
  - Cause of the 2002-2003 SARS Outbreak
    - SARS-CoV
      - discovery, diagnostics, transmission
  - Origin of SARS-CoV In Humans
- Influenza A virus background
  - Evolution and ecology
  - Pandemics
    - Past, present

J. Craig Venter™
INSTITUTE

Coronavirus

- nveloped
  - Large mRNA genome
  - N, S, (HE), M, E
- nteric and/or spiratory
- ndemic or epidemic
- nzootic and epizootic



K.V. Holmes, 2003

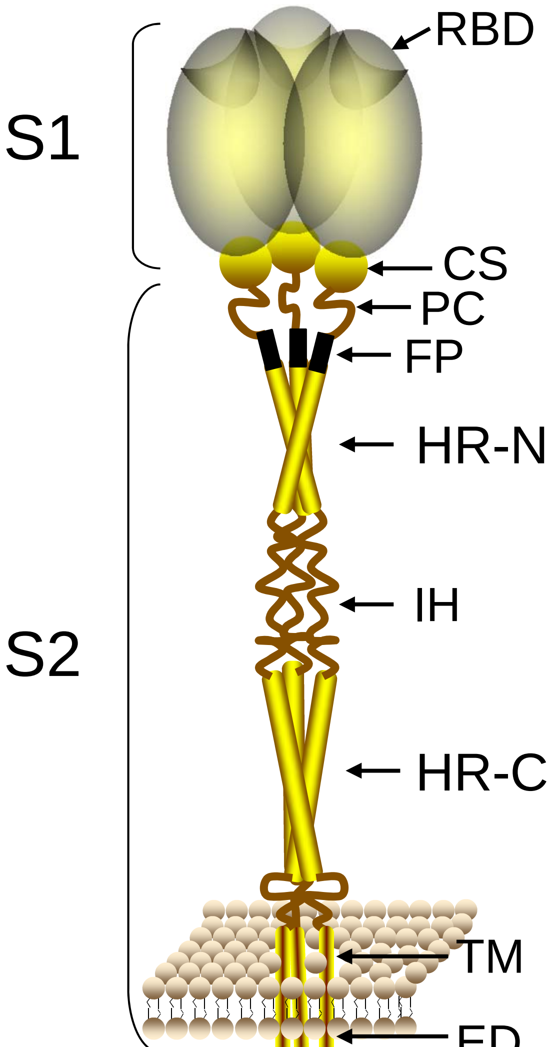
Coronaviruses Naturally Infect Many Species

ronaviruses, Host Range and Disease

Virus	Host	Major Disease
HCoV-229E	human	respiratory infection
HCoV-NL63	human	respiratory infection
TGEV	pig	enteritis and respiratory infection
PRCoV	pig	respiratory infection
FIPV	cat	peritonitis, systemic infection
FECoV	cat	enteritis
CCoV	dog	enteritis
HCoV-OC43	human	respiratory infection
SARS-CoV	human bat/civet	respiratory infection, enteritis
BCoV	cattle	enteritis
TCoV	turkey	enteritis
RCoV	rat	respiratory infection
HEV	pig	respiratory infection, encephalomyelitis
MHV	mouse	respiratory infection, enteritis, hepatitis, encephalitis
IBV	chicken	respiratory infection

oronavirus Spike

- Corona" appearance
- eceptor binding
  - Host range
- embrane fusion
- eutralizing antibodies



Severe Acute Respiratory Syndrome (SARS)

- utbreak of “atypical neumonia”
  - 2002-2003
- apid progression to ARD
- entilator required
- larming death rate
- erson-to-person ansmission

Emergence of SARS

<u>November 2002</u>

Guangdong Province
Southern China

Outbreak of Severe Acute Respiratory Syndrome

2002

Nov. 16

ical pneumonia

2003

Feb. 14

Hotel (HK)

Feb. 28

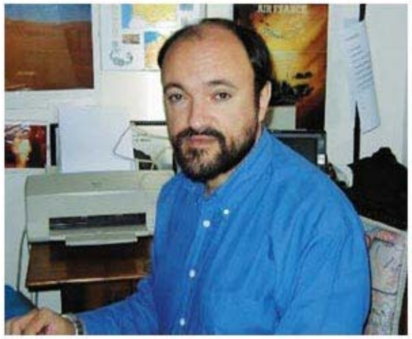
WHO alerted

Mar. 12

Global alert

4 Months → July

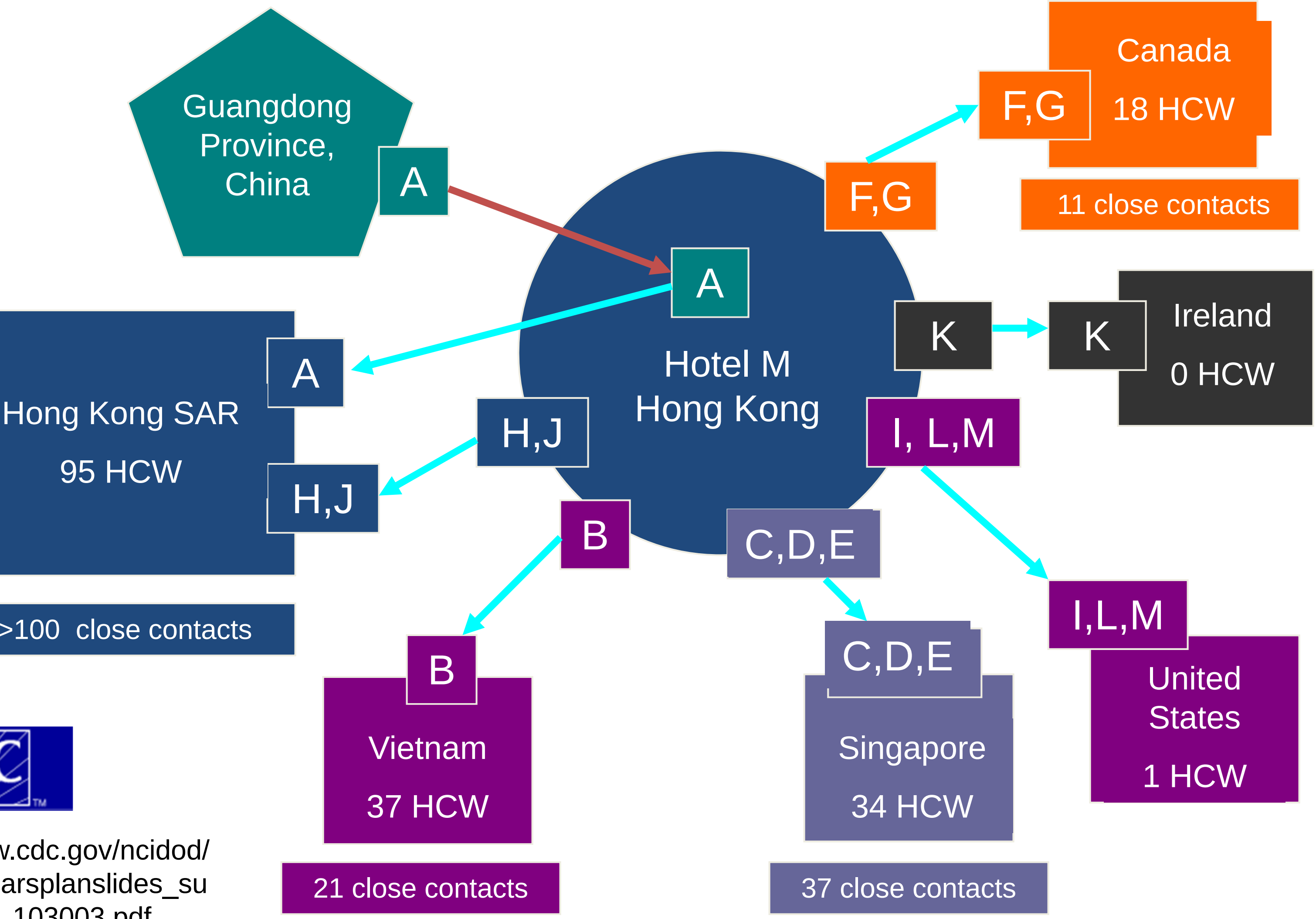
Human chain of transmission broken



Dr. Carlo Urbani
(SARS-CoV/Urbani)

Total of 8,098 cases of SARS and 774 deaths in 30 countries

Spread from Hotel M, Hong Kong February 2003



Guangdong Province, China

A

Hotel M Hong Kong

A

F,G

F,G

Canada

18 HCW

11 close contacts

K

K

Ireland

0 HCW

I, L,M

I,L,M

United States

1 HCW

C,D,E

C,D,E

Singapore

34 HCW

37 close contacts

B

B

Vietnam

37 HCW

21 close contacts

H,J

H,J

A

Hong Kong SAR

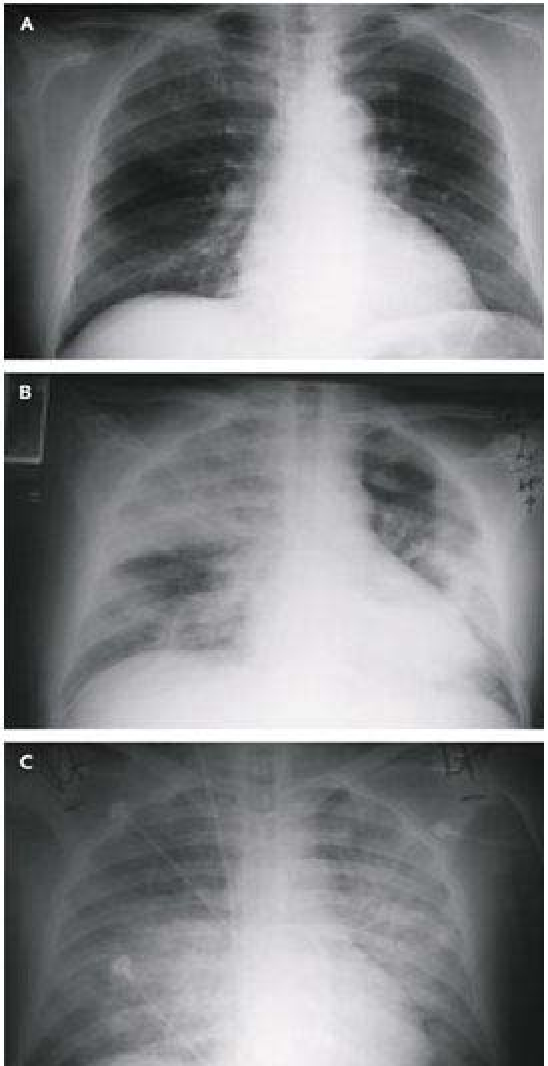
95 HCW

>100 close contacts

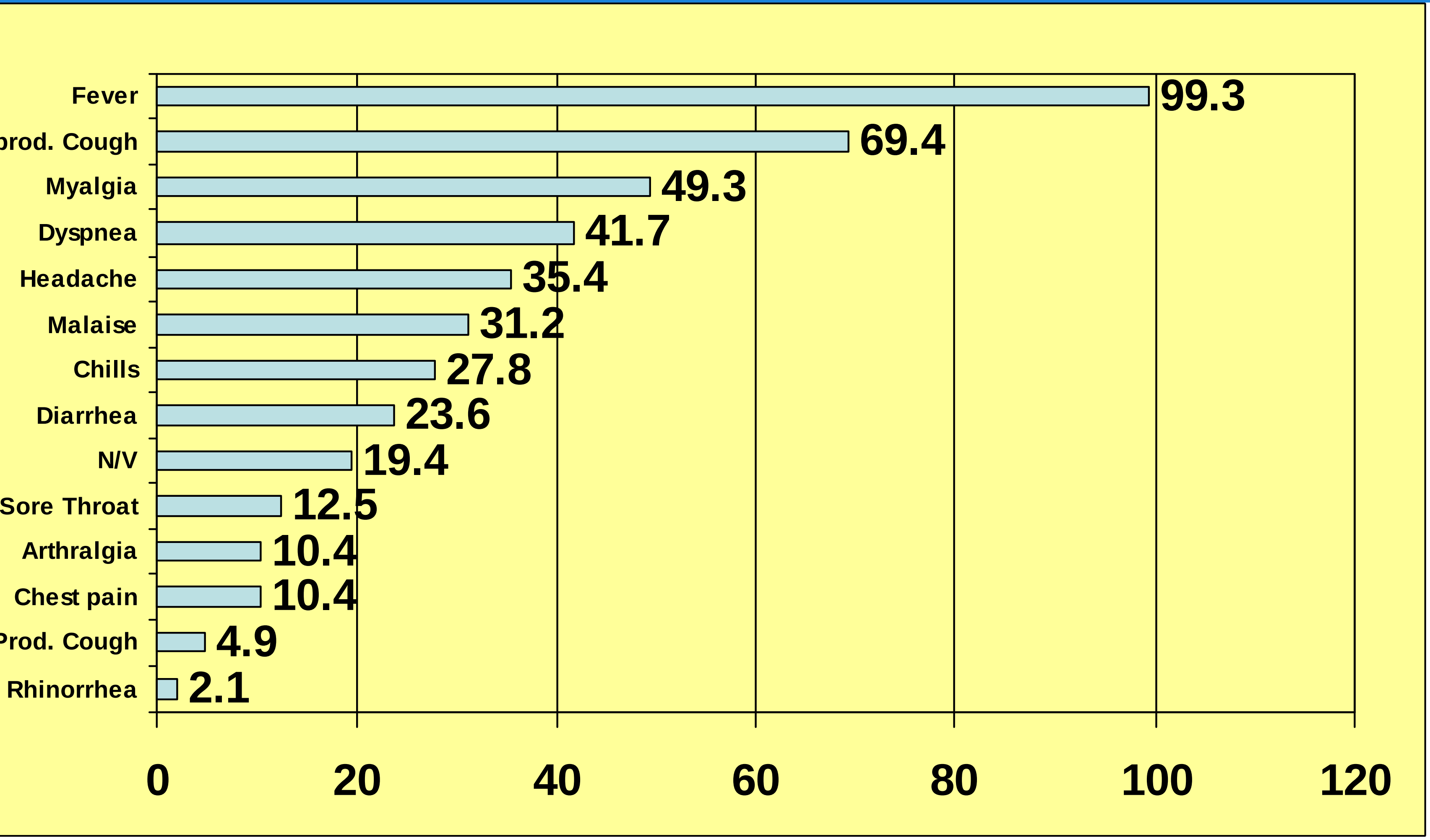
v.cdc.gov/ncidod/
arsplanslides_su
_103003.pdf

SARS-The Disease

- Symptoms
  - Fever
  - Dry cough
  - Diarrhea
  - Acute interstitial pneumonia
- 2-10 day incubation period
- 20% intensive care
- 10% case fatality rate
- Seroconversion
  - 2-4 weeks post-onset
- Transmission
  - respiratory droplets



Symptoms of SARS



JAMA, June 4, 2003 – Vol. 289, No. 21

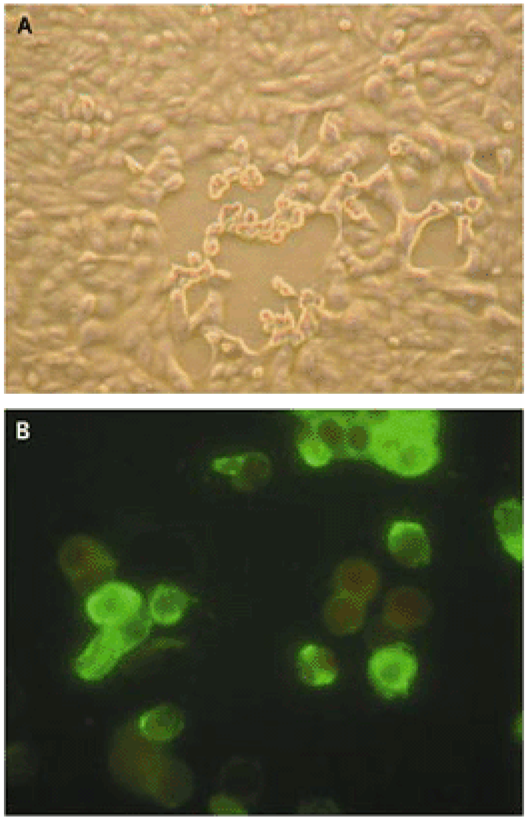
Clinical Outcome

- 20% admitted to ICU
- 5% required mechanical ventilation
- ~10% died
- ncreased risk of death or ICU admission if:
  - Increased age
    - < 60 yo: 6.8%
    - > 60 yo: 55.0%
  - Co-morbidity
  - High LDH
  - High neutrophil count

Tsui et al. EID 2003; 9: 1064-1069
Fowler et al. JAMA 2003; 290: 367-373
Lew et al. JAMA 2003; 290: 374-380
Chan et al. Thorax 2003;58:686-689
Choi et al. Ann Int Med 2003;139:715-723

The Hunt for the Causative Agent of SARS

1. Ruled out known respiratory viruses
2. Inoculated cells with clinical specimen



+ clinical specimen: oropharyngeal washes
respiratory specimen
sputum
lung biopsy
kidney biopsy

*CPE seen only in Vero and FRhK-4

Figure 1. Ksiazek et al. 2003 NEJM 348:1953-1966

RS-Coronavirus

- el CoV isolated from SARS atients’
  - Lungs, sputum, feces, etc.
  - Grown in monkey kidney
    - Vero and pRHMK
- serological cross reaction
  - SARS-CoV not previously circulated in humans
- ome distantly related to known oVs.
- n’s postulates fulfilled

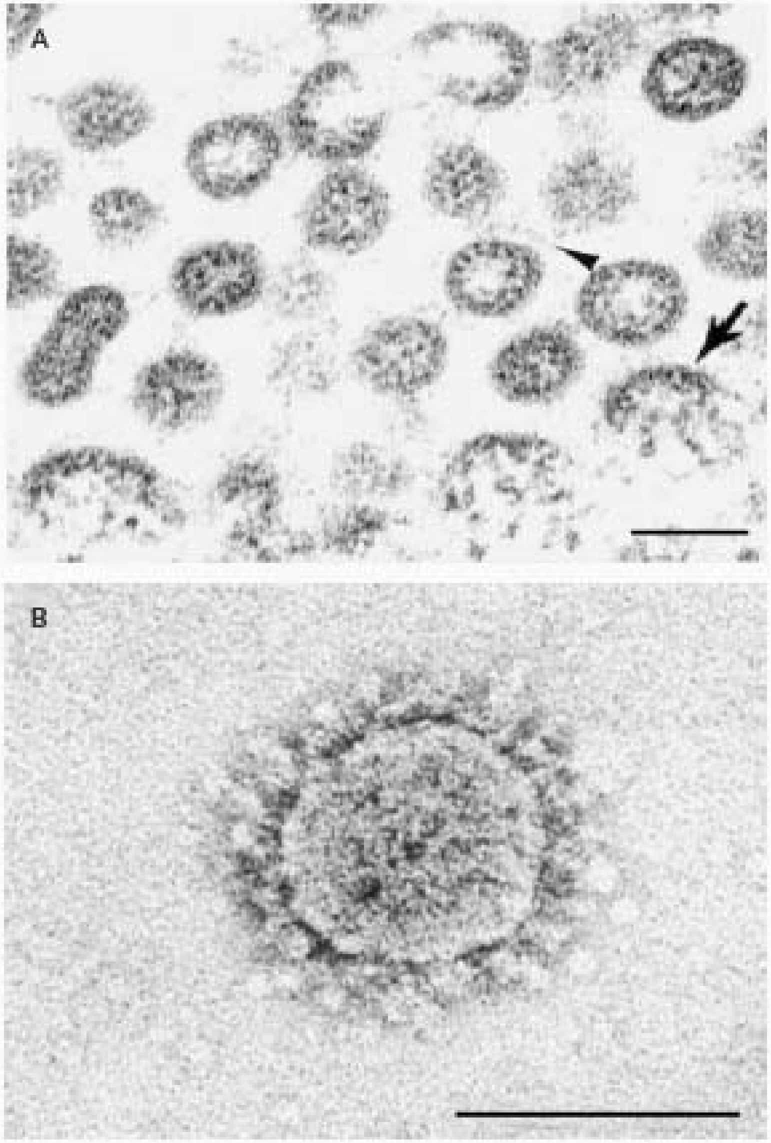


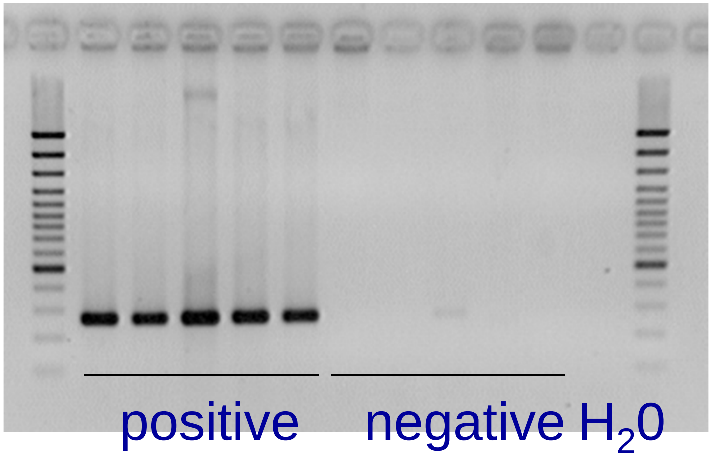
Figure 2. Ultrastructural characteristics of SARS-Associated Coronavirus Grown in Vero E6 cells.

In a short period of time….

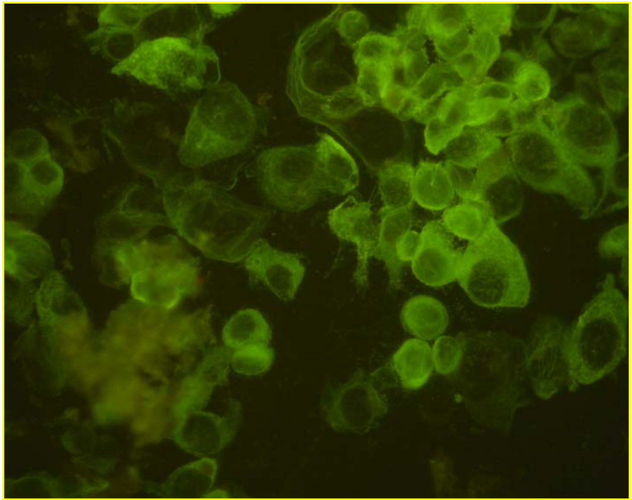
- Virus detected
- Virus amplified
- Virus identified
- Sequenced
- Koch’s postulates

Laboratory diagnosis:
st Generation tests: March 28

- RT-PCR for P gene



- ndirect immuno-fluorescence serology using virus infected cells



courtesy of J. M. Peiris

Problems Providing Laboratory Diagnostics During an Outbreak

o time to evaluate tests in advance;
valuate “on the run”

valanche of specimens: 200/ day

o / little clinical information available in
eal time” to help evaluate results

nrealistic expectations

nknown risk to laboratory workers

courtesy of J. M. Peiris

l Load

t al. Lancet 2003; 361:
2

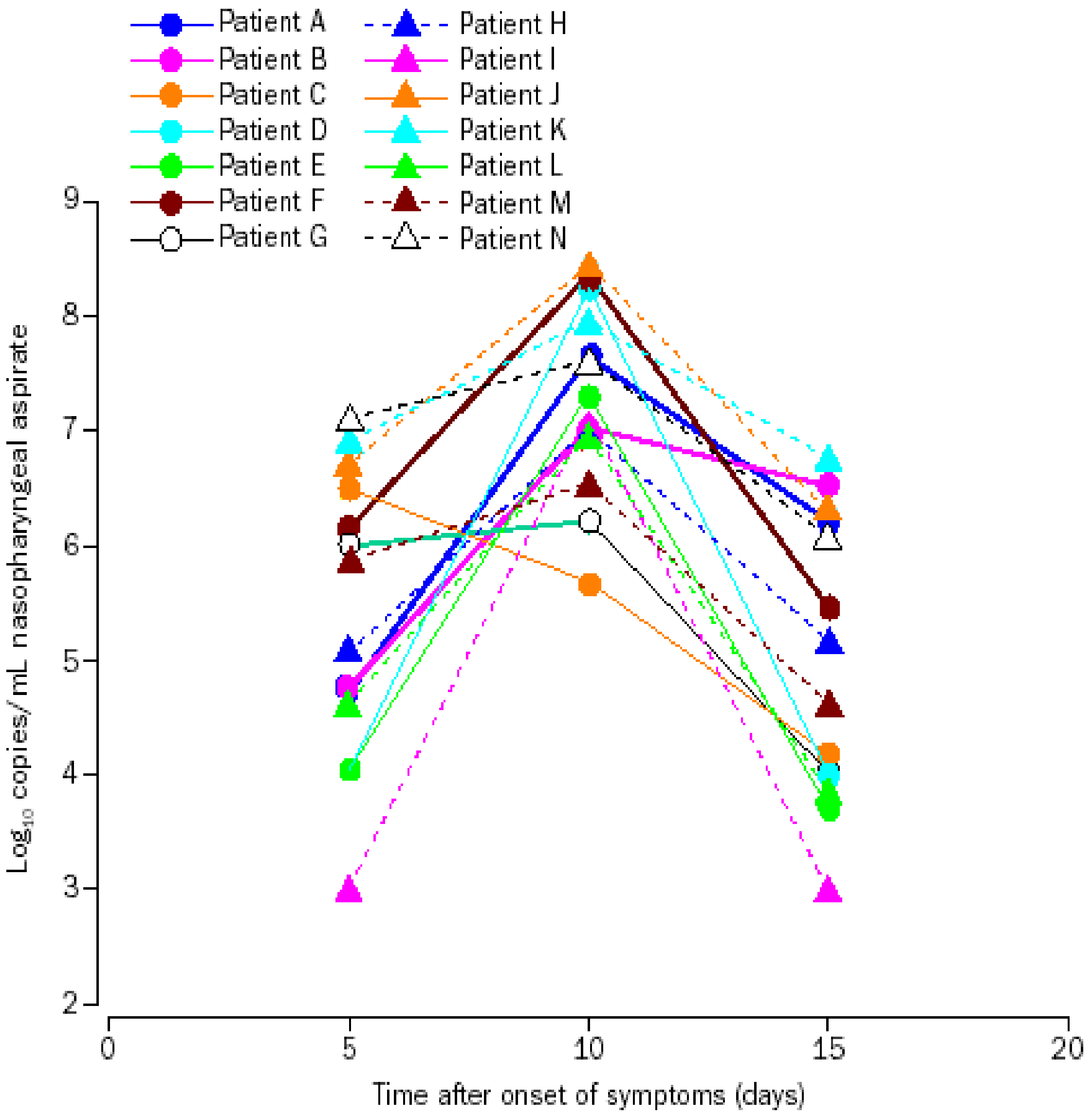


Figure 4: **Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients**

Transmission of SARS

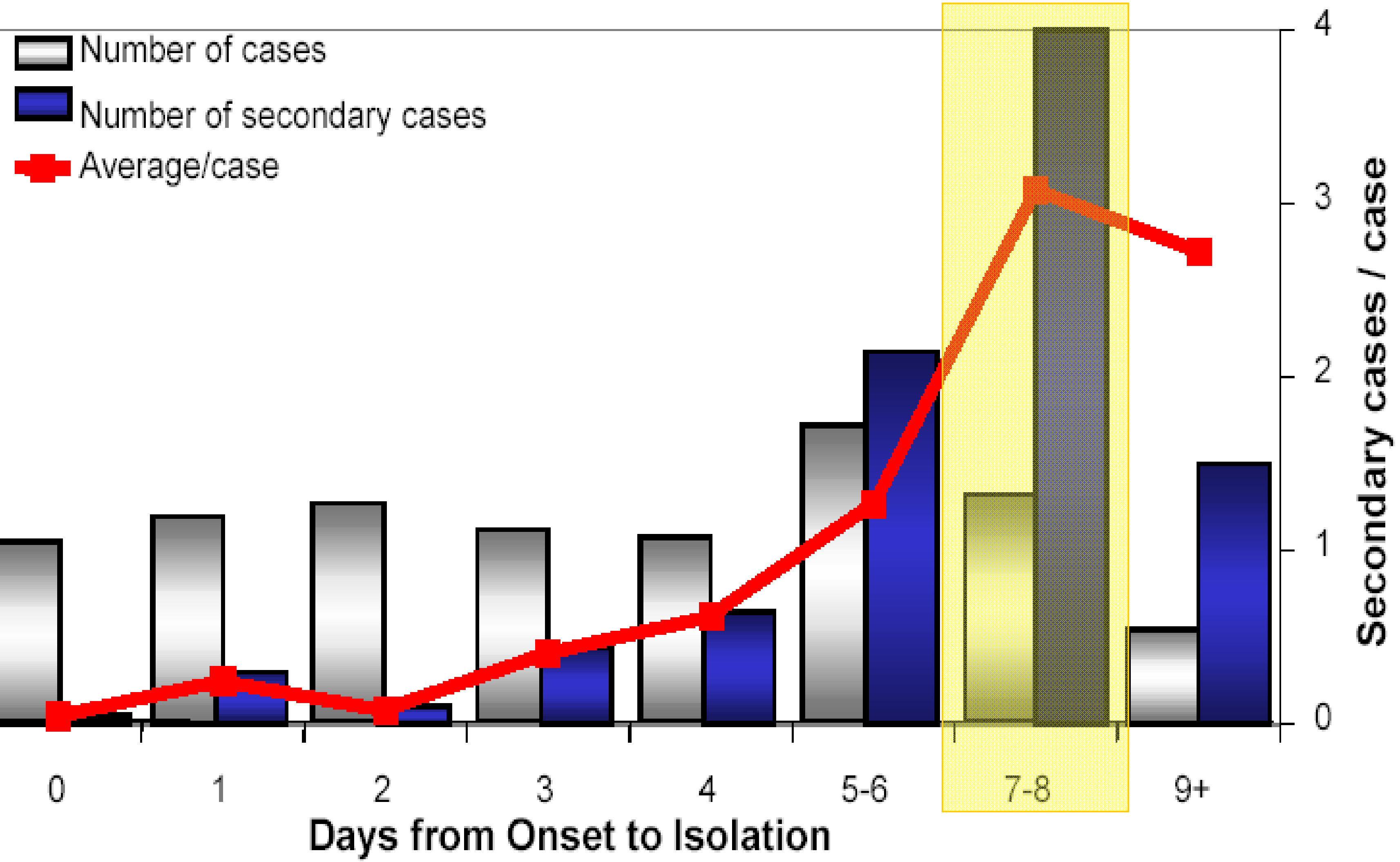
Positive samples: respiratory samples, stool, blood, urine, conjunctival secretions

Droplet transmission
- Respiratory-droplet
- Fecal-droplet (?)

Contact transmission
- Direct > indirect

Period of transmissibility
- Symptom onset to at least 10 days after fever resolution*
  - *virus detectable but no reported transmission

Generation of Secondary Cases



http://www.who.int/csr/sars/en/WHOconsensus.pdf

Spread of SARS

spitals

or amplifiers of virus

% of people infected were HCW

Travel

nternational flights

•37 positive individuals

ghts led to spread

•>60 cases exported by air

Transmission in the Face of Precautions

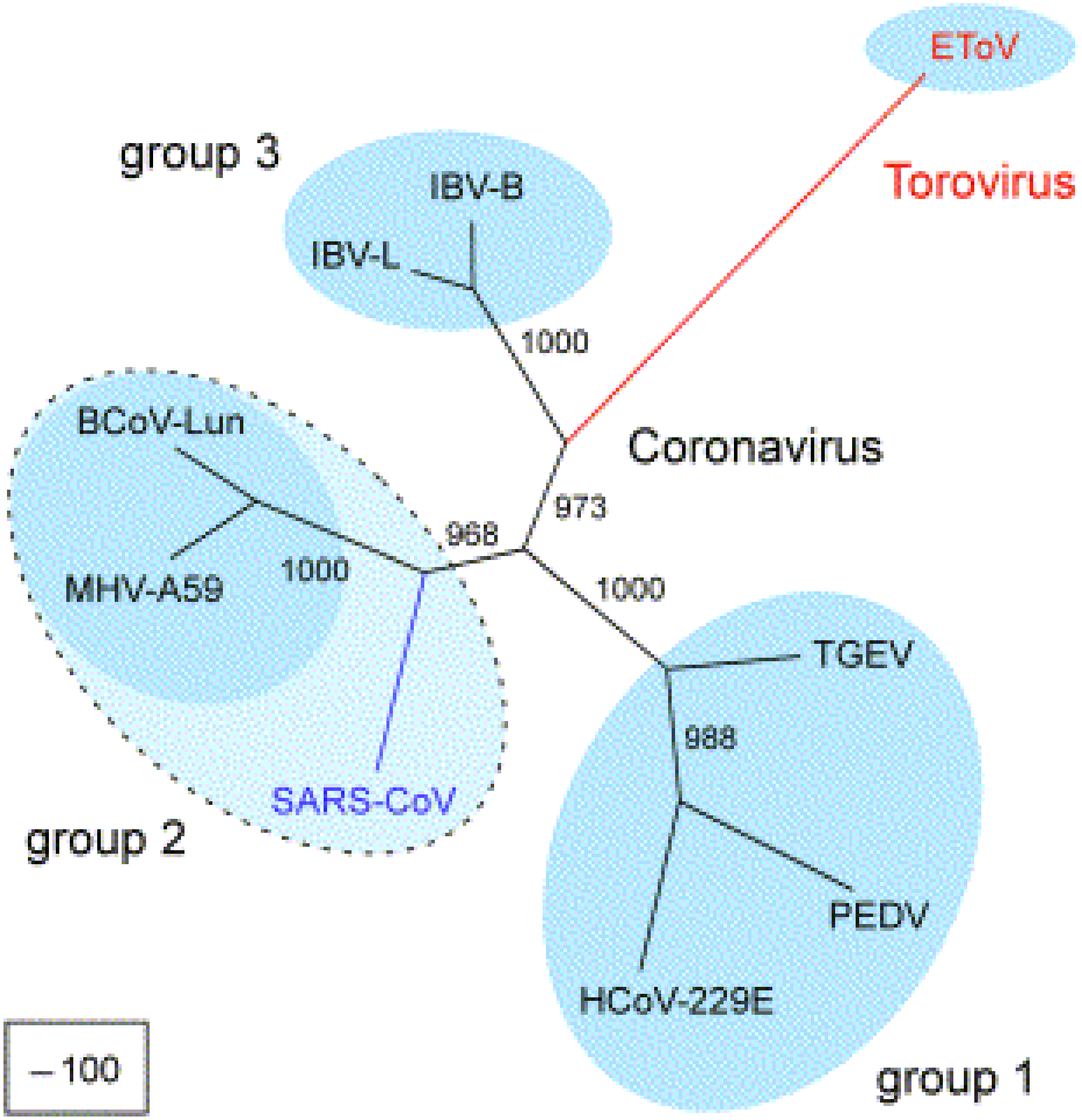
cumstances:

- Severely ill patients
  - Shedding high titer
- Close contact
- Aerosol generating procedures

ential Exposures:

- High level of airborne virus
  - Improper N95 respirators (non fit-tested)
- Break in containment
  - For example, improper removal of PPE

SARS-CoV- Group 2 CoV



ARS coronavirus: Where did it come from?

onotic transmission: Viruses genetically related to SARS-CoV isolated from Himalayan palm civet cats.

n live animal markets

SARS-CoV-like virus

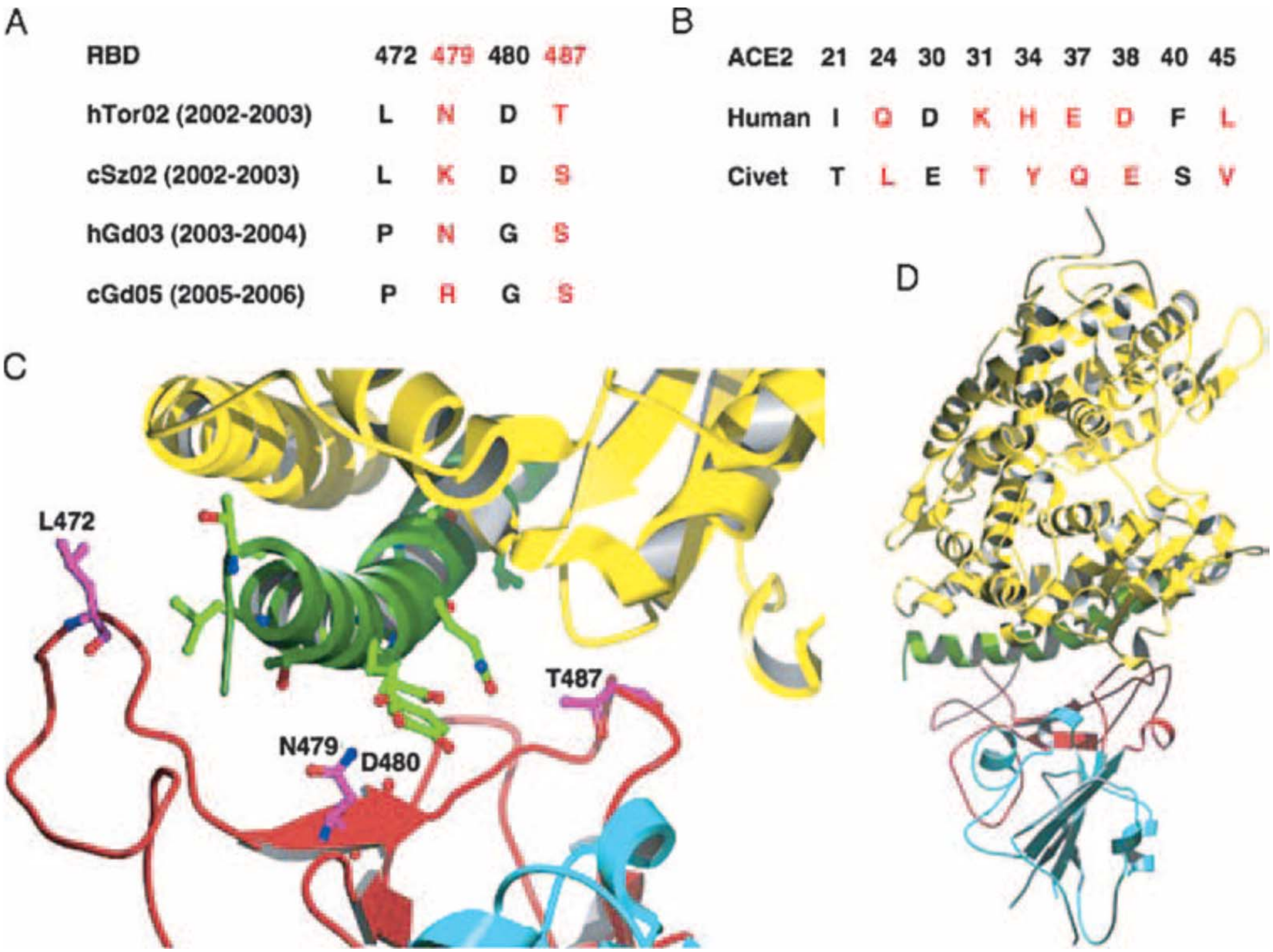
oncurrently identified

- •Y. Guan *et al.*, *Science* 302, 276 (2003).

Not found in wild civet

- •L. L. Poon et al., J. Virol. 79, 2001 (2005)

hanges in Spike Important in Emergence of SARS-CoV?



RBD	472	479	480	487
hTor02 (2002-2003)	L	N	D	T
cSz02 (2002-2003)	L	K	D	S
hGd03 (2003-2004)	P	N	G	S
cGd05 (2005-2006)	P	R	G	S

ACE2	21	24	30	31	34	37	38	40	45
Human	I	Q	D	K	H	E	D	F	L
Civet	T	L	E	T	Y	Q	E	S	V

What is the natural reservoir of SARS-CoV?

ese horseshoe bats: Viruses genetically related to S-CoV have been isolated from animals.

RS-CoV

9% RT-PCR

4% N antibody

ossible Genesis of SARS Outbreak

orseshoe bat

Direct zoonosis

? Intermediate

et al.,

Palm civet

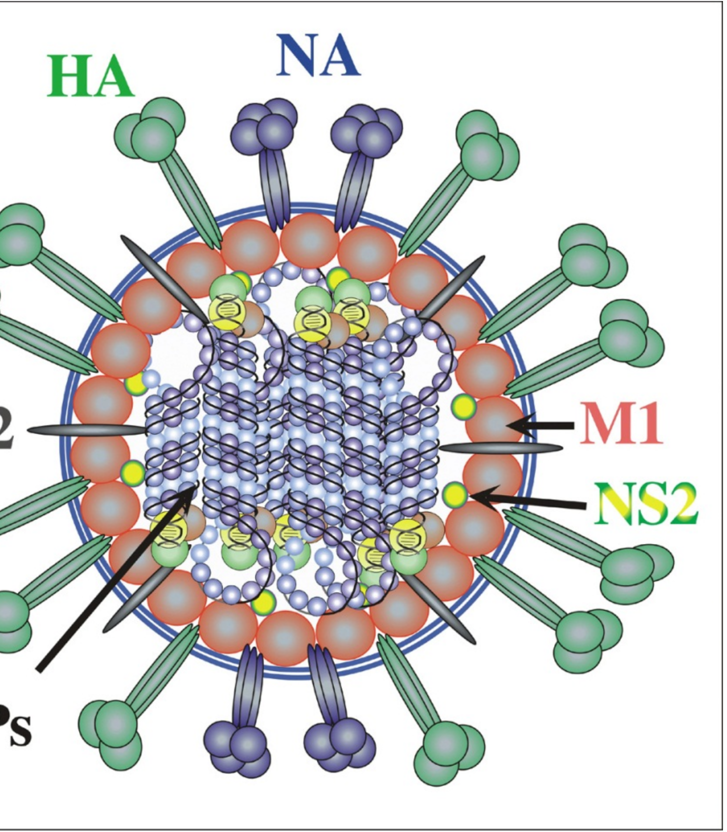
- SARS-CoV antibodies Science 370: 297

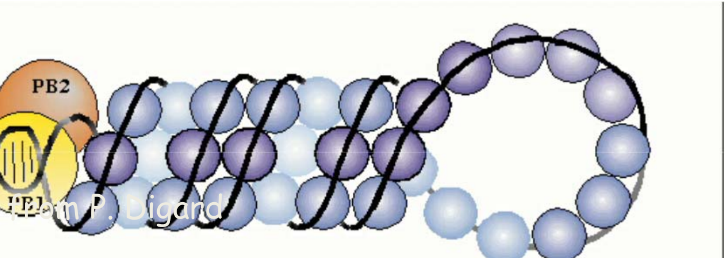
-40% of wild animal traders

-20% of slaughterers

-5% of vegetable traders

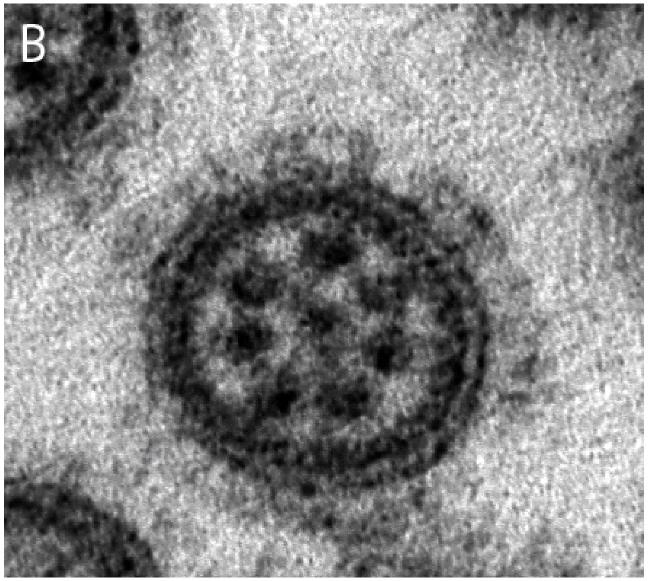
Influenza A Virus





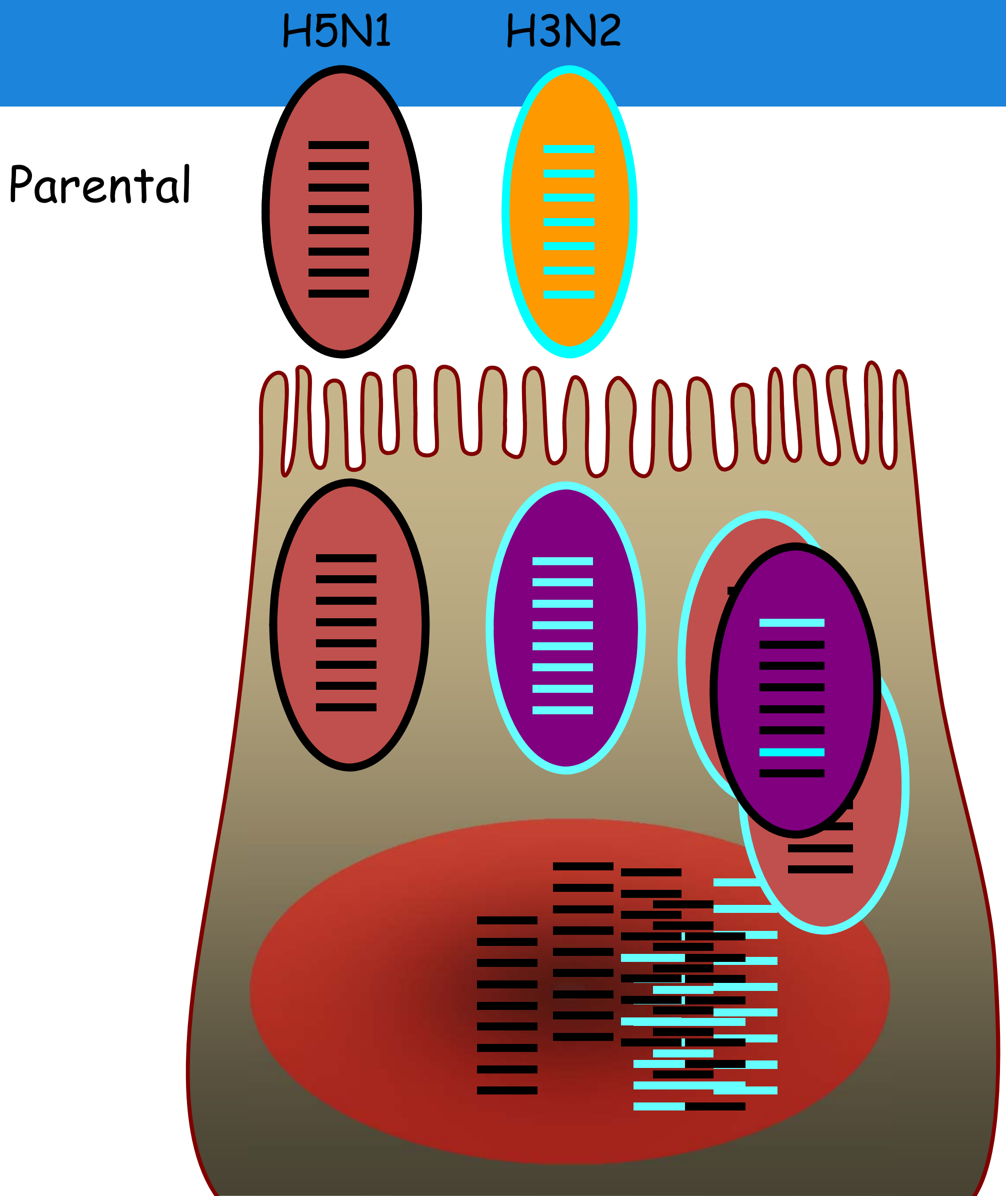
From P. Digard

- 16 distinct HA's +1
  - (H1-H16)
- 9 distinct NA's +1
  - (N1-N9)
- Nomenclature
  - A/Chicken/WI/5/78 (H7N7)



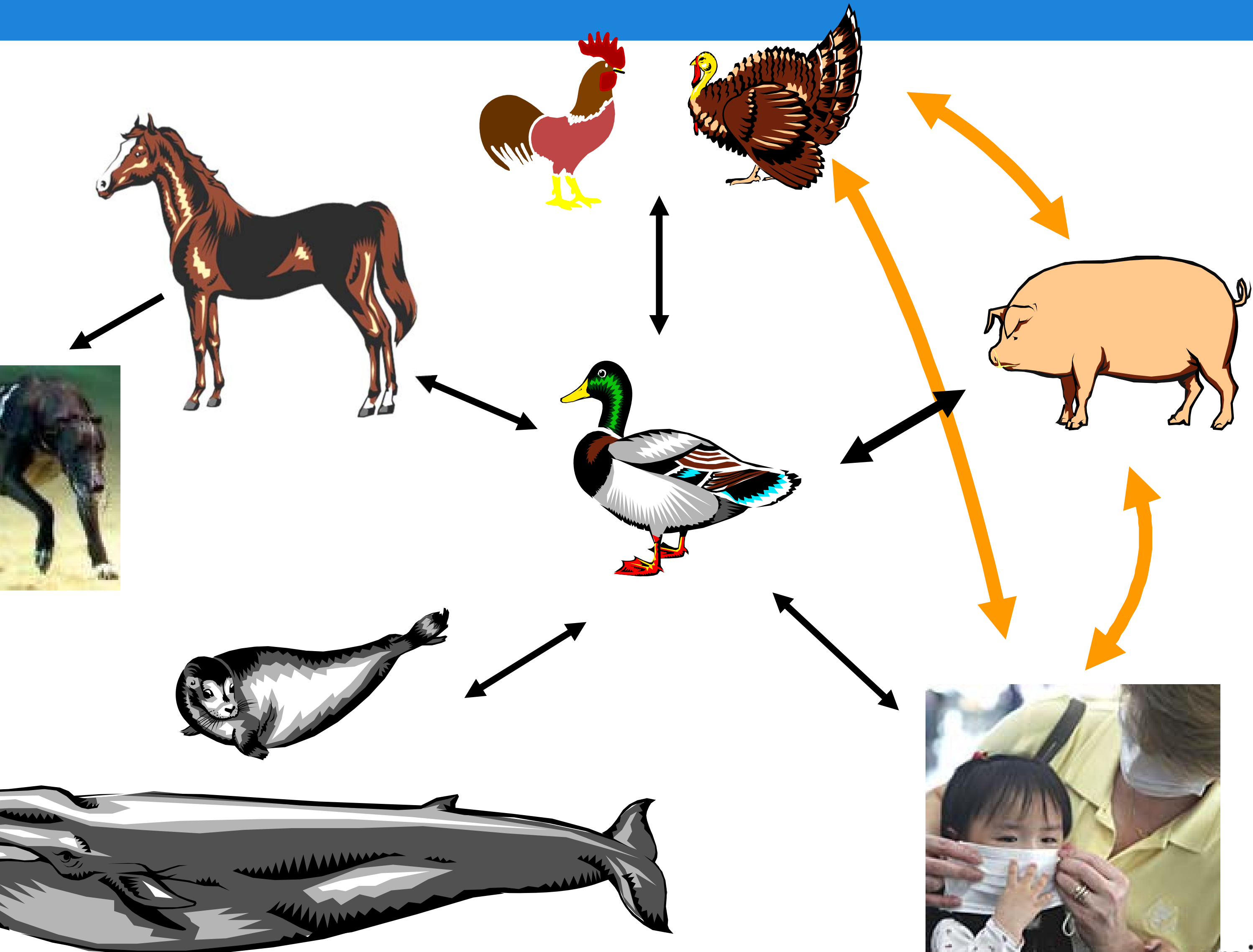
Thin Section EM. T. Noda, et al, Nature 439

Importance of a Segmented RNA Genome



256 Possible
Combinations

Influenza A Ecology and Emergence of Strains with Pandemic Potential



tural Distribution of HA and NA

Hemagglutinin (HA)

Human Beings	Other Mammals	Aquatic Birds
👪	🐖	🦆
👪		🦆
👪	🐖 🐎 🐕	🦆
	🐖 🦭	🦆
Zoonotic		🦆
		🦆
Zoonotic	🐎 🦭	🦆
		🦆
Zoonotic		🦆
		🦆
		🦆
		🦆
		🦆
		🦆

Neuraminidase (NA)

	Human Beings	Other Mammals	Aquatic Birds
N1	👪	🐖	🦆
N2	👪	🐖	🦆
N3			🦆
N4			🦆
N5			🦆
N6		🦭	🦆
N7	Zoonotic	🐎 🦭	🦆
N8	👪	🐎	🦆
N9			🦆

man Influenza A Virus Evolution

genic Drift

H3N2
1968
Hong Kong →

H3N2
1975
Victoria →

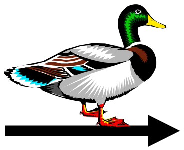
H3N2
1993
Beijing →

H3N2
1997
Sydney →

H3N2
2009
Albany

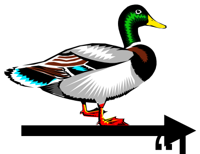
genic Shift

H1N1
1918
Spanish”



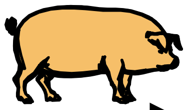
→

H2N2
1957
“Asian”



→

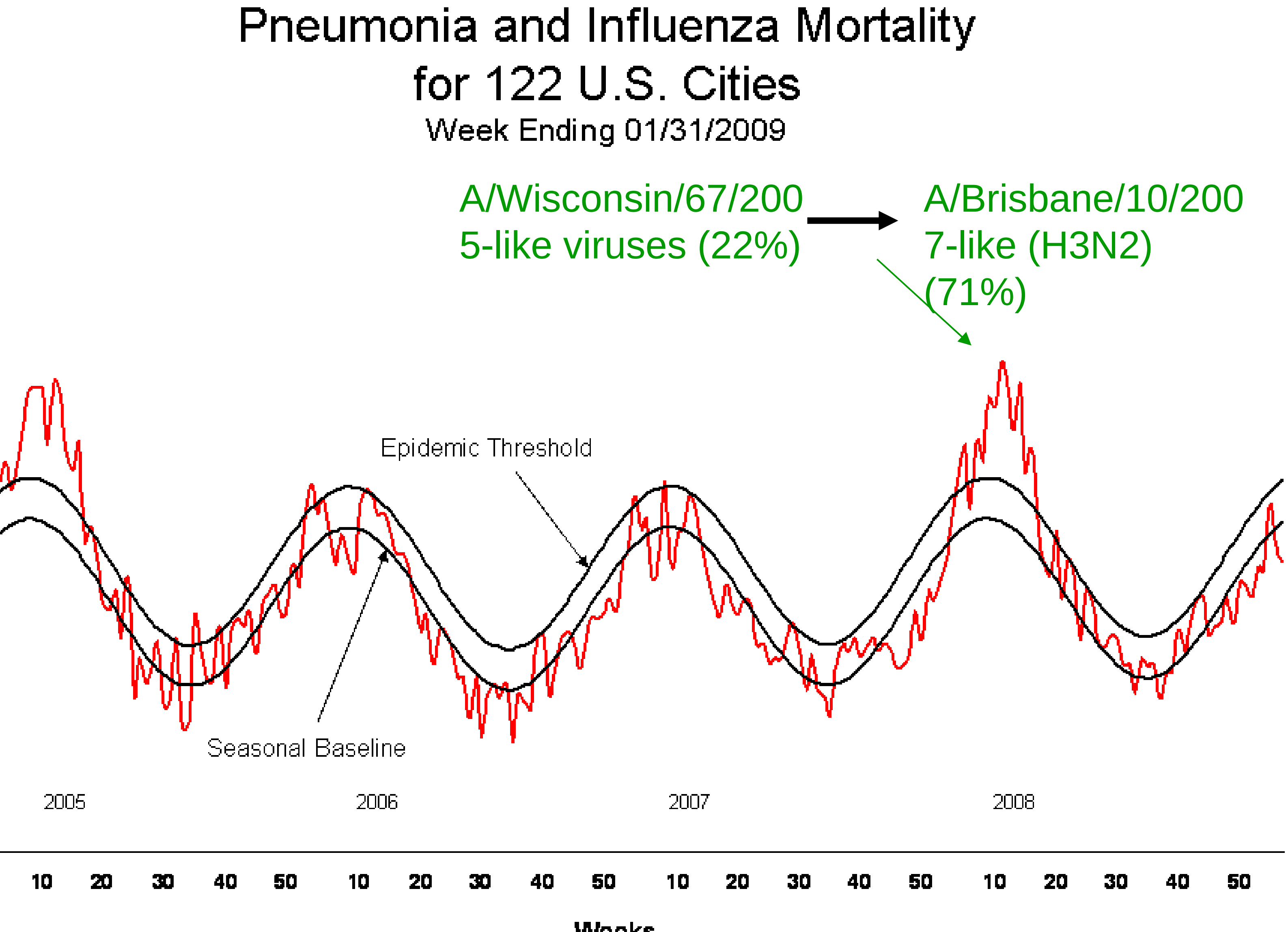
H3N2
1968
“Hong Kong”



→

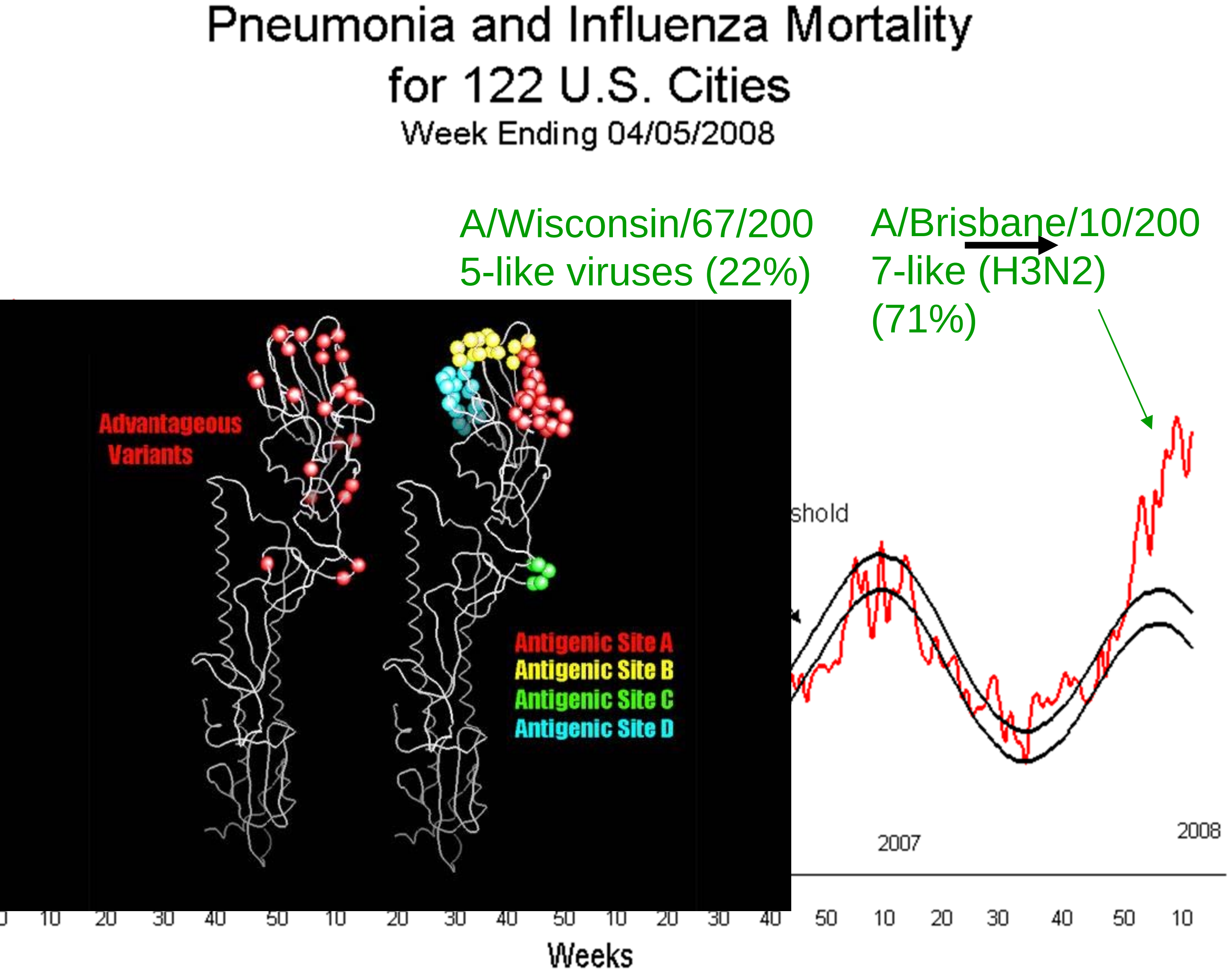
H1N1
2009
“SOIV”

Antigenic Drift Important In 2007/08 Season



A/Wisconsin/67/2005-like viruses (22%) → A/Brisbane/10/2007-like (H3N2) (71%)

rift Important In 2007/08 Season

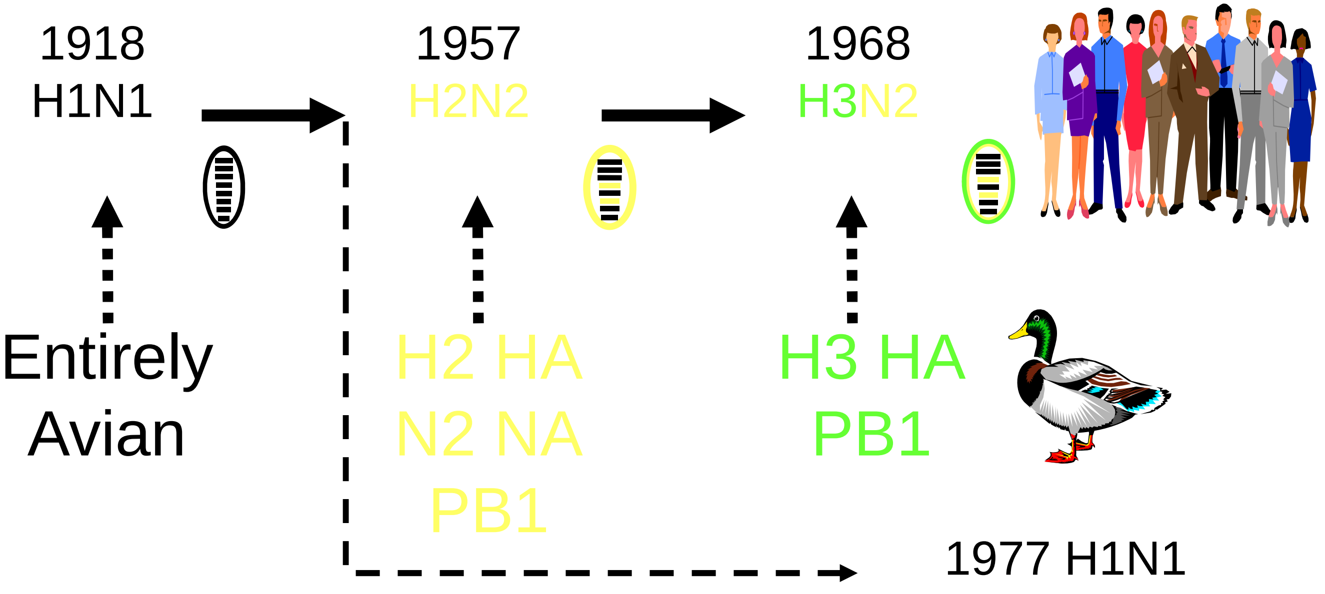


A/Wisconsin/67/2005-like viruses (22%)

A/Brisbane/10/2007-like (H3N2) (71%)

ntigenic Shift and Previous Human Pandemics

 was turned into an emergency infirmary at th
setts during the 1957 "Asian flu" pandemic.
L 306 15 OCTOBER 2004



1918
H1N1

Entirely
Avian

1957
H2N2

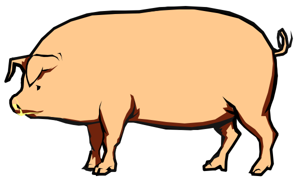
H2 HA
N2 NA
PB1

1968
H3N2

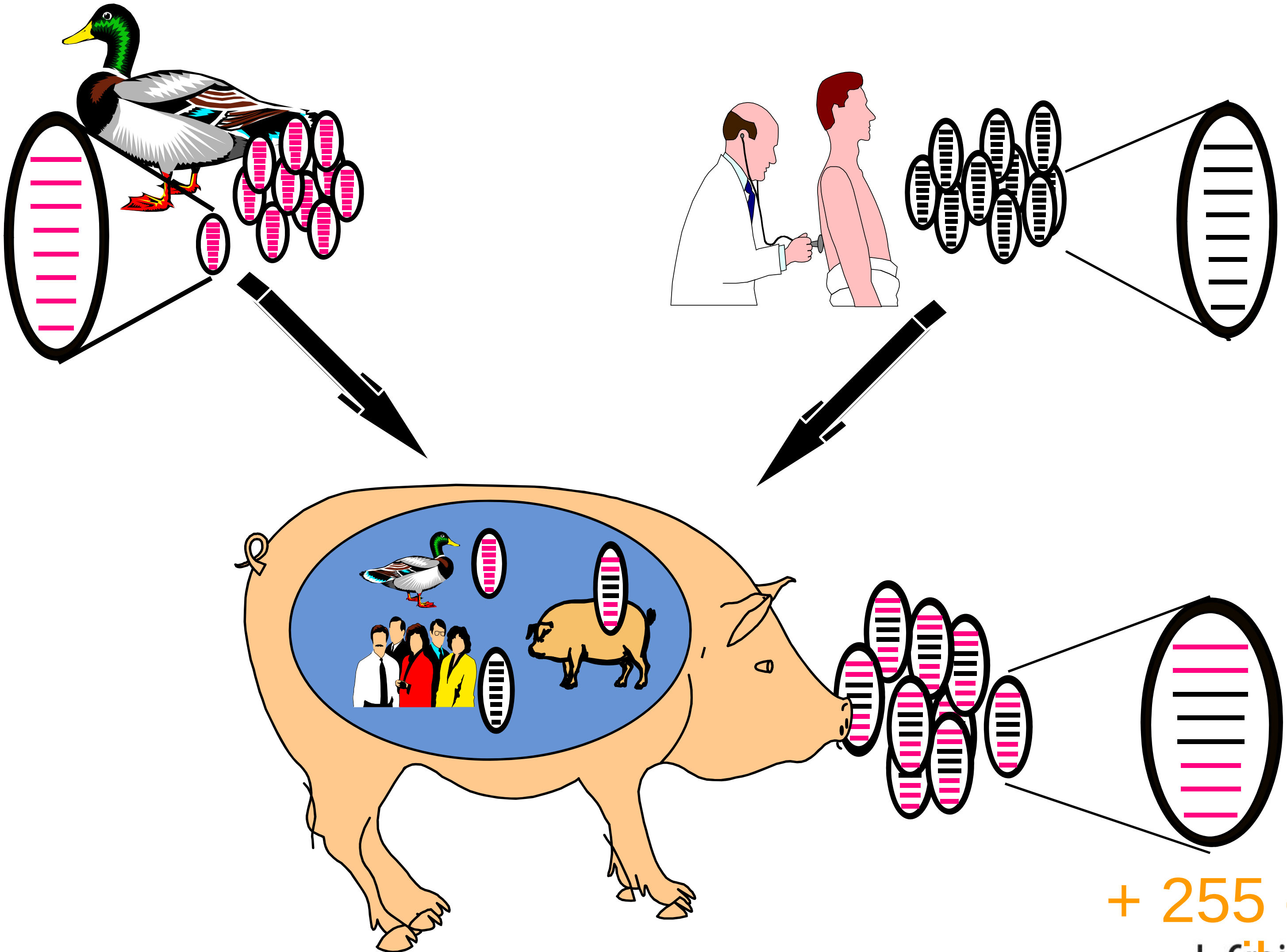
H3 HA
PB1

1977 H1N1

irect interspecies transmission
eassortment between 2 coinfecting strains
eappearance of a previous strain

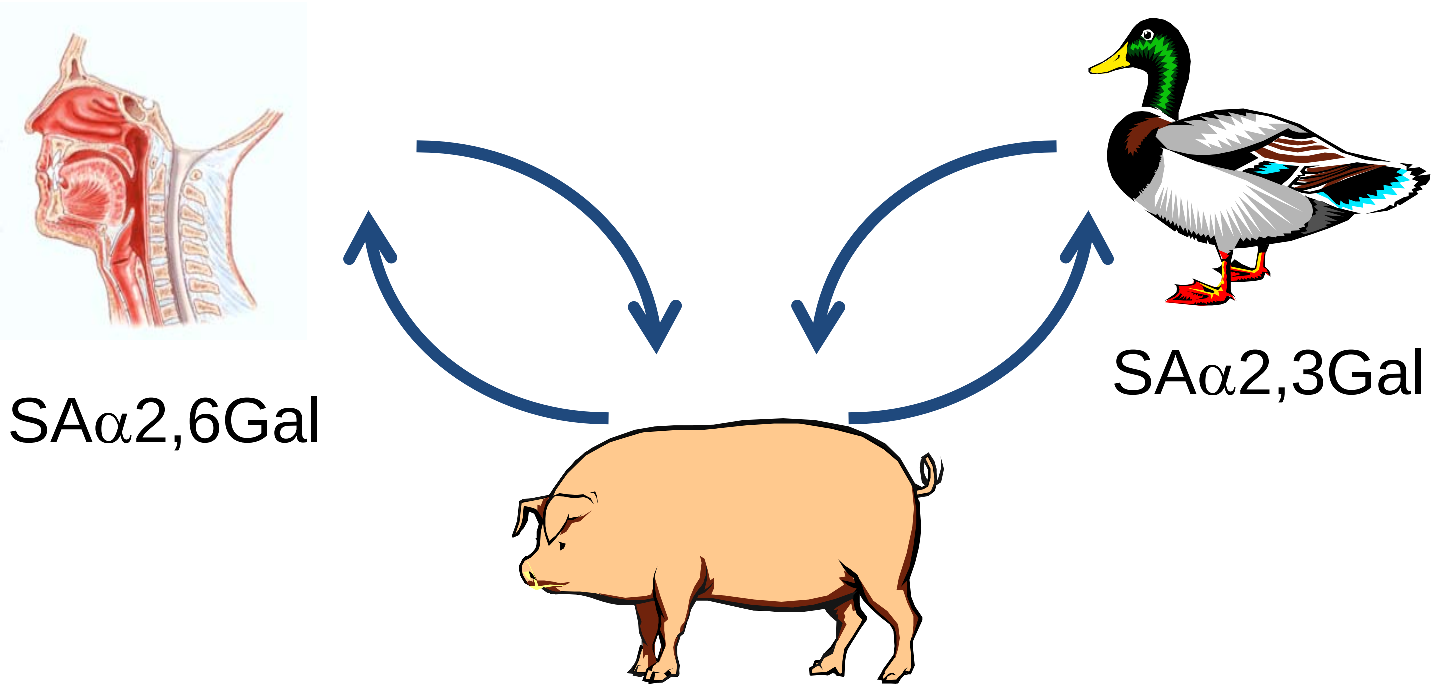


Pigs Are " Mixing Vessels " for Influenza A Viruses



+ 255 other

olecular Mechanism of Mixing Vessel



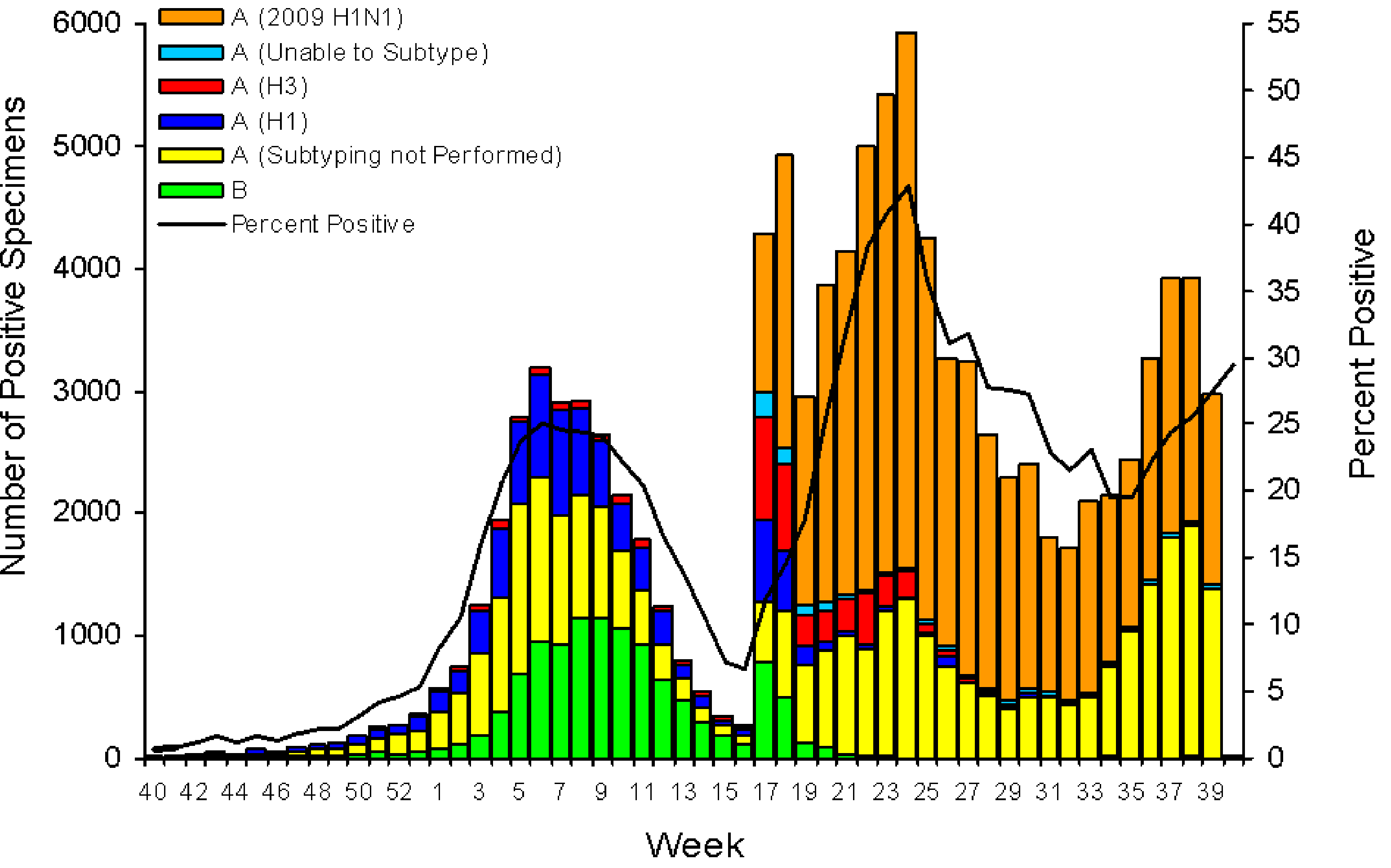
SAα2,6Gal

SAα2,3Gal

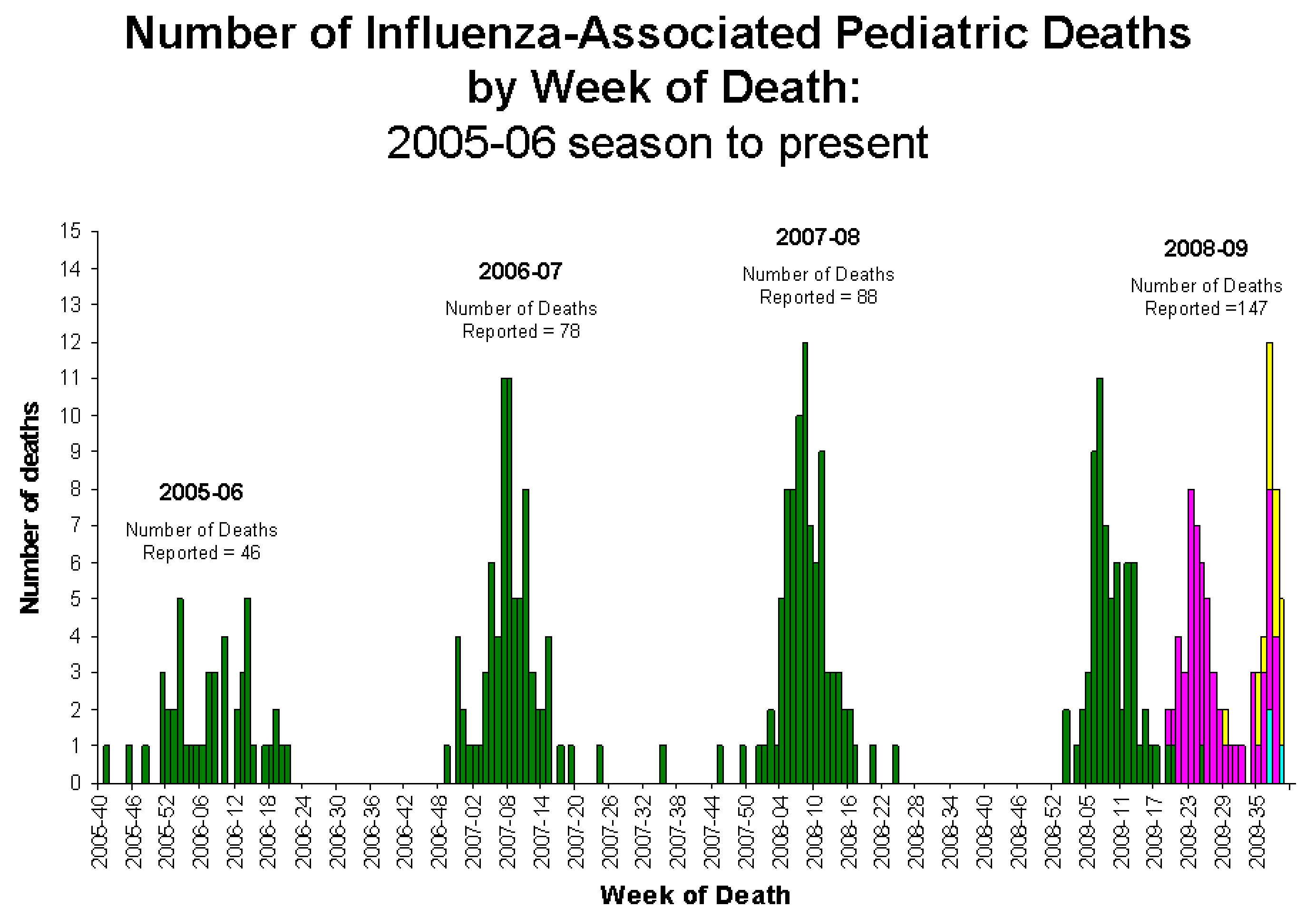
SAα2,3Gal
And
SA α2,6Gal

Emergence of Novel H1N1 Causes Pandemic

Influenza Positive Tests Reported to CDC by U.S. WHO/NREVSS Collaborating Laboratories, National Summary, 2008-09



New H1N1 Causes Out of Season Pediatric Deaths

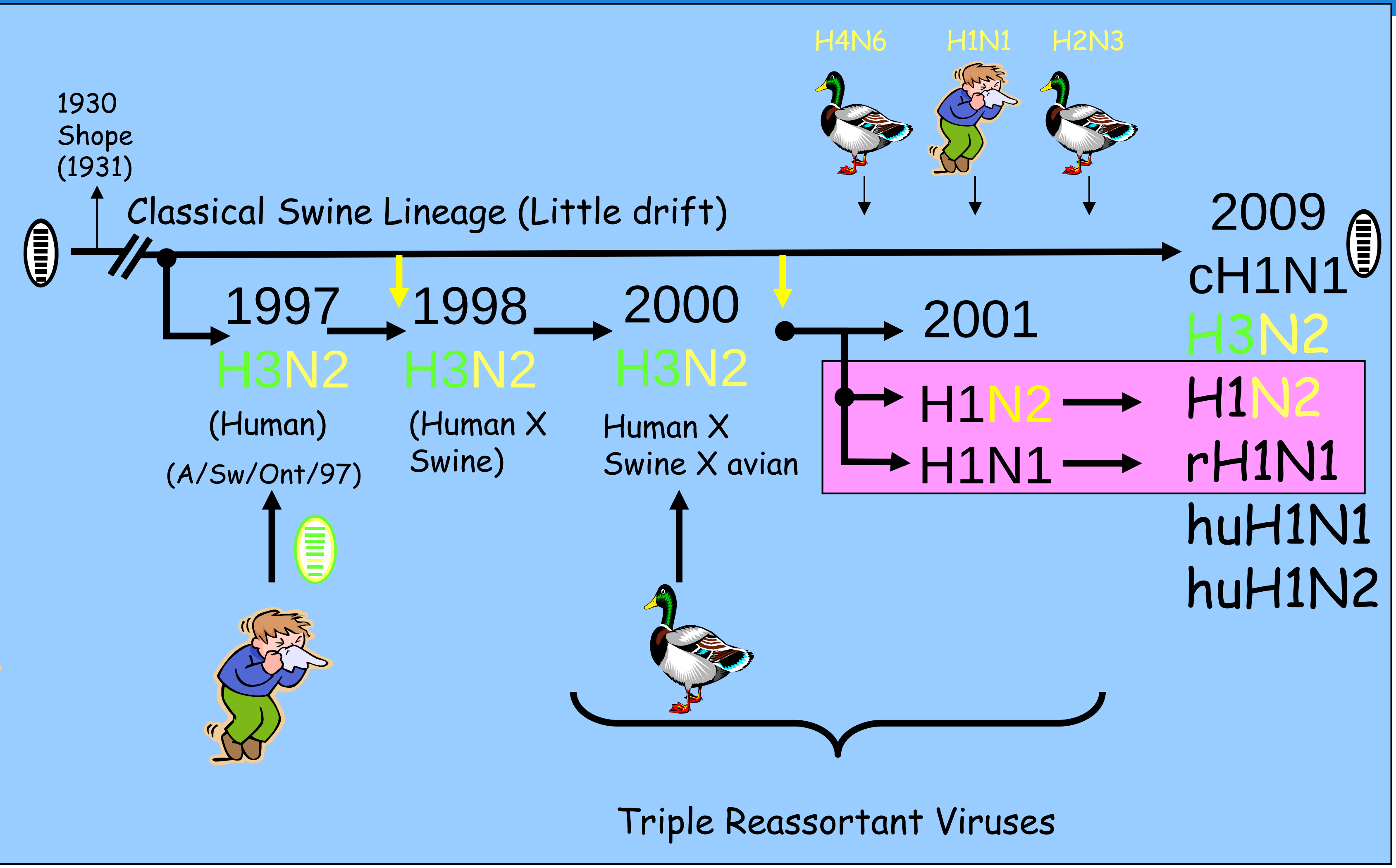


http://www.cdc.gov/flu/weekly/

s of 4 October 2009, more than 375,000 laboratory
onfirmed cases of pandemic influenza H1N1 2009 and over
500 deaths reported to WHO

ere did the 2009 H1N1 pandemic virus come from?

Swine Influenza A Virus in North America



1930
Shope
(1931)

Classical Swine Lineage (Little drift)

H4N6
H1N1
H2N3

1997
H3N2
(Human)
(A/Sw/Ont/97)

1998
H3N2
(Human X
Swine)

2000
H3N2
Human X
Swine X avian

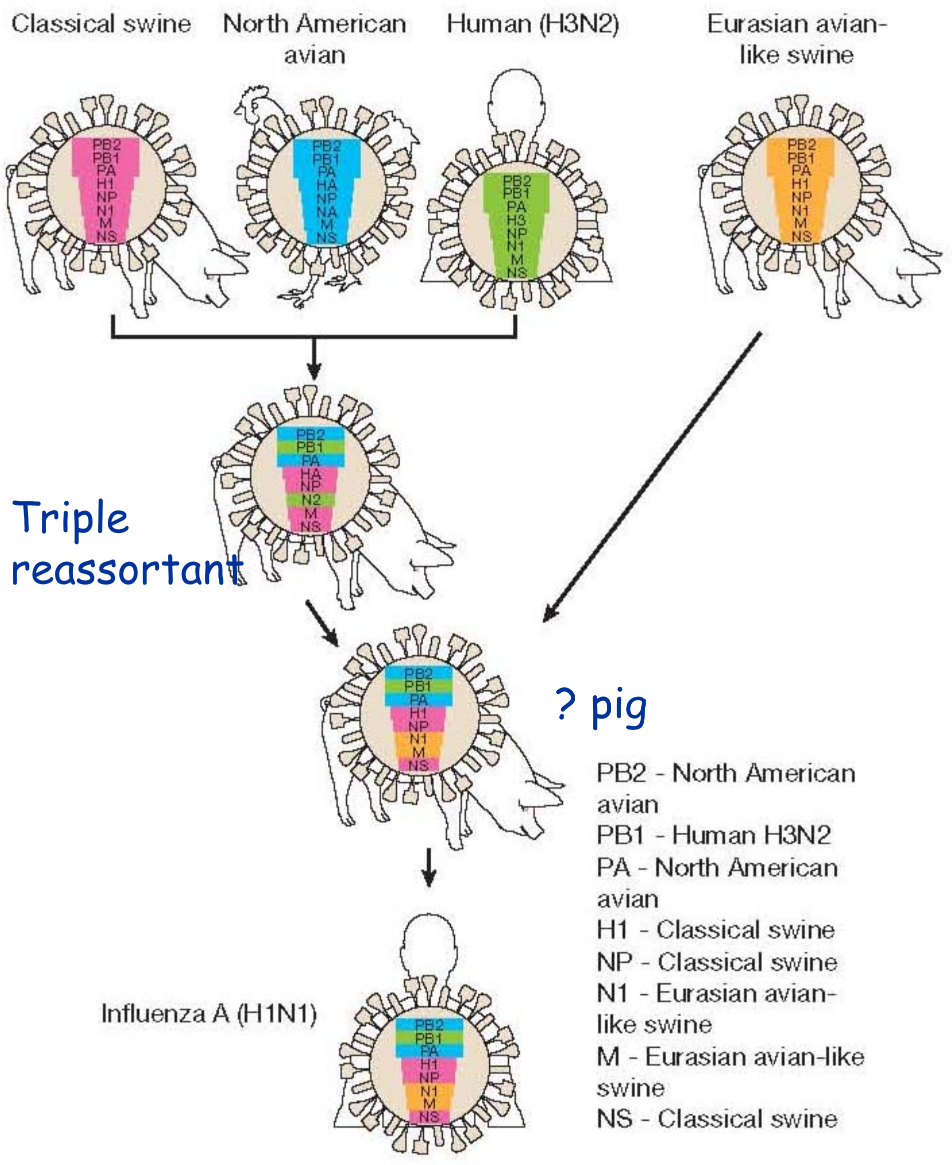
2001
H1N2
H1N1

2009
cH1N1
H3N2
H1N2
rH1N1
huH1N1
huH1N2

Triple Reassortant Viruses

olution of Human
009 H1N1 Virus

eumann, T. Noda, and Y. Kawaoka.
rgence and pandemic potential of
e-origin H1N1 influenza virus.
ure 459 (7249):931-939, 2009.



Triple reassortant

? pig

PB2 - North American avian
PB1 - Human H3N2
PA - North American avian
H1 - Classical swine
NP - Classical swine
N1 - Eurasian avian-like swine
M - Eurasian avian-like swine
NS - Classical swine

Figure 4 | **Genesis of swine-origin H1N1 influenza viruses.** In the late 1990s, reassortment between human H3N2, North American avian, and classical swine viruses resulted in triple reassortant H3N2 and H1N2 swine viruses that have since circulated in North American pig populations. A triple

Emerging and Re-emerging Diseases (NIAID)

o I—Pathogens Newly gnized in the Past Two Decades

anthamebiasis
stralian bat lyssavirus
besia, atypical
rtonella henselae
rlichiosis
cephalitozoon cuniculi
cephalitozoon hellem
terocytozoon bieneusi
ndra or equine morbilli virus
man herpesvirus 8
man herpesvirus 6
rvovirus B19

Group II—Re-emerging Pathogens

- Enterovirus 71
- Clostridium difficile
- Mumps virus
- Streptococcus, Group A
- Staphylococcus aureus

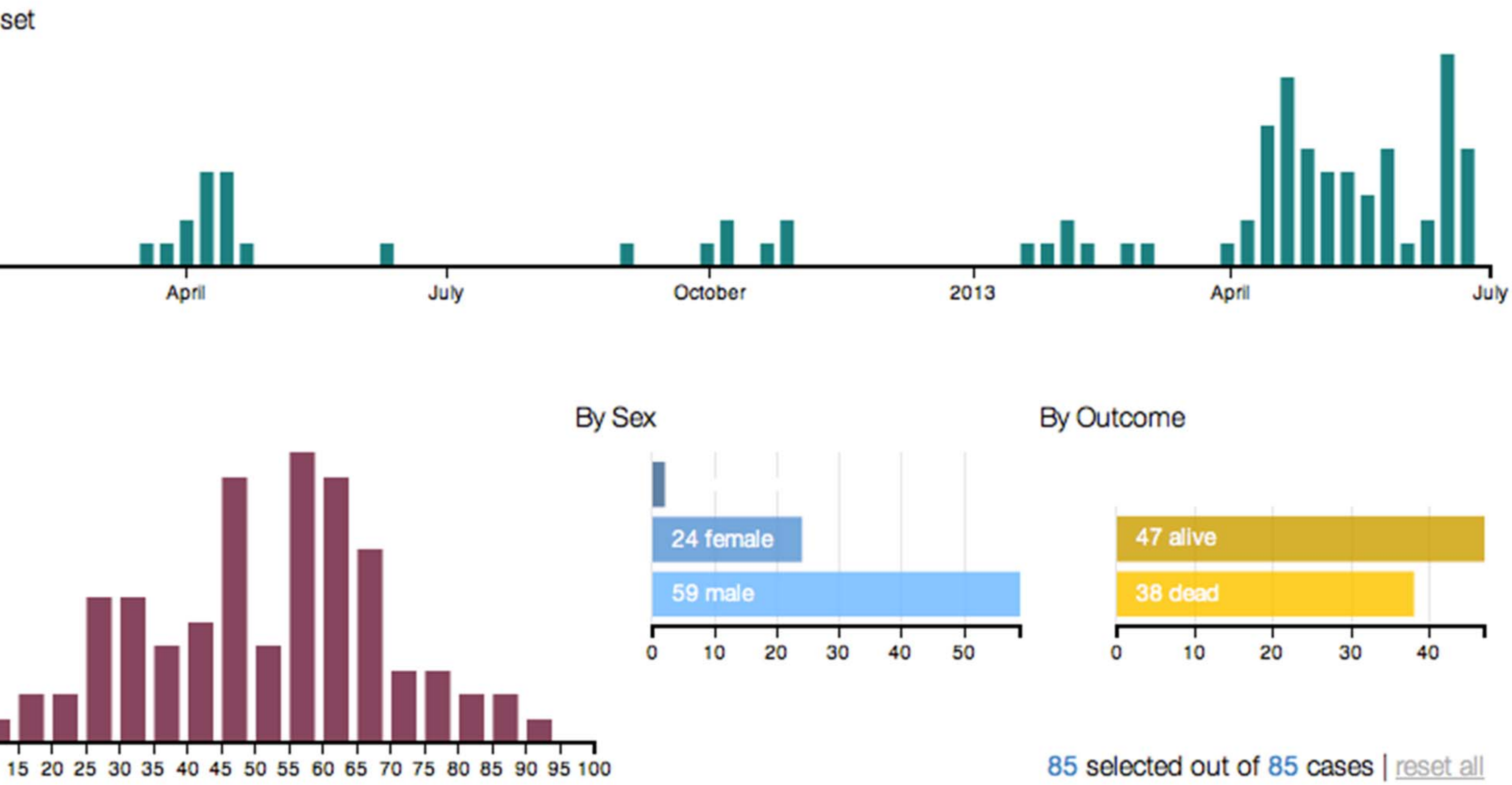
Group III—Agents with Bioterrorism Potential

See the full list of NIAID Category A, B, and C Priority Pathogens.

Most Recent

- Middle East Respiratory Syndrome-Coronavirus (MERS-CoV)
- H7N9-Influenza A Virus

MERS Epidemiology



Clinical Disease Course

SARS

1. Transmission
   -aerosol, fomites
2. Acute Infection
   -virus replication in lung
   -”cytokine storm”
   -mixed inflammatory infiltrates
   -developing lung damage (viral and host)
   -SARS-CoV specific IgG
3. Non-Severe cases (70-80%)
   -decrease titer
   -improved chest x-ray
4. Severe cases (20-30%)
   -intubation
   -ARDS
   -hypoxemia
   -mortality (10% total)

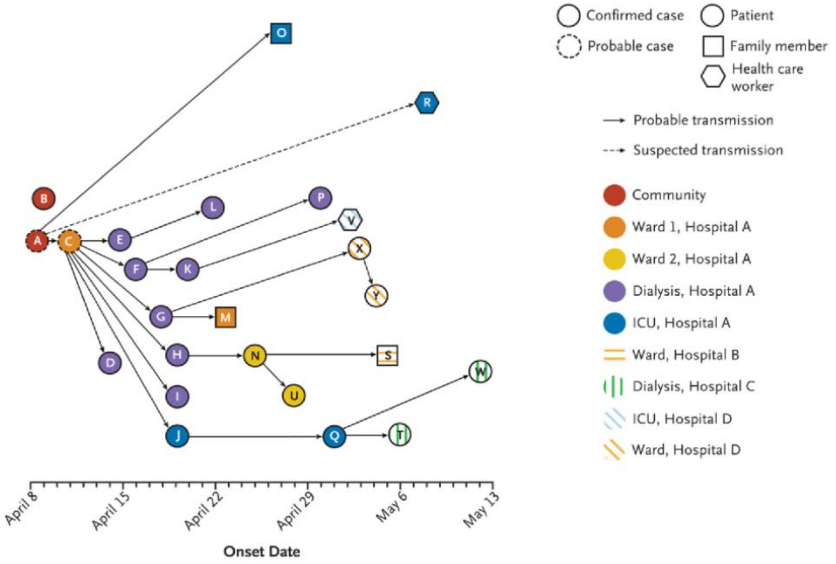
MERS

1. Transmission
   -aerosol, fomites?
2. Acute Infection
   -virus replication in lung (and kidney?)
   -probably ”cytokine storm”
   -mixed inflammatory infiltrates
   -developing lung damage (viral and host)
   -MERS-CoV specific IgG

   ?
3. Non-Severe cases (50%?)
   -decrease titer
   -improved chest x-ray
4. Severe cases (50%?)
   -intubation
   -ARDS
   -hypoxemia
   -mortality (50% total)

MERS Clinical spread

Al Hasa Hospital

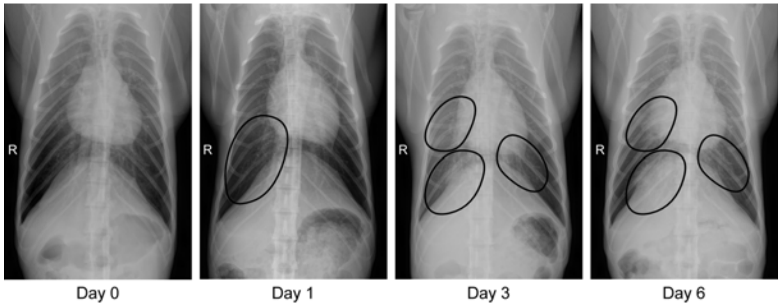


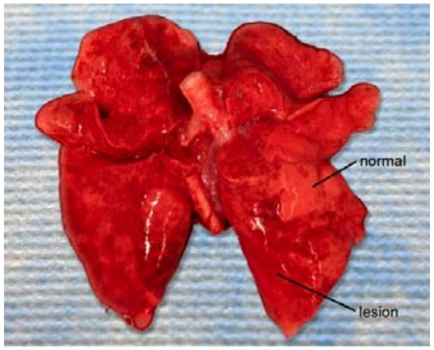
-Spread from some unknown reservoir to people (Camels/Bats suspected)
-Spread from person to person in hospital settings
-Spread by travel, either vacation or for treatment

MERS-CoV replicates efficiently and causes MERS-like linical and pathological symptoms in Rhesus Macaques

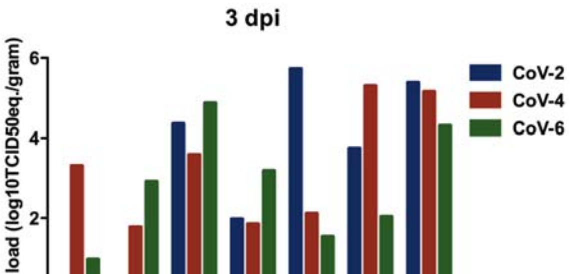
S-CoV replicates in Rhesus Macaques

ncent Munster et al NEJM, 2013.



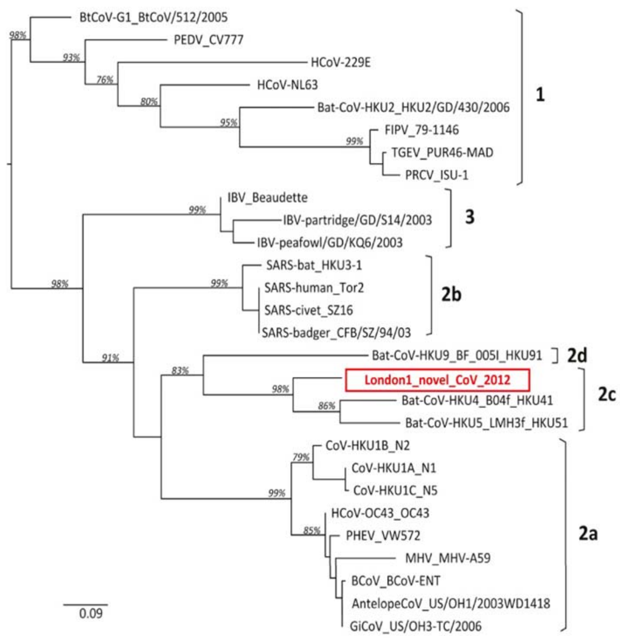


Day 3



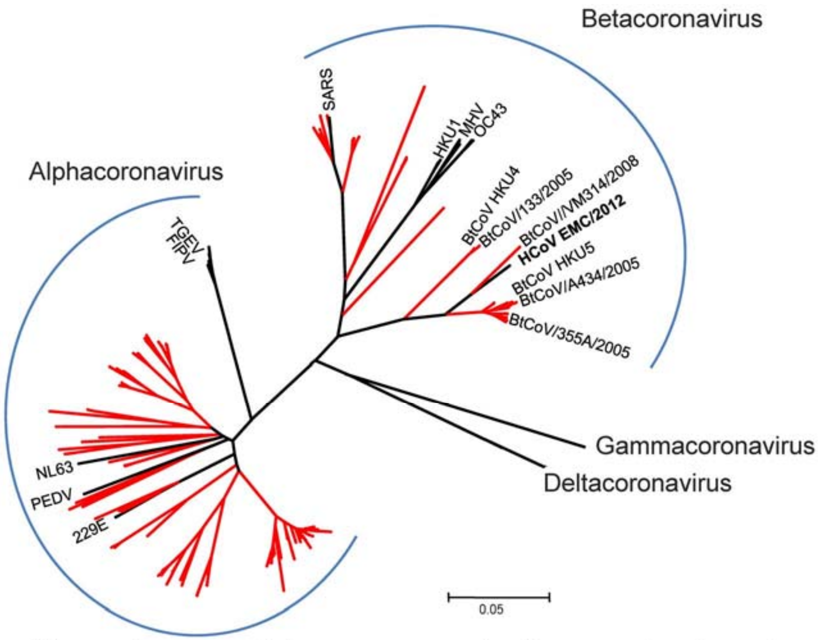
Coronavirus Phylogeny

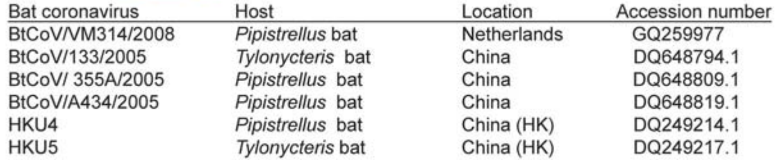
lationships of partial sequences from the polymerase gene (nsp12) of the coronavirus sequence obtained at
ection Agency, together with representative coronaviruses from different groups



ained at the Health Protection Agency has been tentatively named as London1_novel CoV 2012. The phylogenetic tree was
th fastTree software, using the maximum-likelihood method with general time-reversible model of nucleotide substitution.
es were obtained with 1,000 replicates. Coronavirus groups are shown on the right hand side of the tree, with 1, 2 and 3
to Alpha, Beta and Gammacoronaviruses respectively.

(partial NSP12)

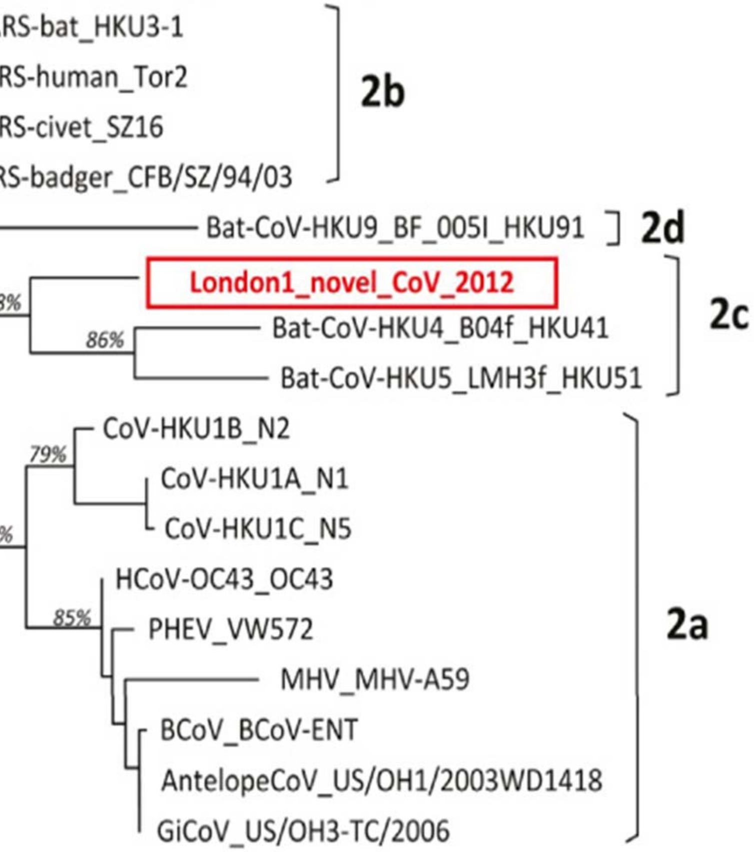


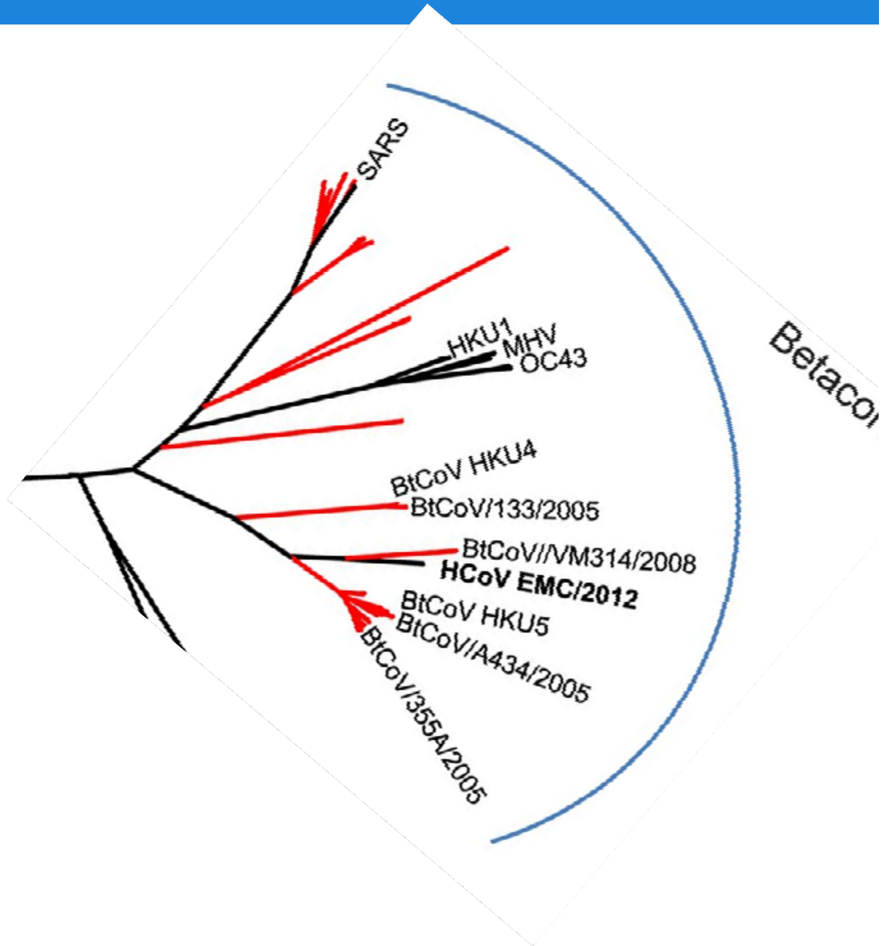


Bat coronavirus	Host	Location	Accession number
BtCoV/VM314/2008	*Pipistrellus* bat	Netherlands	GQ259977
BtCoV/133/2005	*Tylonycteris* bat	China	DQ648794.1
BtCoV/ 355A/2005	*Pipistrellus* bat	China	DQ648809.1
BtCoV/A434/2005	*Pipistrellus* bat	China	DQ648819.1
HKU4	*Pipistrellus* bat	China (HK)	DQ249214.1
HKU5	*Tylonycteris* bat	China (HK)	DQ249217.1

(Partial RdRp)

Coronavirus Phylogeny





Chan et al. MBIO 2013

(Partial RdRp)

oonotic H7N9 Influenza Outbreak

February		March		April		May		June		July		unknown month of onset		Total	
cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
2	2	30	12	88	7	3	0	0	0	2	0	10	23	135	44

dentified unique H7N9 virus in people in late March
ovel subtype for humans
- Antigenic shift -> Pandemic potential

equence of first viruses available April 1
35 cases to date

volution of H7N9

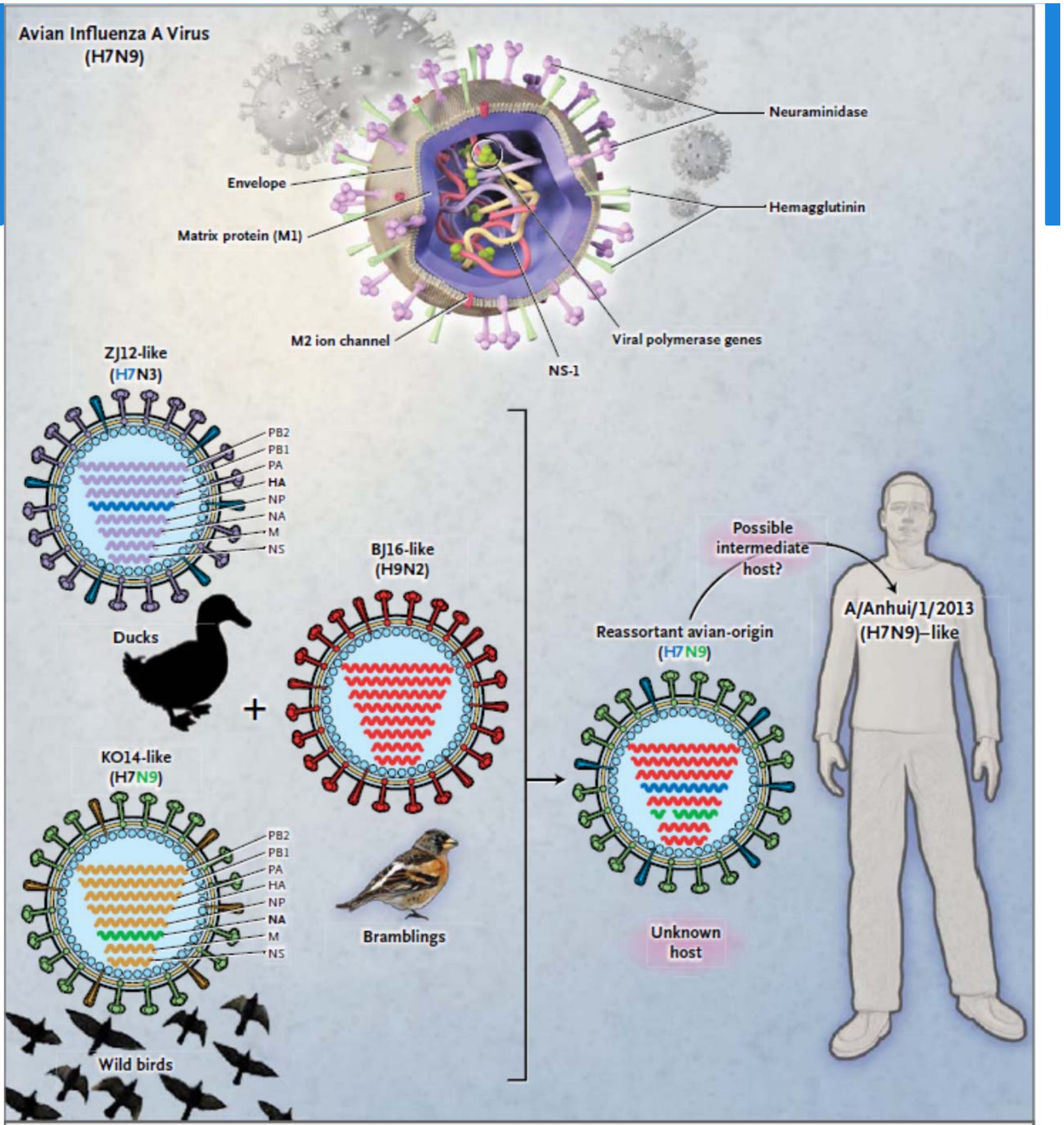
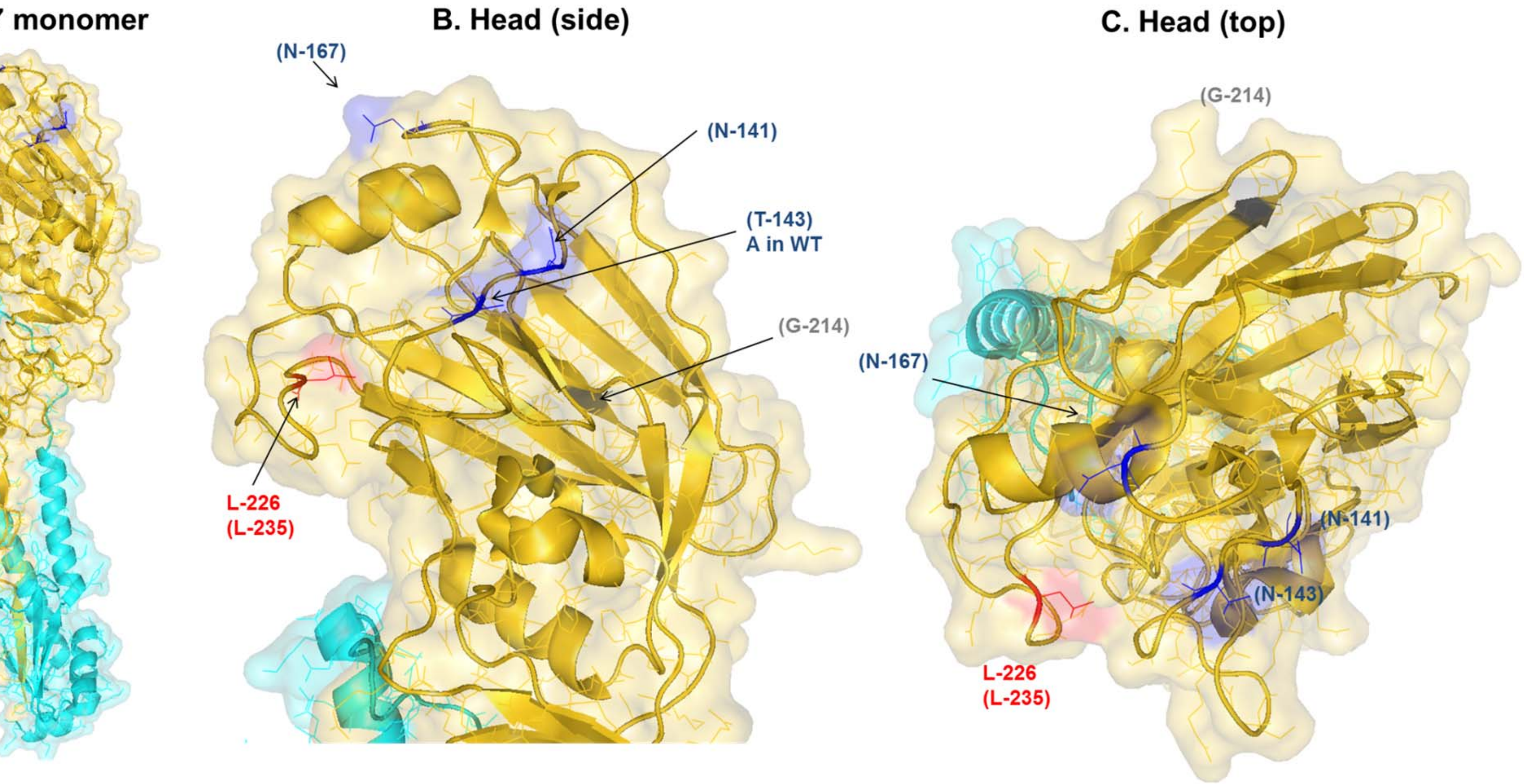


Figure 2. **Hypothetical Host and Lineage Origins of the Gene Segments of the Novel Reassortant Human Influenza A (H7N9) Viruses.** The colors of the gene segments in the ovals indicate their origin. BJ16 denotes A/brambling/Beijing/16/2012, KO14 A/wild bird/Korea/A14/2011, and ZJ12 A/duck/Zhejiang/12/2011.

J. Craig Venter™

Mutations In H7 HA Important in Zoonosis/Transmission

ep sequencing shows mixed population of virus genome in human isolate
R. Fouchier and J. Richt collaboration



Discussion Questions

disease of animals important to humans?

Why/Why not

Examples

do we know SARS-CoV was new to humans?

t profession was dramatically affected by SARS?

t are mechanisms of evolution of influenza A virus?

the new human H1N1 pandemic virus composed
tirely of swine influenza A virus gene segments?

MERS-CoV and H7N9 serious threats?

J. Craig Venter™

Koch’s Postulates

microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

microorganism must be isolated from a diseased organism and grown in pure culture.

cultured microorganism should cause disease when introduced into a healthy organism.

microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.