

INFLUENZA

The Disease & Its Impact

The Virus & Its Origins

Prevention & Control

&

Recent Events

Alan W. Hampson

Interflu Pty Ltd



Influenza - Symptoms

“About the end of April, suddenly a Distemper arose, ... which **laid hold on very many together**: that is some towns, in the space of a week, above a thousand people fell sick together.

The particular symptoms ... was **a troublesome cough, ... accompanied with a feverish distemper, joined with heat and thirst ... and a grievous pain in the back and limbs.**

Men of a more declining age ... not a few died of it.

... the third part of mankind almost should be distempered with the same, in the space of a month.”

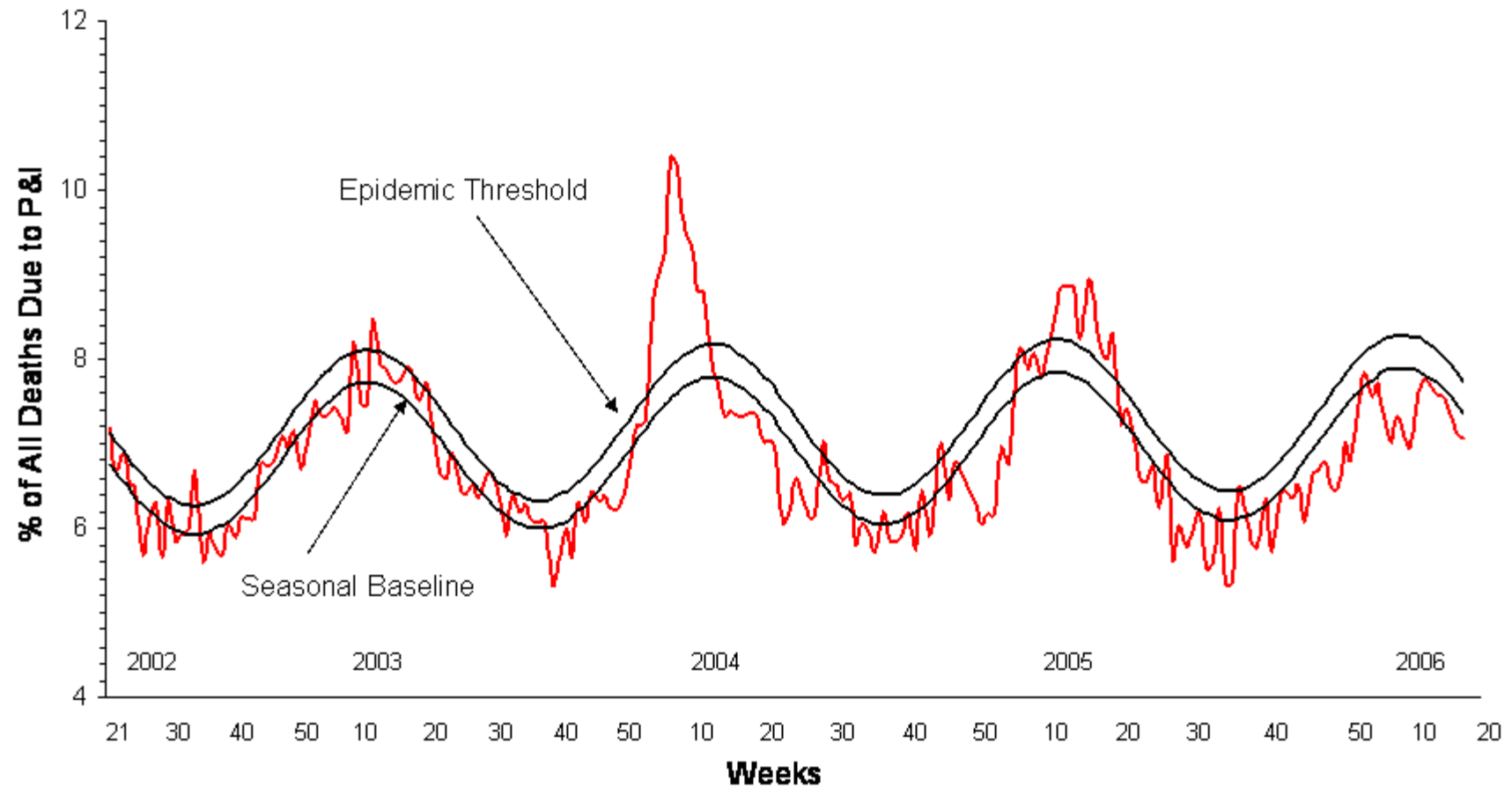
Thomas Willis - 1658 Epidemic in the United Kingdom

Epidemiology & Impact of Influenza

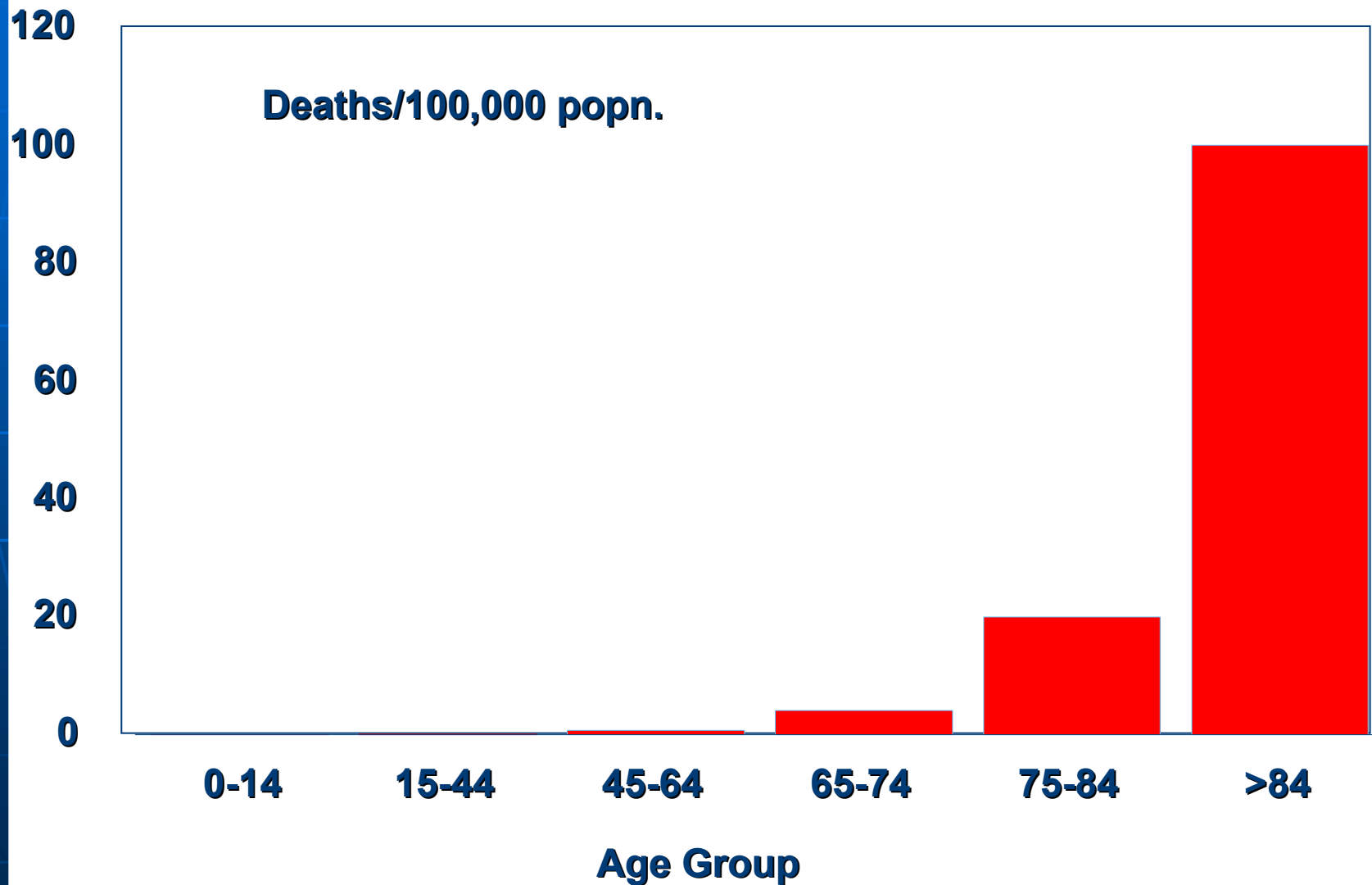
- Repeated infections throughout life
- Seasonal in temperate climates, year round in non-temperate regions
- Rapid spread and high morbidity - often described as “explosive”
- Outbreaks every year
 - regular epidemics
 - mortality in older adults and high risk
 - significant economic impact

Pneumonia and Influenza Mortality for 122 U.S. Cities

Week Ending 04/29/2006



Influenza-Associated Mortality

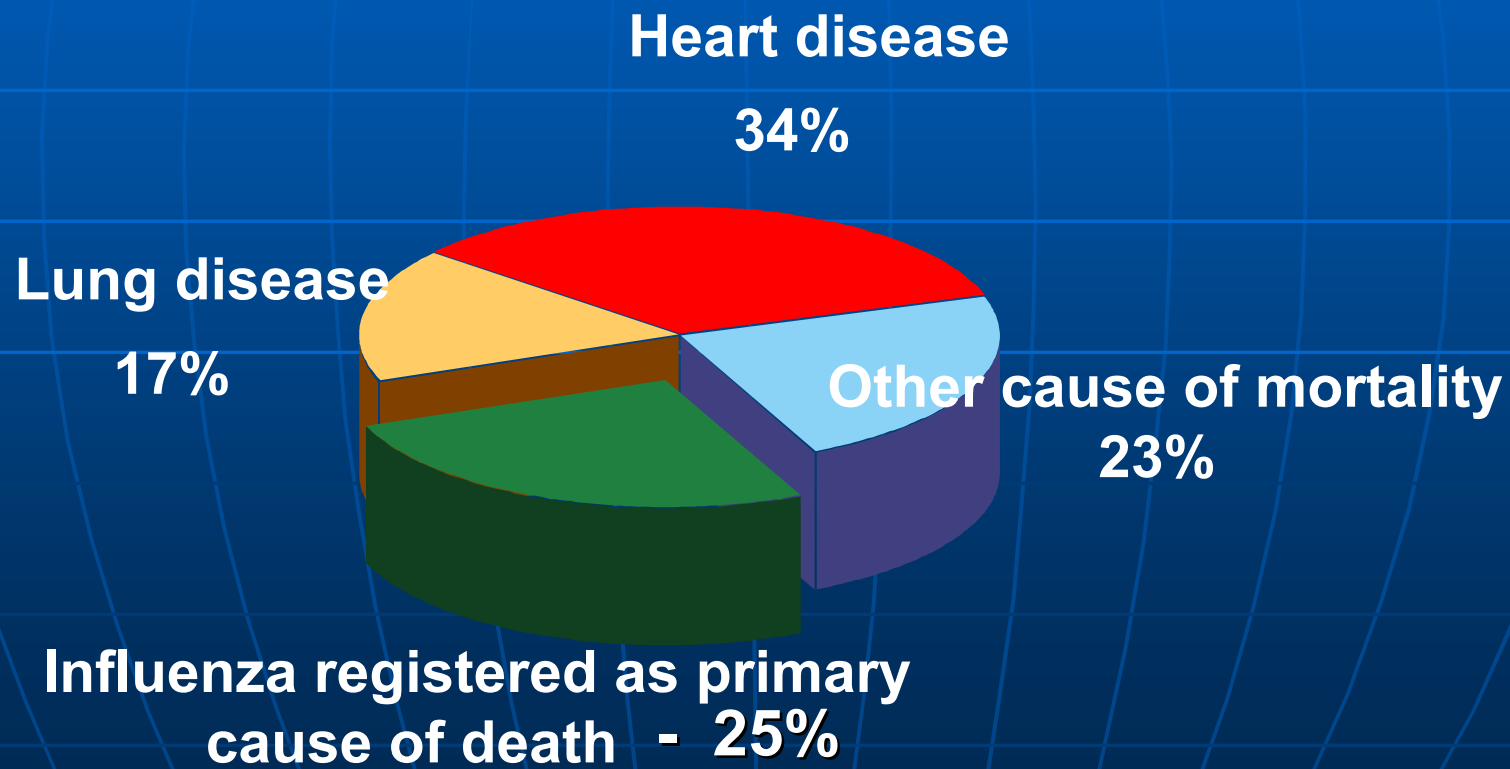


Simonsen et al 1996



The true mortality of influenza

William Farr first described 'excess mortality' associated with influenza in 1848



Annual Illness Impact of Influenza

Deaths 10 per 100,000

Hospitalisations 12 per 10,000

Medically attended 12 per 100

Acute respiratory disease 26 per 100

Infections 31 per 100

Annual Impact of Influenza in Australia (1996)

Percent of population infected	10 - 20%
Medical consultations	Approx. 1 million
Hospitalisations	20 – 40,000
Deaths	1500
Work days lost	1.5 million
Medical cost	\$400 million
Other economic costs	\$200 million
TOTAL ANNUAL COST	\$600 million

From Mills & Yapp 1996



Hospitalization Rates for Influenza By Age and Risk Group*

<u>Age Group</u>	<u>Rate** (high-risk)</u>	<u>Rate** (not high-risk)</u>
0-11 mos	1900	496-1038
1-2 yrs	800	186
3-4 yrs	320	86
5-14 yrs	92	41
15-44 yrs	56-110	23-25
45-64 yrs	392-635	13-23
≥65 yrs	399-518	125-228

* Data from several studies 1972 - 1995

** Hospitalizations per 100,000 population



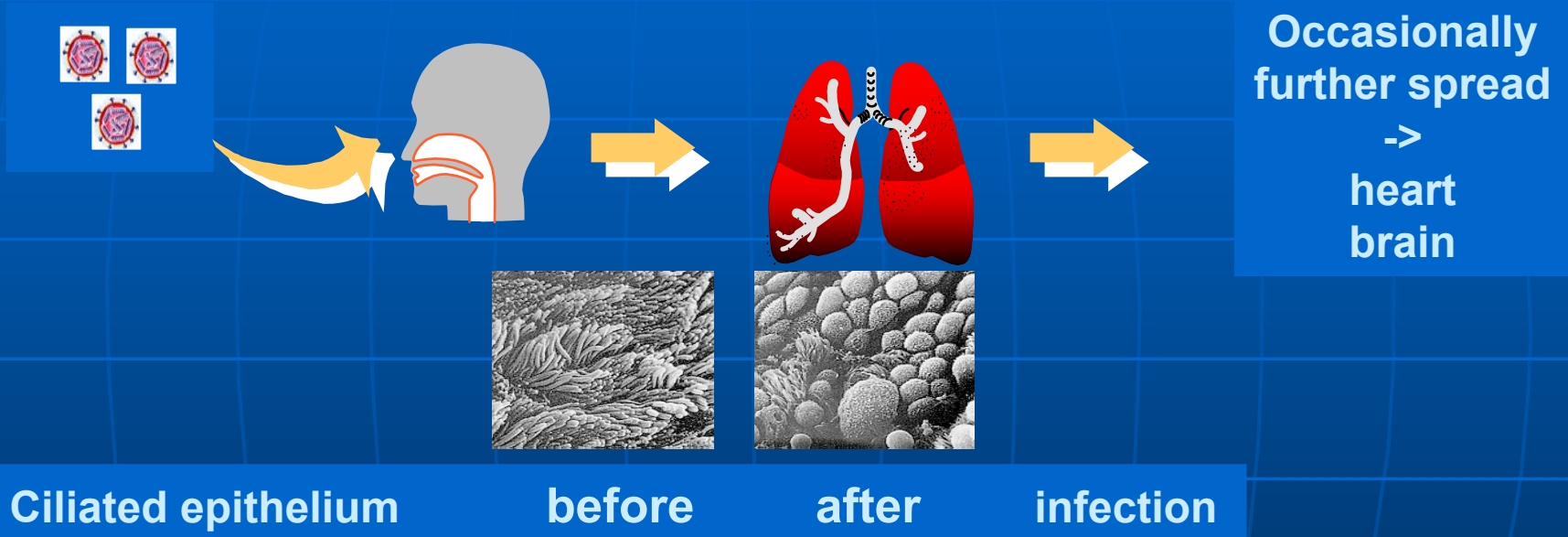


Spread of Influenza Infection

- **Aerosols**
- **Droplets**
- **Surface contact**

Pathology of Influenza Infection

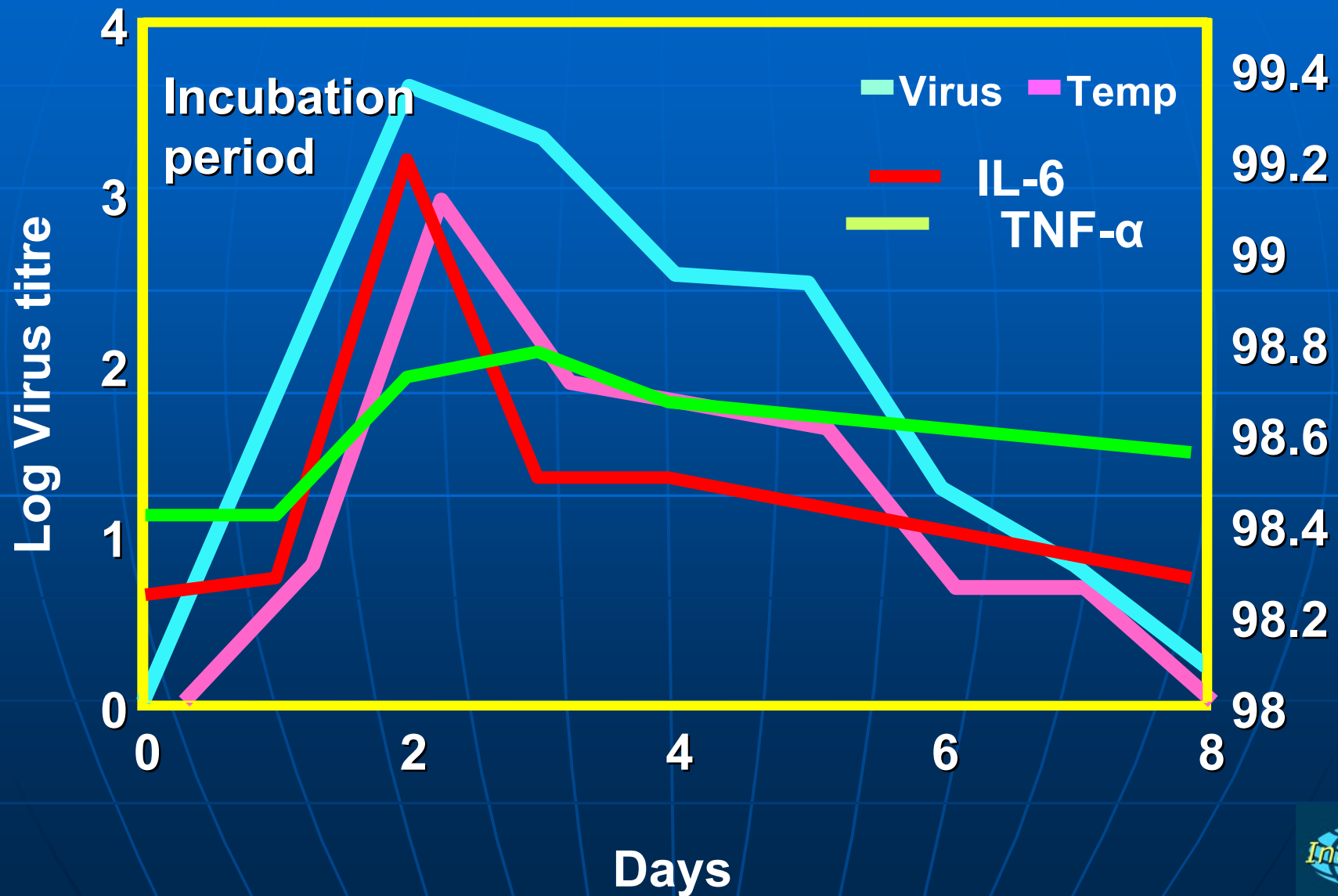
Infection of upper respiratory tract (possibly eyes)
Sometimes direct infection of or spread to lung



Local inflammatory reaction
cytokine release (IL-1, IL-6, TNF α , IFN α)

Systemic symptoms
fever, myalgia etc.

Infection and Symptoms



Influenza Pandemics 1700 - 2000

Year(s)	Origin
1968-69	China
1957	China
1918-19	USA?
1889-90	Asia
1833	Russia
1830-31	China?
1781-82	China?
1732-33	Russia
1729-30	Russia



20th Century Pandemics

Deaths

1918-19

Spanish Influenza

20-50 million

1957

Asian Influenza

approx 1 million

1968-9

Hong Kong Influenza

approx 1 million

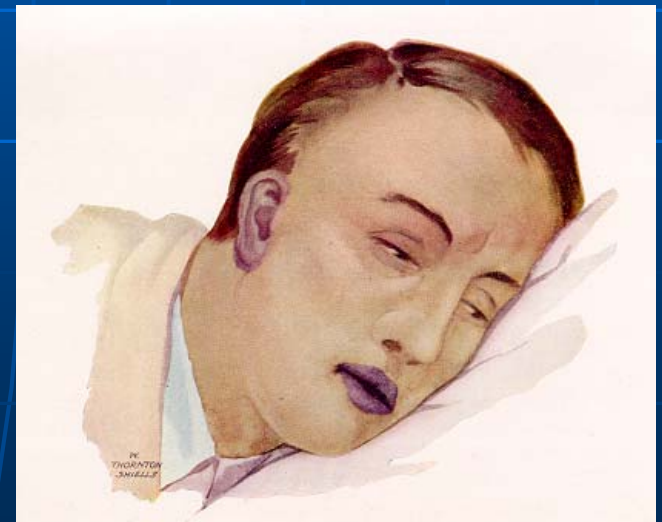
Spanish 'Flu Symptoms

.....the most striking clinical feature of influenza was the colour of the patient in the more severe cases.

..... the patient often became lilac or lavender huedthe whole of the face became lilac in hue.

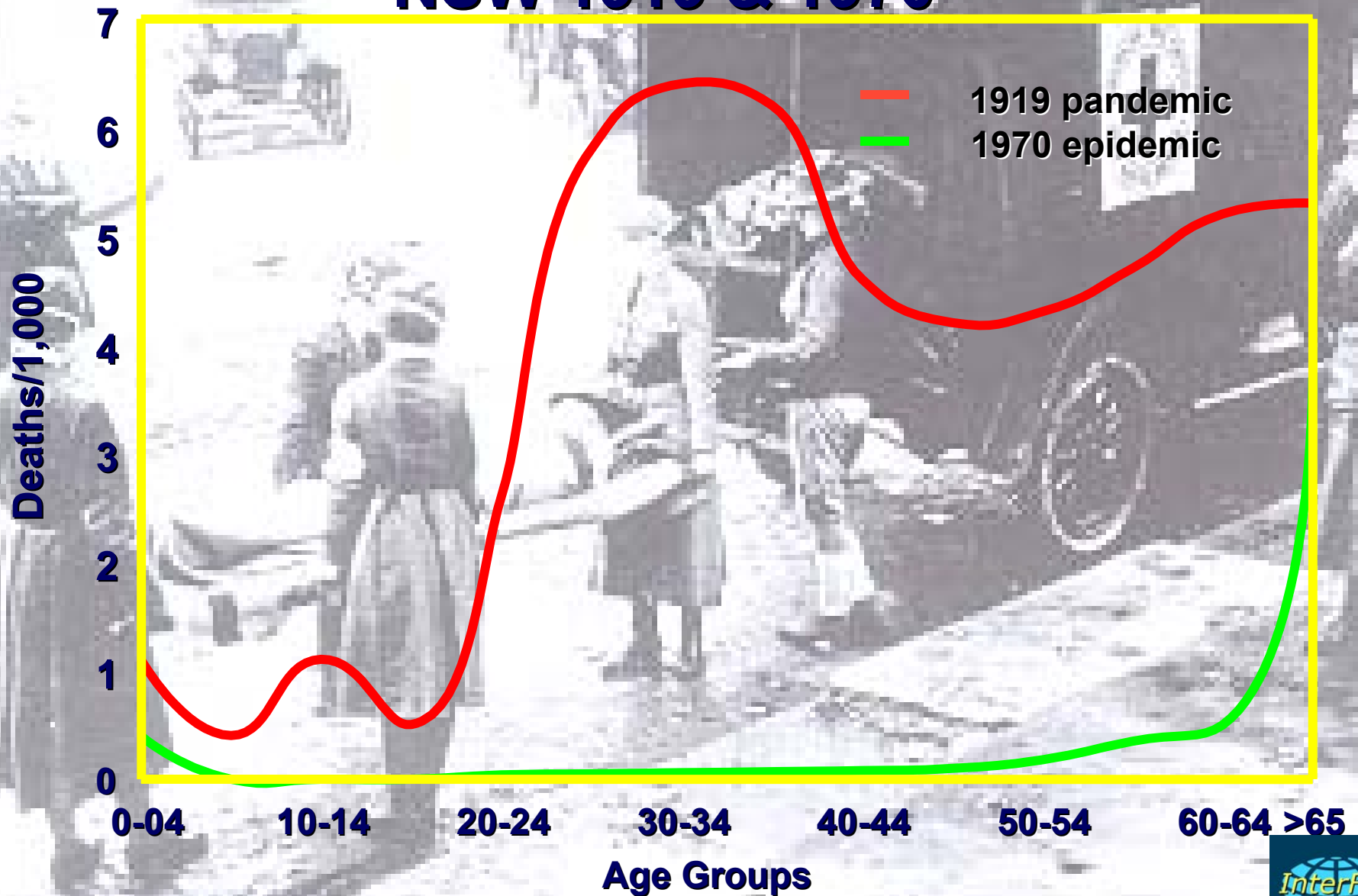
The prognosis in these cases was extremely grave.

From: Report of the Director General of Health, NSW.



Death Rate vs. Age

NSW 1919 & 1970

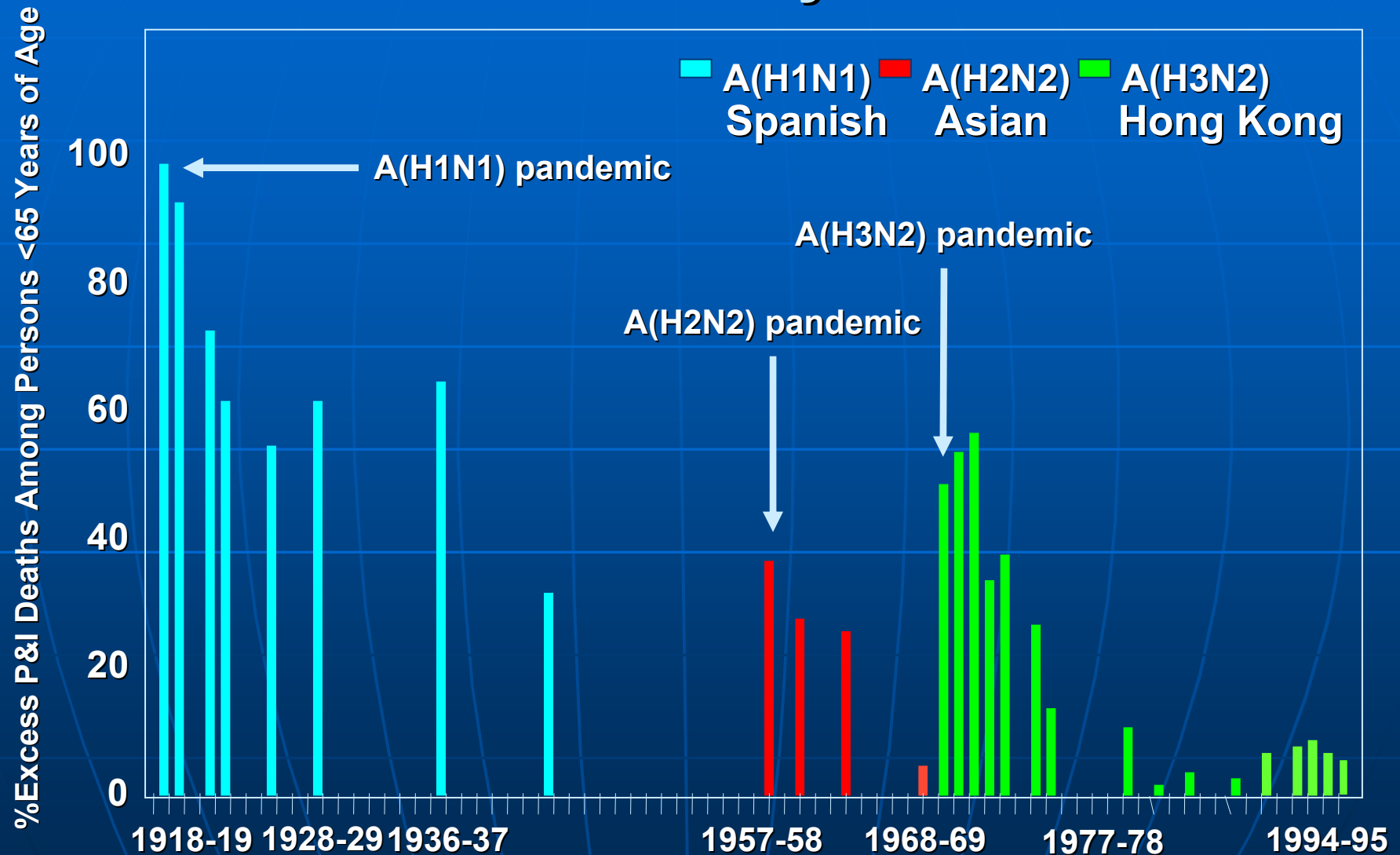


Spread of 'Spanish' Flu: 1918-19



C.W. Potter, Textbook of Influenza, 1998

Pandemic Influenza – Shift in Age Mortality



The Next Pandemic - Global Mortality

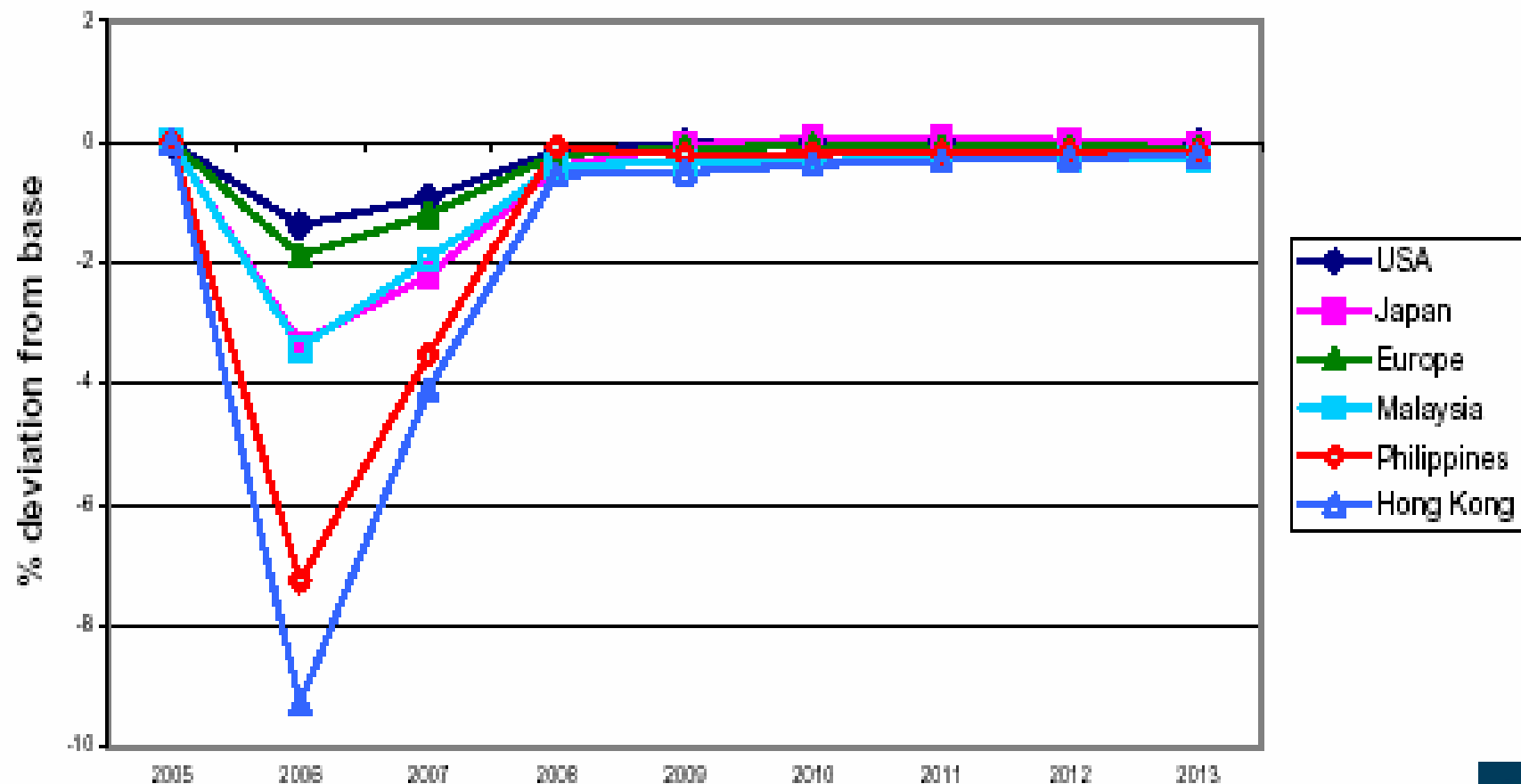
Lowy Institute Estimates (16-02-06)

Scenario	Global Mortality
Mild (HK 68/9)	1.421 m
Moderate (Asian 57)	14.216 m
Severe (Spanish – lower est)	71.08 m
Ultra Severe (Spanish – upper est)	142.16 m

Estimated Pandemic Impact on GDP

Lowy Institute (16-02-06)

Figure 17: Change in GDP in the Moderate Scenario



History of Laboratory Influenza Studies



1933
Transmission of
influenza to
ferrets



1934
Adaption to mice



1940
Growth in
embryonated eggs



1941- Haemagglutination first
described

DEMONSTRATION OF THE EFFICACY OF VACCINATION AGAINST
INFLUENZA TYPE A BY EXPERIMENTAL INFECTION OF
HUMAN BEINGS

WERNER HENLE, GERTRUDE HENLE, AND JOSEPH STOKES, JR.

*From the Department of Pediatrics, School of Medicine, University of Pennsylvania, and
The Children's Hospital of Philadelphia*

Received for publication October 12, 1942

Time after vaccination	Number	Clinical cases	% infected
2 ½ weeks	17	0	} 3%
17 – 19 weeks	27	1	
Controls	28	10	36%



Experience with Vaccination Against Influenza in the Spring of 1947

A Preliminary Report *

THOMAS FRANCIS, JR., M.D., F.A.P.H.A., JONAS E. SALK, M.D., AND J. J. QUILLIGAN, JR., M.D.

Department of Epidemiology and the Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, Mich.

	Vaccinated	Unvaccinated
Total	10,328	7,615
Respiratory Cases	743	489
% Infection	7.19%	8.09%

THE WHO INFLUENZA PROGRAM

- Initiated at 4th session of WHO Interim Commission 1947
- Consisted of -
 - A World Influenza Centre
 - Regional Laboratories
- Objectives:
 - Gain an understanding of the epidemiology of influenza
 - Promptly isolate viruses from outbreaks and distribute them for vaccine production

Influenza Viruses

Viruses of the Family Orthomyxoviridae

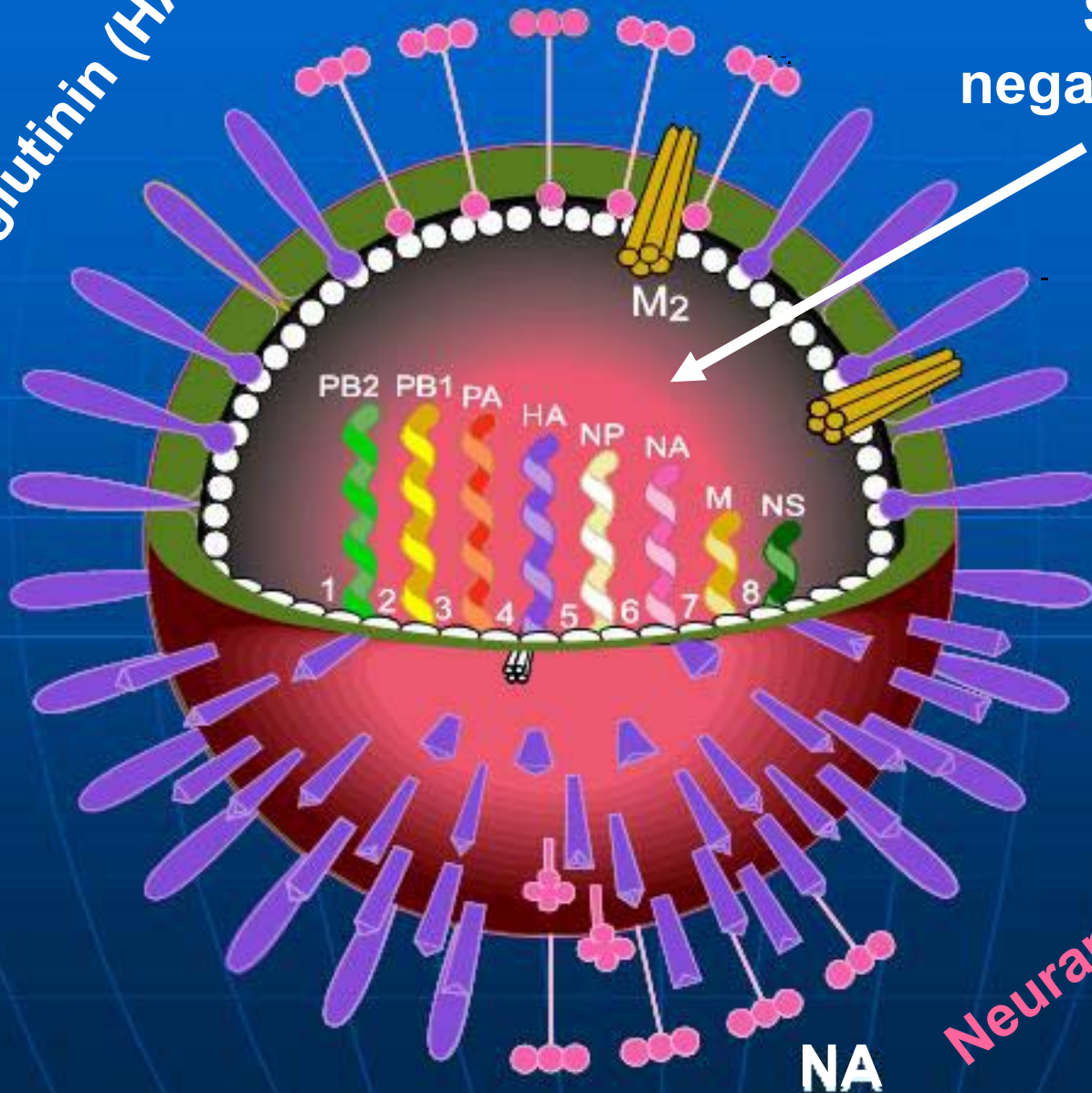
- **3 types: A, B, C**
 - Type A associated with outbreaks epidemics and pandemics
 - Type B associated with outbreaks and epidemics
 - Type C associated with common cold-like infections, mainly in children
- **Antigenically distinct forms (subtypes) of influenza A based on surface antigens**

Influenza Virus

Haemagglutinin (HA)

Segmented
negative sense RNA
genome

HA



Neuraminidase (NA)

NA

Influenza A Gene Segments

Segment	Size (nt)	Polypeptide(s)	Function
1	2341	PB2	Transcriptase: cap binding
2	2341	PB1	Transcriptase: elongation
3	2233	PA	Transcriptase: protease activity
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex; nuclear/cytoplasmic transport of vRNA
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: lining envelope
		M2	Integral membrane protein – ion channel
8	890	NS1	Non-structural: cellular RNA transport, splicing, translation. Anti-interferon.
		NS2	Non-structural: nuclear transport of RNP



Influenza virus replication

NA Glycoprotein

- Enzymically active
- Conserved enzyme active site
- Cleaves terminal sialic acid from carbohydrates
- Releases newly formed virus from cells

HA Glycoprotein

- Major surface antigen
- Binds to cell receptors to initiate cell infection (specific for N-acetyl neuraminic acid residues - human viruses bind to Sialic Acid $\alpha 2,6\text{Gal}$)
- Activated by protease cleavage during maturation
- Fuses with lysosome membrane to release viral genome (cleavage dependent)



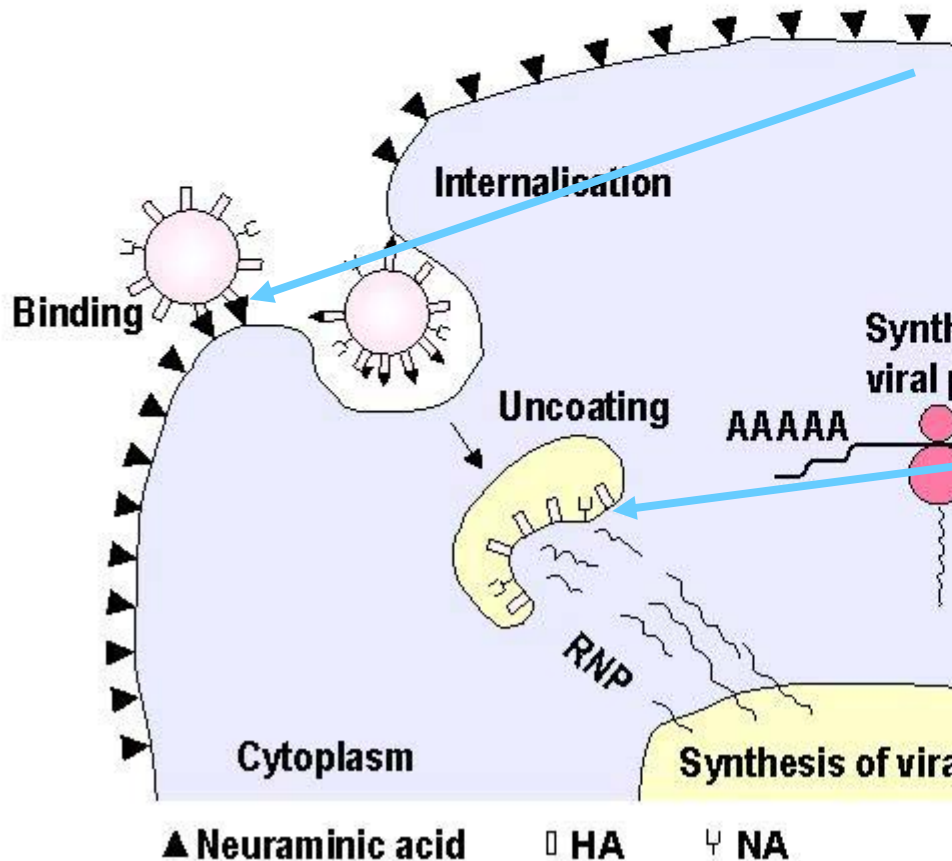
After Gubareva et. al.,



Influenza virus replication

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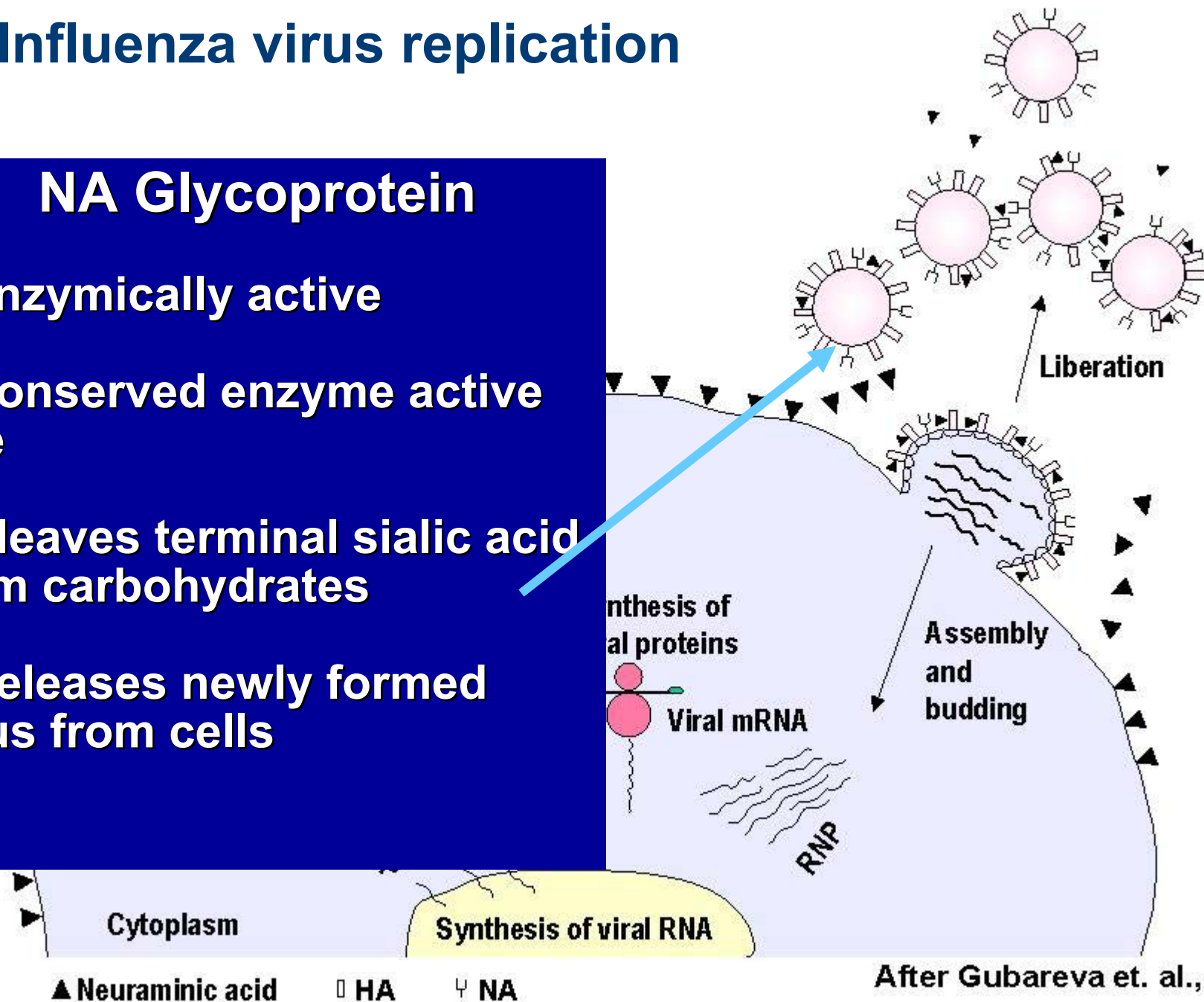
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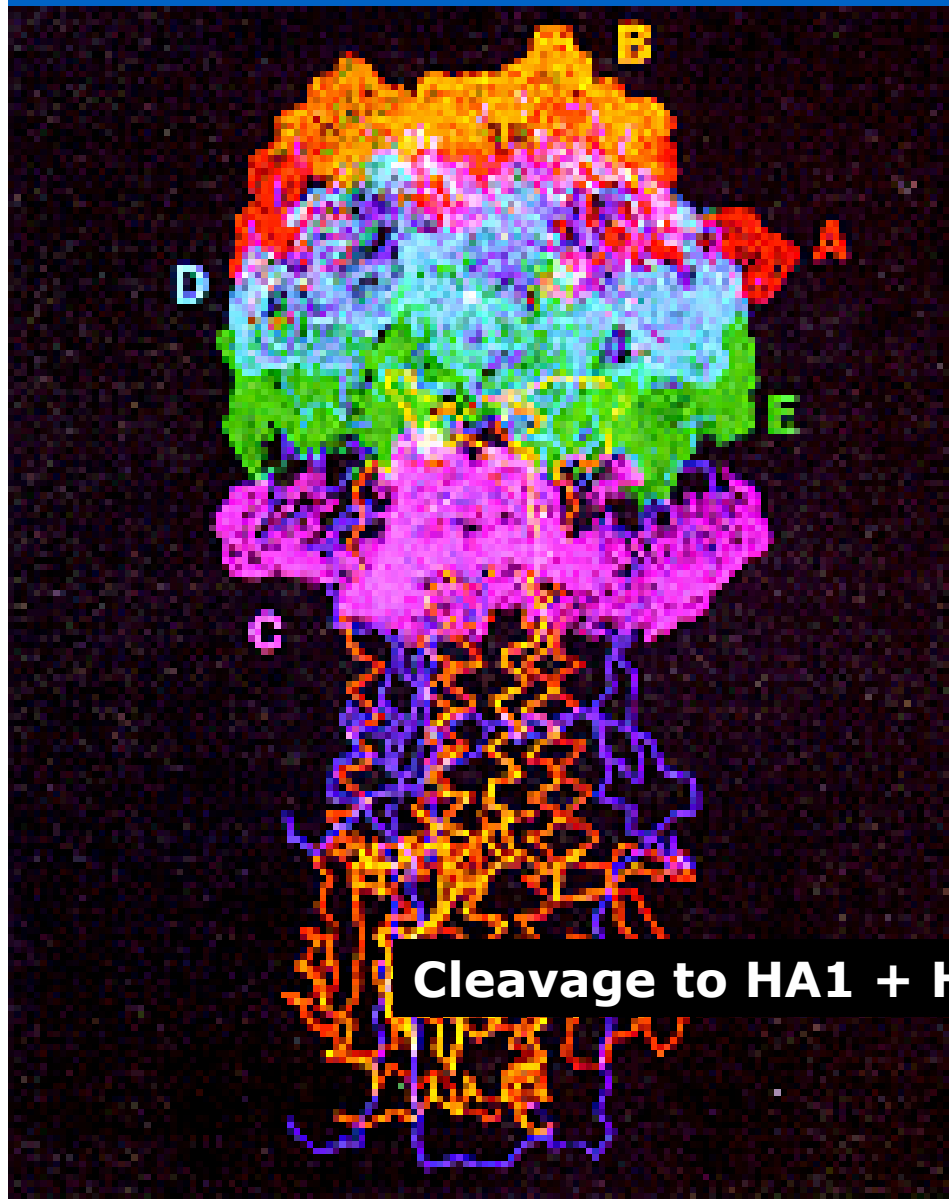
Influenza virus replication

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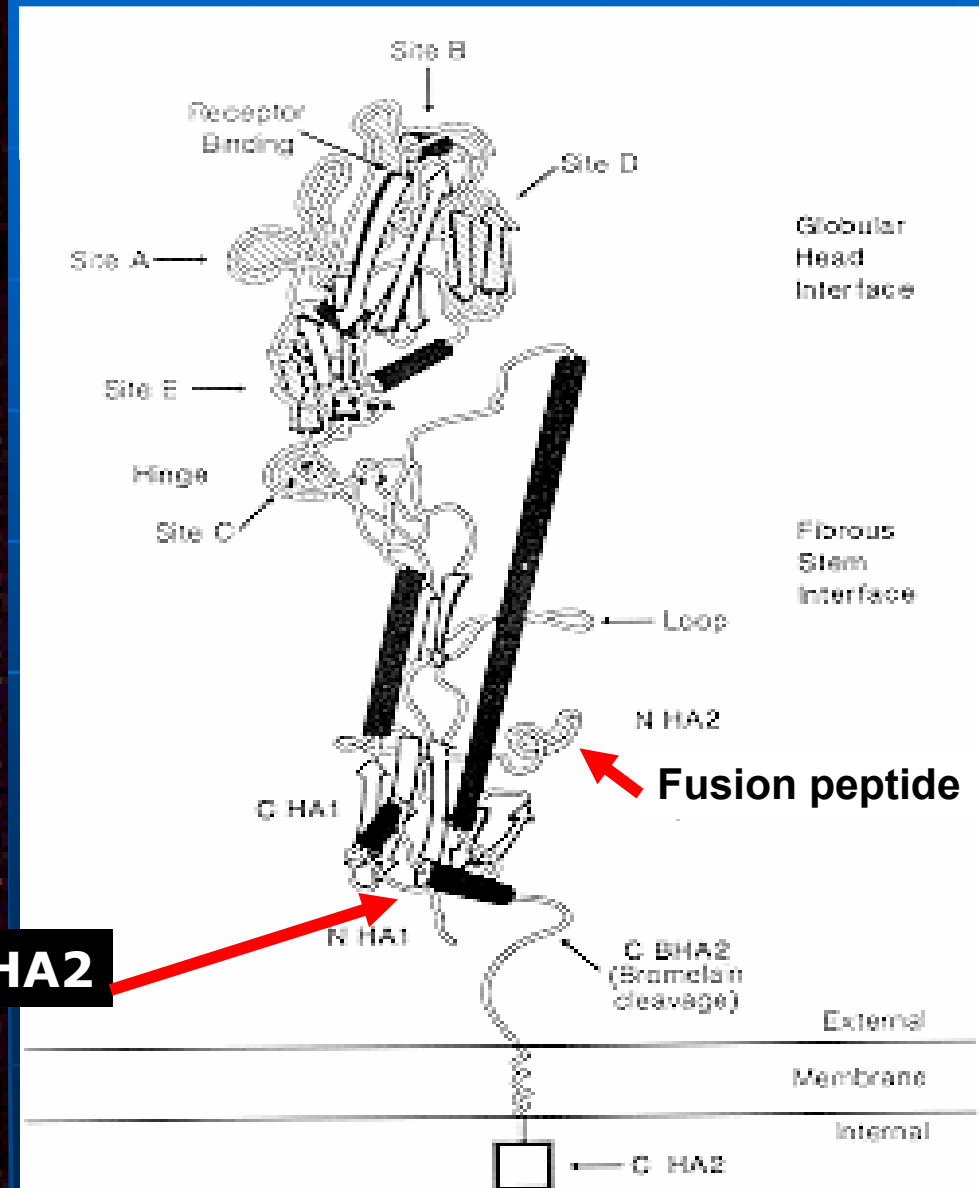
- Enzymically active
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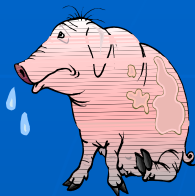
Influenza HA Structure



Cleavage to HA1 + HA2

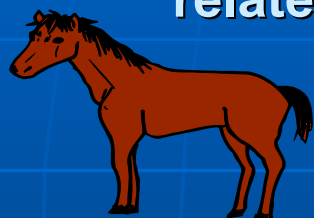


Influenza in Other Species



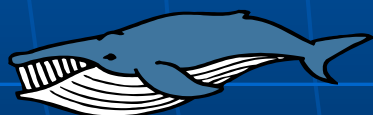
Swine 'Influenza' described 1919, shown to be related to influenza A in 1937

Fowl plague described 1878 in Italy, shown to be related to influenza A in 1956



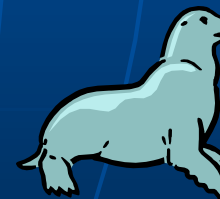
Equine 'flu' described in 18th & 19th centuries shown to be influenza A in 1956

Further antigenically distinct influenza A viruses found in ducks from 1956 onwards



Russian reports of influenza from whales 1975-6 & 1984

Severe epizootics in seals 1979, 1982, 1991



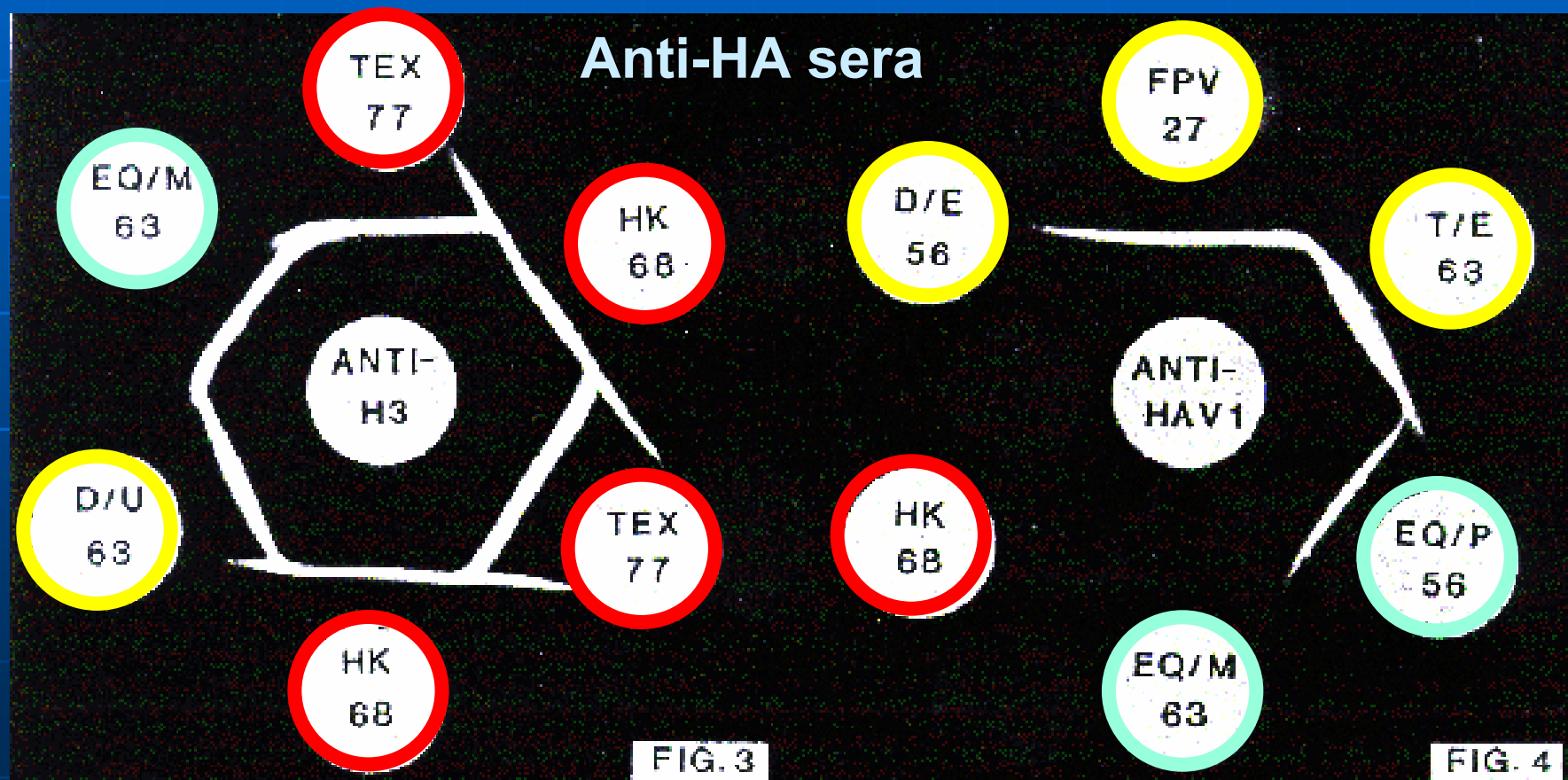
Infection of zoo felids, and experimental infection of domestic cats, with H5N1 2004

Severe equine influenza virus infection in greyhounds in USA 2005



Antigenic Relationships of Influenza Viruses

Analysis by double-immunodiffusion



Occurrence of Influenza Viruses

Influenza A viruses

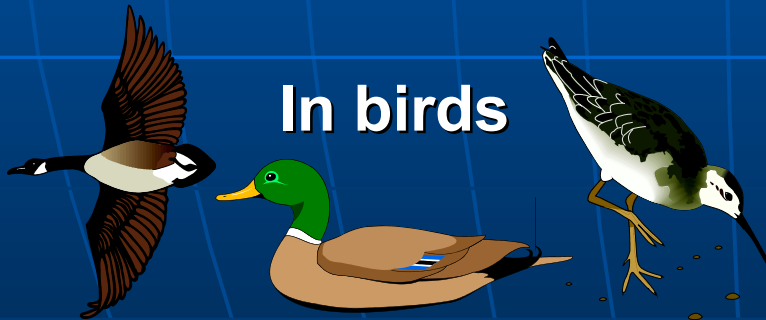
16 antigenically distinct
HA types (subtypes)
9 distinct NA types

Species infected

humans, pigs, horses, birds,
marine mammals

In humans

In birds



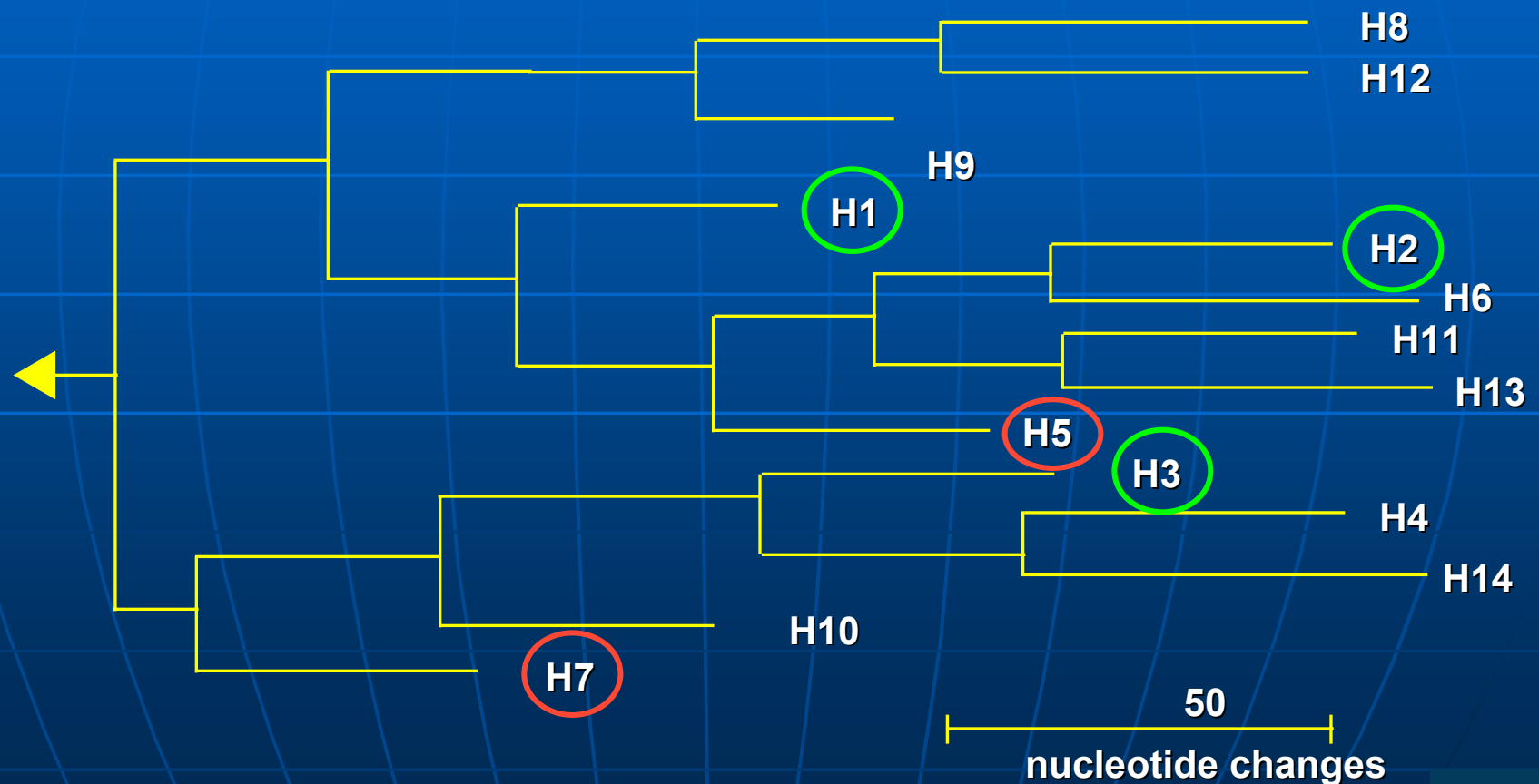
Note –

avian respiratory and gut cells
have sialic acid $\alpha 2,3\text{Gal}$ specificity
human respiratory tract has sialic
acid $\alpha 2,6\text{Gal}$
pig respiratory tract has both

Influenza B viruses

Highly adapted to humans,
no subtypes

Relatedness of Influenza A Virus Subtypes – by sequence analysis of HA



ANCESTRAL VIRUS

→ PARAINFLUENZA VIRUSES - MEASLES, MUMPS ETC.

→ INFLUENZA C VIRUSES

AVIAN INFLUENZA VIRUSES

→ HUMAN INFLUENZA B VIRUSES

→ SUBTYPES H1-H12

→ EQUINE VIRUSES

→ HUMAN INFLUENZA A VIRUSES

→ H13 VIRUSES

→ H14 VIRUSES

→ FPV (H7N7) viruses

→ EQUINE 2 VIRUSES

1890 → H3N8 HUMAN VIRUSES

1900 → H2N8 HUMAN VIRUSES

1910-1918 → H1N1 HUMAN and SWINE VIRUSES

1957 → H2N2 HUMAN VIRUSES

1968 → H3N2 HUMAN VIRUSES

1977 → REEMERGENT H1N1 HUMAN VIRUSES

1979 → H1N1 AVIAN-LIKE SWINE VIRUSES

1989 → EQUINE 3 (H3N8) VIRUSES

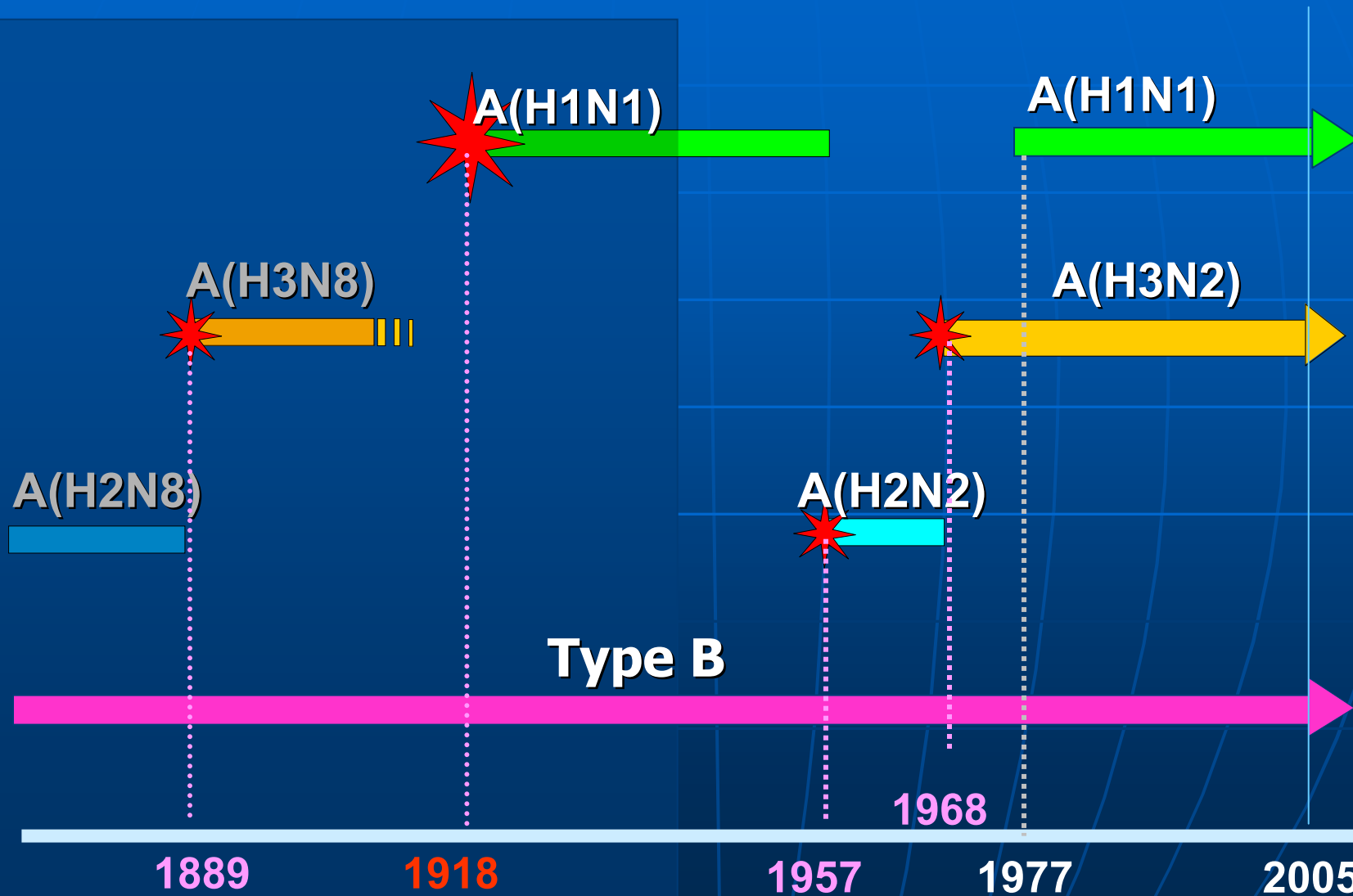
?

Avian Virus Host Reservoir

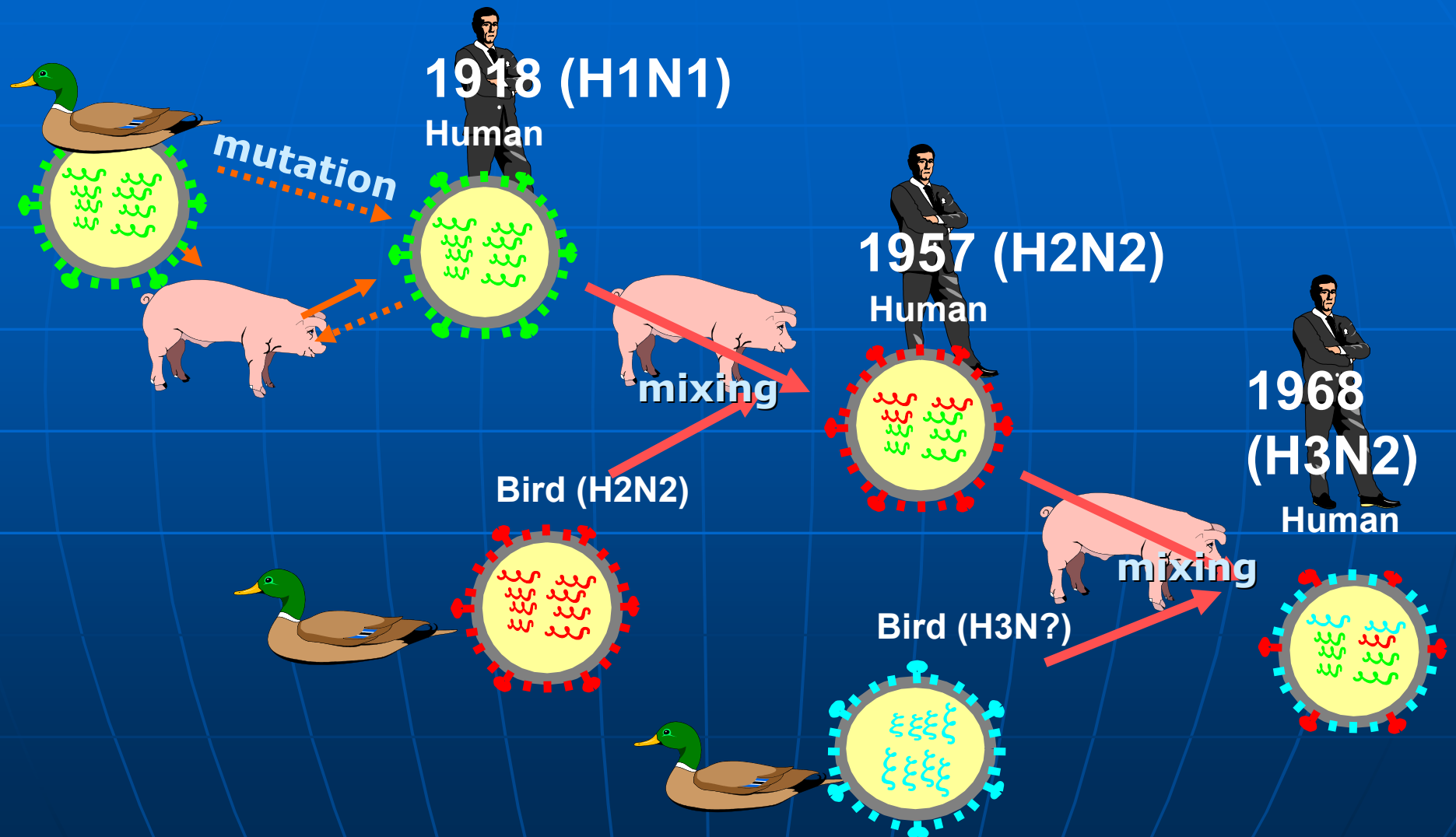
From Webster

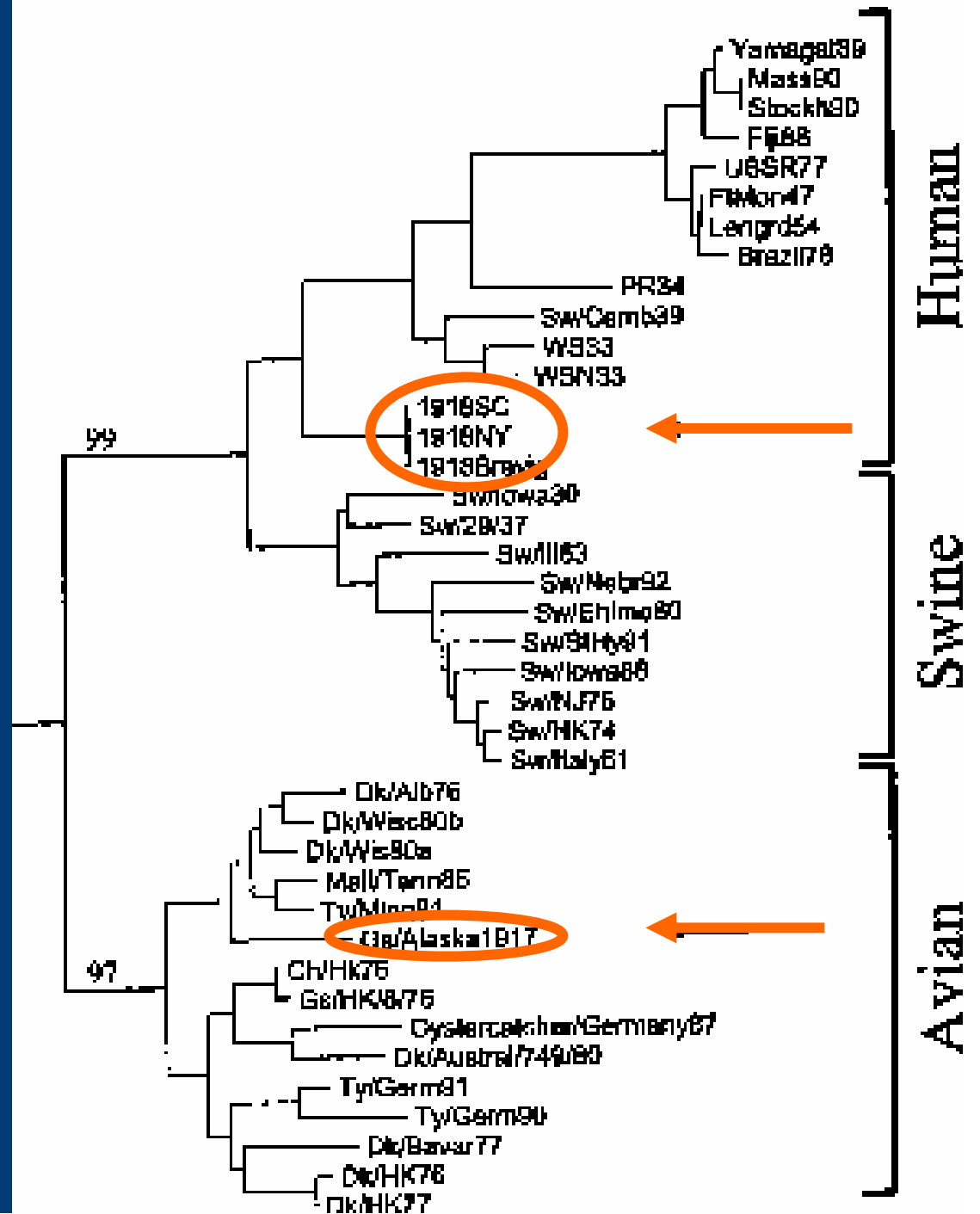


Eras of Human Influenza Viruses

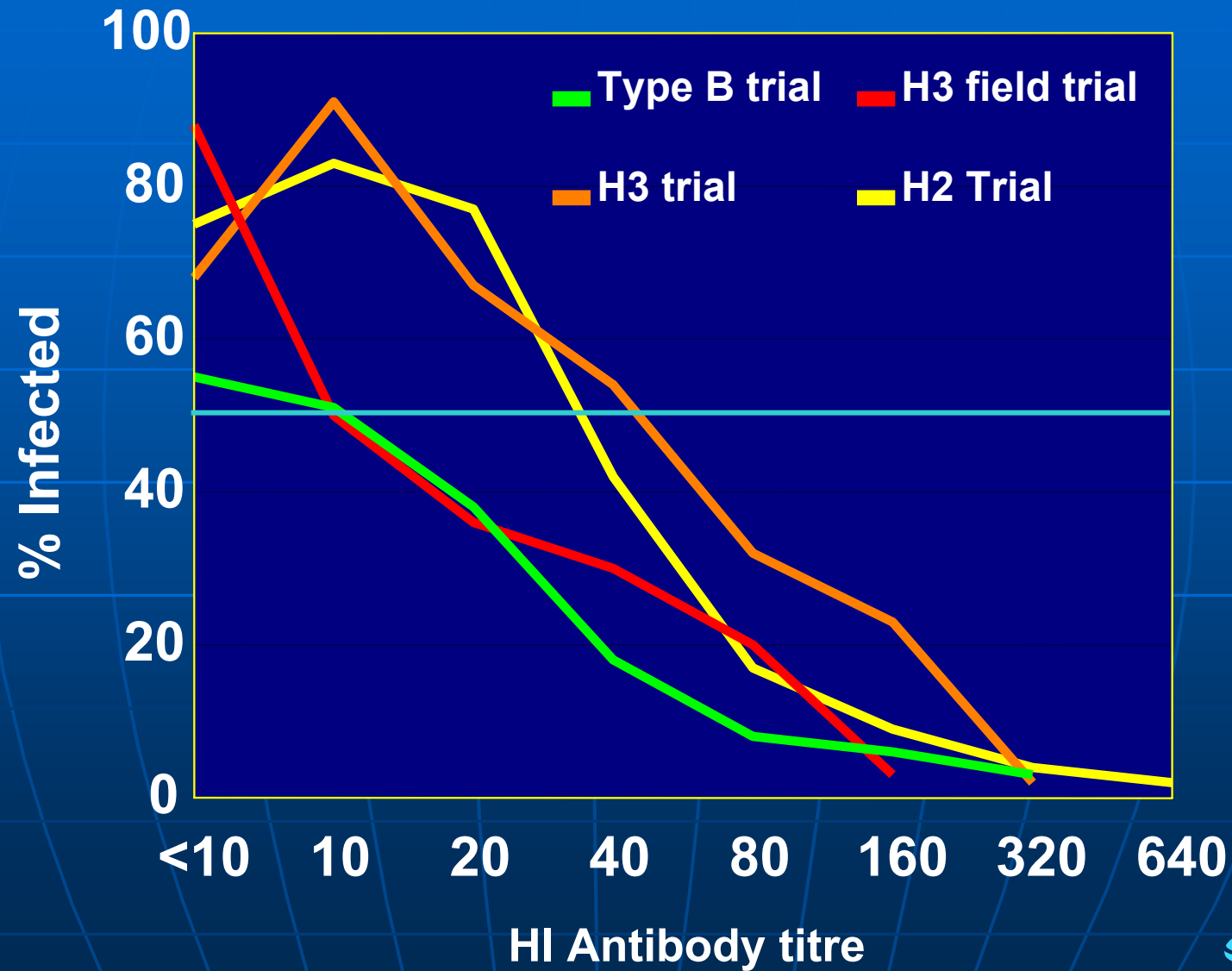


Evolution of Pandemic Viruses





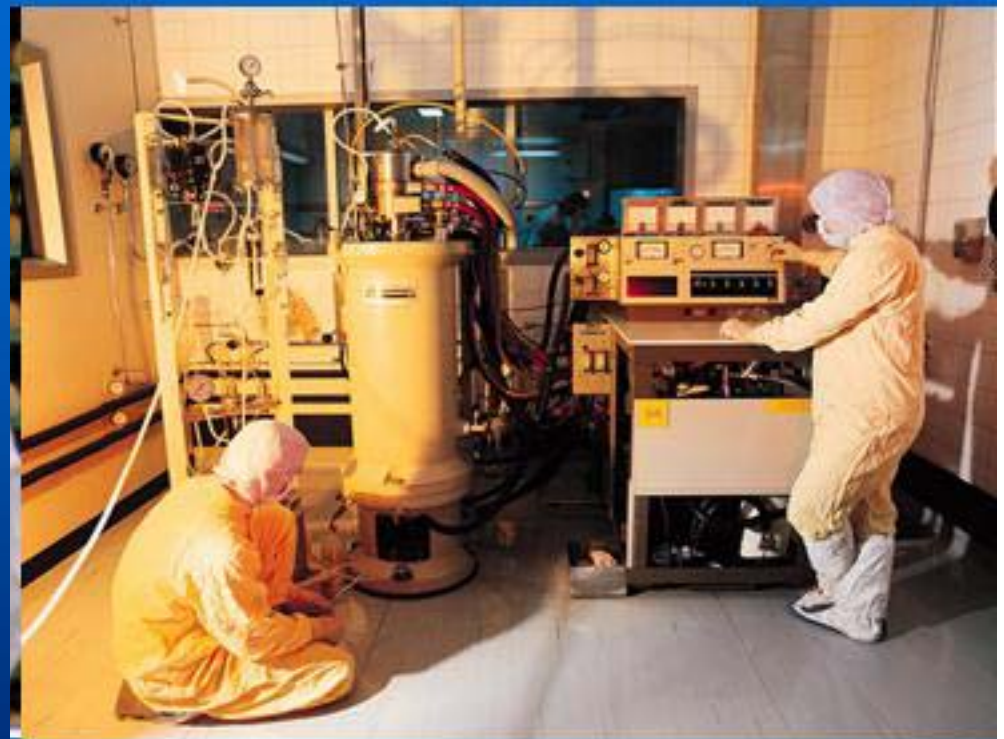
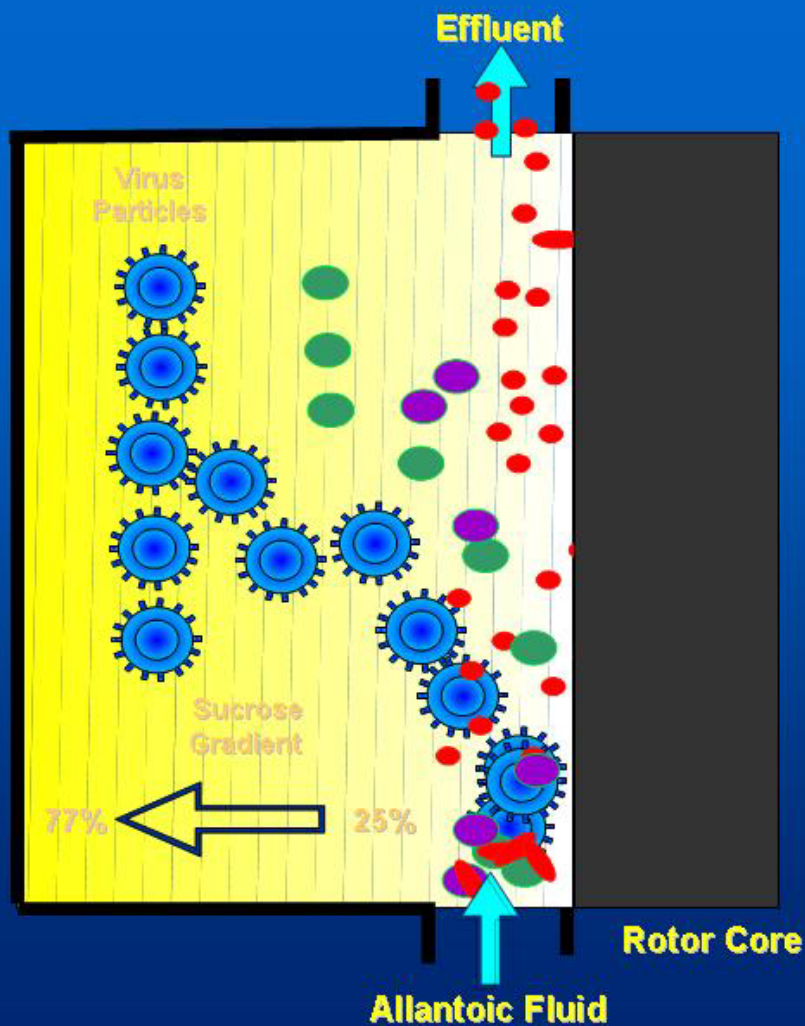
Antibody-based Immunity to Influenza



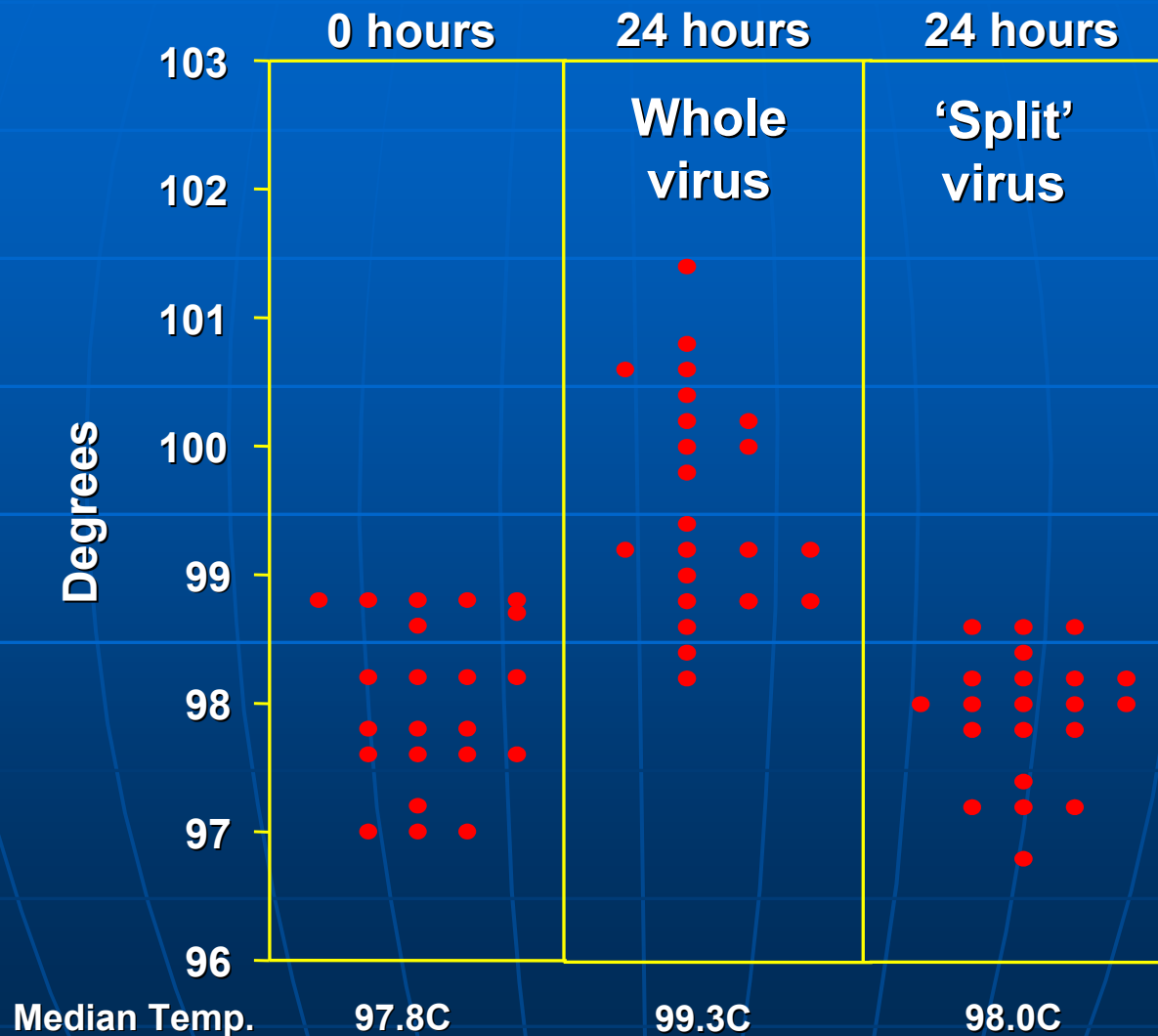
Influenza Infection – Recovery & Immunity

- Recovery from infection
 - cytotoxic-T cell dependent
- **Protection against disease/infection is:**
 - **antibody mediated by anti-HA antibodies**
 - **subtype specific**
 - **largely strain specific**
- Anti-NA immunity
 - sparing effect, not protective
- In addition there may be
 - short-term mucosal immunity
 - short-lived cell-mediated, cross-subtype immunity (explains subtype replacement)

Current Production of Influenza Vaccine - Zonal Ultracentrifuge



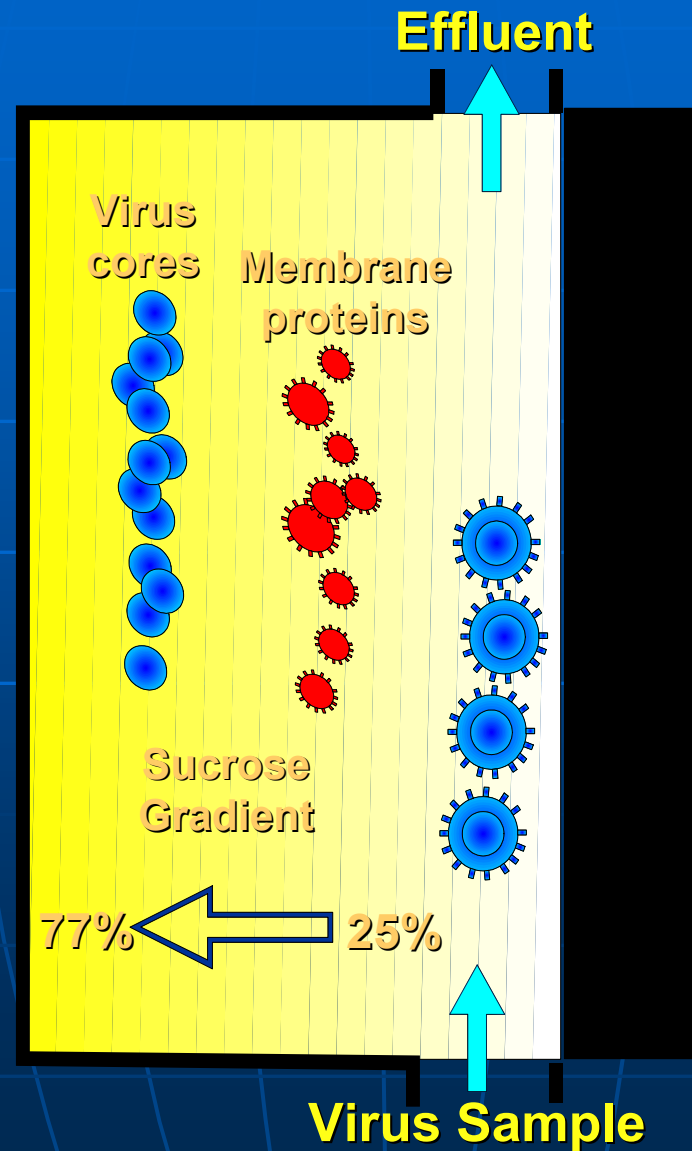
Febrile Reaction to Influenza Vaccine



Davenport et al 1964

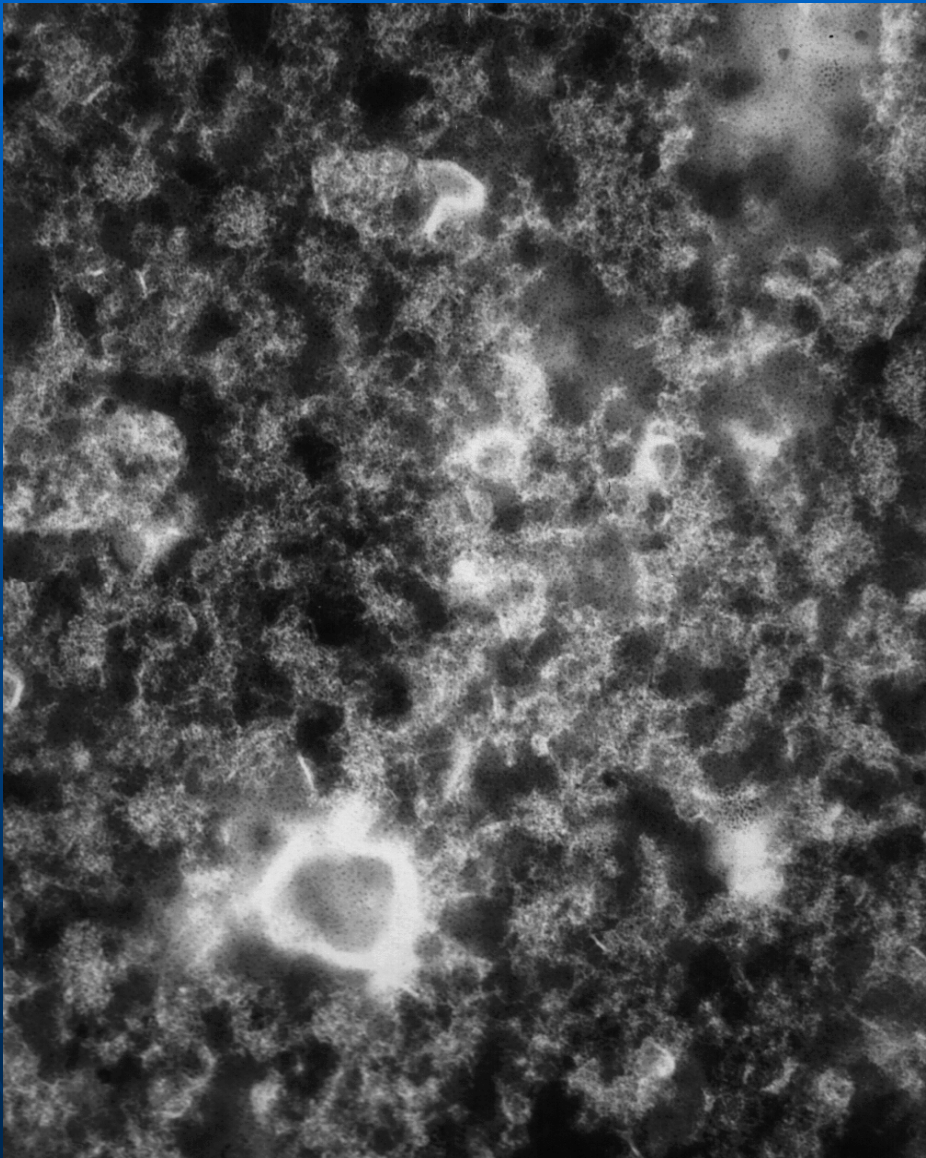


Preparation of Influenza Virus Subunits

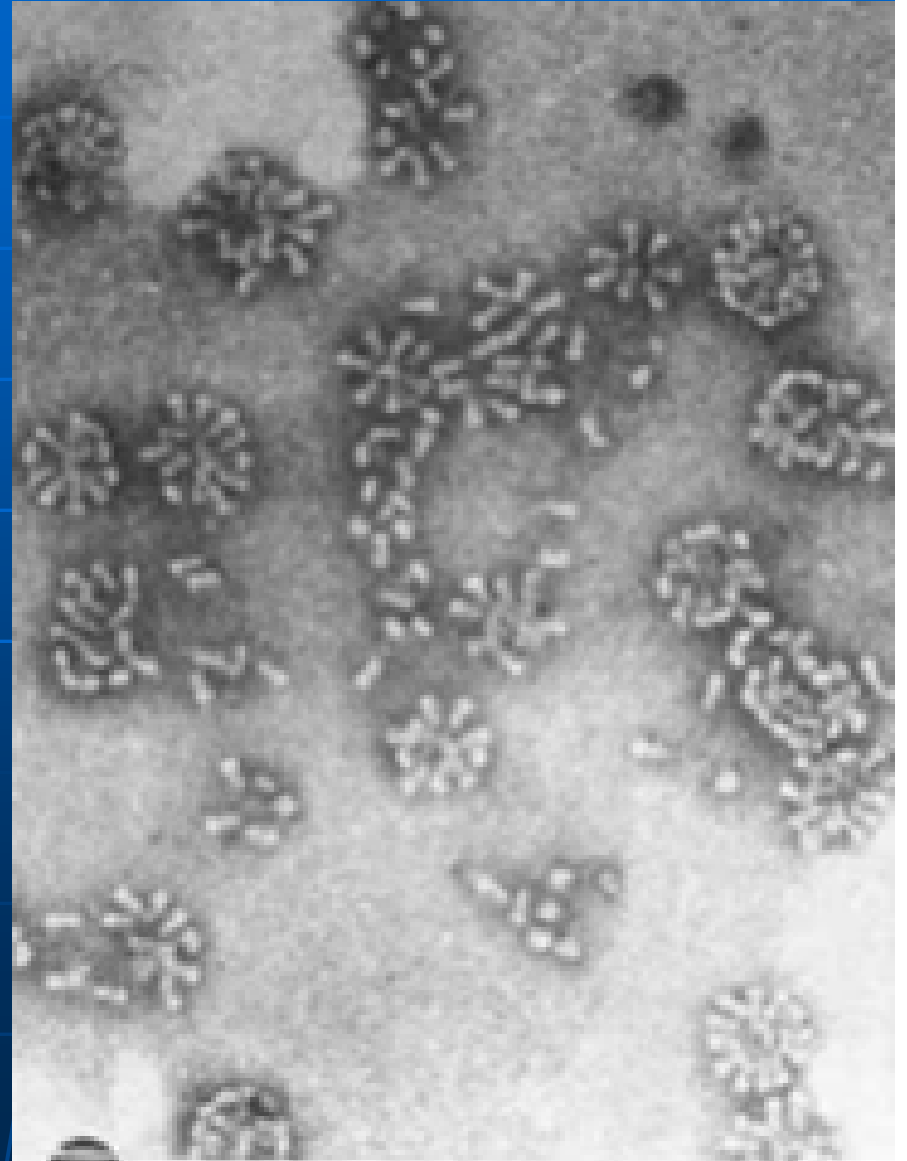


Electron Micrographs – Influenza Vaccines

Split vaccine
all virus components present



Purified sub-unit vaccine
Essentially only surface components



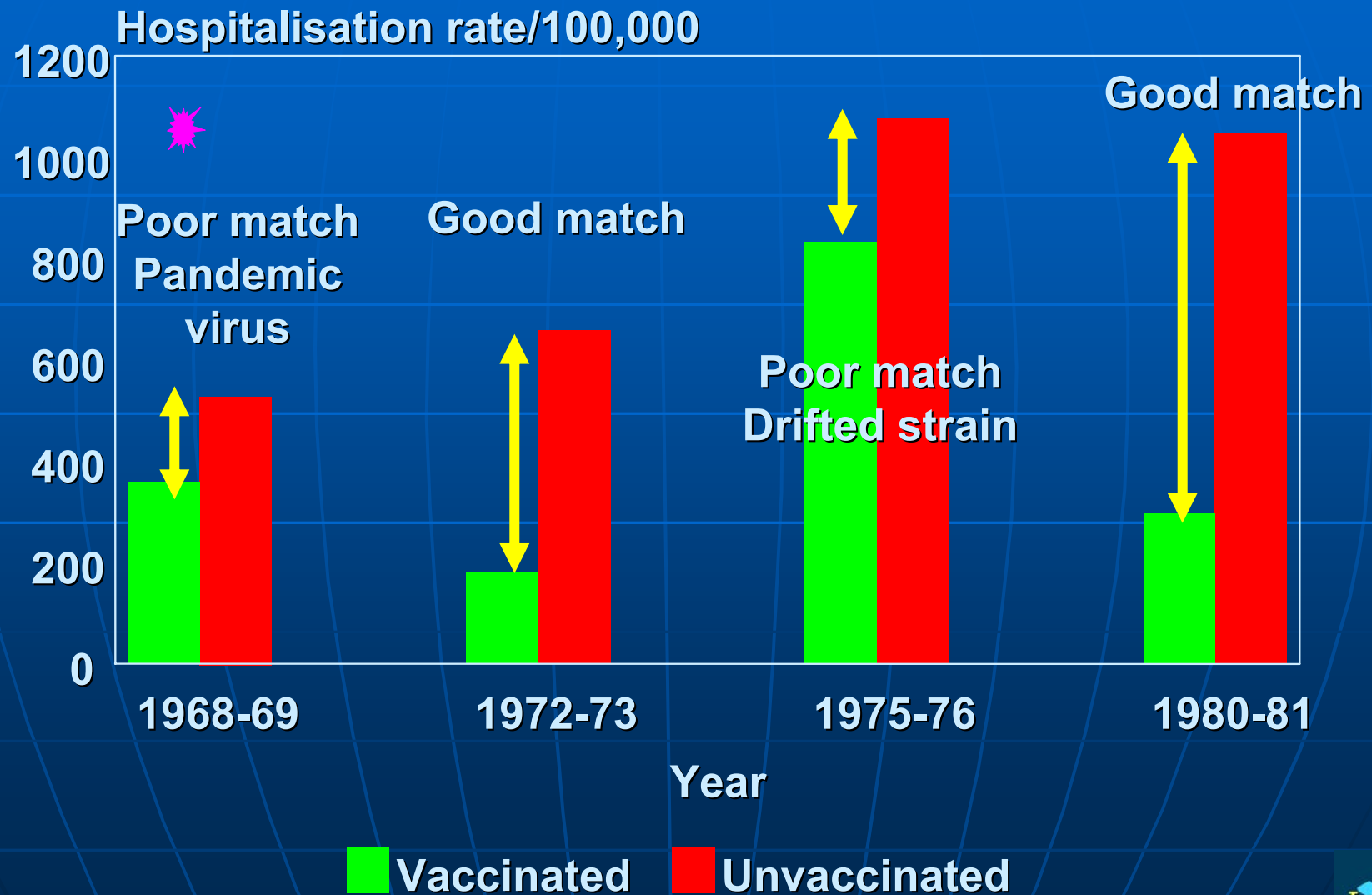
Febrile Reactions in Children

Percentage with fever 103 F		
Age (years)	Purified Whole Virus	Split Virus
1-4	36	11
5-9	6	0
>9	17	0
Total	59	11

After Gross et al 1977

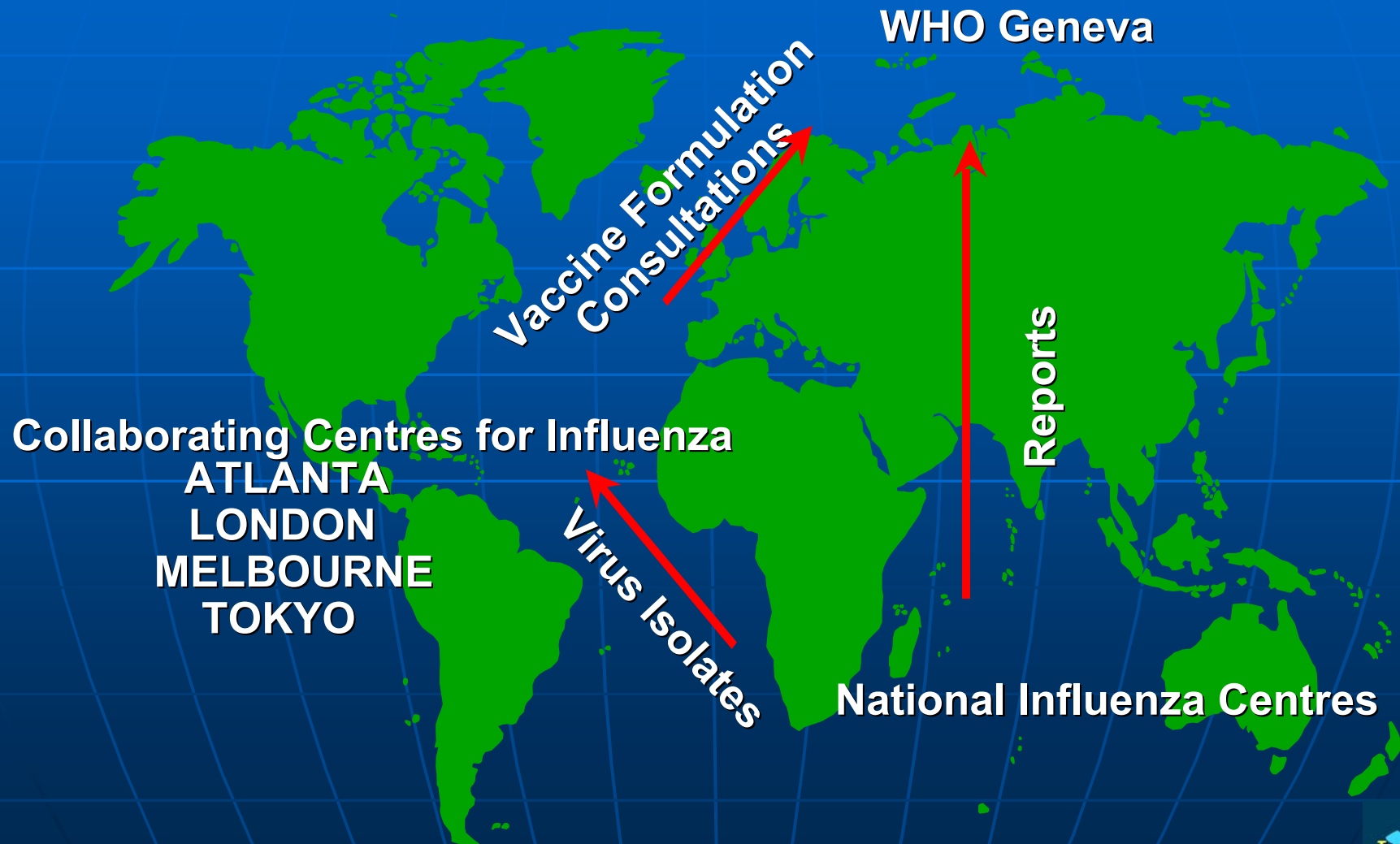


Protection of Older Vaccine Recipients Against Hospitalisation



WHO Influenza Network

Commenced 1947



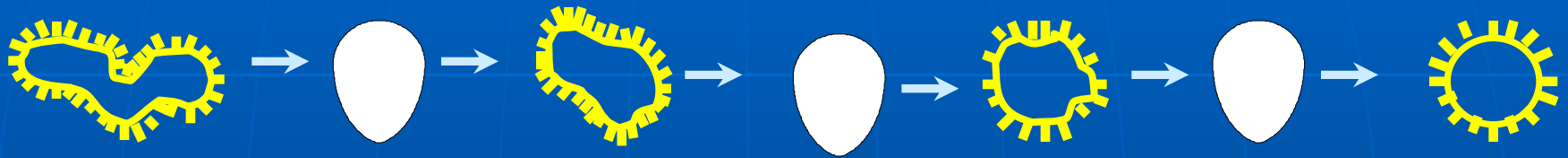
Vaccination Formulation and Use

WHO Consultations	February	September
Confirmatory (country/regional) meetings	March	Sept – Oct
Vaccine Available	August	February
Suitable for outbreaks commencing	Sept - Oct	March - April

Preparation of Influenza A Vaccine Strains

Adaption by Passaging

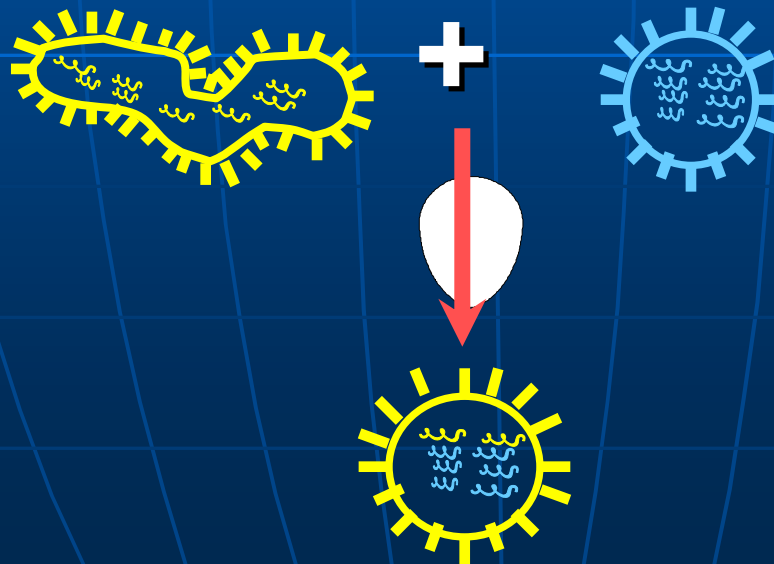
New Isolate



Adaption by Reassortment

New Isolate

Laboratory Strain (A/PR-8)



Standardisation of Vaccines

By HA Antigen Content

SRID test introduced 1978-79

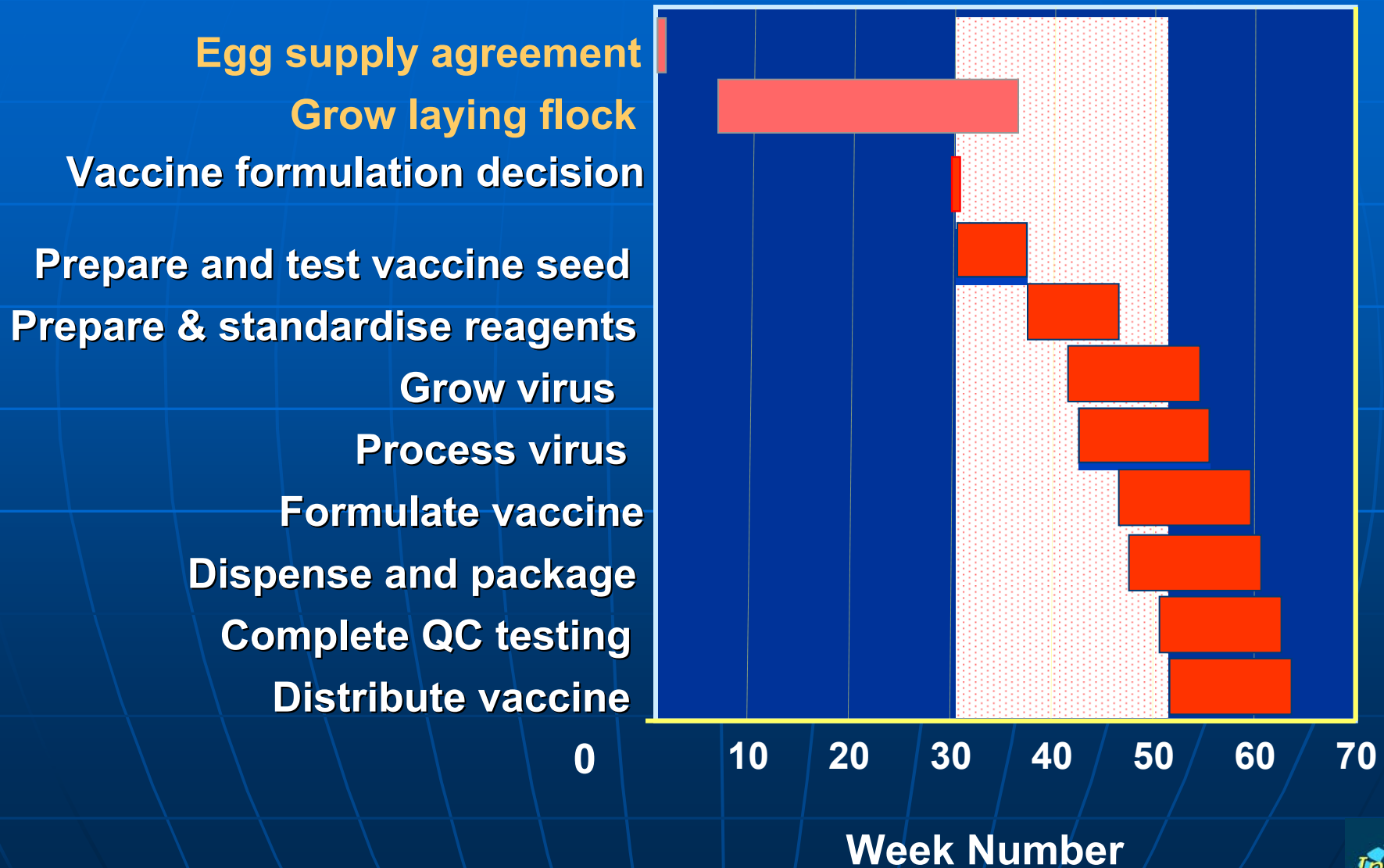
- antigen content recommended 7ugm/dose

Amended antigen content 1981-82

- 15 ug/dose

ACIP: "... one way to improve vaccine effectiveness against viruses that have undergone some antigenic drift is to increase the concentration of related antigens in the vaccine."

Typical Influenza Vaccine Production Schedule



Weaknesses of Current Influenza Vaccines

- **Narrow breadth of immunity – must be good antigenic match**
- **Short-lived immunity**
- **Reduced responsiveness in high-risk individuals**
- **Long production lead-time**
- **Limited global production capacity, not readily scalable (around 400 million doses/yr)**
- **Still dependant on eggs**

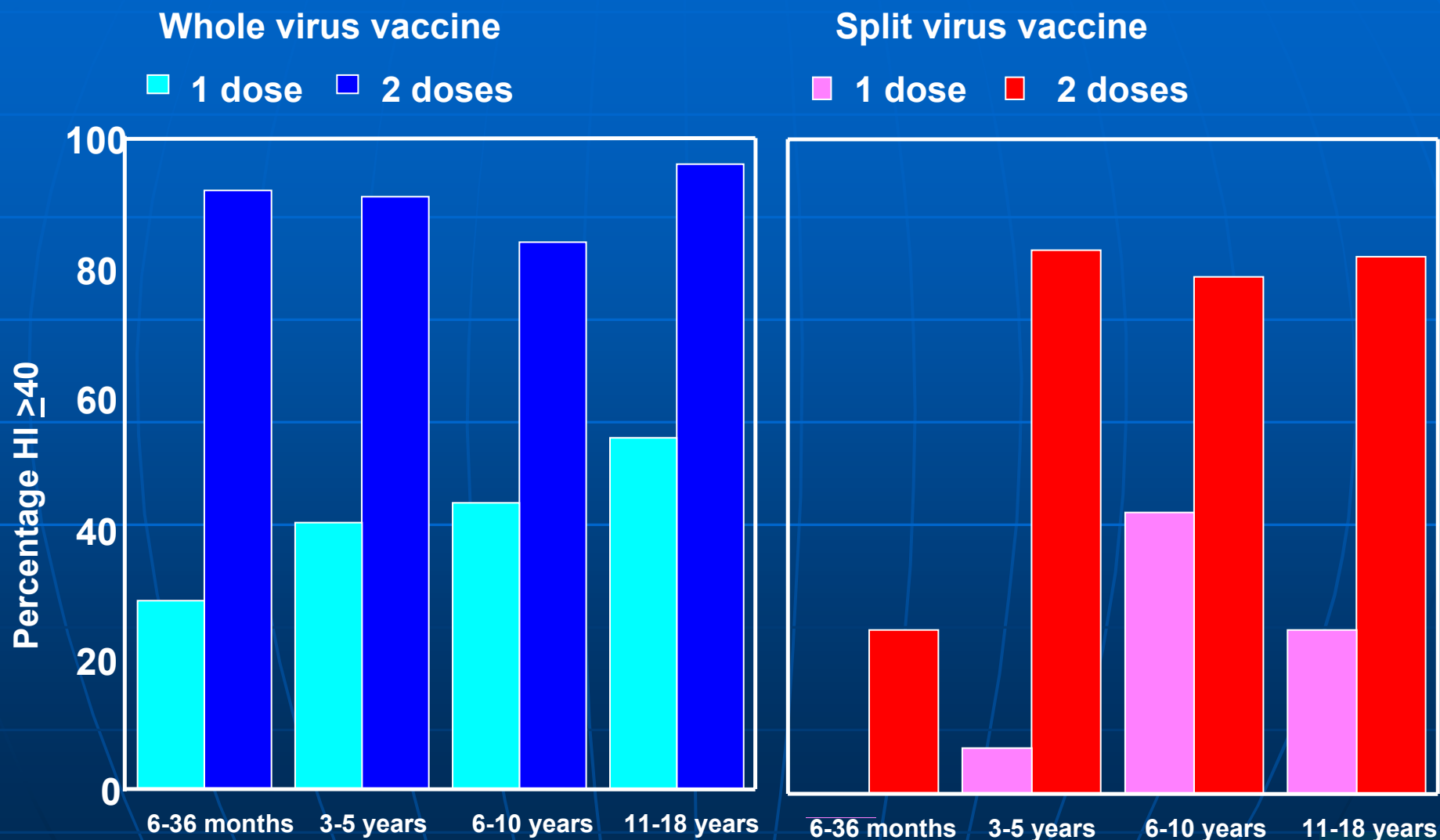
RESPONSE IN NAIVE CHILDREN TO A SINGLE DOSE OF VACCINE A/New Jersey/76 (H1N1)



Wright et al 1977



Response in Naïve Children to A/New Jersey/76 (H1N1) Vaccine

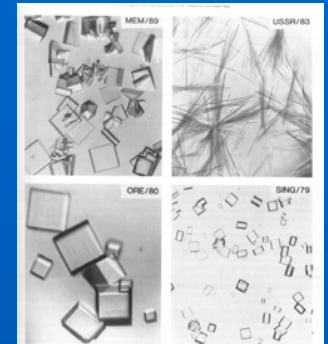
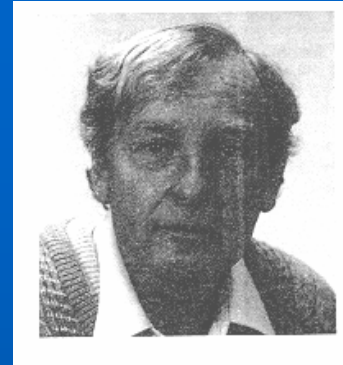


Wright et al 1977

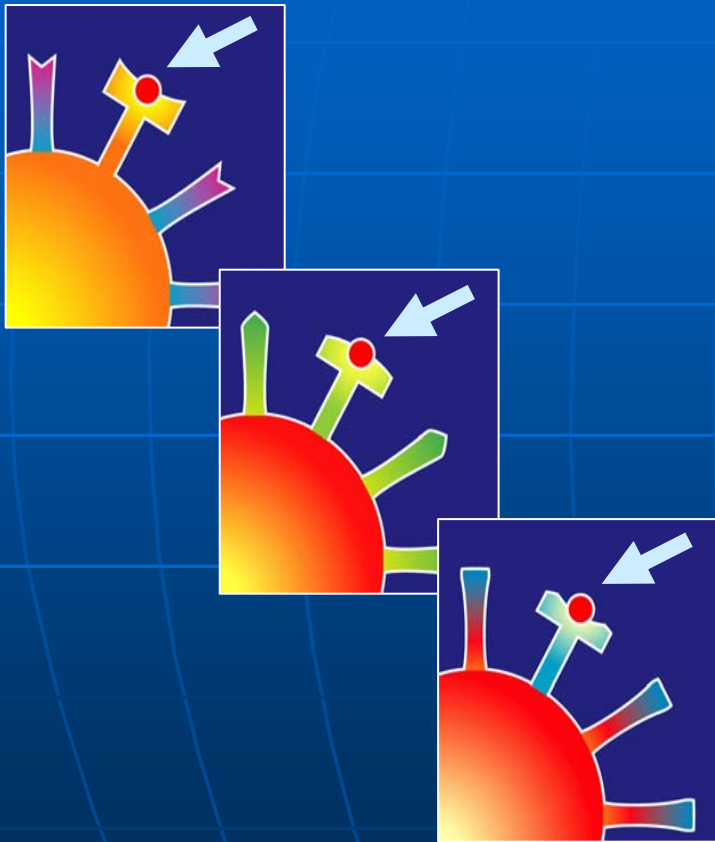


Treating Influenza

- Symptomatic relief
- 1965 Amantadine (Symmetrel)
(blocks M2 ion-channel)
 - 'flu A only
 - unwanted side-effects
 - viral resistance
- Neuraminidase Inhibitors
(inhibit neuraminidase enzyme)
 - Zanamivir (1999)
 - Oseltamivir (2001)

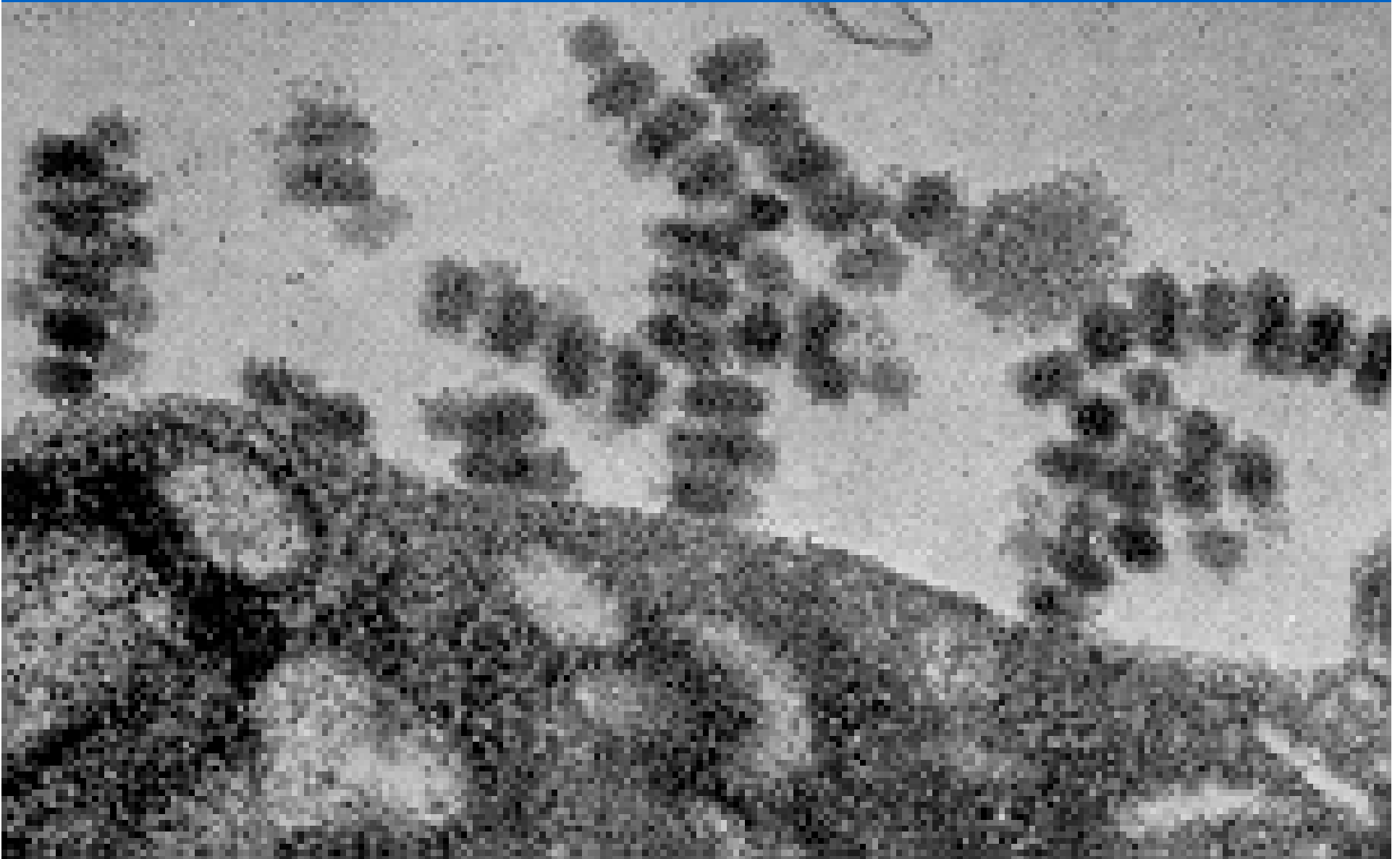


Influenza Neuraminidase



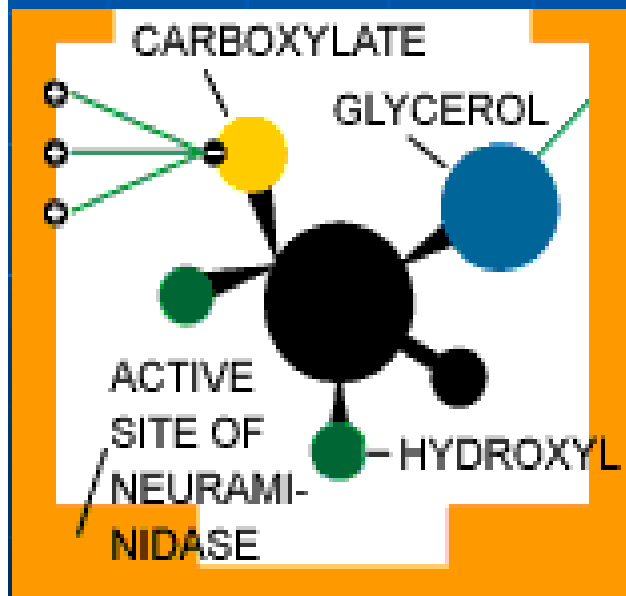
- **active site is highly conserved**
- **essential for virus replication and infectivity in humans**
- **an ideal target for antiviral intervention**

Aggregation of Influenza Virus by Inhibition of Neuraminidase Activity

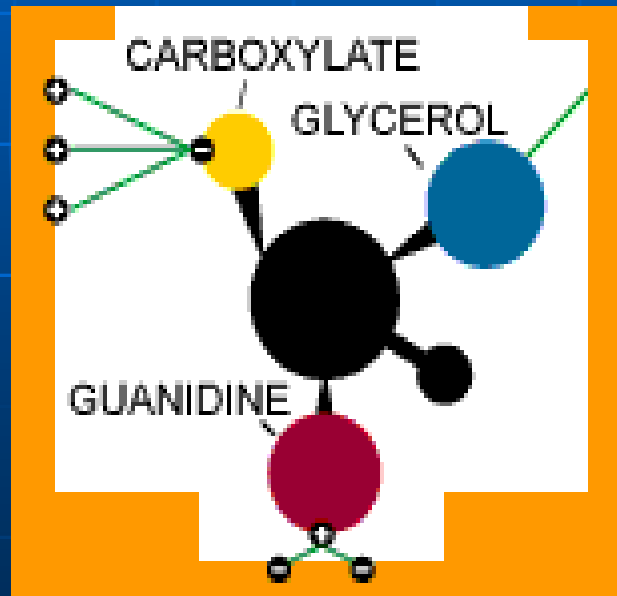


Fit of molecules into the influenza neuraminidase active site

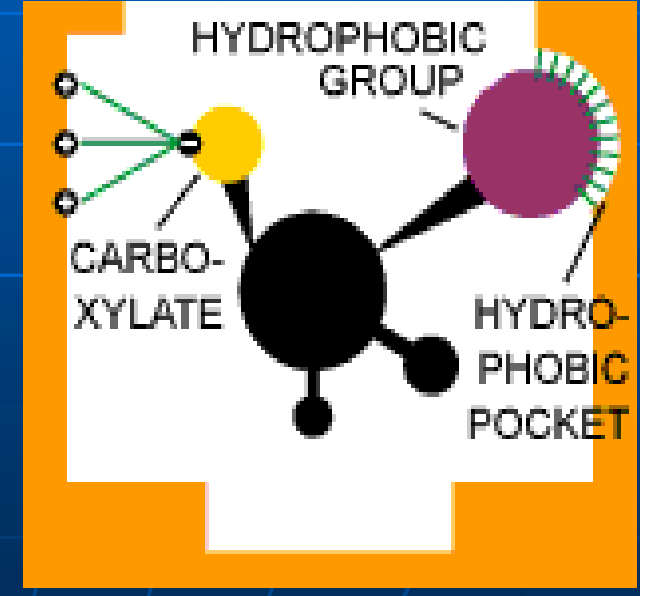
Natural substrate Sialic Acid



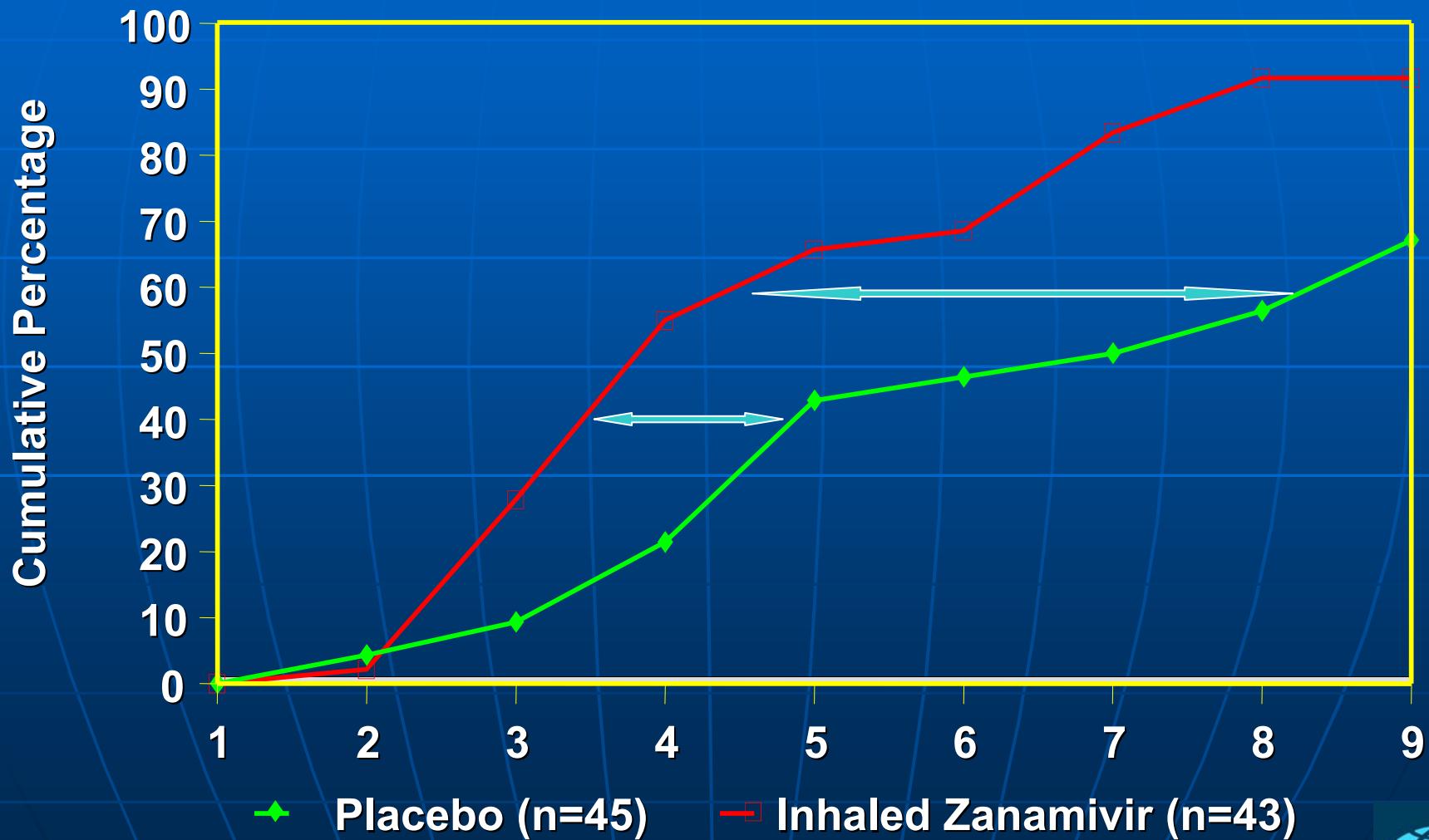
Zanamivir



GS 4107 Oseltamivir

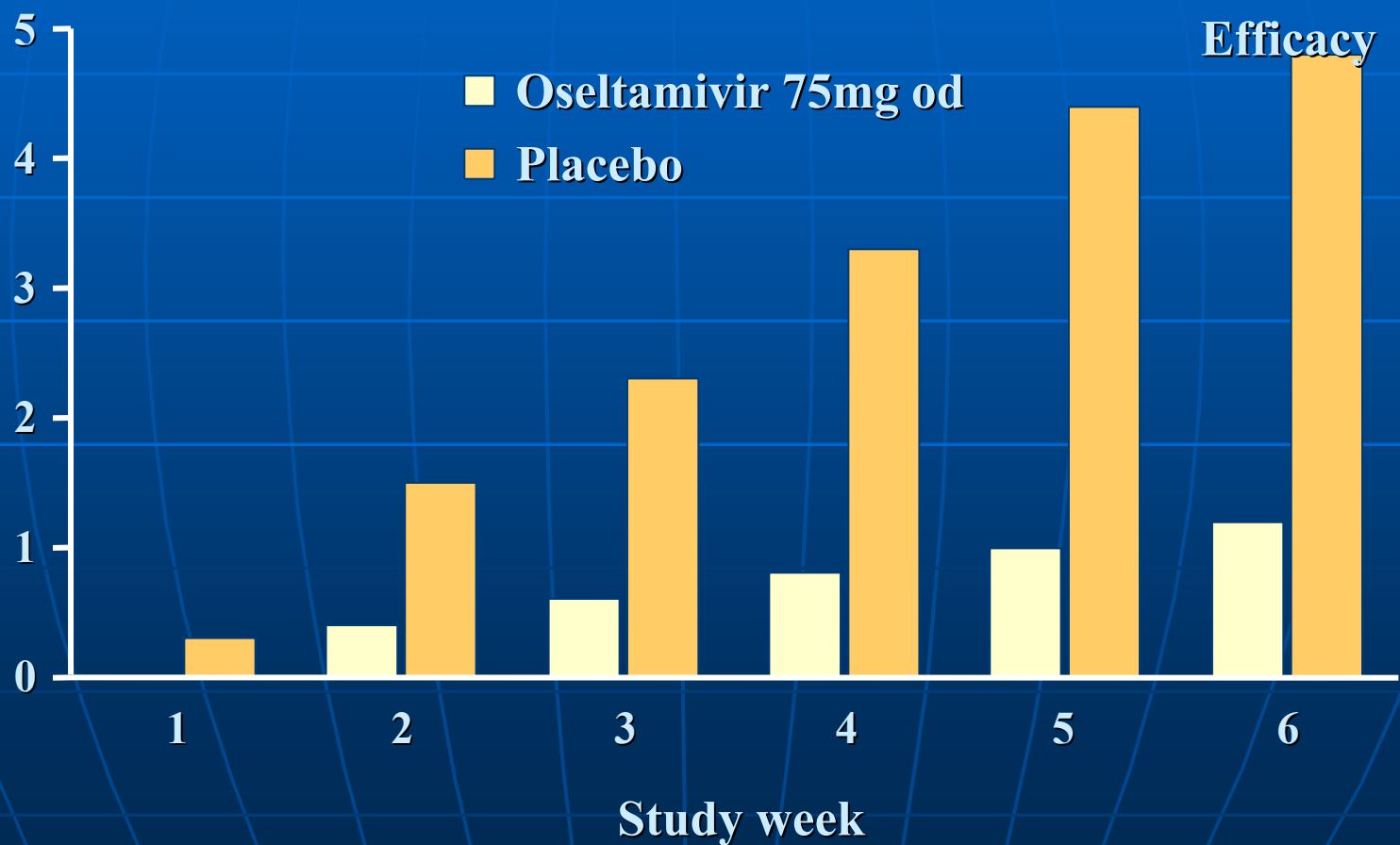


Alleviation of Symptoms By NI in Patients Infected with Influenza A or B Virus



Long-term prophylaxis with an NI

Cumulative % with confirmed clinical influenza



84%
Protective
Efficacy



Available Neuraminidase Inhibitors



- Good safety profile.
- Reduce time & severity of illness if given early (within 48hrs).
- ~86% effective prophylactically
- Limited evidence of resistance (no clinically significant resistance to date)



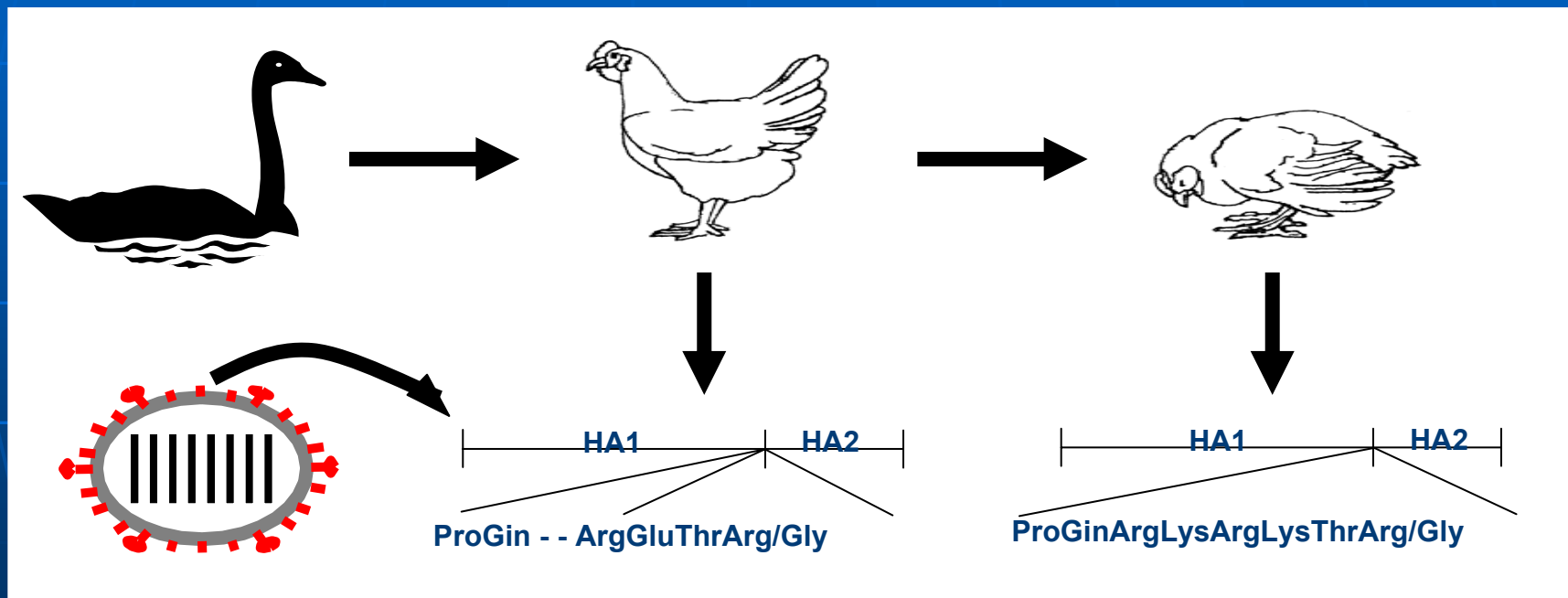
Characteristics of Avian Influenza

Until 1990s

- **Not pathogenic in wild birds** (*one exception*)
- **Two subtypes (H5 & H7) caused occasional severe disease in domestic poultry in Europe, Americas, Australia - but NOT Asia.**
- **No direct evidence of infection in humans** (*one exception with H7 from poultry*)
 - **- some antibody evidence**

Pathogenicity of H5 and H7 in domestic poultry

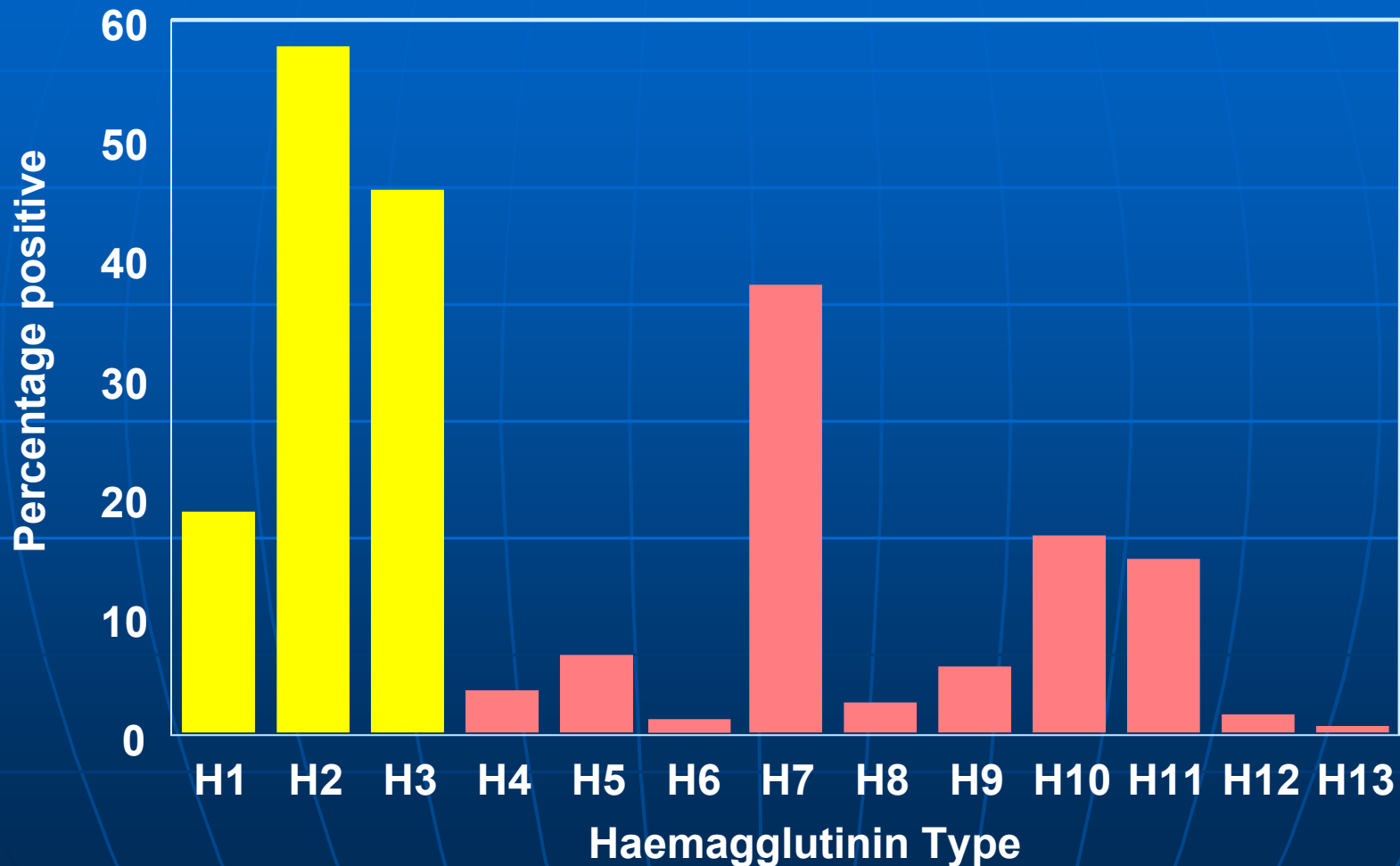
Associated with additional basic amino acids at HA cleavage site.



HA now cleaved by weak proteases found throughout the body and the virus spreads to other organs.

Serological Evidence of Avian Influenza Infection

Antibodies in Rural Population - Jiangsu Province China



From Shortridge 1988



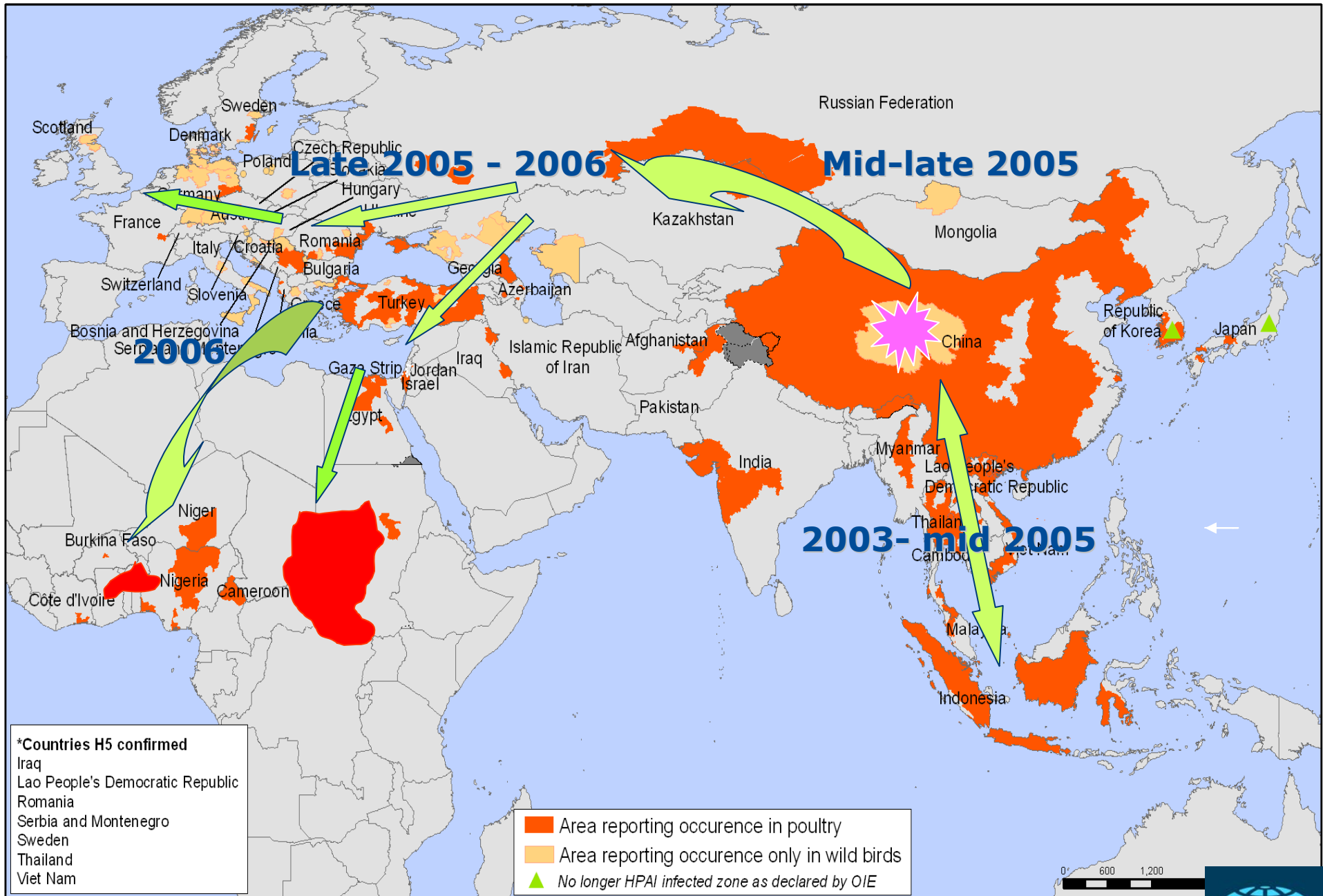
1997 H5 'Chicken' Flu Incident

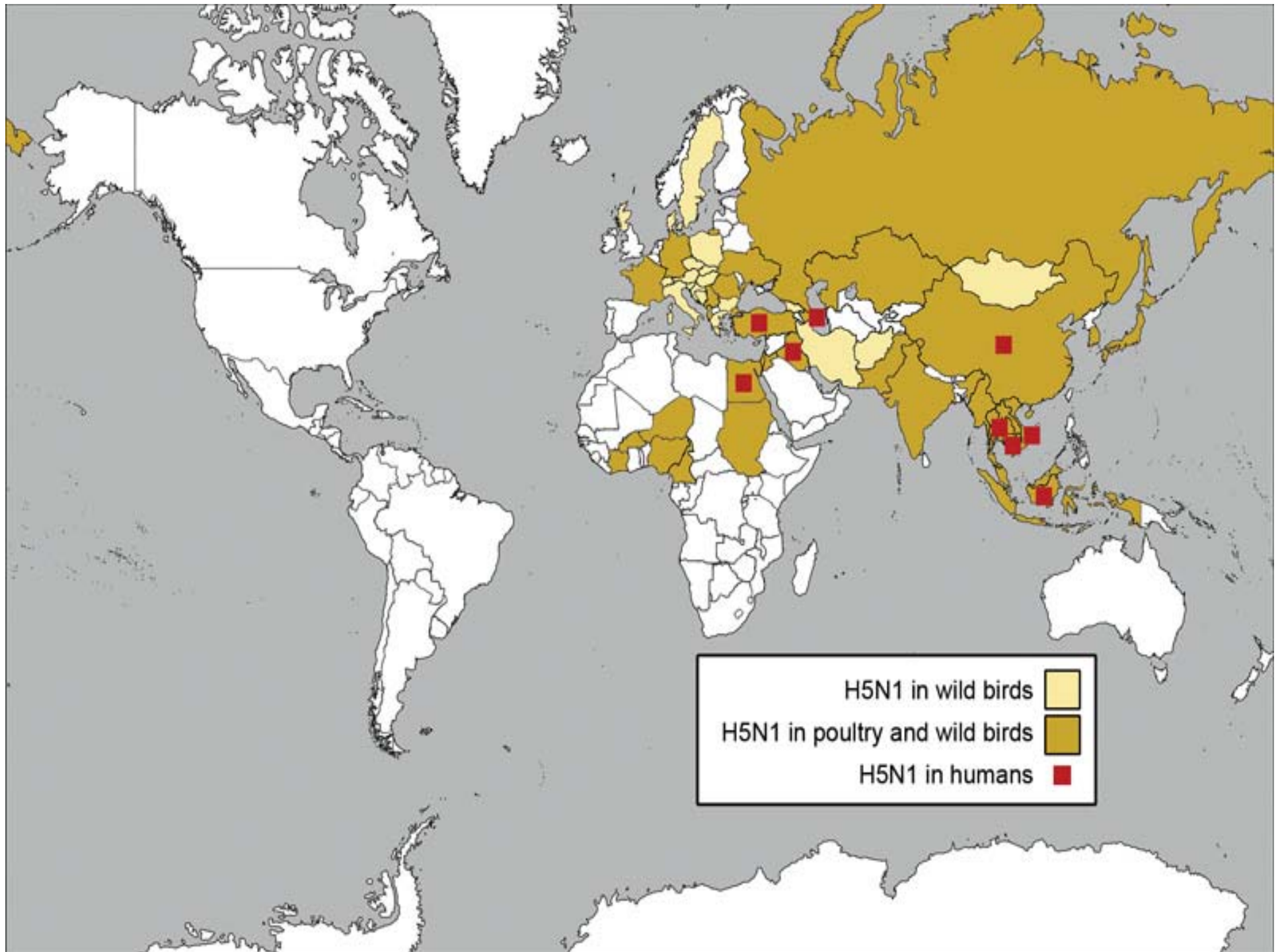
- 18 people hospitalised in Hong Kong
- Influenza H5N1 diagnosed
- Serious illness - 6 deaths
- Virus found in chickens - cases associated with poultry contact (markets)
- Poultry culled on 29 December 1997 - no more cases



Recent History of Avian Influenza

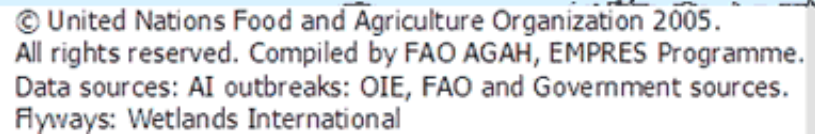
- Poultry outbreaks of H5N1 commencing late 2003 spreading through Asia and beyond
- Associated sporadic human H5N1 cases with high mortality - **207 confirmed cases with 115 deaths** (at 8 May 2006)
- Poultry outbreaks of H7 influenza
- Human cases of H7, H9 and H10





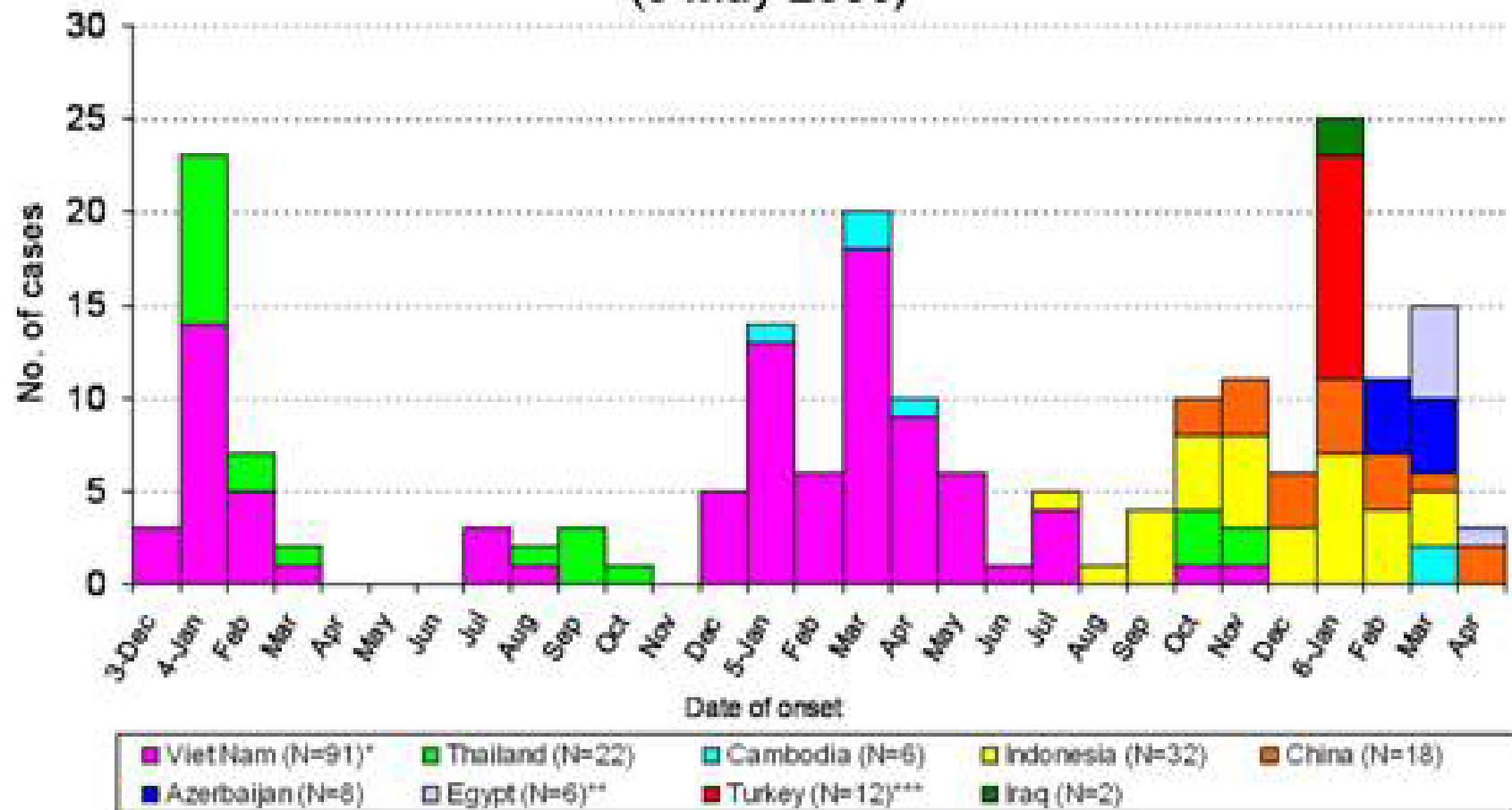
Situation on 30 August 2005

Situation on 30 August 2005



Human Avian Influenza A/H5N1 Cases by Onset Date and Country

(5 May 2006)



As of 5 May 2006, total of 206 cases were reported officially to WHO.

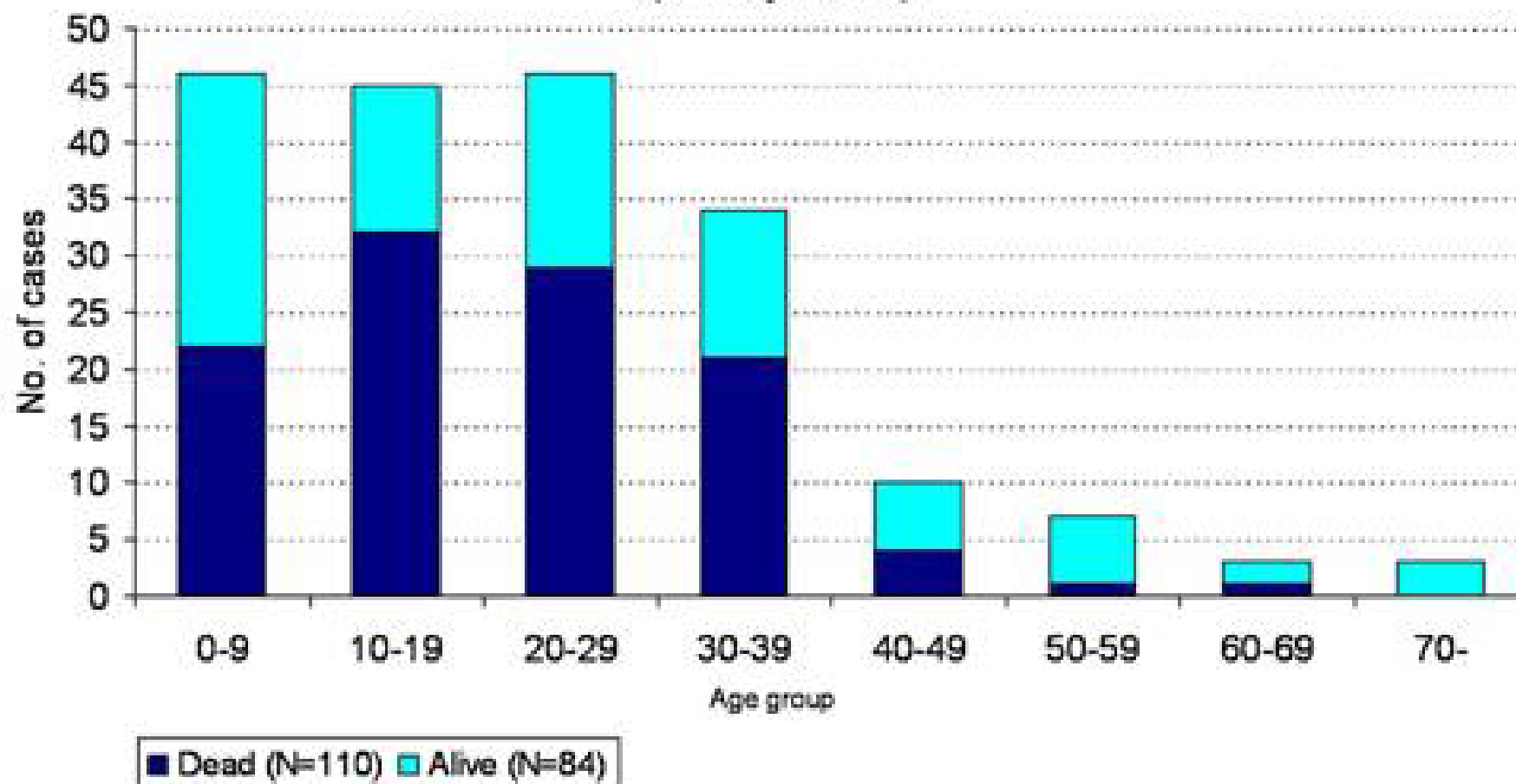
* The 2 asymptomatic cases in Viet Nam were excluded.

** The 7 cases in Egypt without reported date of onset were excluded.

*** Date of onset for Turkey are based on reporting date.

Human Avian Influenza A/H5N1 Cases by Age Group and Outcome

(5 May 2006)



- As of 5 May 2006, total of 206 cases were reported officially to WHO.
- The 12 cases in Turkey were excluded.

Why Does H5N1 Infect but Not Transmit from Person to Person?

There may be several reasons. However, the H5 HA has a specificity for receptors present on avian cells but which have recently been demonstrated on cells in the human lower respiratory tract. (ie. only lower RT penetration infects, virus not present in URT secretions)

HOWEVER

This could possibly change with a single mutation.

Of two reconstructed sequences for the HA of the Spanish influenza:

- one had one amino acid difference from the avian virus at the receptor-binding site
- the other had two amino acid differences

Why Does H5N1 Have Such a High Mortality in Humans?

Deaths occur due to

- ARDS
- Multiple organ failure
- It may induce an excessive cytokine cascade (as the Spanish Influenza may also have done).
- It may spread to other organs as it does in poultry.

Some Questions

- **What is the chance of a pandemic?**
 - **Will it evolve from the current H5N1?**
- **How severe might such a pandemic be?**
- **What has been done to minimise the impact?**
- **Can a pandemic be averted?**

The Chance of a Pandemic?

- The current situation is unique
- There has been extensive exposure with no ongoing human transmission – does this mean that it won't happen?
- Areas for concern:
 - Continuing evolution in poultry including domestic ducks
 - Spread by migratory birds
 - Infection of cats
 - Recent spread into Africa (possible interaction with HIV?) and India
 - Lab experiments show no incompatibility of H5 for reassortment with current human strains

Severity

- **'Spanish Flu' had mortality of 2-5% mainly in younger adults**
- **From 2003-2006 H5N1 has had an overall mortality of ~56% mainly in children & young adults**
- **Severity may be due to specific genes (M, NS), and effects such as cytokine induction, and these could be lost on reassortment or modified by mutation**

Reverse Genetics for Influenza Virus

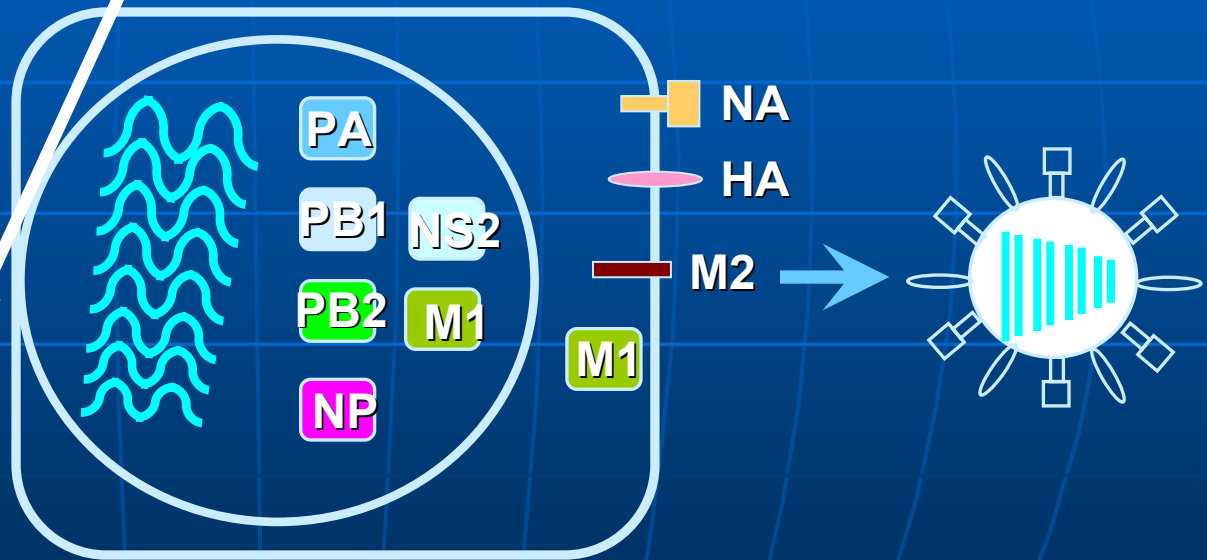
Plasmids expressing
influenza viral RNAs



Gene segments can be:

- constructed from sequence data
- modified at will

Plasmids expressing
influenza viral proteins



Reassortment vs Mutation

1918-19	Adaptation from avian ? New HA & NA	Severe
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1957	Genetic reassortment New HA & NA	Moderate
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1968-9	Genetic reassortment New HA unchanged NA	Mild
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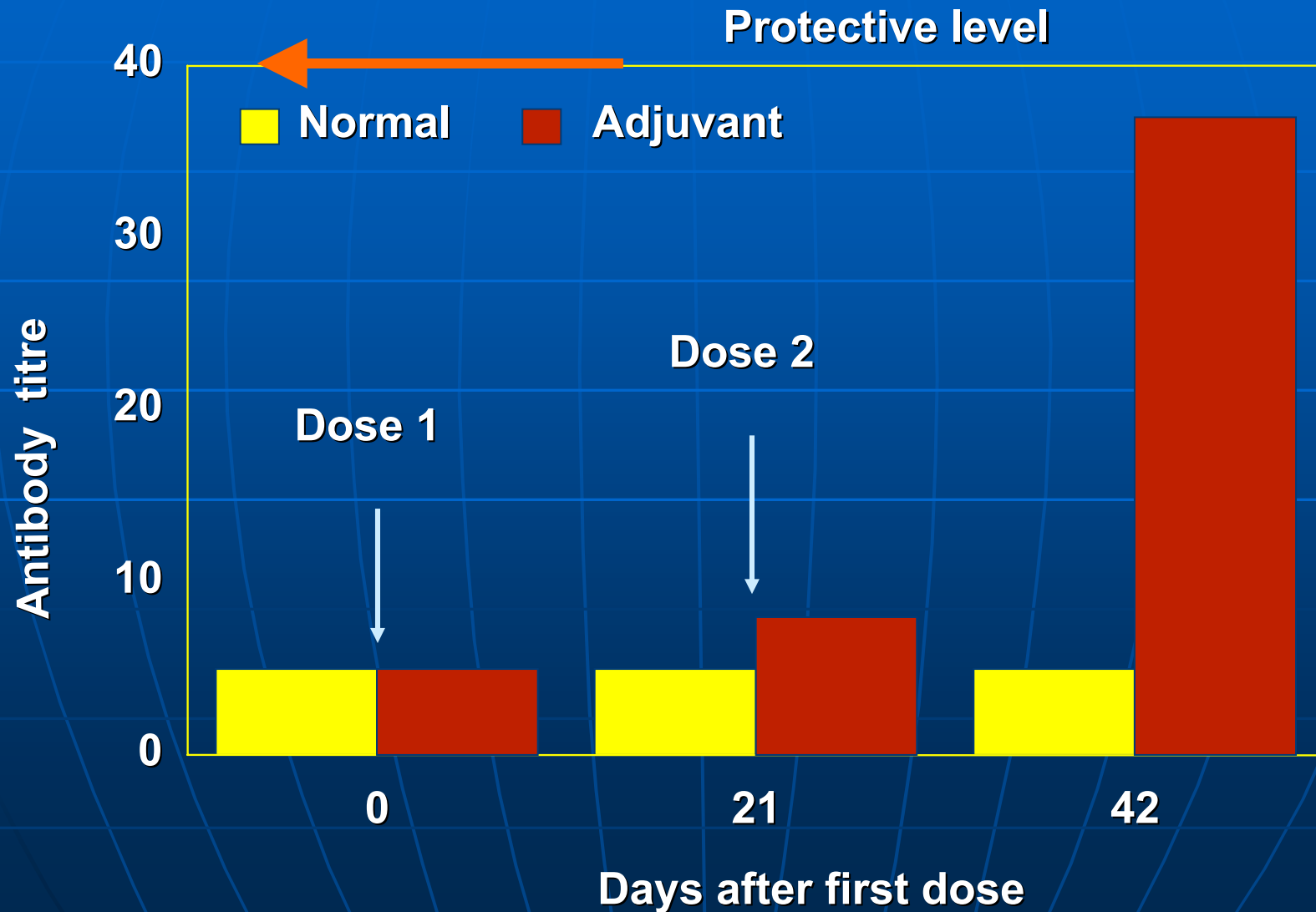
Australian Government Response since 2003

- Updated Australian plan
- Office of Health Protection formed
- Purchase of antivirals stockpile (to approx 7M courses)
- Development of pandemic vaccine contracts
- Funding pandemic vaccine trials *
- \$6.5M Urgent research funds

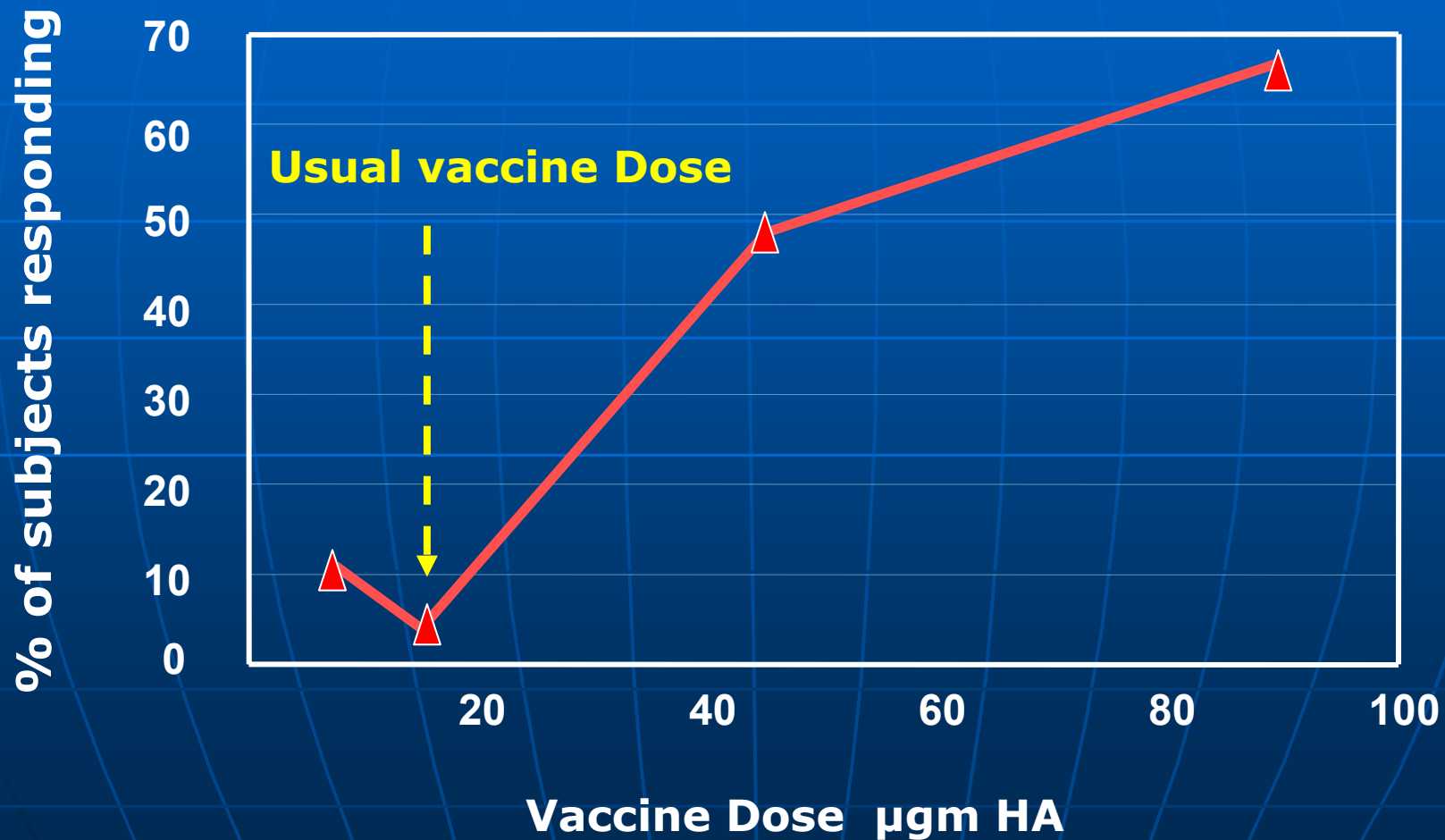
Particular Problems Relating to H5N1 Vaccines

- Early human trials with H5 vaccines showed poor responses to conventional vaccines
- The recent H5N1 viruses are unsuitable for vaccine production because of:
 - danger to workers
 - killing egg embryos
 - potential escape into the environment
- Therefore vaccine strains must be 'engineered' genetically to make them more safe for workers and eggs
 - Subject to IP licencing
 - Become GMOs requiring BSL-2 containment production facilities
- Evidence of antigenic drift and multiple lineages in domestic poultry – a strain prepared today may have reduced effectiveness in a few months

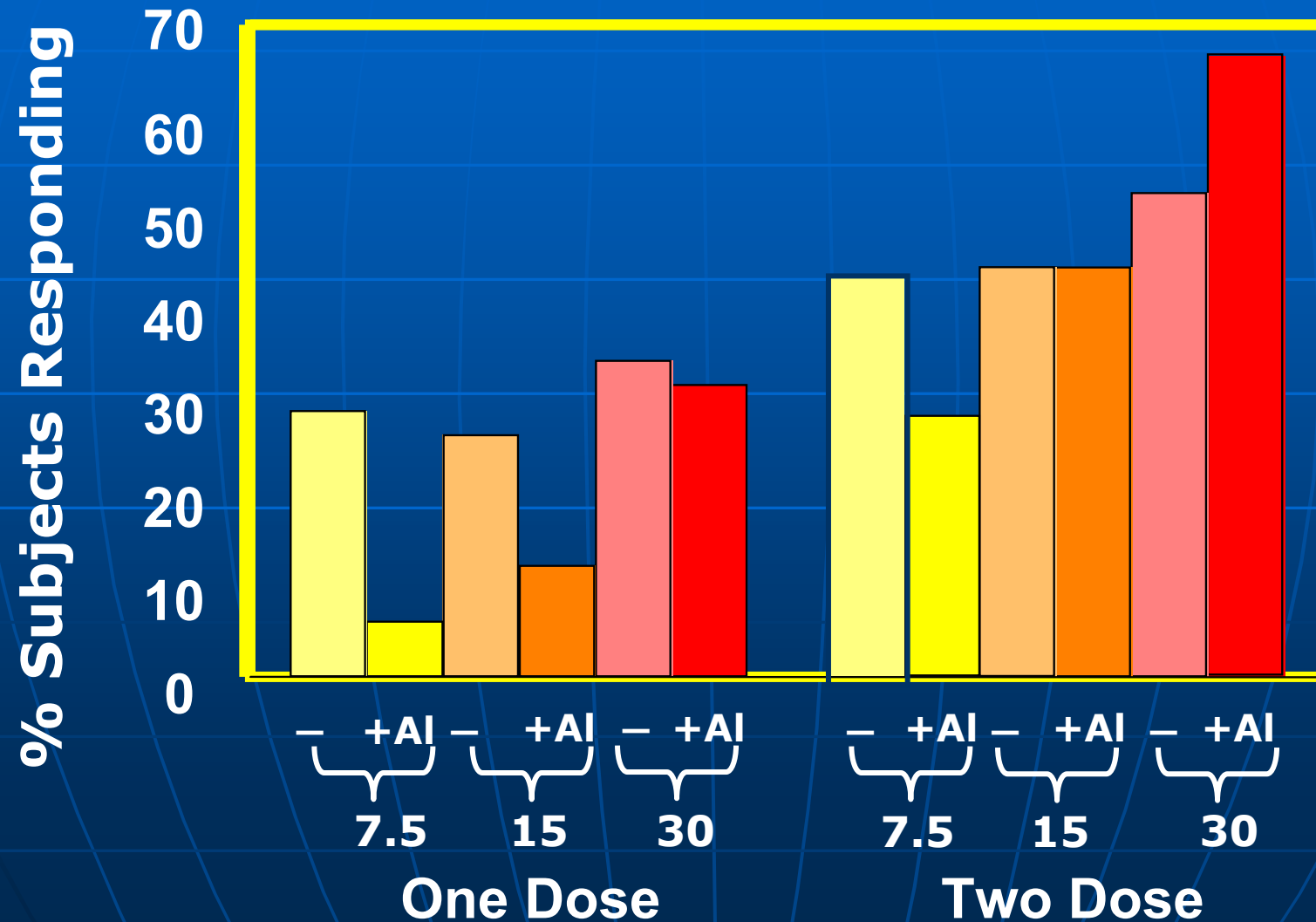
Early Clinical Studies of Vaccines vs H5 Influenza

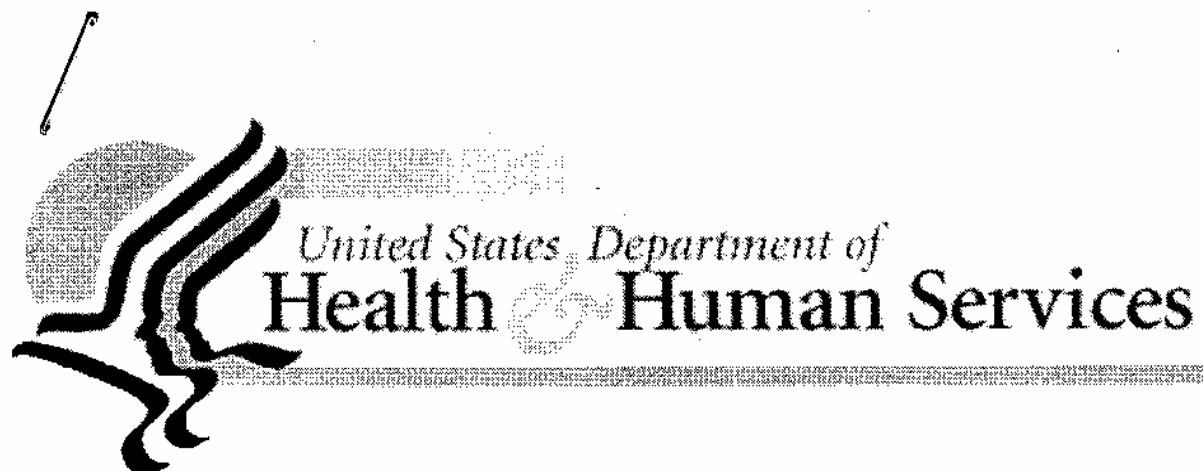


Response in Human Volunteers to Conventional H5N1 Vaccine (AVietnam/1203/04)



Response to Al(OH)₃ Adjuvanted A/Vietnam/1194/2004 (H5N1) Vaccine





News Release

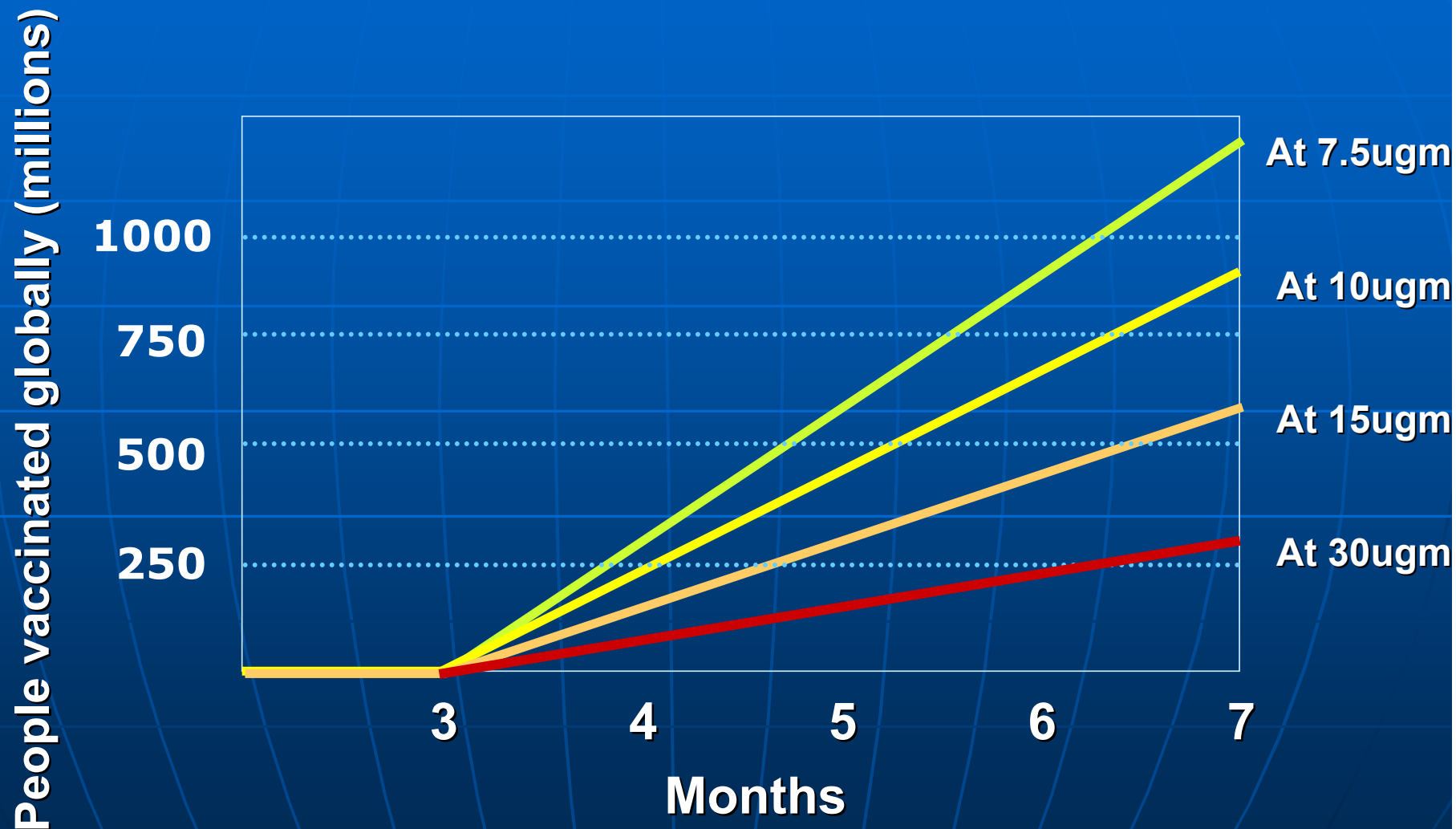
FOR IMMEDIATE RELEASE
Thursday, May 4, 2006

Contact: HHS Press Office
(202) 690-6343

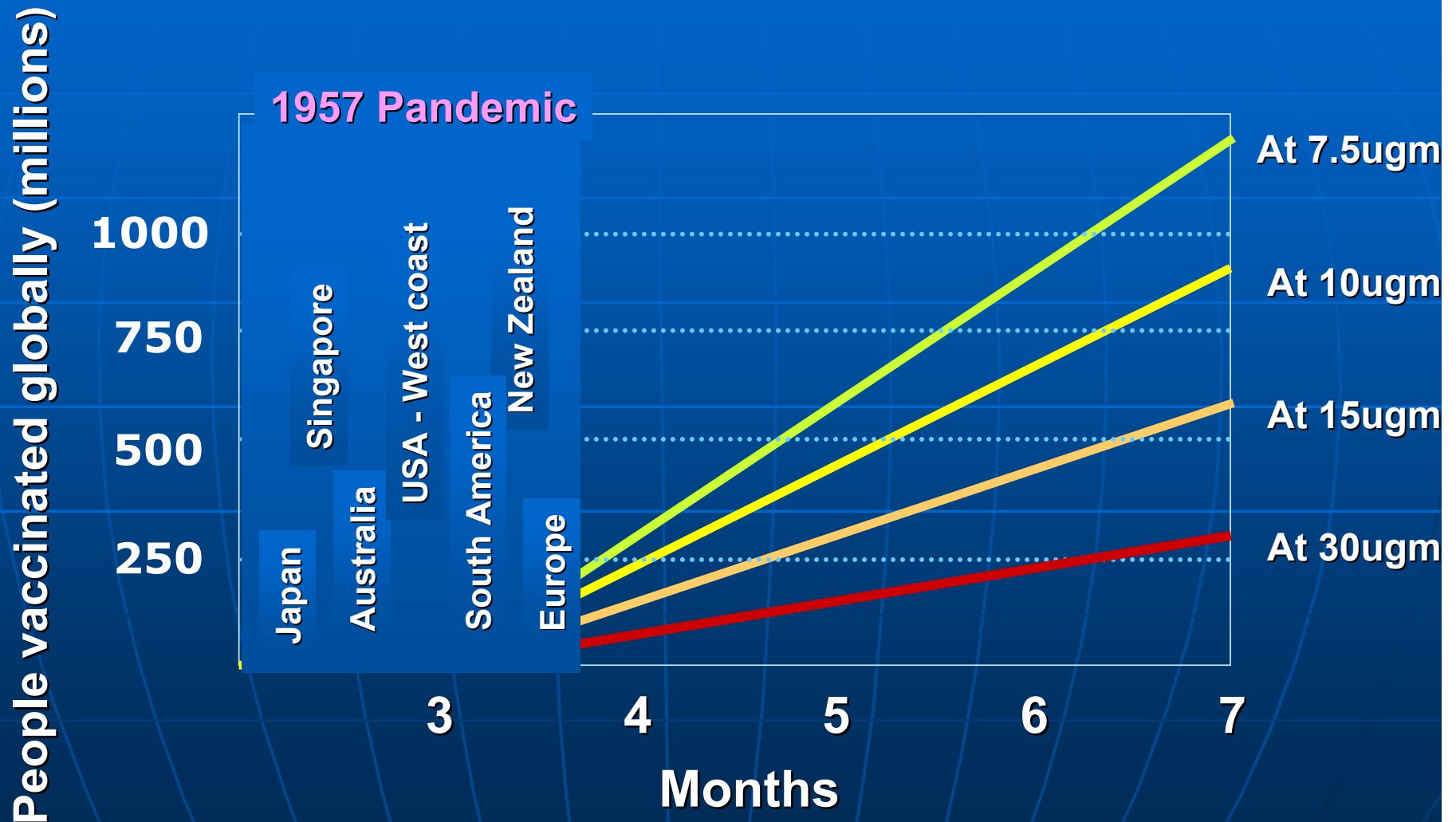
HHS Awards Contracts Totaling More Than \$1 Billion To Develop Cell-Based Influenza Vaccine

As part of the President's plan to prepare for a pandemic, HHS Secretary Mike Leavitt today awarded more than \$1 billion to accelerate development and production of new technologies for influenza vaccines within the U.S. These five contracts support the advanced development of cell-based production technologies for influenza vaccines and will help to modernize and strengthen the nation's influenza vaccine production by creating an alternative to producing influenza vaccines in eggs. These funds are part of \$3.3 billion proposed by the President and appropriated by Congress to HHS for fiscal year 2006 to help the nation prepare for a pandemic.

Potential Global Pandemic Vaccine Availability



Potential Global Pandemic Vaccine Availability



Can a pandemic be Averted?

Some recent studies show that if the virus starts to spread in people and:

- It is detected early
- Spread isn't very fast
- It can be effectively treated with antivirals (ie. the WHO stockpile is effectively deployed) and
- Local quarantine can be enforced

then an H5N1 pandemic may be averted

However, history suggests that in all probability there will be another influenza pandemic in the not too distant future!