Influenza antivirals: surveillance and resistance

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Influenza Surveillance - Global

- Global Influenza Surveillance Network - GISN
- Established in 1952
- Comprises
  - 4 WHO Collaborating Centers (WHO CCs)
  - 121 WHO NICs in 93 countries
  - NICs collect specimens, perform primary virus isolation and preliminary antigenic characterization à
  - Ship newly isolated strains to WHO CCs for comprehensive antigenic and genetic analysis à
  - Basis for WHO recommendations on the composition of influenza vaccine for the Northern and Southern Hemispheres each year
- Alerts emergence of new virus variants (antigenic, drug-resistant) and/or viruses with pandemic potential

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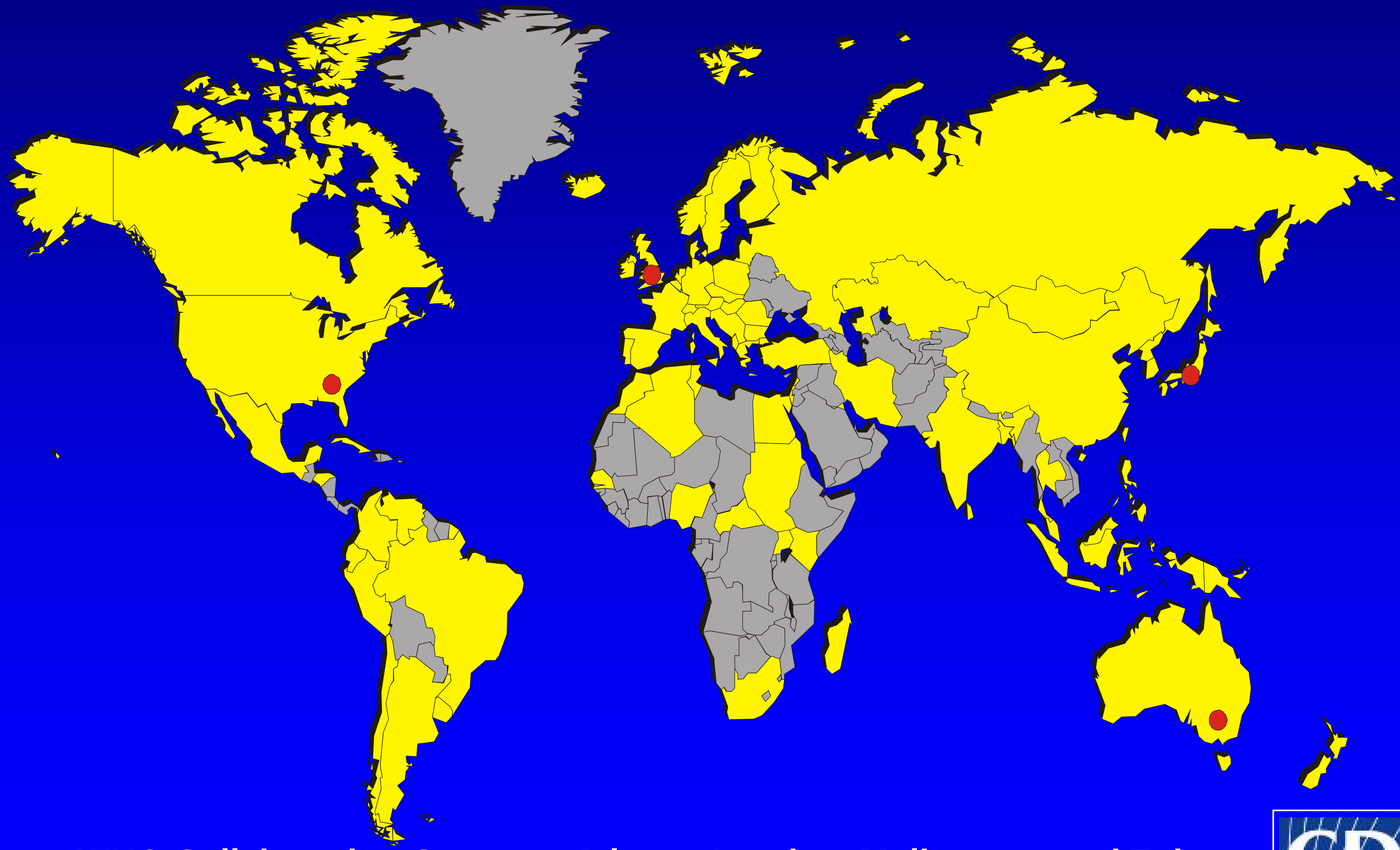
WHO Collaborating Centers for Influenza



● WHO Collaborating Centers - Atlanta, London, Melbourne, and Tokyo
■ Countries containing at least 1 WHO influenza laboratory

Human Infections with Avian Influenza Detected by GISN

1997 - H5N1 - Hong Kong, SAR China - 18 (6 fatal)
1998 - H9N2 - China - 6
1999 - H9N2 - Hong Kong, SAR China - 2
2002 - H7N2 - Virginia - 1
2003 - H5N1 - Hong Kong, SAR China - 2 (1 fatal)
2003 - H5N1 - China, Vietnam - 4 (4 fatal)
2003 - H7N7 - Netherlands - 89 (1 fatal)
2003 - H9N2 - Hong Kong, SAR - 1
2003 - H7N2 - New York - 1
2004 - H7N3 - Canada - 2
2004 - H5N1 - Thailand, Vietnam - 46 (32 fatal)
2005 - H5N1 - Cambodia, China, Indonesia, Thailand, Vietnam - 98 (43 fatal)
2006 - H5N1 - Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey - 115 (79 fatal)
2007 - H5N1 - Cambodia, China, Egypt, Indonesia, Laos, Myanmar, Nigeria, Pakistan, Thailand, Vietnam - 85 (58 fatal)
2007 - H7N2 - United Kingdom - 4

Most cases – direct contacts with infected poultry
Limited H-to-H transmission

(January 3, 2008)

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WHO CC for Surveillance, Epidemiology and Control of Influenza, Atlanta, USA

- Identification and characterization of circulating influenza viruses using serologic and molecular techniques
  - Monitoring appearance of new antigenic or potentially pandemic variants
  - WHO and FDA vaccine strain selection
  - Monitoring resistance to FDA-approved drugs
    - Adamantanes (M2 blockers) - amantadine, rimantadine
    - NA inhibitors - zanamivir, oseltamivir
- Pandemic preparedness
- Develop methodology for influenza diagnosis
  - Preparation and distribution of reagents
    - Serological (antigenic drift; antigenic shift)
    - Molecular (seasonal; animal; pandemic)
- Laboratory training
- Research

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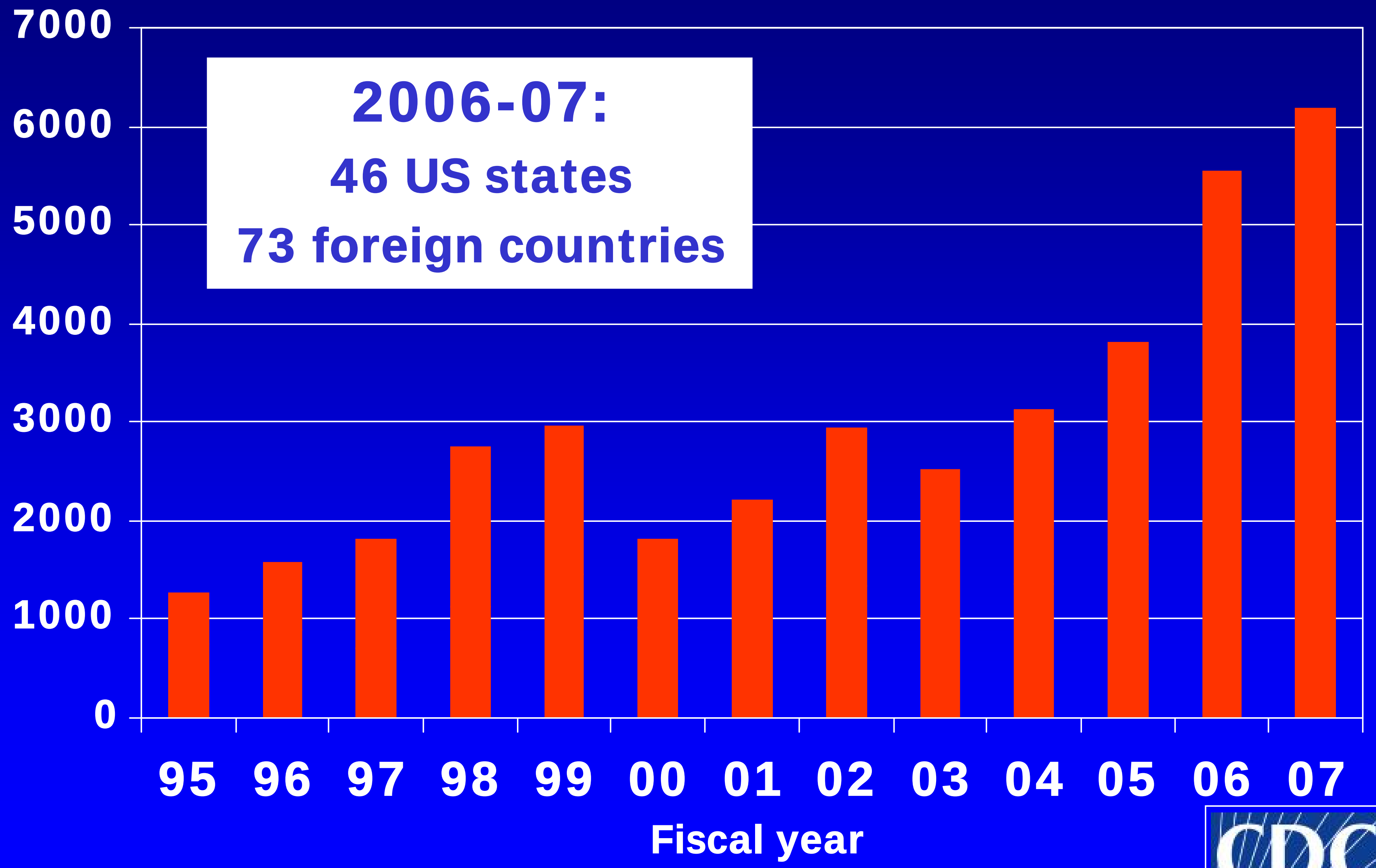
Specimens Received by Influenza Division, CDC



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Two Classes of FDA Approved Drugs

- Adamantanes or M2 blockers
    - amantadine and rimantadine

- Neuraminidase inhibitors
    - oseltamivir (Tamiflu) and zanamivir (Relenza)

- While both classes are effective at treating and preventing influenza illness, they differ by mechanisms of action, resistance development and transmission

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Adamantanes (M2 blockers)

- Amantadine, rimantadine (influenza A only)
  - Decrease Symptoms and shedding by 1-2 days
  - Prophylaxis - 70-90% effective
  - Cross-resistance (Amantadine & Rimantadine)
  - Rapid development of resistance
    - 10-40% in 2-5 days
  - Mechanisms of action:
    - blocking virus "uncoating" at stage of infection (H^+ flow across the M2 channel)
  - Molecular markers of resistance:
    - AA changes within transmembrane domain of M2 (26F/I, V27A, A30V/T, S31N, G34E)

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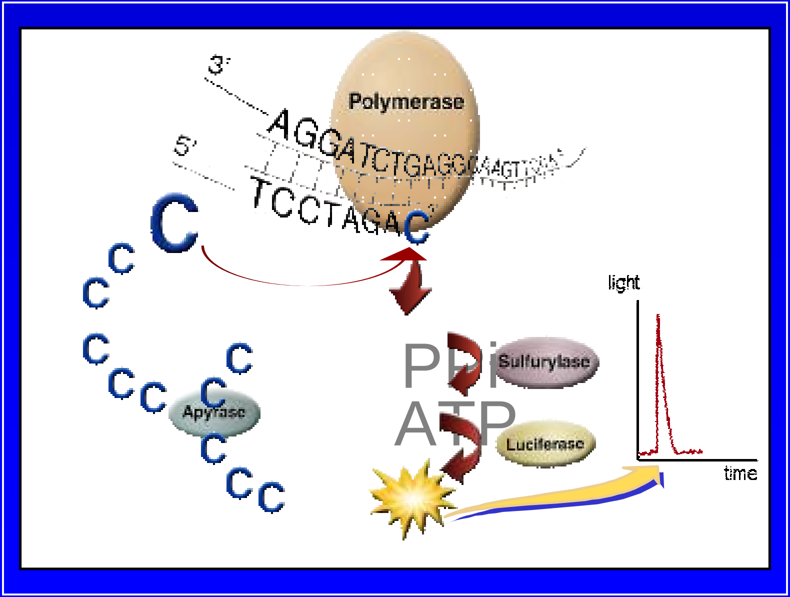
Monitoring Resistance to M2 Blockers: Seasonal and Avian Influenza Viruses

- Detection of established molecular markers of resistance in the virus genome (M2 gene)

- Pyrosequencing is current assay of choice:
  - High throughput
    - CDC: >11,000 viruses tested
  - Could be done with clinical specimen
  - Rapid
  - Accurate



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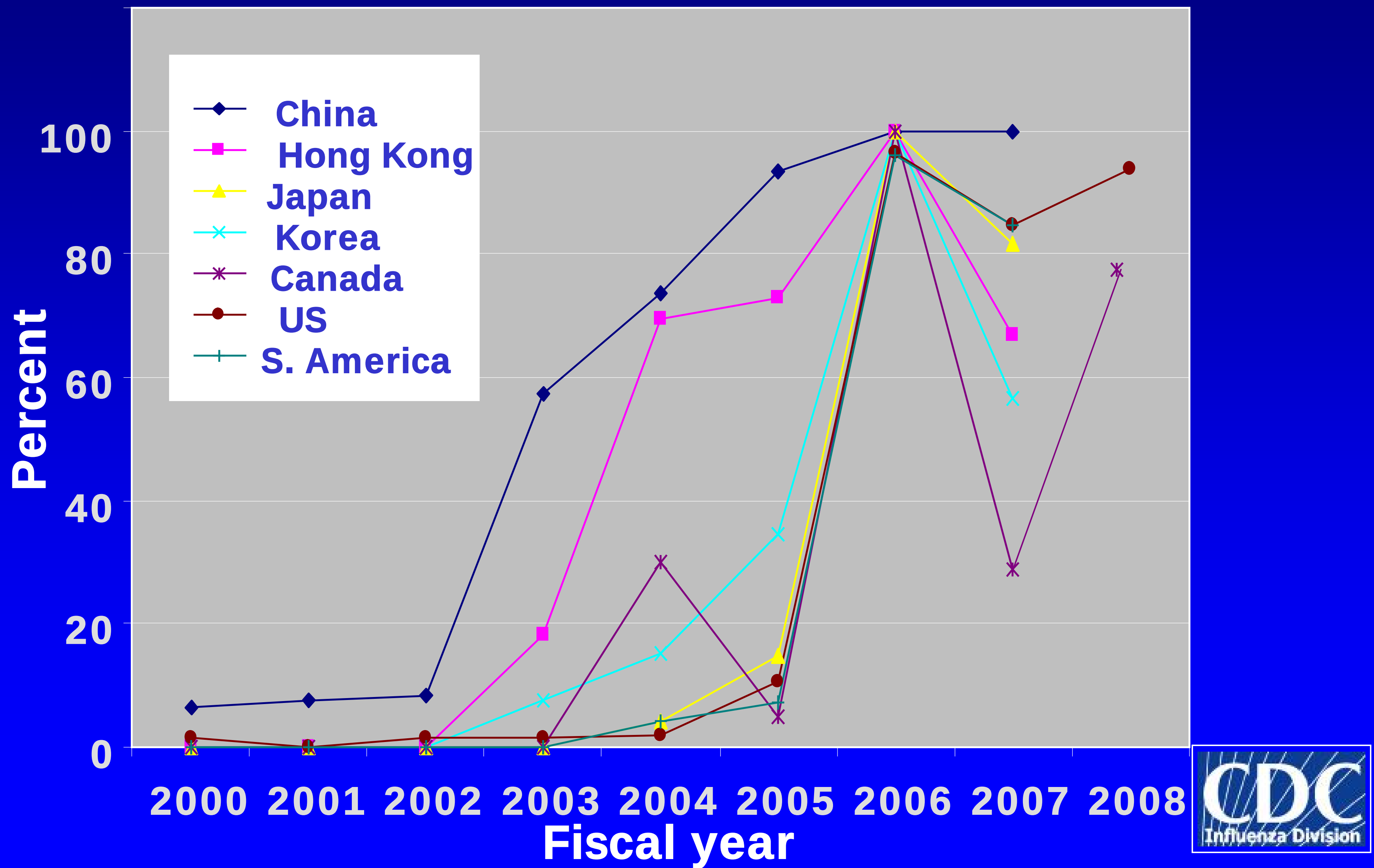
Rapid Spread of Adamantane Resistance in A(H3N2) Viruses

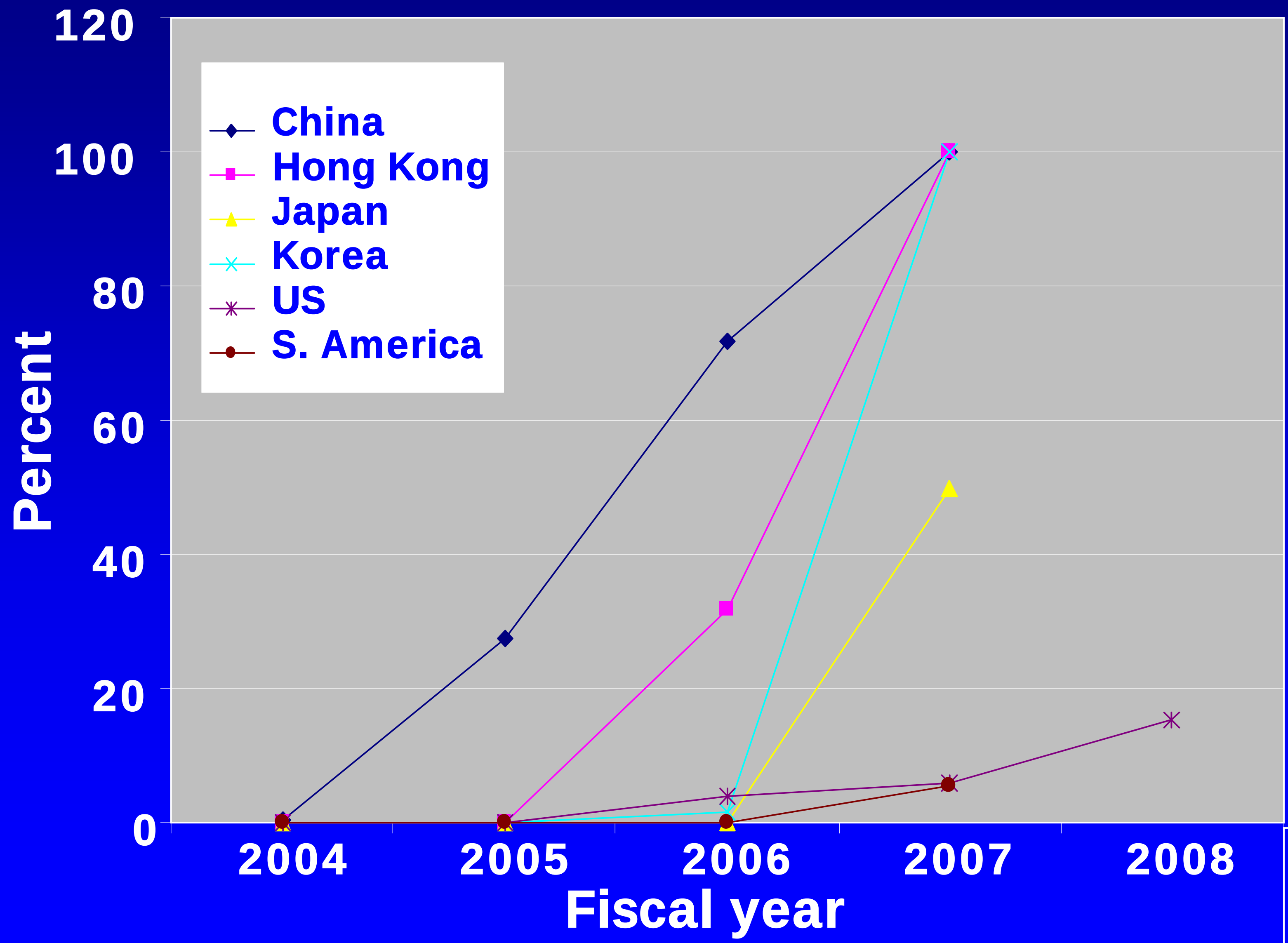


Rapid Spread of Adamantane Resistance in A(H3N2) Viruses

- Present system of surveillance allowed us to timely detect a rise of M2 resistance

- Rapid change in policy recommendations
  - CDC advised against prescribing amantadine and rimantadine in 2005-06, 2006-07, and 2007-08 seasons

Spread of Adamantane Resistance in A(H1N1) Viruses



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Adamantane resistance in 2006-07: geographic diversity

	A(H3N2)		A(H1N1)	
	No.	%	No.	%
Asia	353	63	180	83
China	69	100	62	100
S. Korea	101	57	42	100
Europe	100	39	58	52
Canada	143	30	27	0
US	395	86	551	6
Global	1348	72	892	24

Resistance emergence in H1N1 is an independent event
No evidence for reassortment between H3N2 and H1N1 viruses

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Resistance to M2 Blockers: Seasonal Influenza

- Rapid development of influenza A drug resistance under treatment
- Rapid increase of resistance among influenza A viruses in recent years
- Transmissibility of resistant mutants in humans
- Several independent introductions of resistant viruses into human population
  - Different from bacterial resistance
- Unaltered fitness of drug resistant viruses

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Global spread of M2 resistant seasonal influenza A viruses – factors ?

- Increased use of drugs
  - due to SARS outbreak and bird flu scare ?
- Accessibility of drugs
  - no prescription needed in some countries
  - cheap
- Changes in other genes may facilitate spread of resistant viruses
  - HA antigenic change
  - favorable gene constellation ?
    - Holmes et al., 2005; Simonsen et al., 2007;
    - CDC unpublished data

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NA Inhibitors

- Zanamivir, Oseltamivir (Influenza A and B)
  - Treatment*:
    - decrease symptoms by 1-2 d, hospitalizations by ~50%; seasonal mortality among elderly
  - Prophylaxis (seasonal)*: 50-70% effective
  - H5N1 cases - small numbers
    - early treatment may reduce mortality
  - Mechanism of action:
    - block virus release from infected cells
    - different from M2 blockers
  - Molecular markers of resistance:
    - not well defined (especially for H5N1)
    - changes in NA structure
      - drug specific; virus type/subtype specific

* Cooper at al., 2003

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NA Inhibitors

- Zanamavir, oseltamivir (influenza A and B)
  - Emergence of resistance during treatment
    - 5.5% - US children (Whitley et al, 2001)
    - 18% - Japanese hospitalized children (Kiso et al, 2004)
    - 0.2% - adults (Treanor et al, 2000)
- Resistant viruses appear to be less transmissible and possibly less viable, but human data are limited
  - Reduced transmissibility in ferrets (seasonal)
    - only a few mutants tested (R292K; E119V; H274Y)
- Cross-resistance is variable
  - mutations in NA catalytic site confer cross-resistance
  - mutations in framework (e.g. H274Y) do not

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Monitoring NAI-susceptibility: seasonal and H5N1 influenza

- Changes in the interactions between NA **enzyme** and **inhibitor**
  - IC_{50}, a concentration of NAI which reduces the enzyme activity by 50% (nM)
- <u>High throughput screening</u> method:

  NA inhibition assay with chemiluminescent substrate (**NAStar kit, Applied Biosystems**)
  - **>5,000** influenza A and B viruses tested at CDC
  - Limitations:
    - **Complicated data interpretation**
    - Artificial, small substrate
    - Requires a grown virus

- Confirmation: Sequence analysis of NA to detect mutations at NA active site

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Detected NAI resistant viruses - seasonal

- **2004-05**
  - **1 oseltamivir-resistant virus:**
    - **H274Y: from the US (no epi data)**
  - **1 oseltamivir- and zanamivir-resistant virus:**
    - **R371K: from China (no epi data)**
- **2005-06**
  - **1 oseltamivir-resistant virus:**
    - **H274Y (H1N1) from China**
- **2006-07**
  - **US: 5 oseltamivir-resistant viruses**
    - **4 H274Y (H1N1)**
      - **1 from oseltamivir-treated, 3 unknown**
    - **1 E119V (H3N2): oseltamivir-treated**
  - **China: 1 oseltamivir-resistant virus**
    - **H274Y (H1N1) (no epi data)**

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Frequency of oseltamivir resistance among seasonal influenza A and B viruses

	FY 2007	FY 2008*
US	5/1350 (0.4%)	3/80 (3.8%)
Foreign	1/797 (0.1%)	-

* October 1 – December 31, 2007

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Resistance to NI Inhibitors: Seasonal Influenza

- Frequency of resistance to NA inhibitors is low

  In 2006-07
  - 2121 isolates were tested using NAStar Kit
  - 859 H3N2; 776 H1N1; 486 B
  - 0.5% oseltamivir-resistance
  - all but one sensitive to zanamivir

- H3N2 viruses (0.04-5.2 nM) were the most sensitive to oseltamivir, followed by H1N1 (0.16-3.9 nM) and B (0.19–10.5 nM) viruses

- H1N1 (0.15 – 11.6 nM) viruses were the most sensitive to zanamivir, followed by B viruses (0.03–12.0 nM) and H3N2 (0.15–24.5 nM)

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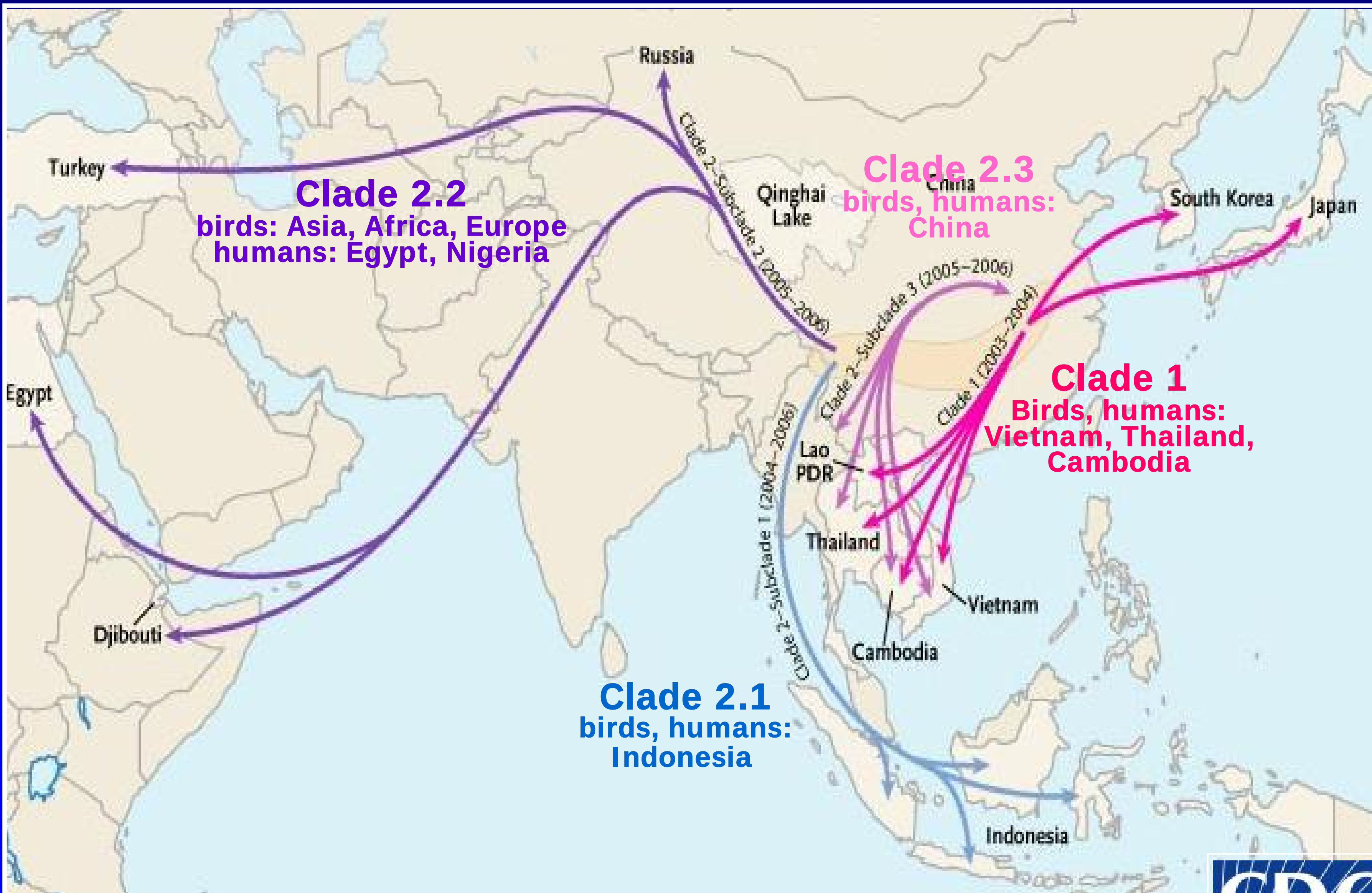
Influenza H5N1 Viruses *



* Webster & Govorkova. N Engl J Med. 2006; 355: 2174-7.

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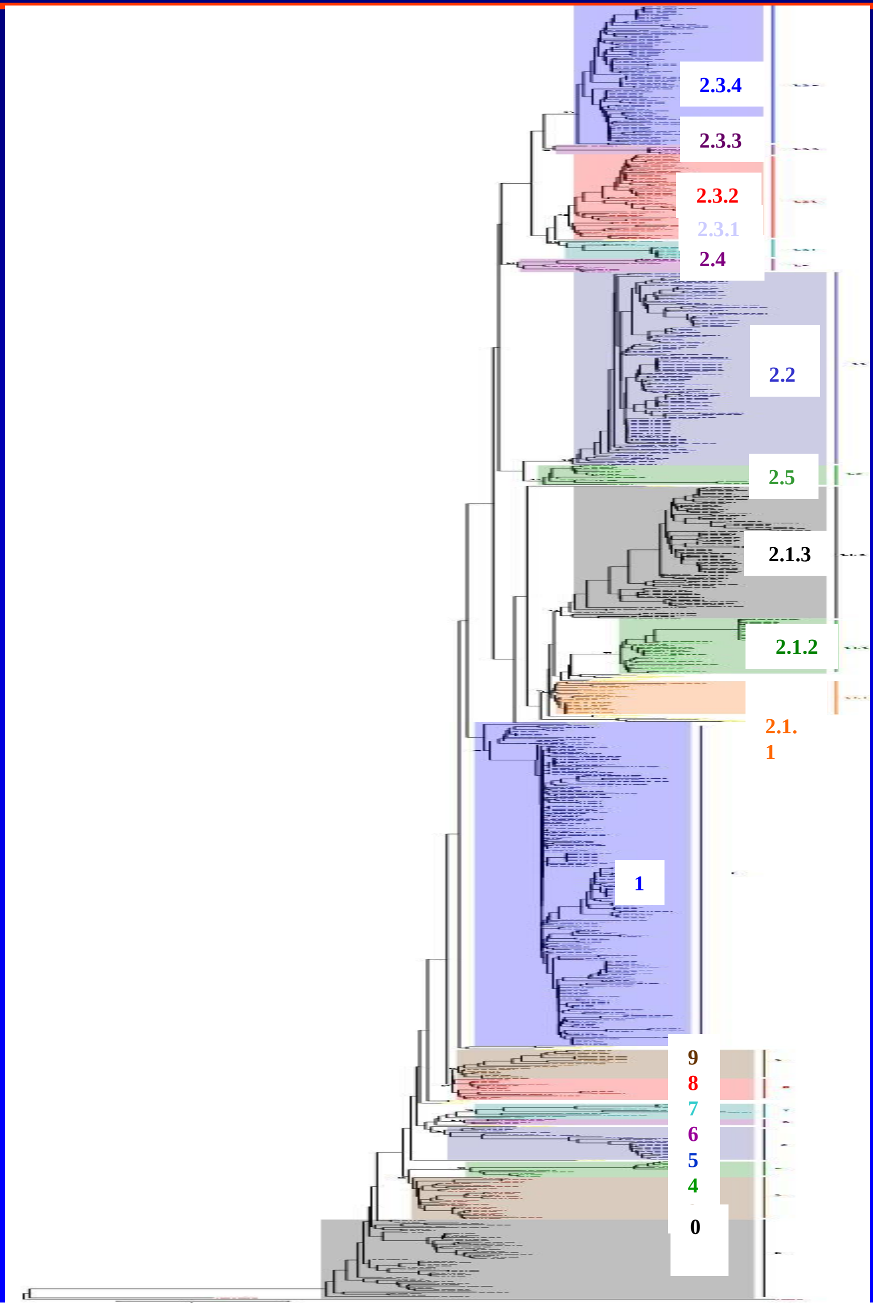
Evolution of the H5 HA Gene

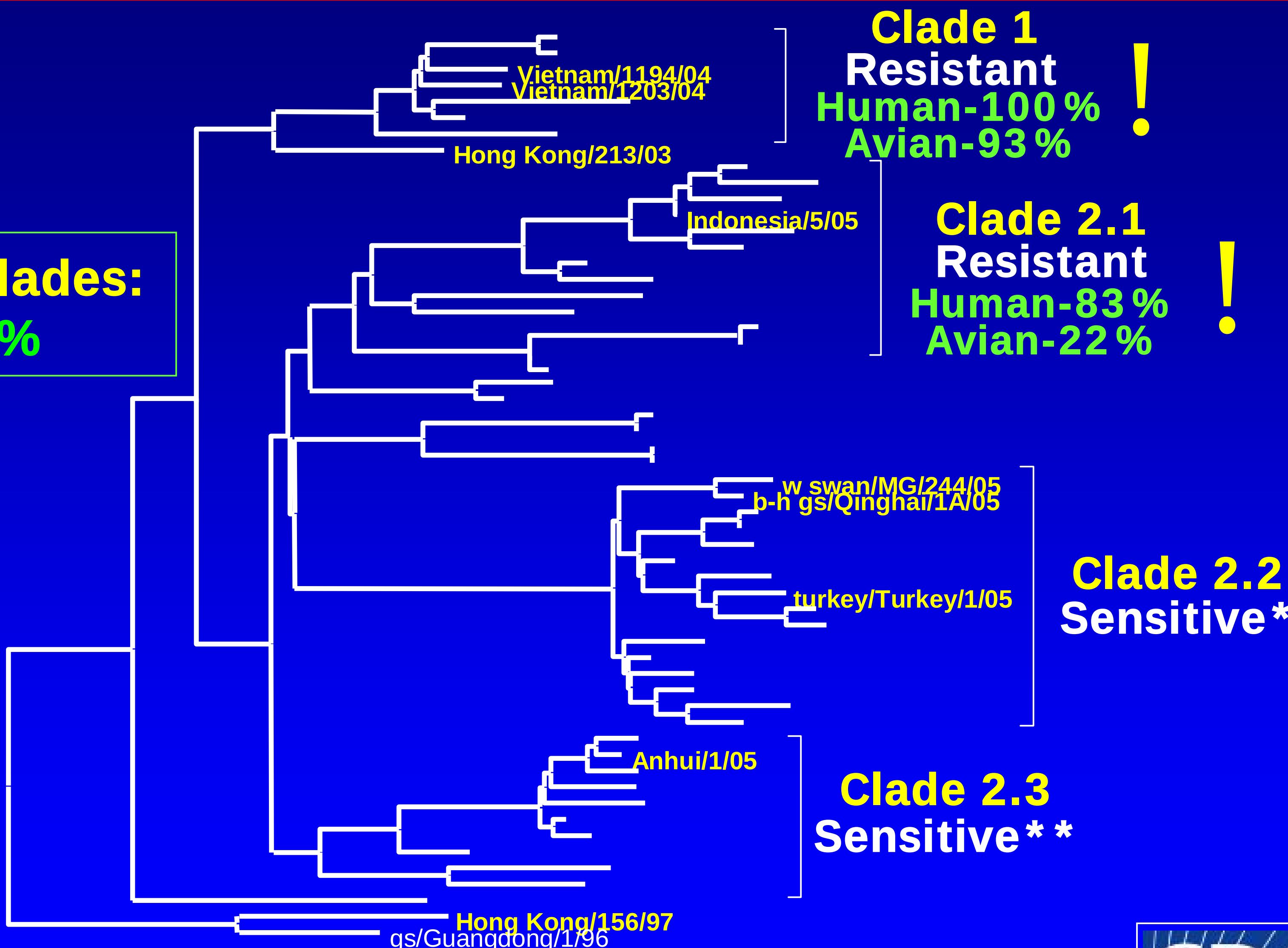


884 full length (1-1659) ORF of H5 HA sequences

Resistance of H5N1 Viruses to M2 blockers

Published M gene sequencing data

Other clades:
17 %



* 1% resistant (avian)
** 5% resistant (avian)

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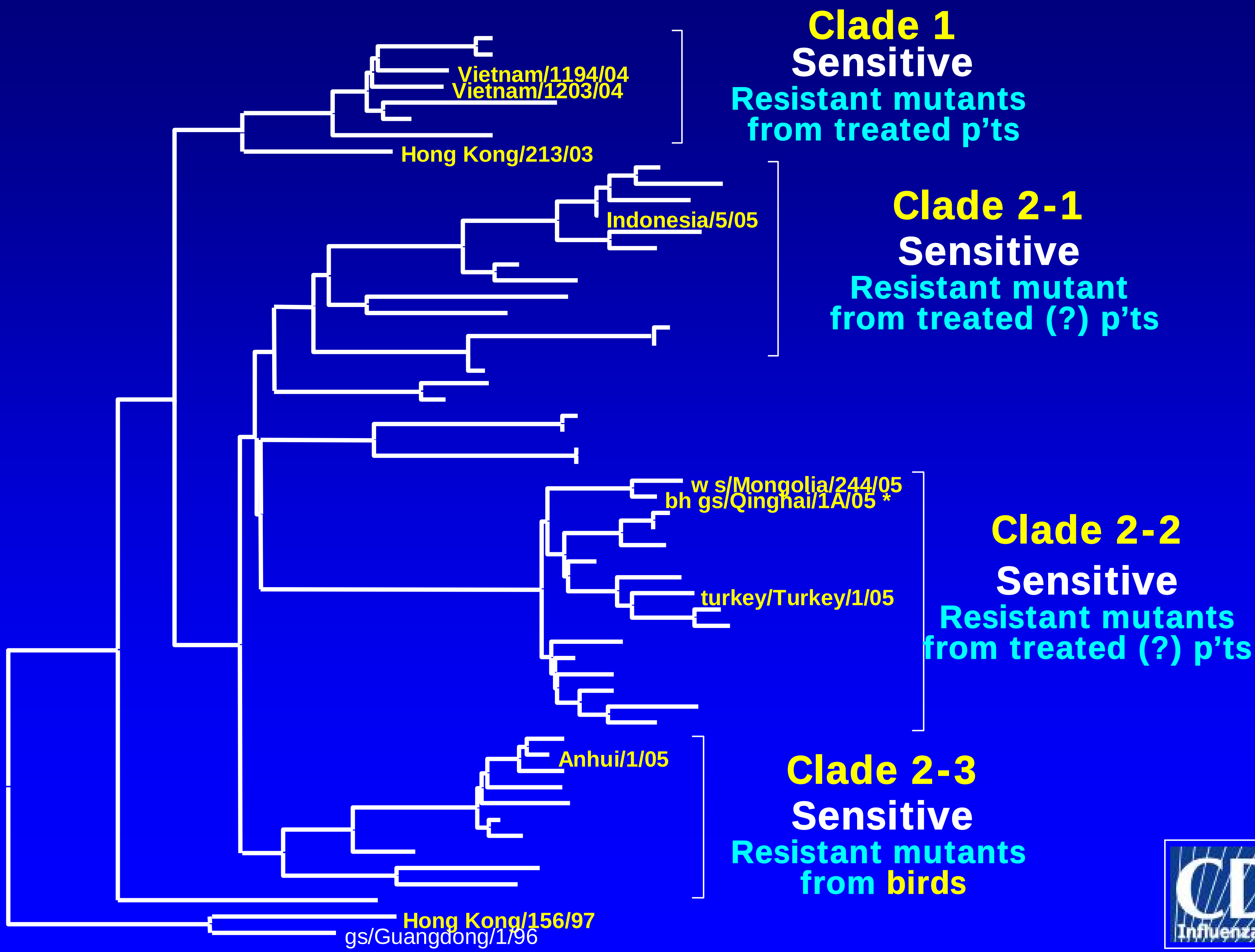
Sensitivity of H5N1 Viruses to NIs *



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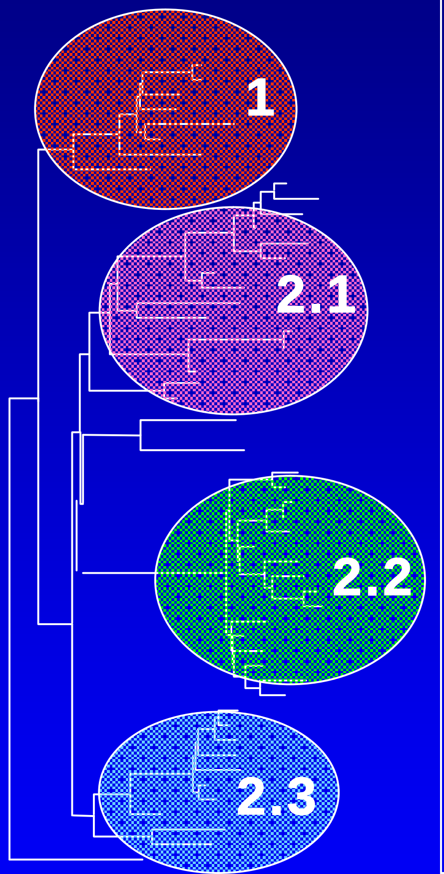
H5N1 Viruses Resistant to Oseltamivir (chemiluminescent assay)*



Clade	#R/#total	IC_{50}	NA mutation
1	1/33	23 vs 0.4 (~57 fold)	274
2.1	1/33	29 vs 1.4 (~21 fold)	136**
2.2	2/19	13 vs 0.8 (~15 fold)	294
2.3	2/58	10-40 vs 1.5 (7-27 fold)	150+222***

Clinical significance is not clear

* CDC preliminary data
** Resistant to zanamavir (~270 fold)
*** Avian isolates

H5N1: IC_{50} - Fluorescent NAI assay

Clade	N	Oseltamivir	Zanamivir
1	18	0.4 ± 0.2	1.9±2.2
2.1	35	7.1± 4.7	3.2±2.2
2.2	2	1.3; 7.2	0.8; 1.8
2.3	46	10.3±7.1	1.1±0.2

Clinical significance of elevated IC_{50} values is not clear

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H5N1: IC_{50} - Chemiluminescent NAI assay

Clade	N	Oseltamivir	Zanamivir
1	20	0.7 ± 1.5	1.3±2.7
2.1	31	1.4± 0.9	1.2±0.7
2.2	11	0.7± 0.3	1.0±0.4
2.3	50	1.5±0.7	0.5±0.2

Clinical significance of elevated IC_{50} values is not clear

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H5N1 viruses - Conclusions

- H5N1 viruses remain a pandemic threat but have not yet developed the ability to be transmitted efficiently from person-to-person
- Distinct geographical distribution of H5N1 genetic and antigenic variants have been identified
- It is not known which, if any, H5N1 variant might acquire the ability to be transmitted efficiently
- Difficulties in WHO pre-pandemic vaccine recommendations

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H5N1 viruses - Conclusions

- **High level of resistance to amantadine and rimantadine detected in some genetic groups**
  - **clade 1; clade 2.1**
- **Low level of resistance to NIs**
  - **several resistant mutants identified**
  - **clinical significance of some R mutants is not clear**
- **New techniques for rapid detection of known mutations is needed**
  - **pyrosequencing (274, 294)**
- **Continued global surveillance for antiviral resistance is critical**
  - **even without H5N1 pandemic threat (seasonal influenza, other potentially pandemic subtypes)**

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