



# **Antiviral Resistance Monitoring – seasonal, pandemic and high pathogenic influenza viruses**

**Aeron Hurt**

**WHO Collaborating Centre for Reference and Research on Influenza,  
Melbourne**



# Overview



- Brief introduction to influenza antivirals
- Influenza antiviral resistance in:
  - Seasonal influenza viruses
    - NA inhibitors
    - Adamantanes
  - Pandemic A(H1N1) 2009 influenza viruses
    - NA inhibitors
    - Adamantanes
  - Highly pathogenic A(H5N1) viruses
    - NA inhibitors
    - Adamantanes
- Summary



# Influenza antiviral drugs



- Two major classes of influenza antiviral drugs are available

**Neuraminidase (NA) inhibitors**

**Adamantanes**



# Influenza antiviral drugs



- Two major classes of influenza antiviral drugs are available

## Neuraminidase (NA) inhibitors

Inhibit neuraminidase

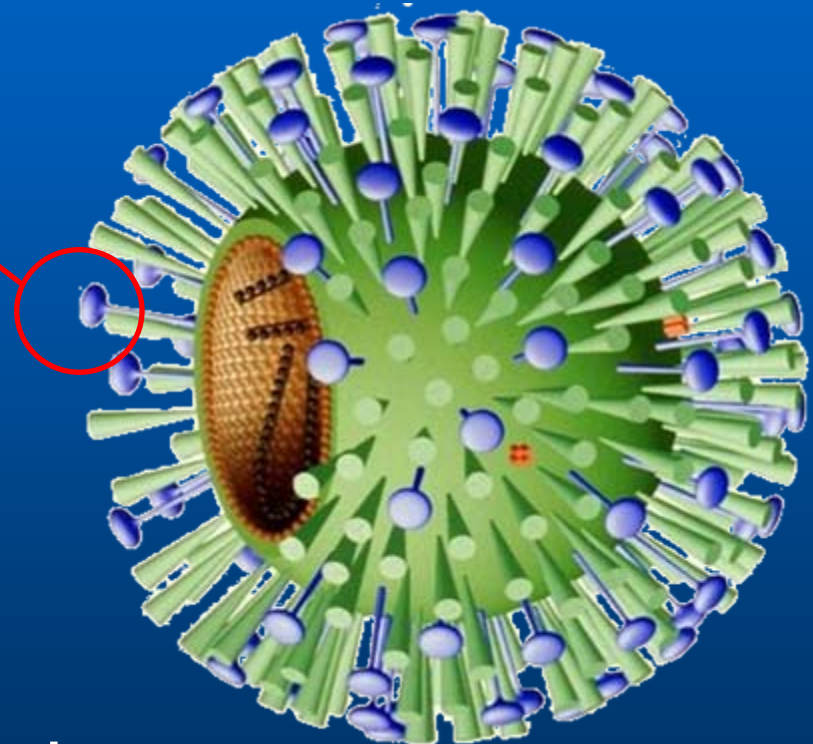
Used since 1999

Zanamivir and Oseltamivir  
(Relenza™ and Tamiflu™)

Effective for influenza A and B

Large volumes stocked for pandemic use

Prior to 2007, resistance was uncommon





# Influenza antiviral drugs



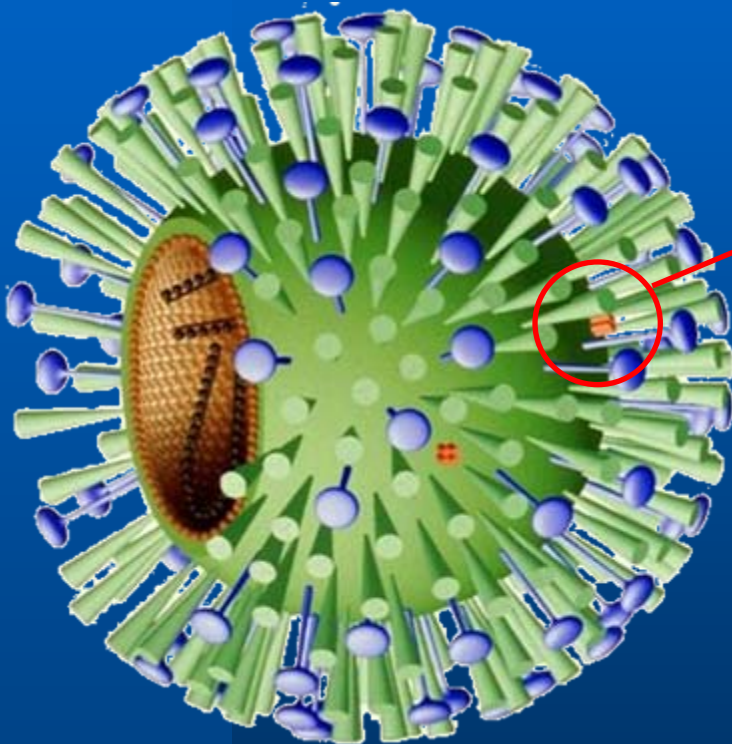
- Two major classes of influenza antiviral drugs are available

## Adamantanes

Inhibit M2 channel protein

Used since 1967

Amantadine and rimantadine  
(Symmetrel™ and Flumadine™)



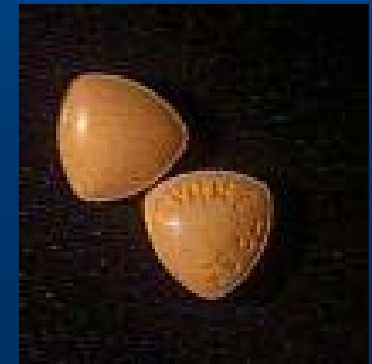
**Rimantadine**  
ANTIVIRAL DRUG

**\$149**  
BUY NOW

28x100mg



Prevent Influenza this  
Flu Season





# Influenza antiviral drugs



- Two major classes of influenza antiviral drugs are available

## Adamantanes

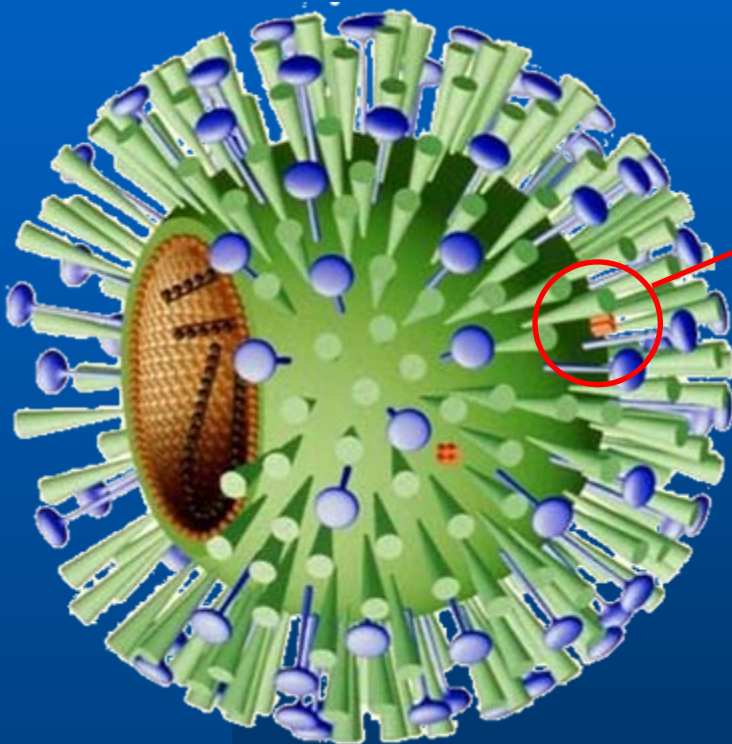
Inhibit M2 channel protein

Used since 1967

Amantadine and rimantadine  
(Symmetrel™ and Flumadine™)

Rapidly select for resistance

Effective only for influenza A





# Overview



- Brief introduction to influenza antivirals
- Influenza antiviral resistance in:
  - **Seasonal influenza viruses**
    - NA inhibitors
    - Adamantanes
  - Pandemic A(H1N1) 2009 influenza viruses
    - NA inhibitors
    - Adamantanes
  - Highly pathogenic A(H5N1) viruses
    - NA inhibitors
    - Adamantanes
- Summary



# NA inhibitor resistance



- Prior to 2007,
  - resistance to NA inhibitors was considered uncommon
  - 1-2% of adults and 5-6% of children under oseltamivir treatment shed resistant viruses
    - Reports of up to 18% in oseltamivir treated Japanese children
  - Only one case of zanamivir resistance (immuno-compromised patient) following treatment
  - Analysis of community isolates (majority untreated) detected <1% resistance
- Although A(H3N2) and influenza B viruses continue to remain sensitive to the NA inhibitors
  - High proportions of oseltamivir resistant seasonal A(H1N1) viruses have recently been detected



# Emergence of oseltamivir resistance



- European 2007/2008 influenza season
  - Oseltamivir resistant seasonal A(H1N1) isolates first detected in France, UK and Norway in late 2007
    - H274Y mutation (N2 numbering) in NA responsible for the resistance



# Emergence of oseltamivir resistance



- European 2007/2008 influenza season
  - Oseltamivir resistant seasonal A(H1N1) isolates first detected in France, UK and Norway in late 2007
    - H274Y mutation (N2 numbering) in NA responsible for the resistance

***What impact does the H274Y have on resistance?***

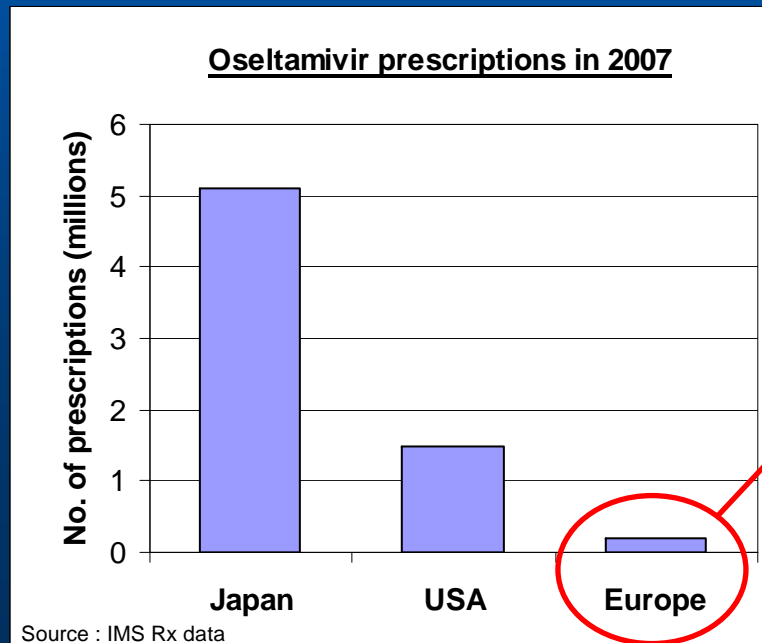
	<b>Change in IC<sub>50</sub></b>
<b>Oseltamivir</b>	<b>↑ 1500-fold</b>
<b>Zanamivir</b>	<b>No change</b>



# Emergence of oseltamivir resistance



- European 2007/2008 influenza season
  - Oseltamivir resistant seasonal A(H1N1) isolates first detected in France, UK and Norway in late 2007
    - H274Y mutation (N2 numbering) in NA responsible for the resistance
  - From untreated patients - low oseltamivir usage in Europe



Germany: 49 %  
France: 38 %  
Greece: 1 %  
Finland: 3 %  
Belgium: 7 %  
Austria: 2 %

Other countries with negligible use



# Emergence of oseltamivir resistance



- European 2007/2008 influenza season
  - Oseltamivir resistant seasonal A(H1N1) isolates first detected in France, UK and Norway in late 2007
    - H274Y mutation (N2 numbering) in NA responsible for the resistance
  - From untreated patients - low oseltamivir usage in Europe
  - Subsequent testing revealed spread of mutant throughout Europe, USA and then to the Southern Hemisphere



# Speed of H274Y spread

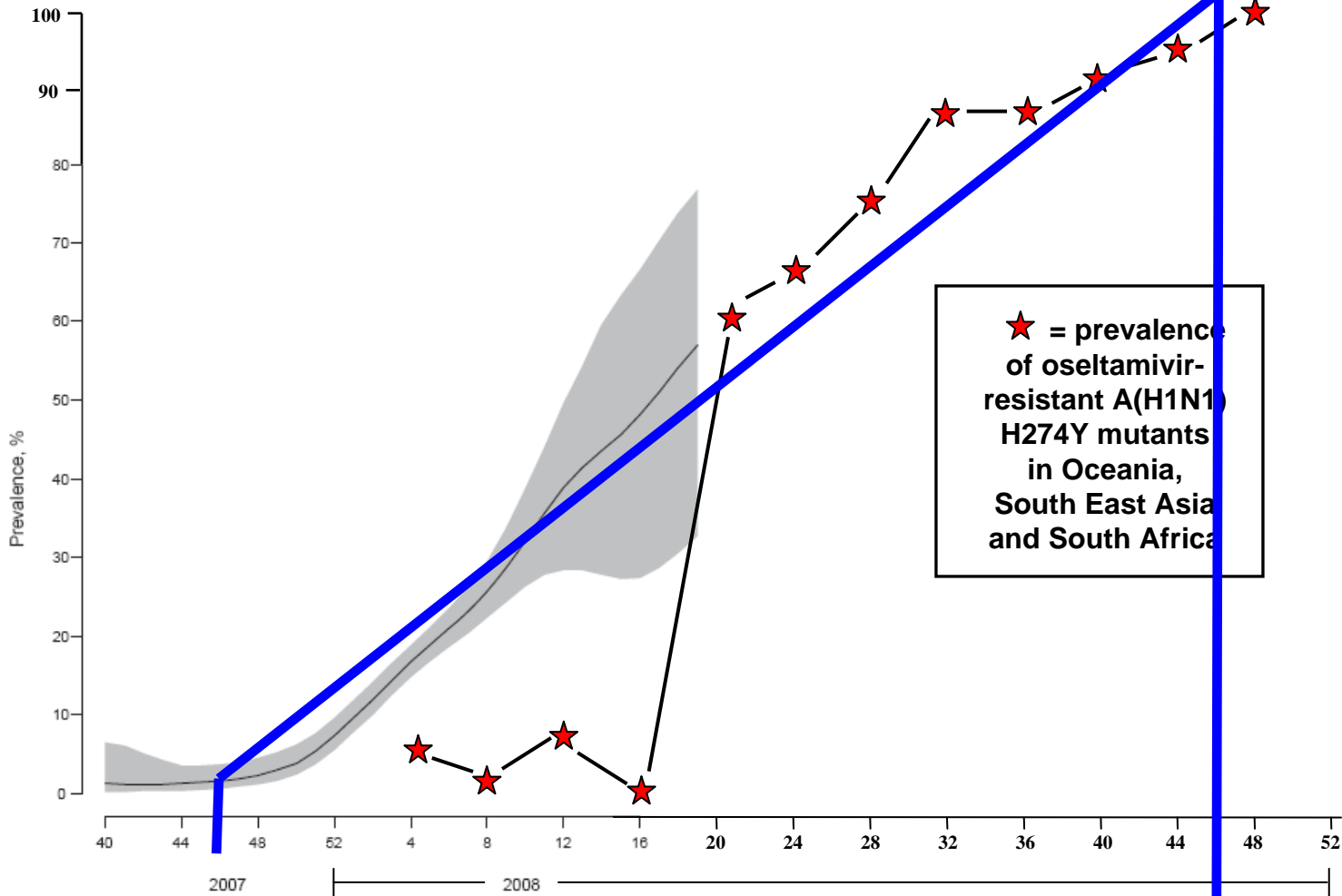


Figure 5. Weighted influenza viruses A ( region indicates the

**From 0 to 100% in one year!**



# ..and more recently...



## % Oseltamivir resistance in A(H1N1) viruses globally Oct 2008 – Jan 2009

WHO region	% of H1N1s that were Oseltamivir resistant
AMRO (Americas)	98% (n=301)
EURO (Europe)	98% (n=204)
SEARO (South East Asia)	92% (n=12)
WPRO (Western Pacific)	94% (n=822)
<b>TOTAL</b>	<b>95% (n=1362)</b>

Insufficient data available for other WHO regions



# Emergence of oseltamivir resistance



- European 2007/2008 influenza season
  - Oseltamivir resistant seasonal A(H1N1) isolates first detected in France, UK and Norway in late 2007
    - H274Y mutation in NA responsible for the resistance
  - From untreated patients - low oseltamivir usage in Europe
  - Subsequent testing revealed spread of mutant throughout Europe and then to Southern Hemisphere
  - **No difference in clinical symptoms and disease severity between resistant and sensitive seasonal A(H1N1) strains**



# Emergence of oseltamivir resistance



- European 2007/2008 influenza season
  - Oseltamivir resistant seasonal A(H1N1) isolates first detected in France, UK and Norway in late 2007
    - H274Y mutation in NA responsible for the resistance
  - From untreated patients - low oseltamivir usage in Europe
  - Subsequent testing revealed spread of mutant throughout Europe and then to Southern Hemisphere
  - No difference in clinical symptoms and disease severity between resistant and sensitive seasonal A(H1N1) strains
  - High prevalence of circulating resistant virus demonstrates that H274Y mutants are 'fit' viruses
    - Dismissing the theory that all NA inhibitor viruses have compromised fitness



# Overview



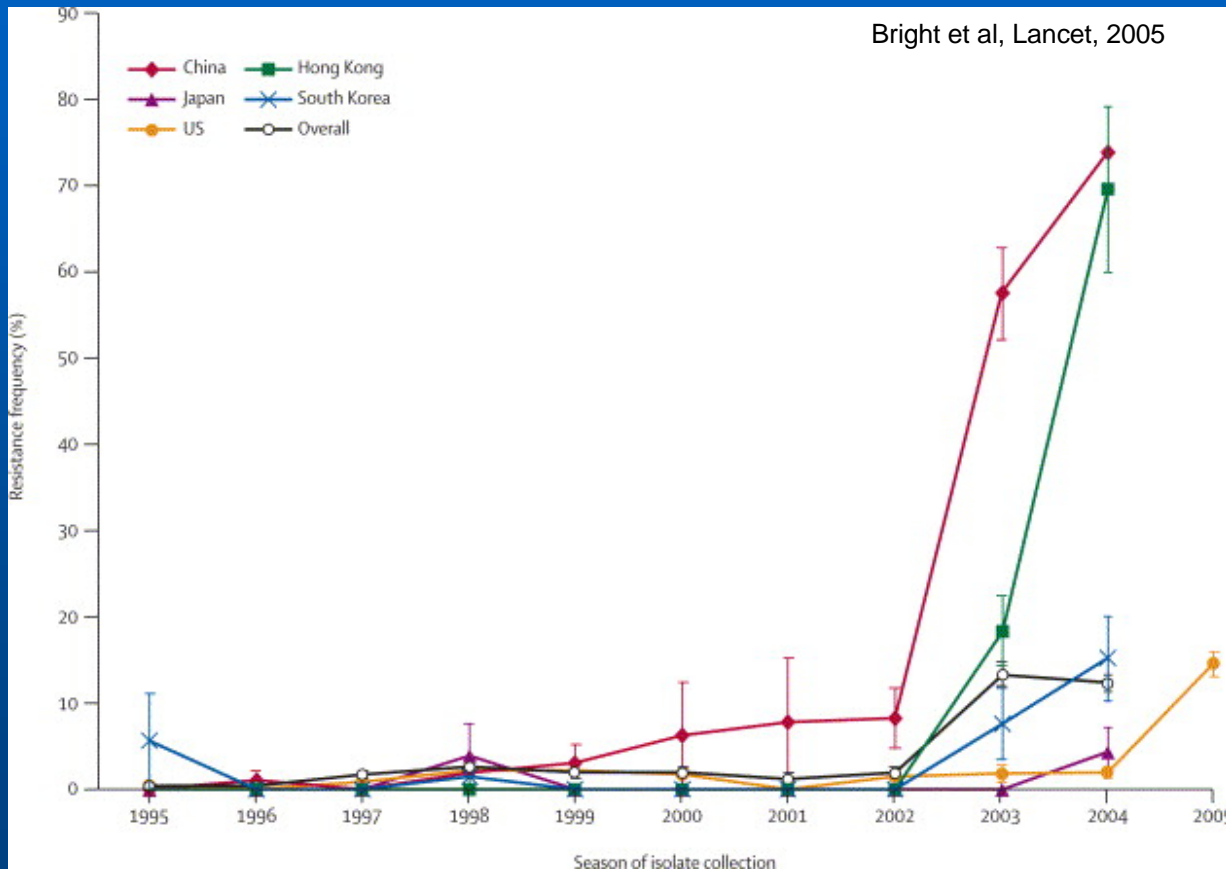
- Brief introduction to influenza antivirals
- Influenza antiviral resistance in:
  - **Seasonal influenza viruses**
    - NA inhibitors
    - **Adamantanes**
  - Pandemic A(H1N1) 2009 influenza viruses
    - NA inhibitors
    - Adamantanes
  - Highly pathogenic A(H5N1) viruses
    - NA inhibitors
    - Adamantanes
- Summary



# Emergence of adamantane resistance in A(H3N2) viruses



- Adamantane resistance in A(H3N2) viruses
  - Emerged in 2002: China and Hong Kong; 2004 in USA



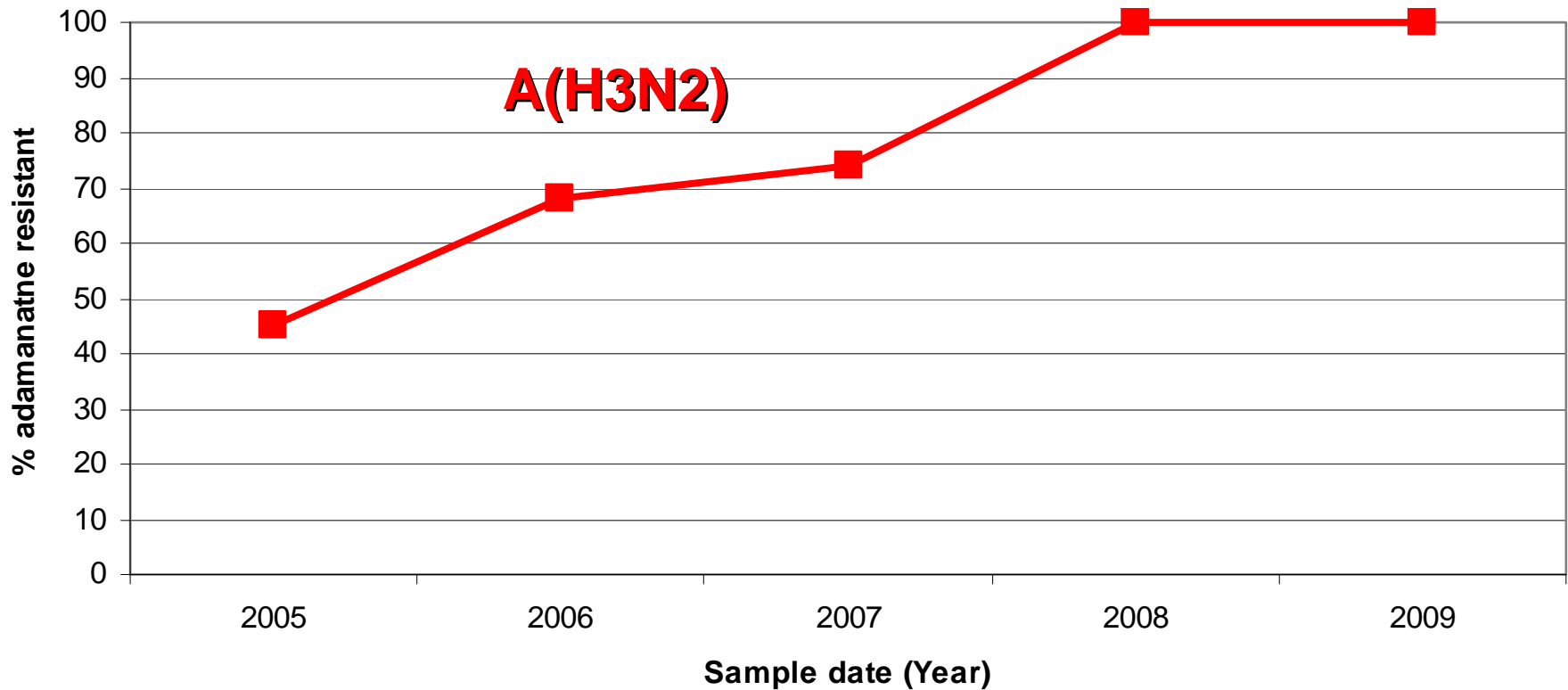
- Mostly associated with S31N mutation in M2 gene



# Adamantane resistance in seasonal viruses



- Overall – Australasia and South East Asia



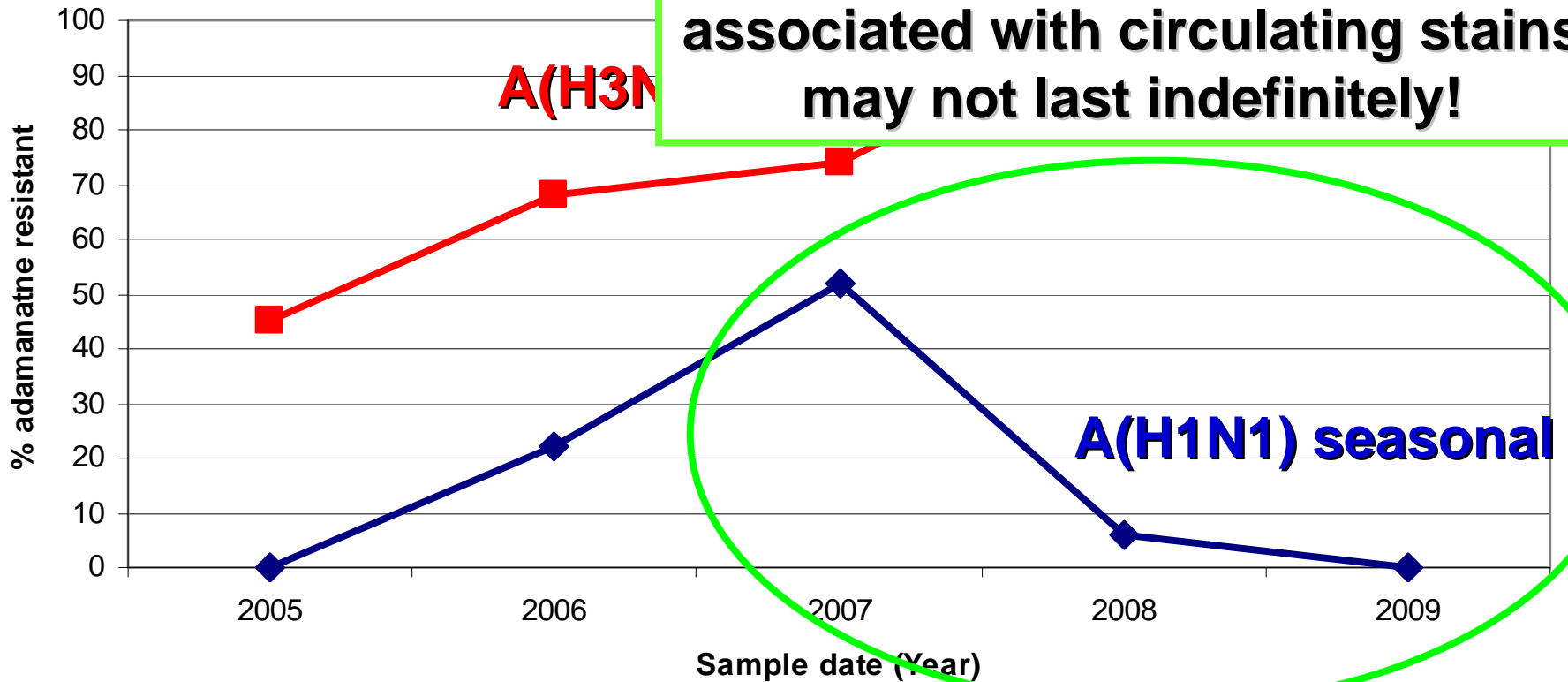


# Adamantane resistance in seasonal viruses



- Overall – Australia

Encouraging that resistance mutations that become associated with circulating stains may not last indefinitely!





# Overview



- Brief introduction to influenza antivirals
- Influenza antiviral resistance in:
  - Seasonal influenza viruses
    - NA inhibitors
    - Adamantanes
  - **Pandemic A(H1N1) 2009 influenza viruses**
    - **NA inhibitors**
    - Adamantanes
  - Highly pathogenic A(H5N1) viruses
    - NA inhibitors
    - Adamantanes
- Summary



# Pandemic H1N1 2009



Pandemic (H1N1) 2009

Status as of 31 July 2009

Countries, territories and areas with lab confirmed cases and number of deaths as reported to WHO



The boundaries and names shown on this map do not imply the endorsement of the World Health Organization on the part of the World Health Organization. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Map produced: 04 August 2009 13:00 GMT

World Health Organization  
© WHO 2009. All rights reserved



# Oseltamivir resistant cases



Denmark, Japan (4),  
Canada, China, HK

Singapore

USA (2)  
(Immuno-  
compromised  
Patients)

Hong Kong

Patients under  
oseltamivir  
prophylaxis

Already infected?  
Infection during  
prophylaxis?



# Oseltamivir resistant cases



Denmark, Japan (4),  
Canada, China, HK

Singapore

USA (2)  
(Immuno-  
compromised  
Patients)

Hong Kong

Patients under  
oseltamivir  
prophylaxis

Already infected?  
**Infection during  
prophylaxis?**



# The Denmark case



Likely that infection occurred during prophylaxis ?

Oseltamivir  
prophylaxis started

Patient developed  
symptoms

Sample PCR neg. and  
patient asymptomatic

Sample PCR  
pos. with H274Y  
mutation

Most likely infection  
occurred during prophylaxis

2<sup>nd</sup>  
June

3<sup>rd</sup>  
June

4<sup>th</sup>  
June

5<sup>th</sup>  
June

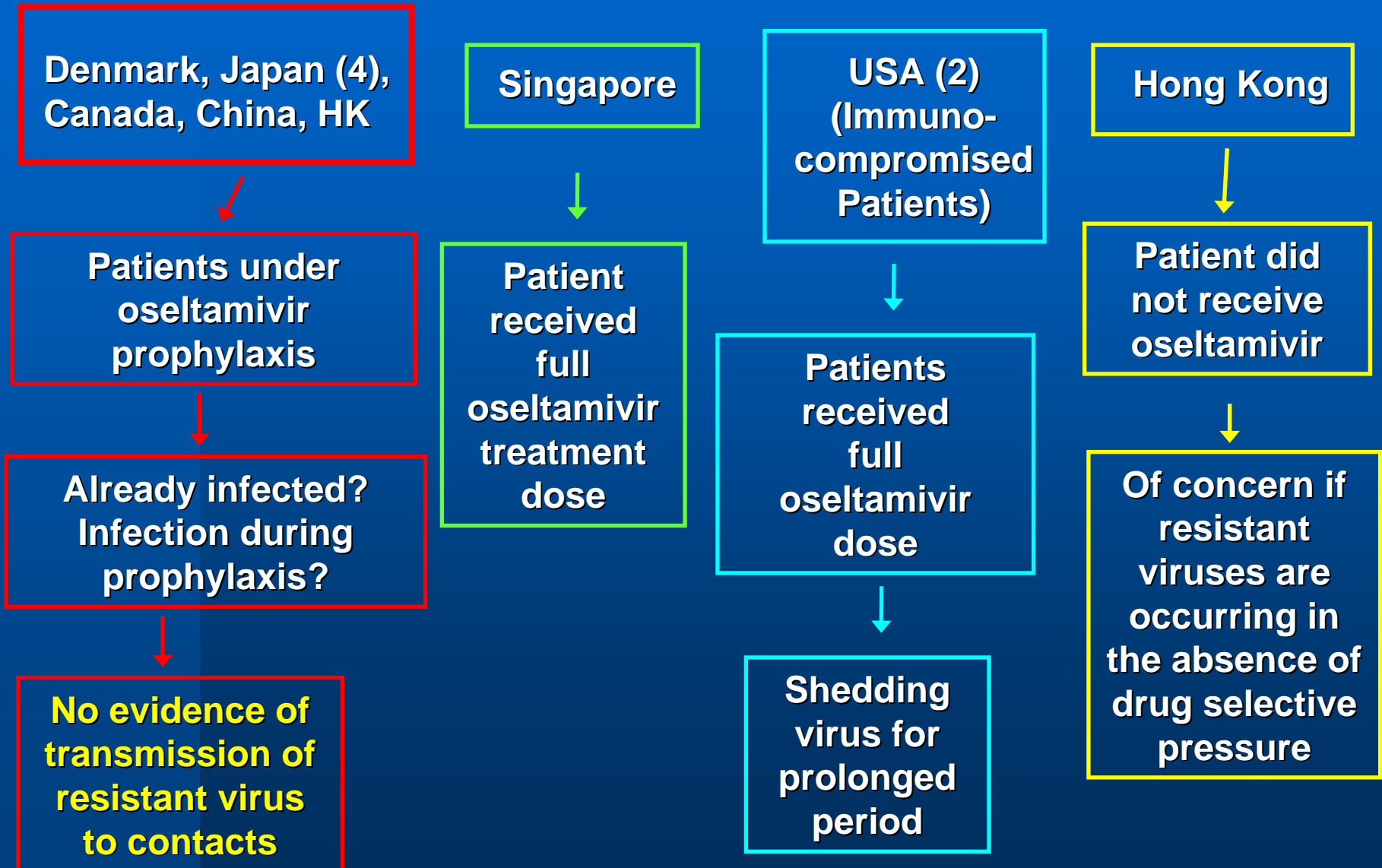
6<sup>th</sup>  
June

7<sup>th</sup>  
June

8<sup>th</sup>  
June



# Oseltamivir resistant cases





# Overview



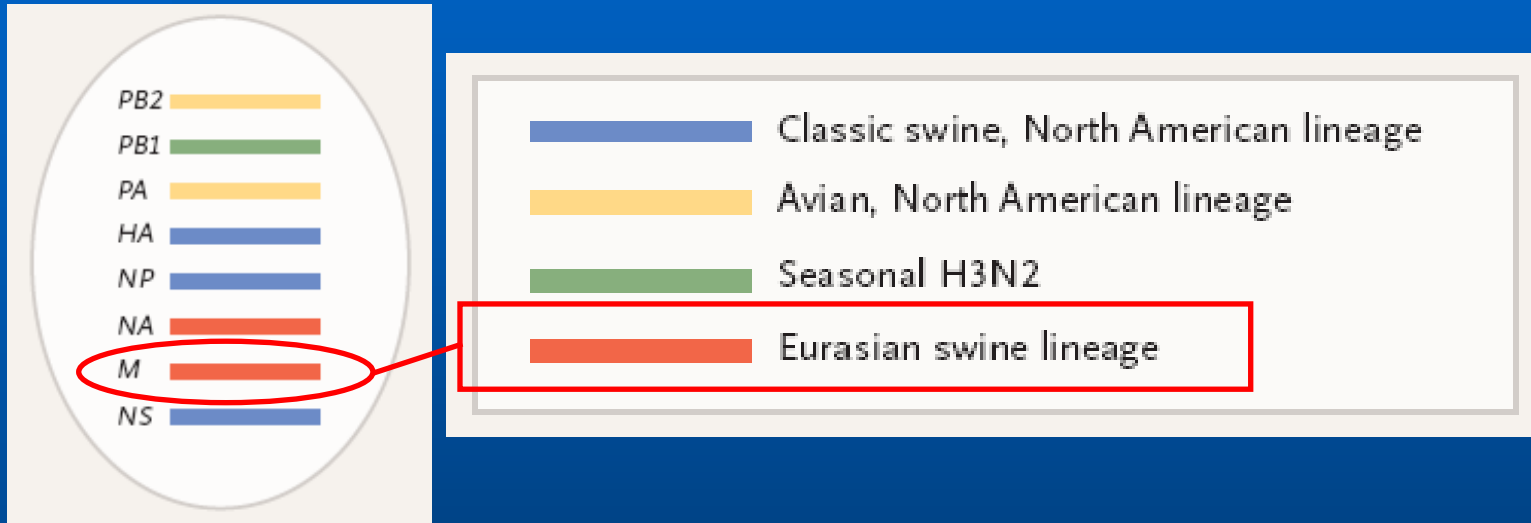
- Brief introduction to influenza antivirals
  
- Influenza antiviral resistance in:
  - Seasonal influenza viruses
    - NA inhibitors
    - Adamantanes
  - **Pandemic A(H1N1) 2009 influenza viruses**
    - NA inhibitors
    - **Adamantanes**
  - Highly pathogenic A(H5N1) viruses
    - NA inhibitors
    - Adamantanes
  
- Summary



# A(H1N1) pdm is adamantane resistant



- Pandemic A(H1N1) virus is a reassortant with genes derived from various viruses



- Matrix gene derived from Eurasian swine lineage virus
  - contains the S31N mutation that confers adamantane resistance
- All pandemic A(H1N1) viruses reported to date have continued to contain the S31N adamantane resistance mutation



# Overview



- Brief introduction to influenza antivirals
- Influenza antiviral resistance in:
  - Seasonal influenza viruses
    - NA inhibitors
    - Adamantanes
  - Pandemic A(H1N1) 2009 influenza viruses
    - NA inhibitors
    - Adamantanes
  - **Highly pathogenic A(H5N1) viruses**
    - **NA inhibitors**
    - Adamantanes
- Summary



# NA inhibitors and A(H5N1)



- NA inhibitors have been essential in the treatment of H5N1 due to the severe illness in infected patients
- Oseltamivir resistance has occurred on occasions in patients



# NA inhibitors and A(H5N1)



- NA inhibitors have been essential in the treatment of H5N1 due to the severe illness in infected patients
- Oseltamivir resistance has occurred on occasions in patients

<b>NA Mutation</b>	<b>Oseltamivir resistance</b>	<b>Under treatment?</b>	<b>Patient outcome?</b>	<b>Ref</b>



# NA inhibitors and A(H5N1)



- NA inhibitors have been essential in the treatment of H5N1 due to the severe illness in infected patients
- Oseltamivir resistance has occurred on occasions in patients

<b>NA Mutation</b>	<b>Oseltamivir resistance</b>	<b>Under treatment?</b>	<b>Patient outcome?</b>	<b>Ref</b>
<b>H274Y</b>	<b>High (1000-fold)</b>	<b>Yes</b>	<b>Fatal (2 patients)</b>	<b>de Jong et al (2005)</b>



# NA inhibitors and A(H5N1)



- NA inhibitors have been essential in the treatment of H5N1 due to the severe illness in infected patients
- Oseltamivir resistance has occurred on occasions in patients

<b>NA Mutation</b>	<b>Oseltamivir resistance</b>	<b>Under treatment?</b>	<b>Patient outcome?</b>	<b>Ref</b>
<b>H274Y</b>	<b>High (1000-fold)</b>	<b>Yes</b>	<b>Fatal (2 patients)</b>	<b>de Jong et al (2005)</b>
<b>N294S</b>	<b>Low (12-15 fold)</b>	<b>No</b>	<b>Fatal (2 patients)</b>	<b>Saad et al (2007)</b>



# NA inhibitors and A(H5N1)



- NA inhibitors have been essential in the treatment of H5N1 due to the severe illness in infected patients
- Oseltamivir resistance has occurred on occasions in patients

<b>NA Mutation</b>	<b>Oseltamivir resistance</b>	<b>Under treatment?</b>	<b>Patient outcome?</b>	<b>Ref</b>
<b>H274Y</b>	<b>High (1000-fold)</b>	<b>Yes</b>	<b>Fatal (2 patients)</b>	<b>de Jong et al (2005)</b>
<b>N294S</b>	<b>Low (12-15 fold)</b>	<b>No</b>	<b>Fatal (2 patients)</b>	<b>Saad et al (2007)</b>
<b>H274Y N294S (mixed popn)</b>	<b>High / Low</b>	<b>Yes (prophylaxis)</b>	<b>Recovered (after t'ment dose)</b>	<b>Le et al (2005)</b>

- Although cases of resistance have been detected following treatment majority of avian A(H5N1) strains remain sensitive to NA inhibitors



# Overview



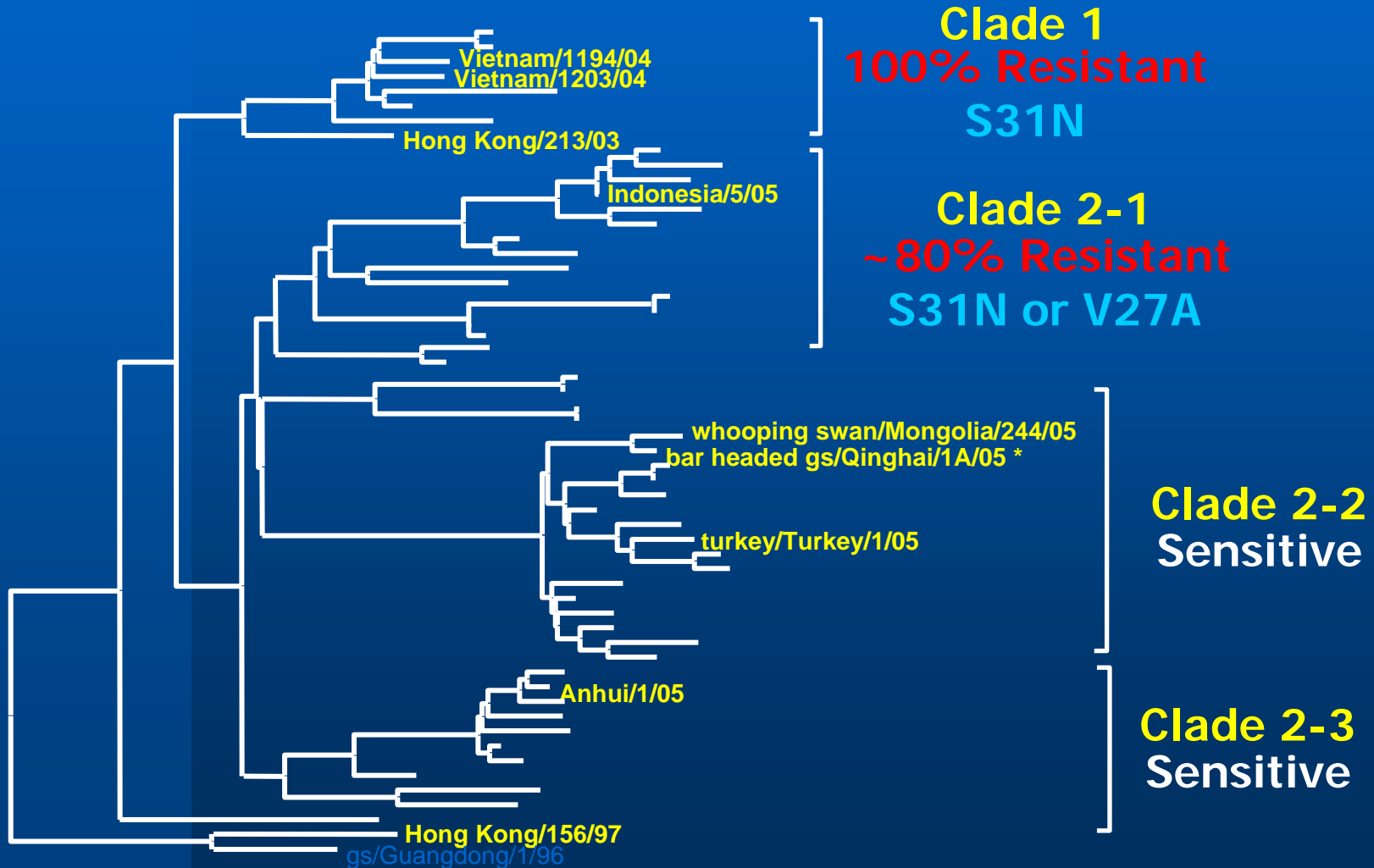
- Brief introduction to influenza antivirals
- Influenza antiviral resistance in:
  - Seasonal influenza viruses
    - NA inhibitors
    - Adamantanes
  - Pandemic A(H1N1) 2009 influenza viruses
    - NA inhibitors
    - Adamantanes
  - **Highly pathogenic A(H5N1) viruses**
    - NA inhibitors
    - **Adamantanes**
- Summary



# Resistance of H5N1 Viruses to Adamantanes



- Variable frequency of adamantane resistance among A(H5N1) viruses





# Summary



- Virtually 100% of seasonal A(H1N1) viruses are oseltamivir resistant
- However the number of seasonal A(H1N1) viruses circulating has decreased significantly since the emergence of pandemic A(H1N1)
  - Will the seasonal A(H1N1) continue to circulate?
- Large volumes of NA inhibitors have been used to treat pandemic A(H1N1) infections
  - Encouragingly only a few cases of resistance have been detected with no evidence of further transmission
  - A(H1N1) pandemic viruses are adamantane resistant
- Continued resistance monitoring of pandemic A(H1N1) viruses is essential, particularly in patients under treatment and their contacts



# Summary



- A(H5N1) viruses are susceptible to NA inhibitors
- Further work necessary to optimise treatment to:
  - Improve patient outcome
  - Reduce the generation of resistance
- Current strategies to address these issues include:
  - Longer treatment duration
  - Higher dose
  - Parenteral delivery
  - Combination therapies



# Acknowledgements



## Submission of influenza isolates and specimens

Thank you to all WHO National Influenza Centres and other submitting laboratories for the provision of influenza isolates and epidemiological data

## WHO CC Influenza, Melb staff

Prof Anne Kelso and Dr Ian Barr

Jo Ernest – NA inhibition assays

Dr Yi-Mo Deng – Pyrosequencing (rapid confirmation of mutations)

Pina Iannello and Naomi Komadina – Conventional sequencing

Robert Shaw and Helen Sjogren – Culture of viruses

The WHO Collaborating Centre for Reference and Research on Influenza, Melbourne is supported by the Australian Government Department of Health and Ageing



***Thank You !***