

Antiviral resistance surveillance: a global program

2nd Asia-Pacific Influenza Summit,
10-11 June 2015, Melia Hotel, Hanoi, Viet Nam

Aeron C. Hurt



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and Research on Influenza, VIDRL, Melbourne

WHO Collaborating Centre
for Reference and
Research on Influenza
VIDRL



Overview

- Introduction to neuraminidase inhibitor resistance and why 'viral fitness' matters
- WHO Global Influenza Program and influenza antiviral resistance surveillance data
- What we might expect in the coming years



The NA inhibitors

Oseltamivir

- Oral, IV(?)
- Global



Zanamivir

- Inhaled, IV(?)
- Global



Peramivir

- IV
- Japan, S.Korea, China, US



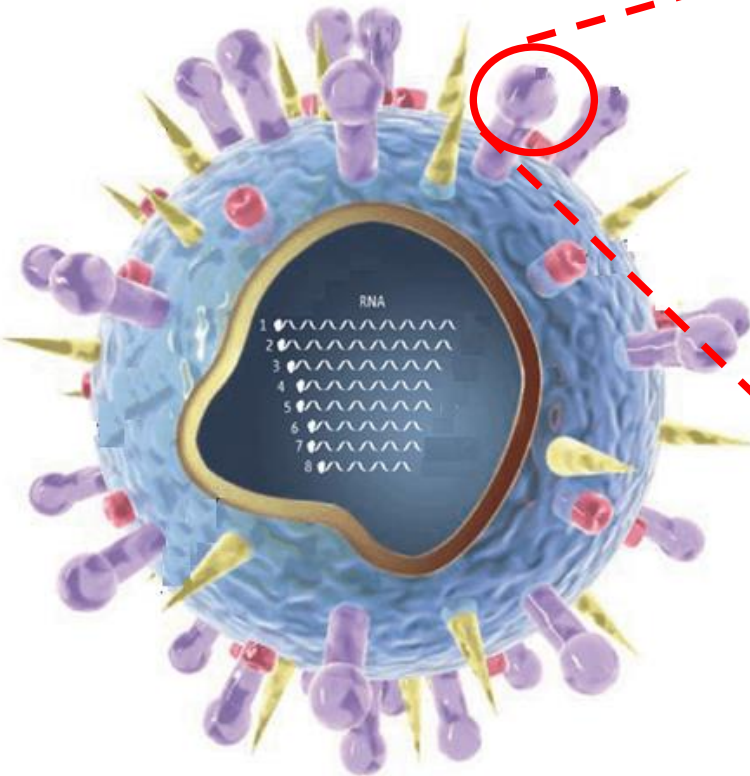
Laninamivir

- Inhaled (single)
- Japan



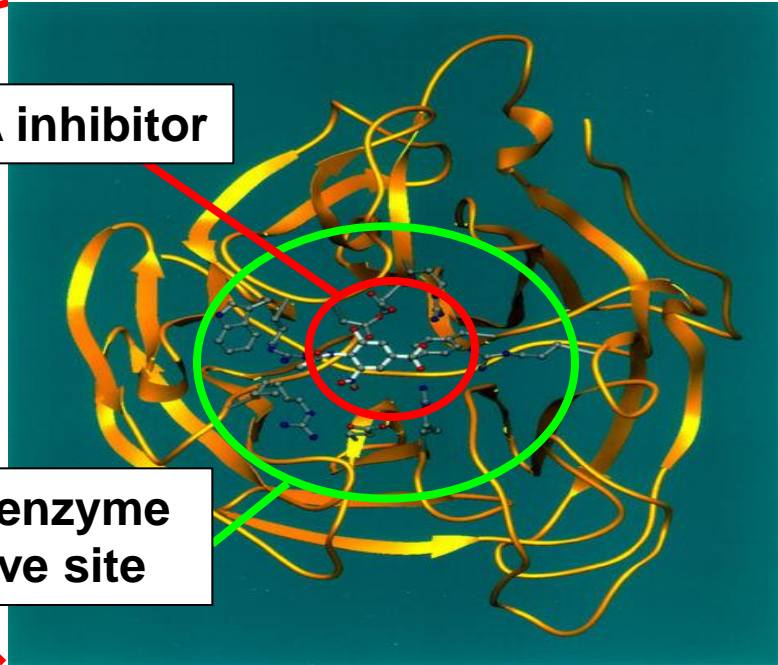
NA inhibitor binding

Top view of NA



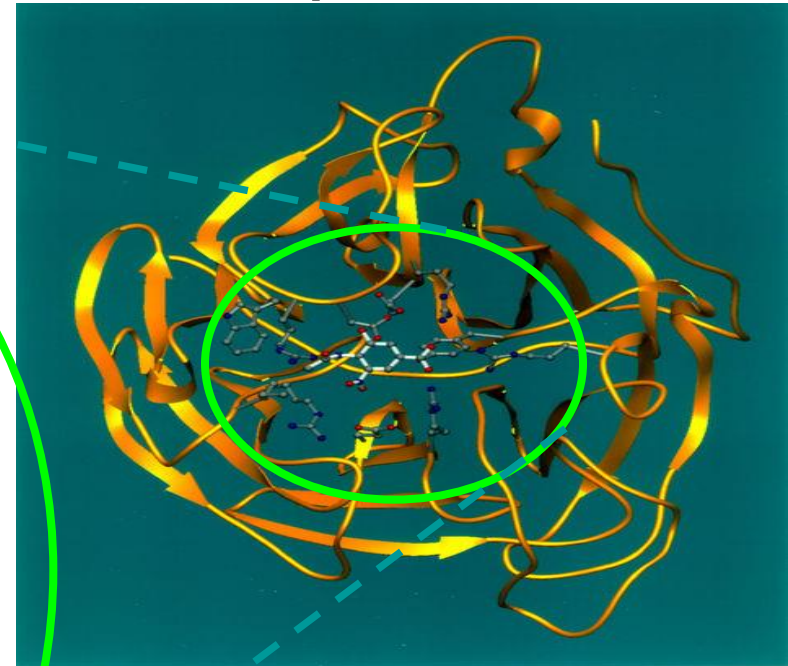
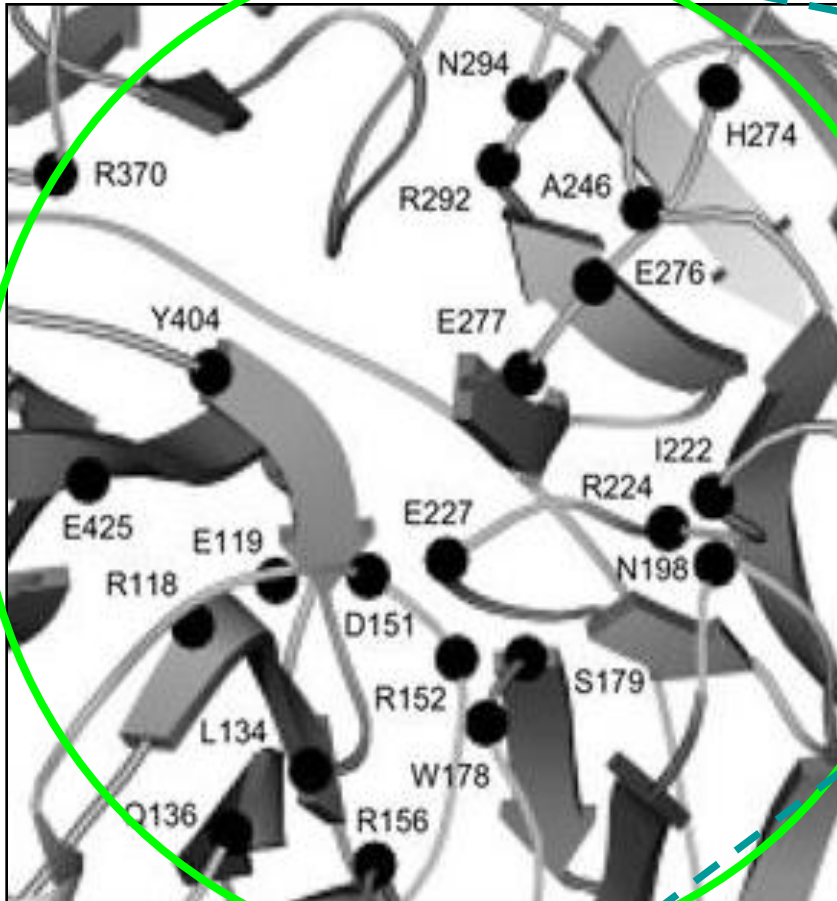
NA inhibitor

NA enzyme active site



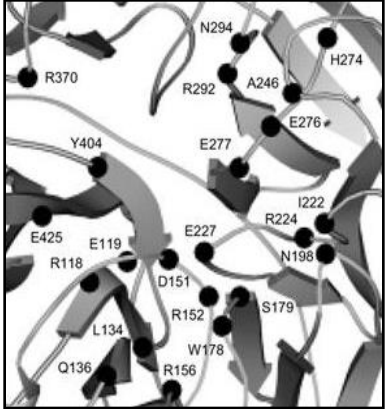
NA enzymatic site

Top view of NA

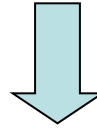


Amino acids located within and around the NA enzyme active site

NA enzymatic site

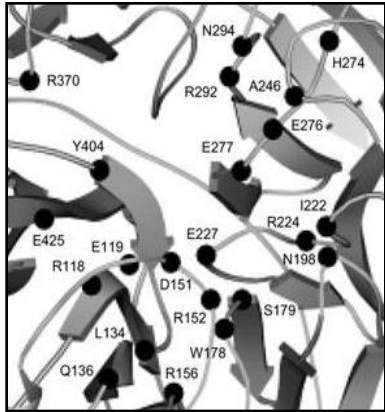


Mutations in the enzymatic site

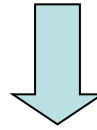


**Prevent NA inhibitor binding
(resistance)**

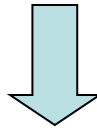
NA enzymatic site



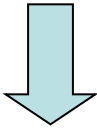
Mutations in the enzymatic site



**Prevent NA inhibitor binding
(resistance)**



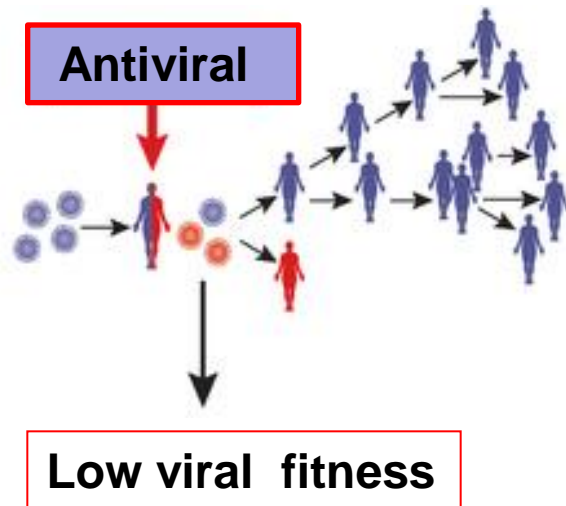
**Potential impact on the normal
function of the enzyme**



**Effect on the replication and
transmission of the resistant virus
(viral fitness)**

The effect on viral fitness can depend on 1) the particular substitution that occurs, and 2) whether other permissive mutations exist within the virus

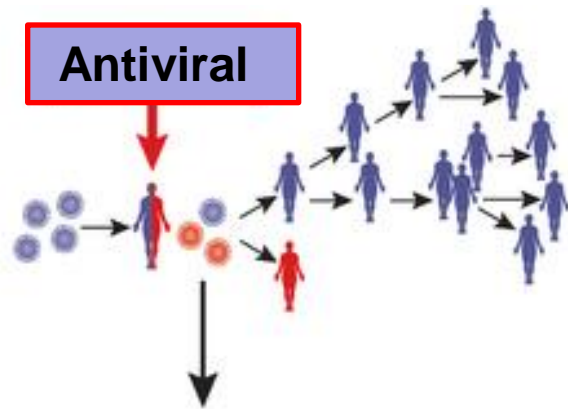
Is a resistant virus likely to spread to the community from a treated patient?



Little or no spread to the community

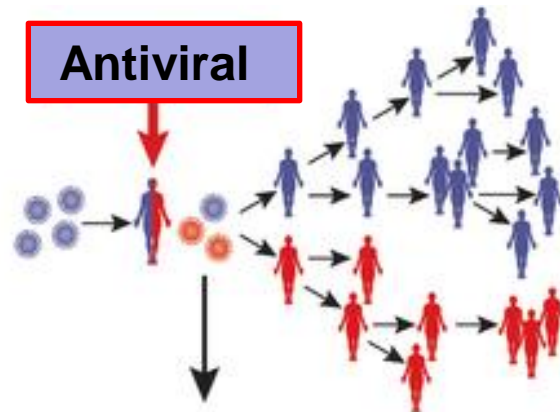
The antiviral remains appropriate for treating subsequent cases

Is a resistant virus likely to spread to the community from a treated patient?



Little or no spread to the community

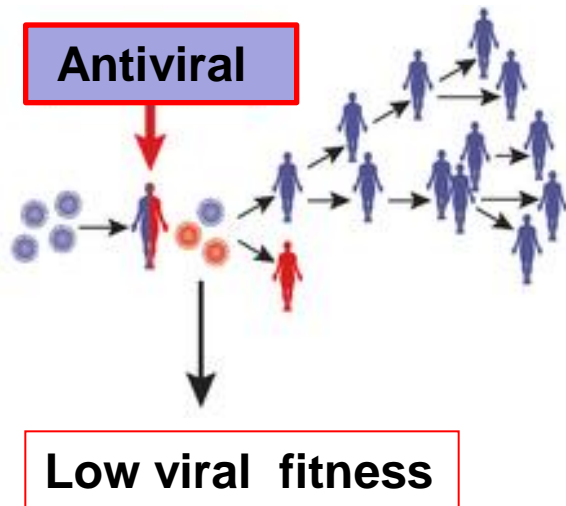
The antiviral remains appropriate for treating subsequent cases



Cluster of resistant cases in the community

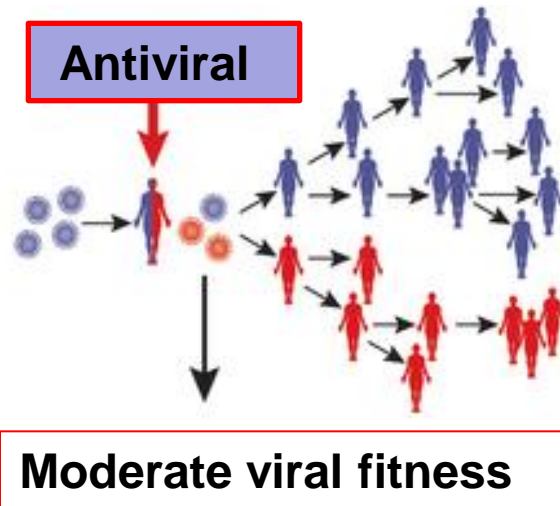
Alternative antivirals are required for treating the cluster of cases

Is a resistant virus likely to spread to the community from a treated patient?



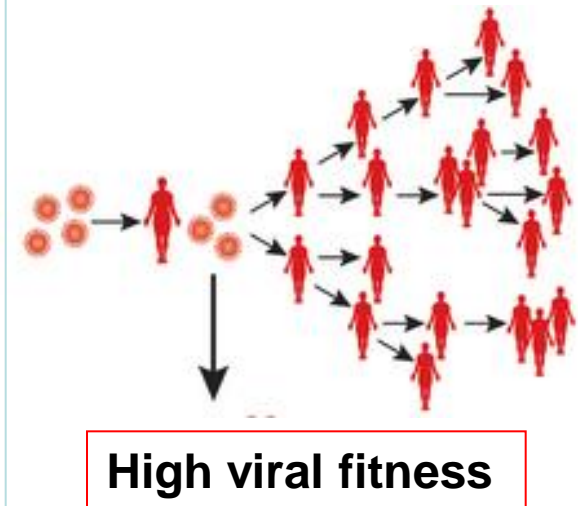
Little or no spread to the community

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Cluster of resistant cases in the community

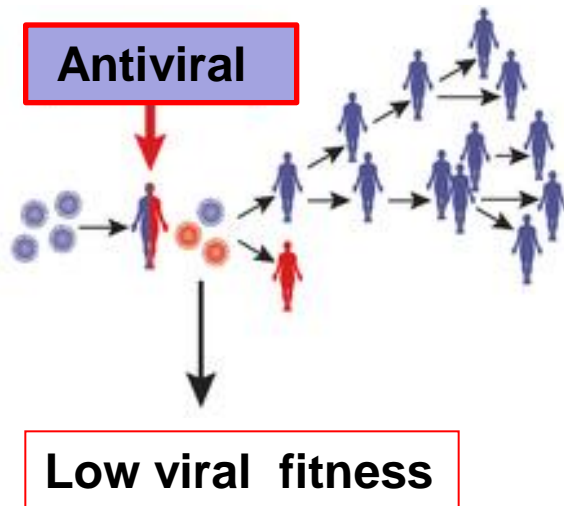
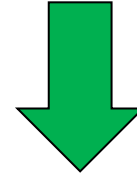
Alternative antivirals are required for treating the cluster of cases



Widespread resistance

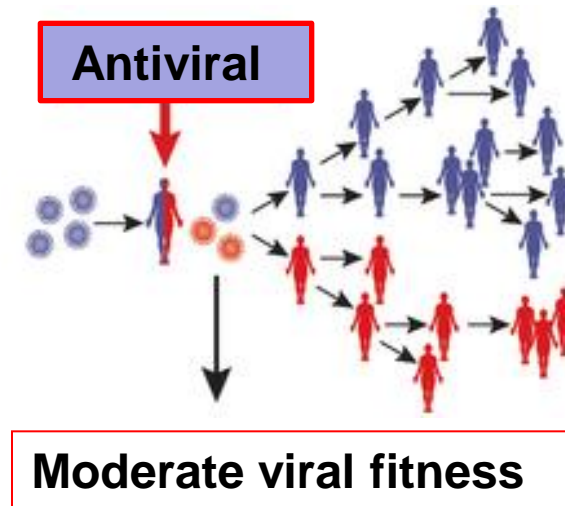
The antiviral is no longer suitable for treatment of all cases in the community

Adamantane resistant viruses



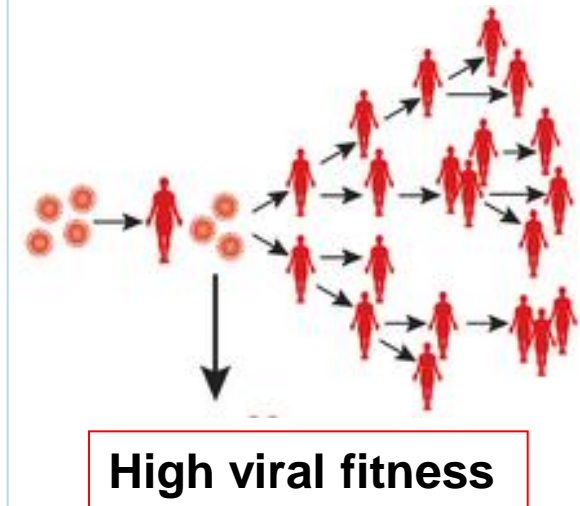
Little or no spread to the community

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Cluster of resistant cases in the community

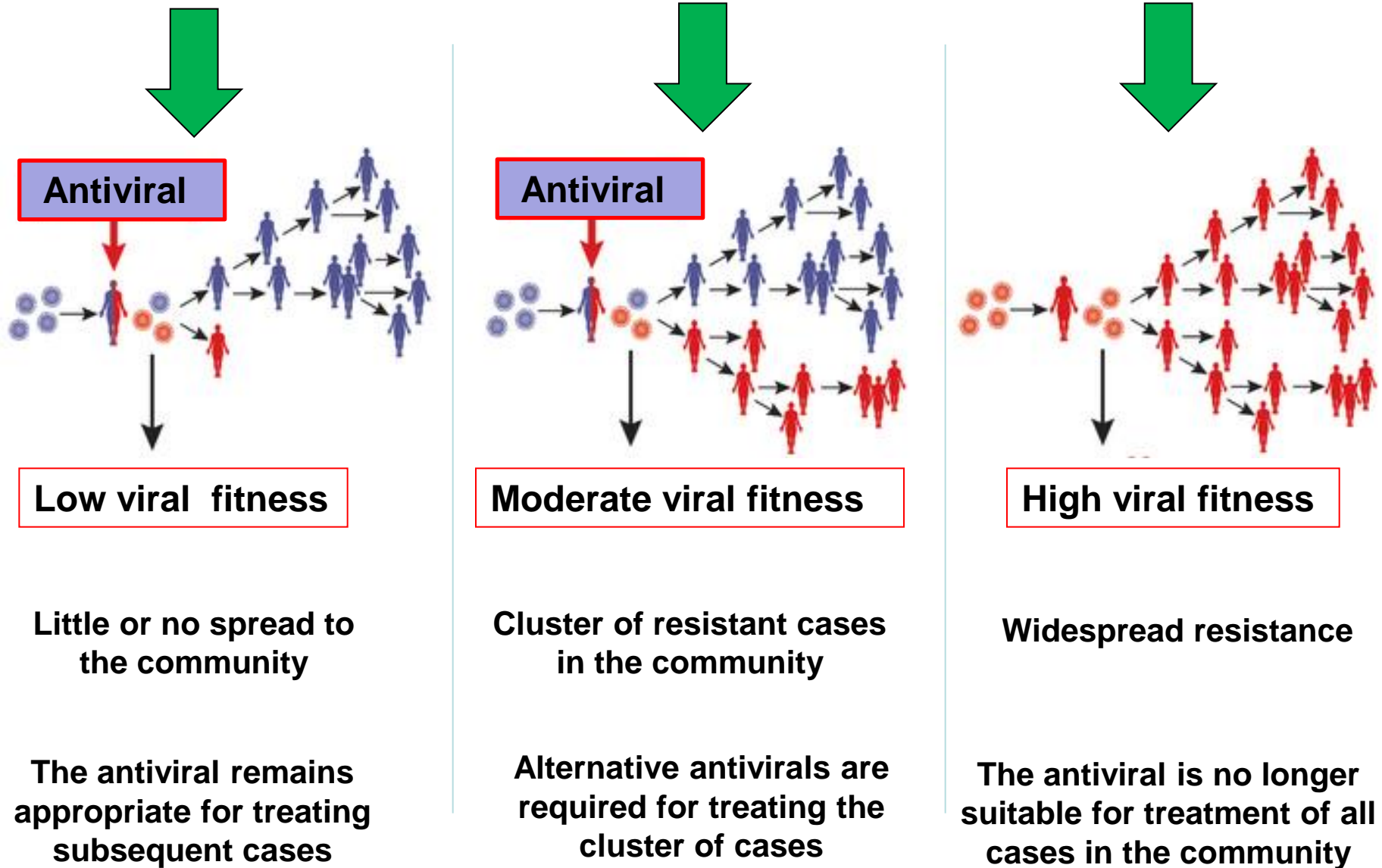
Alternative antivirals are required for treating the cluster of cases



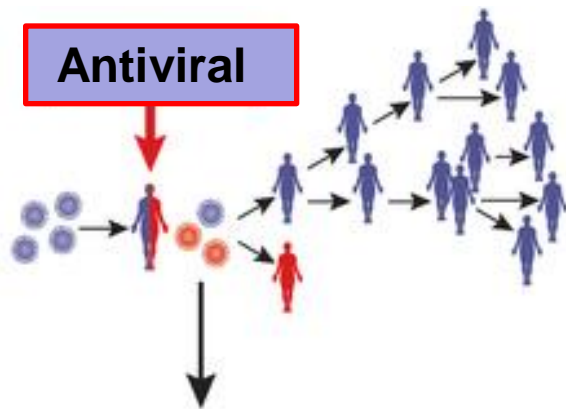
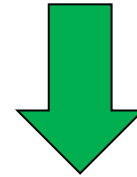
Widespread resistance

The antiviral is no longer suitable for treatment of all cases in the community

NA inhibitor resistant viruses



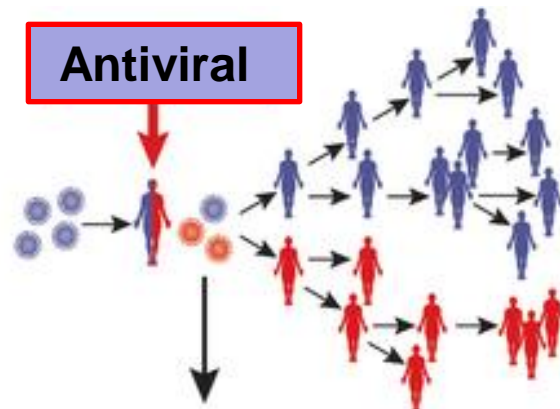
**2008 seasH1N1
H275Y variants**



Low viral fitness

**Little or no spread to
the community**

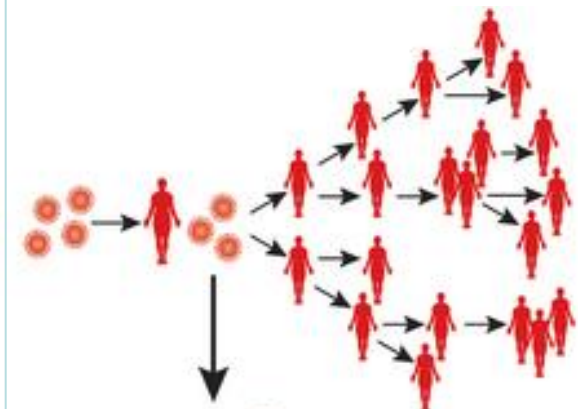
**The antiviral remains
appropriate for treating
subsequent cases**



Moderate viral fitness

**Cluster of resistant cases
in the community**

**Alternative antivirals are
required for treating the
cluster of cases**

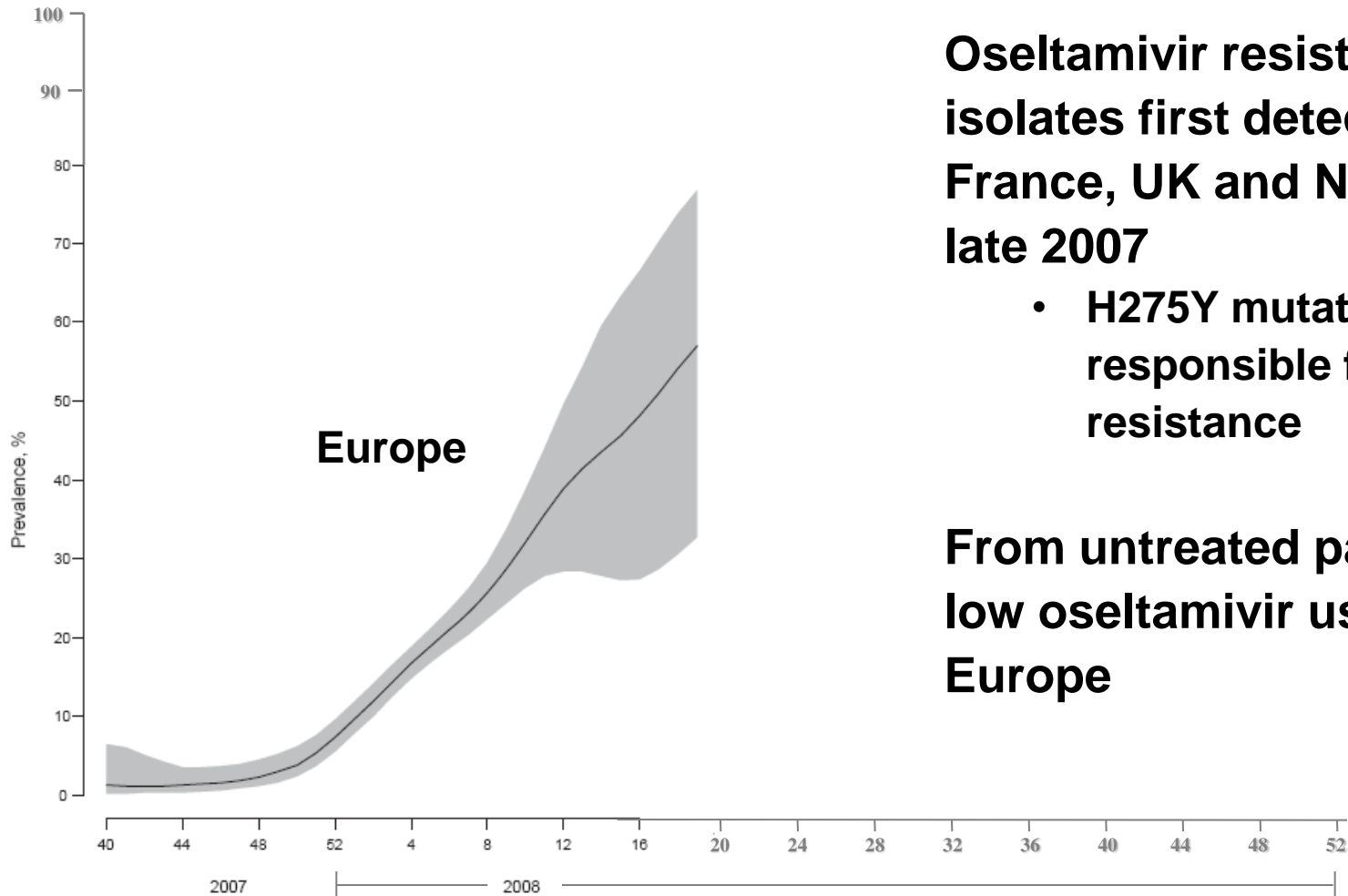


High viral fitness

Widespread resistance

**The antiviral is no longer
suitable for treatment of all
cases in the community**

Emergence of H275Y global spread in 2007/08



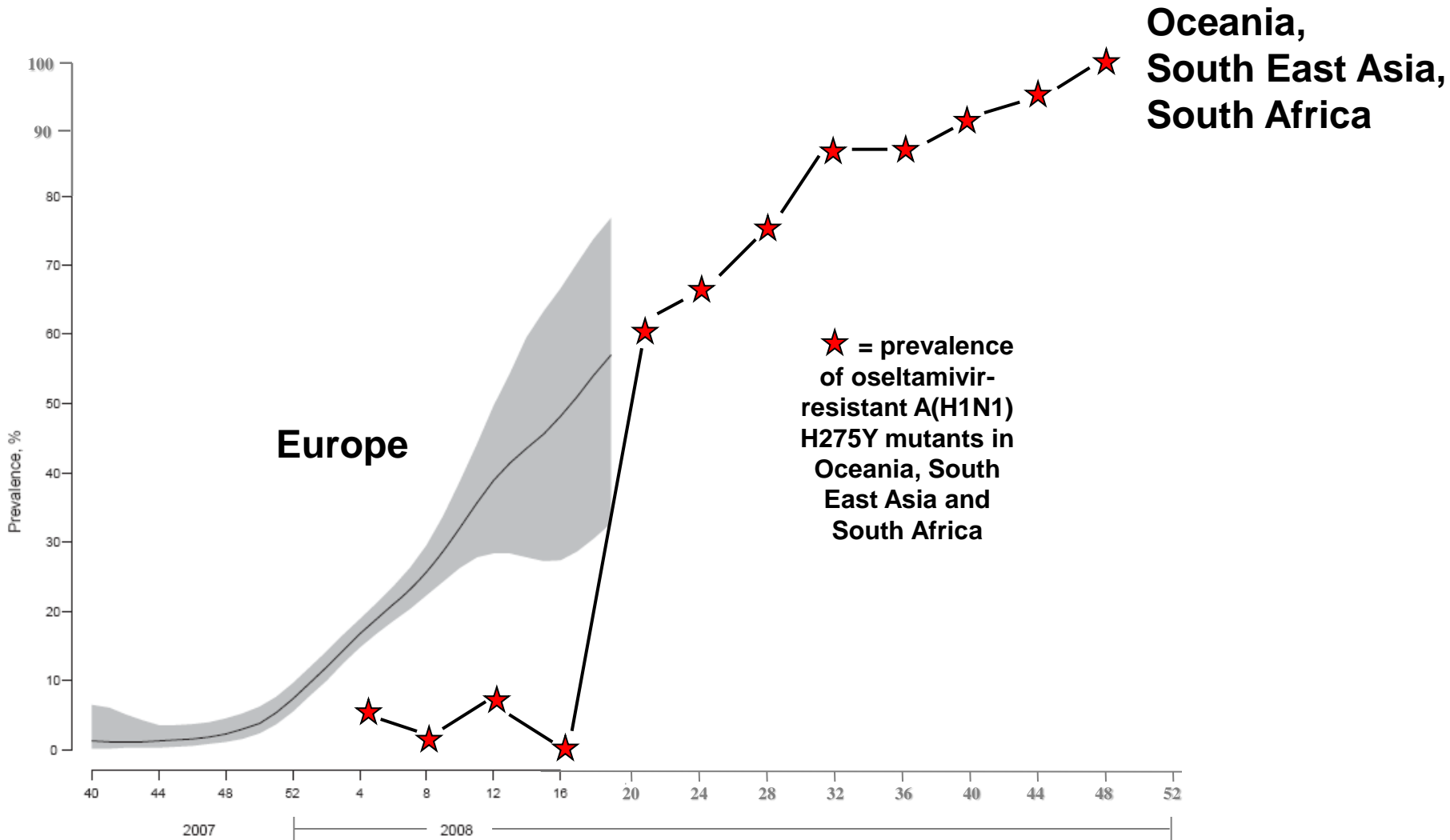
Oseltamivir resistant isolates first detected in France, UK and Norway in late 2007

- **H275Y mutation in NA responsible for the resistance**

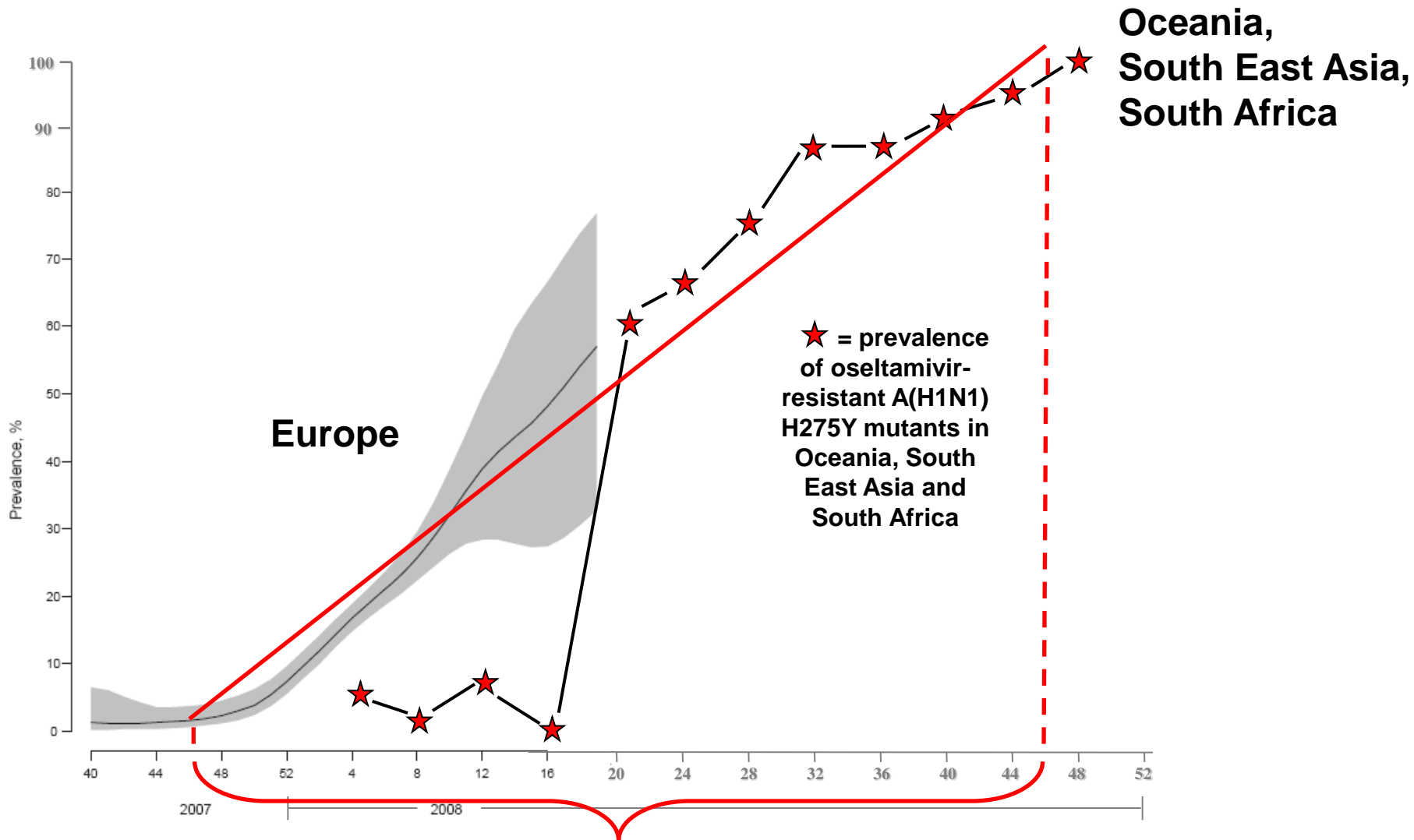
From untreated patients - low oseltamivir usage in Europe

Figure 5. Weighted average prevalence of oseltamivir-resistant influenza viruses A (H1N1), Europe, winter 2007–08. The light gray region indicates the 95% confidence interval. **Meijer et. al., EID, 15 (4), 2009**

Emergence of H275Y global spread in 2007/08



Emergence of H275Y global spread in 2007/08

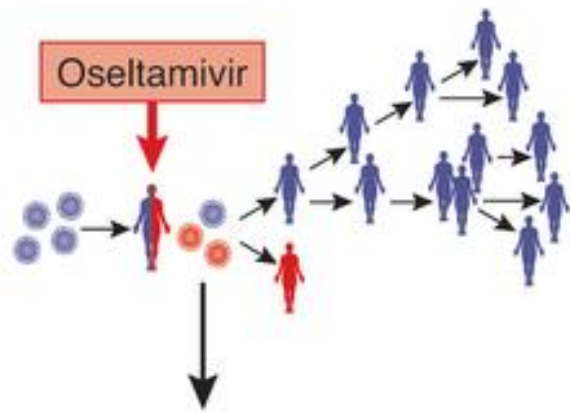
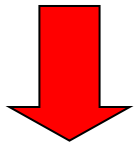


Globally from 0 to 100% in one year!

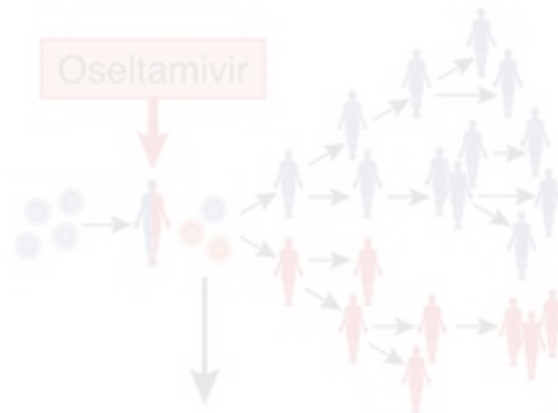
So what was it that allowed these viruses to still be 'fit' and spread ?

- Early studies of seasonal A(H1N1) H275Y virus demonstrated that the resistance mutation decreased the ability of the virus to transmit and replicate

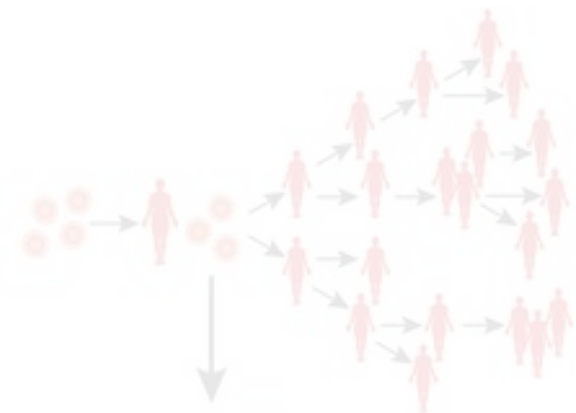
Pre 2007 H1N1 H275Y viruses



Low viral fitness



Moderate viral fitness



High viral fitness

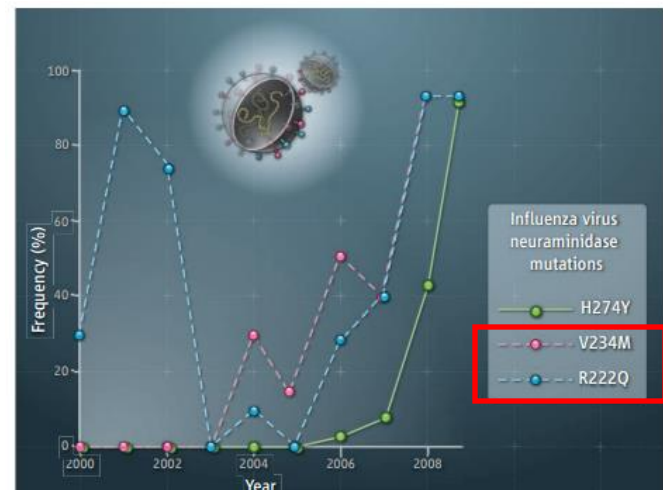
So what was it that allowed these viruses to still be 'fit' and spread ?

- Early studies of seasonal A(H1N1) H275Y virus demonstrated that the resistance mutation decreased the ability of the virus to transmit and replicate
- But analysis of the strains that spread globally in 2008 showed that 'permissive' NA mutations that were present in the virus before the H275Y meant the virus could still replicate and transmit with the H275Y mutation

Permissive Secondary Mutations Enable the Evolution of Influenza Oseltamivir Resistance

Jesse D. Bloom, Lizhi Ian Gong, David Baltimore*

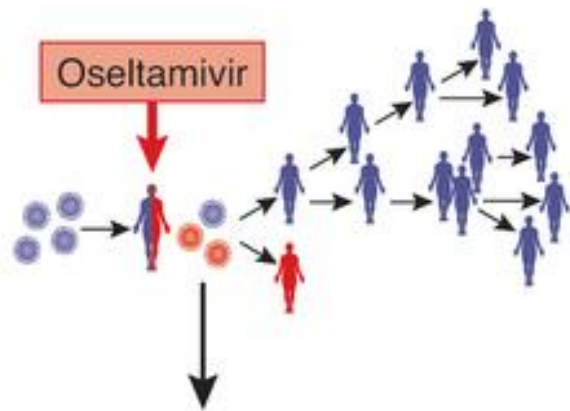
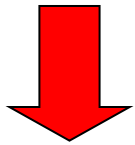
4 JUNE 2010 VOL 328 SCIENCE www.sciencemag.org



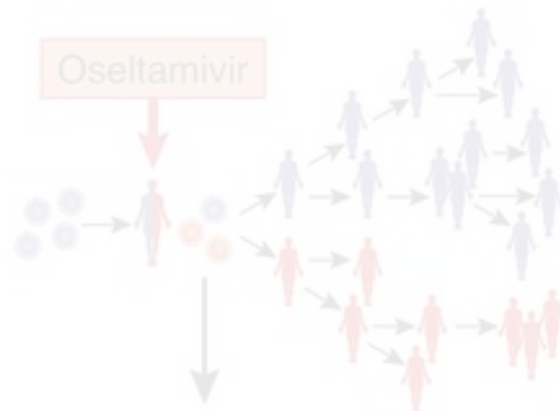
Holmes, E. Science. Vol 328

The presence of these 'permissive mutations' altered the fitness of this virus

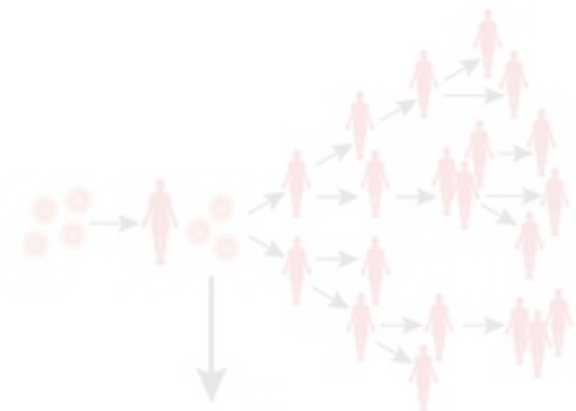
Pre 2007 H1N1
H275Y viruses



Low viral fitness

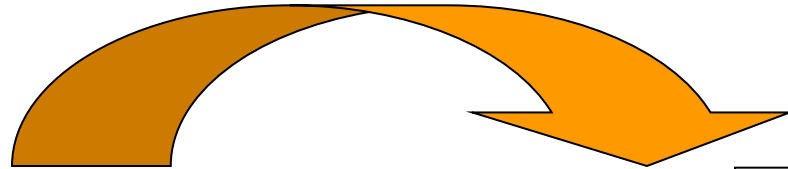


Moderate viral fitness

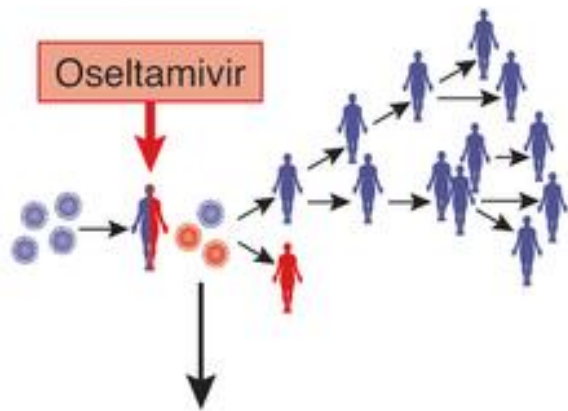
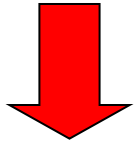


High viral fitness

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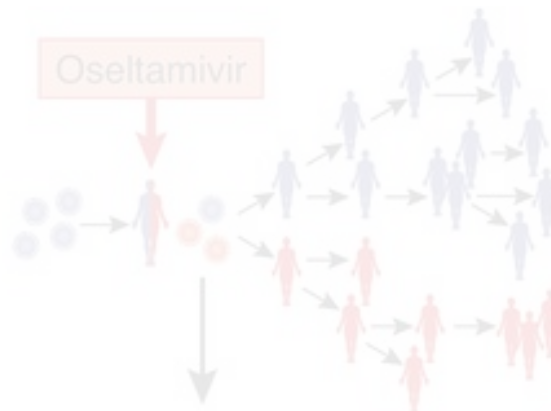
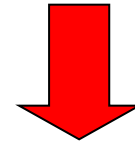


**Pre 2007 H1N1
H275Y viruses**

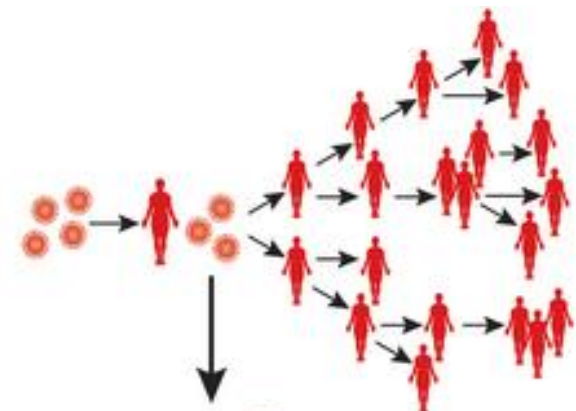


Low viral fitness

**2007/2008 H1N1
H275Y viruses**

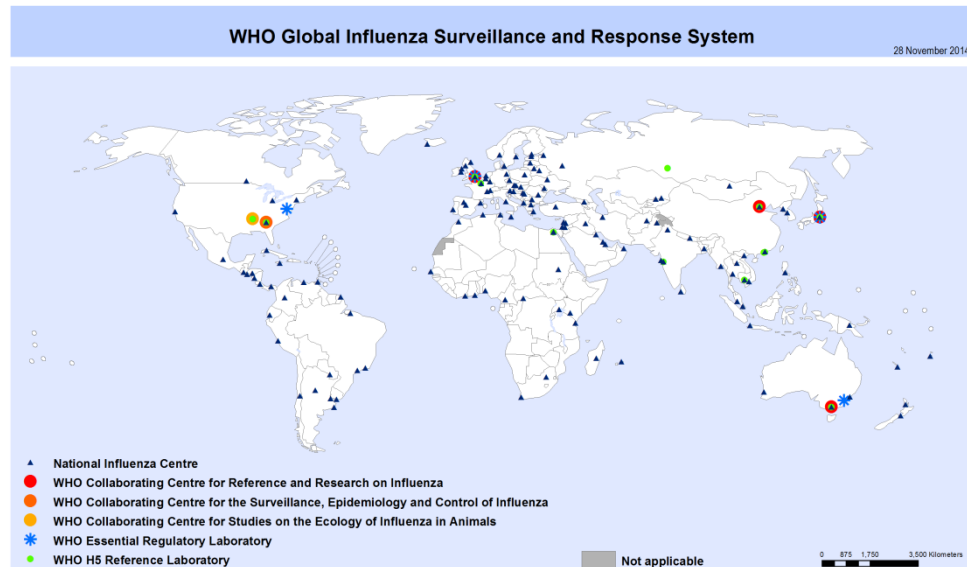


Moderate viral fitness



High viral fitness

Global surveillance for NAI susceptibility via the WHO Global Influenza Surveillance and Response System



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: Global Influenza Surveillance and Response System (GISRS), WHO
Map Production: WHO GISRS Team
World Health Organization



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London

Beijing

Tokyo



2014

Atlanta



Melbourne



- ▲ National Influenza Centre
- WHO Collaborating Centre for Reference and Research on Influenza
- WHO Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza
- WHO Collaborating Centre for Studies on the Ecology of Influenza in Animals
- * WHO Essential Regulatory Laboratory
- WHO H5 Reference Laboratory

Not applicable

WHO Collaborating Centres receive viruses from National Influenza Centres and conduct NAI susceptibility testing

WHO CCs have begun reporting combined NAI susceptibility data

Antiviral Research 110 (2014) 31–41



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Antiviral Research

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Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2012–2013



Adam Meijer^{a,*}, Helena Rebelo-de-Andrade^{b,c,1}, Vanessa Correia^{b,c}, Terry Besselaar^d, Renu Drager-Dayal^d, Alicia Fry^{e,1}, Vicky Gregory^f, Larisa Gubareva^{e,1}, Tsutomu Kageyama^{g,1}, Angie Lackenby^{h,1}, Janice Lo^{i,1}, Takato Odagiri^{g,1}, Dmitriy Pereyaslov^j, Marilda M. Siqueira^{k,1}, Emi Takashita^{g,1}, Masato Tashiro^{g,1}, Dayan Wang^{l,1}, Sun Wong^{i,1}, Wenqing Zhang^d, Rod S. Daniels^{f,1}, Aeron C. Hurt^{m,n,1}

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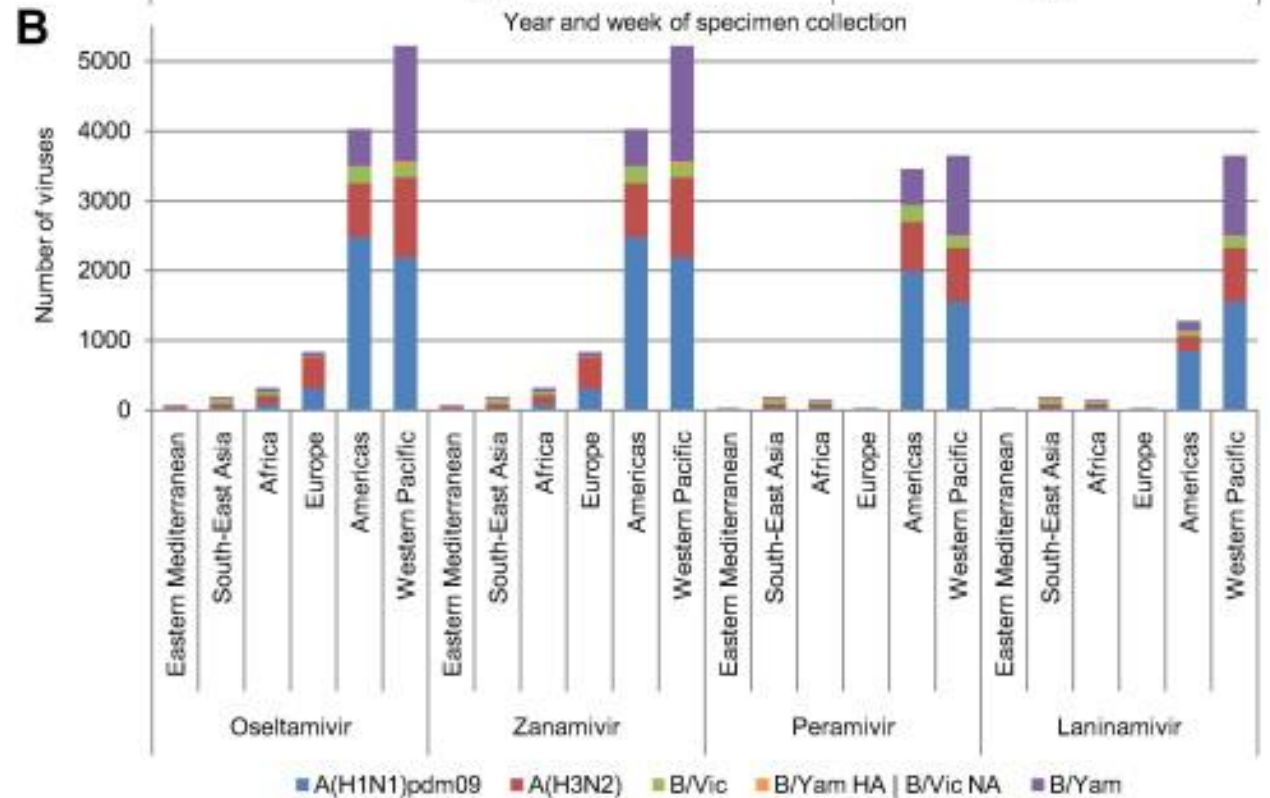
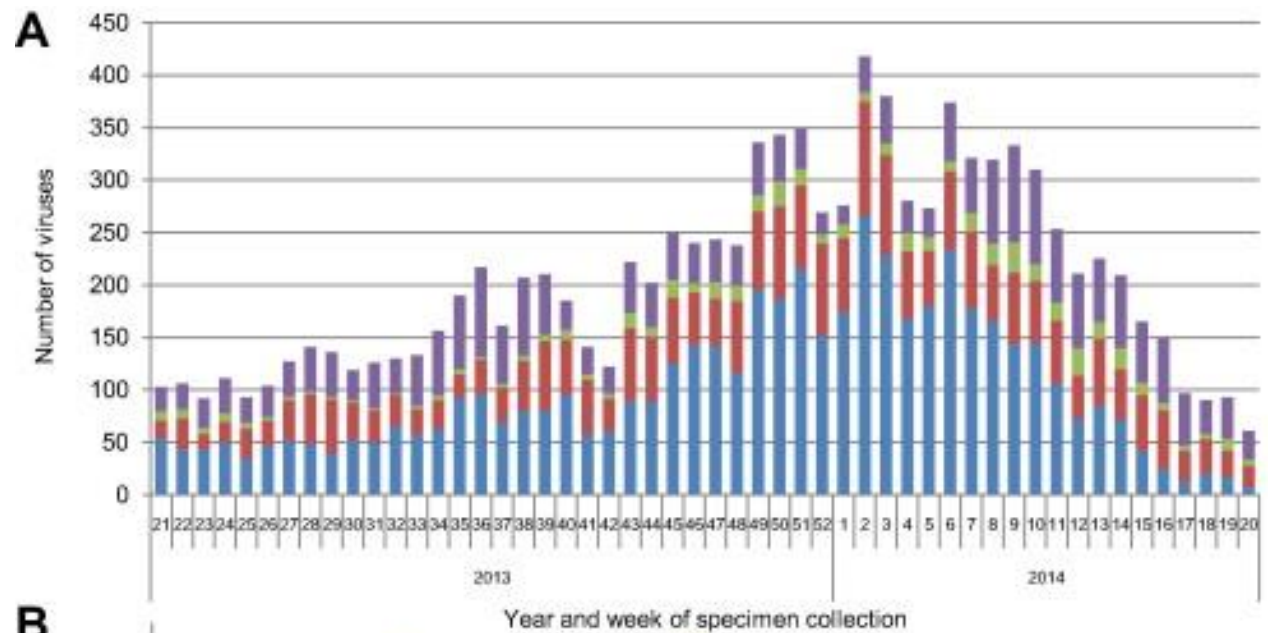


Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014

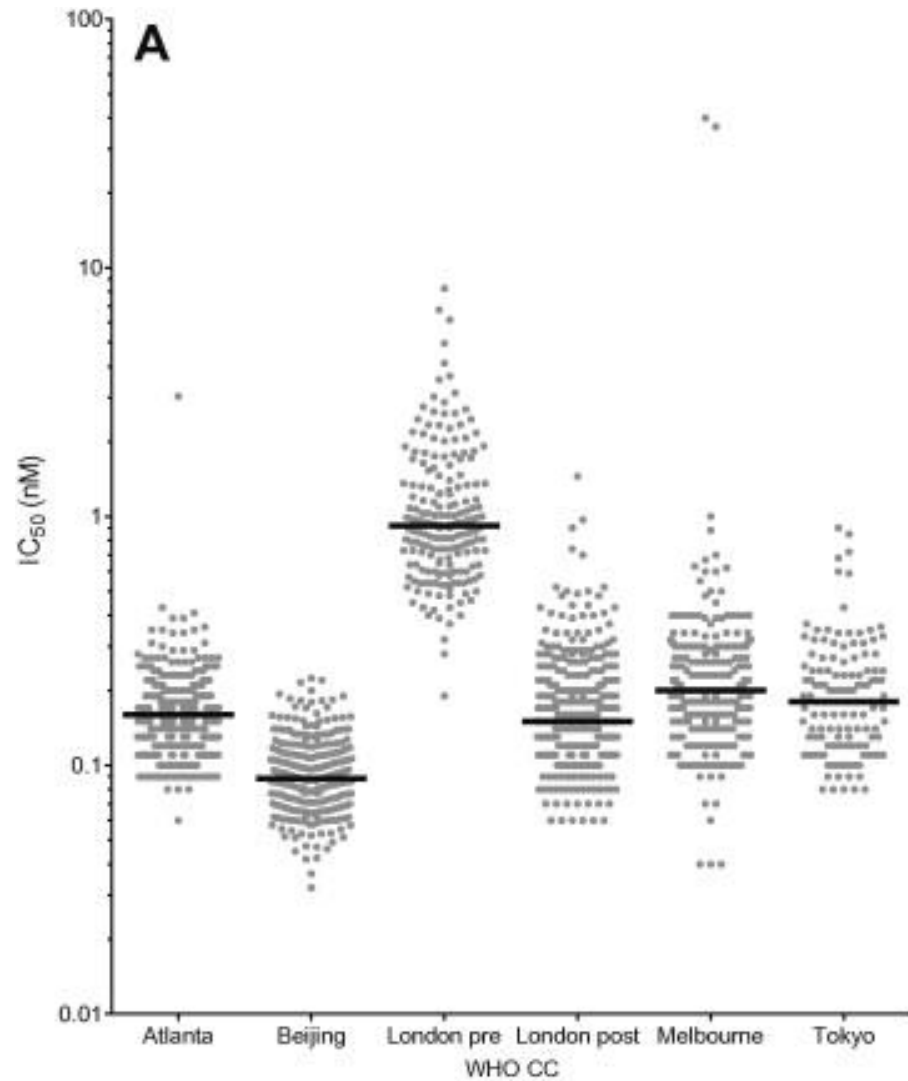


Emi Takashita^{a,1}, Adam Meijer^{b,1}, Angie Lackenby^{c,1}, Larisa Gubareva^{d,1}, Helena Rebelo-de-Andrade^{e,f,1}, Terry Besselaar^g, Alicia Fry^{d,1}, Vicky Gregory^h, Sook-Kwan Leangⁱ, Weijuan Huang^j, Janice Lo^{k,1}, Dmitriy Pereyaslov^l, Marilda M. Siqueira^{m,1}, Dayan Wang^{j,1}, Gannon C. Mak^{k,1}, Wenqing Zhang^g, Rod S. Daniels^{h,1}, Aeron C. Hurt^{i,n,1}, Masato Tashiro^{a,*},^{1,2}

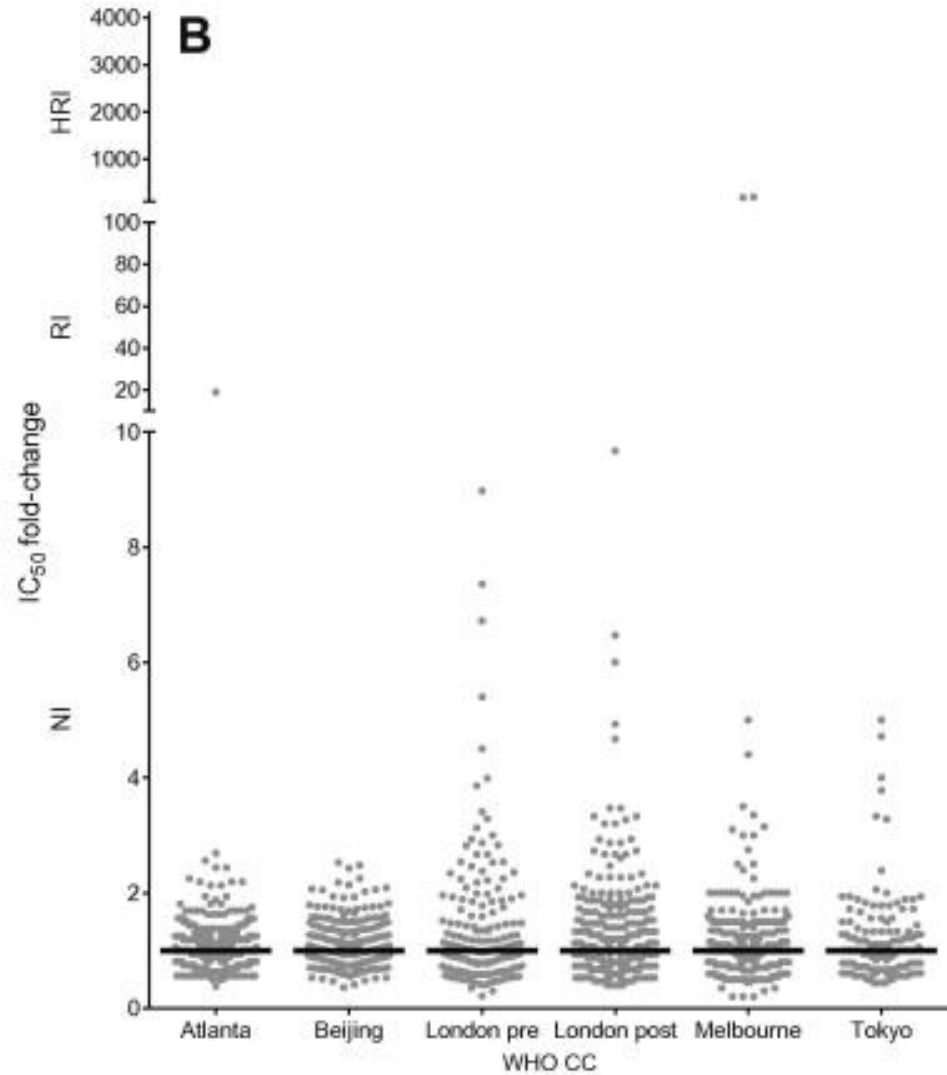
- Large geographic coverage
- Strong predominance of viruses from Western Pacific and Americas



Challenges with combining data and the need to normalise and compare fold differences



Raw IC₅₀ data



Normalised IC₅₀ data

Developed new criteria for describing susceptibility

Influenza A

Influenza B

Highly reduced inhibition

>100-fold

>50-fold

HRI



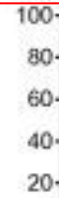
B

Reduced inhibition

>10-fold

>5-fold

RI



Normal inhibition

<10-fold above median

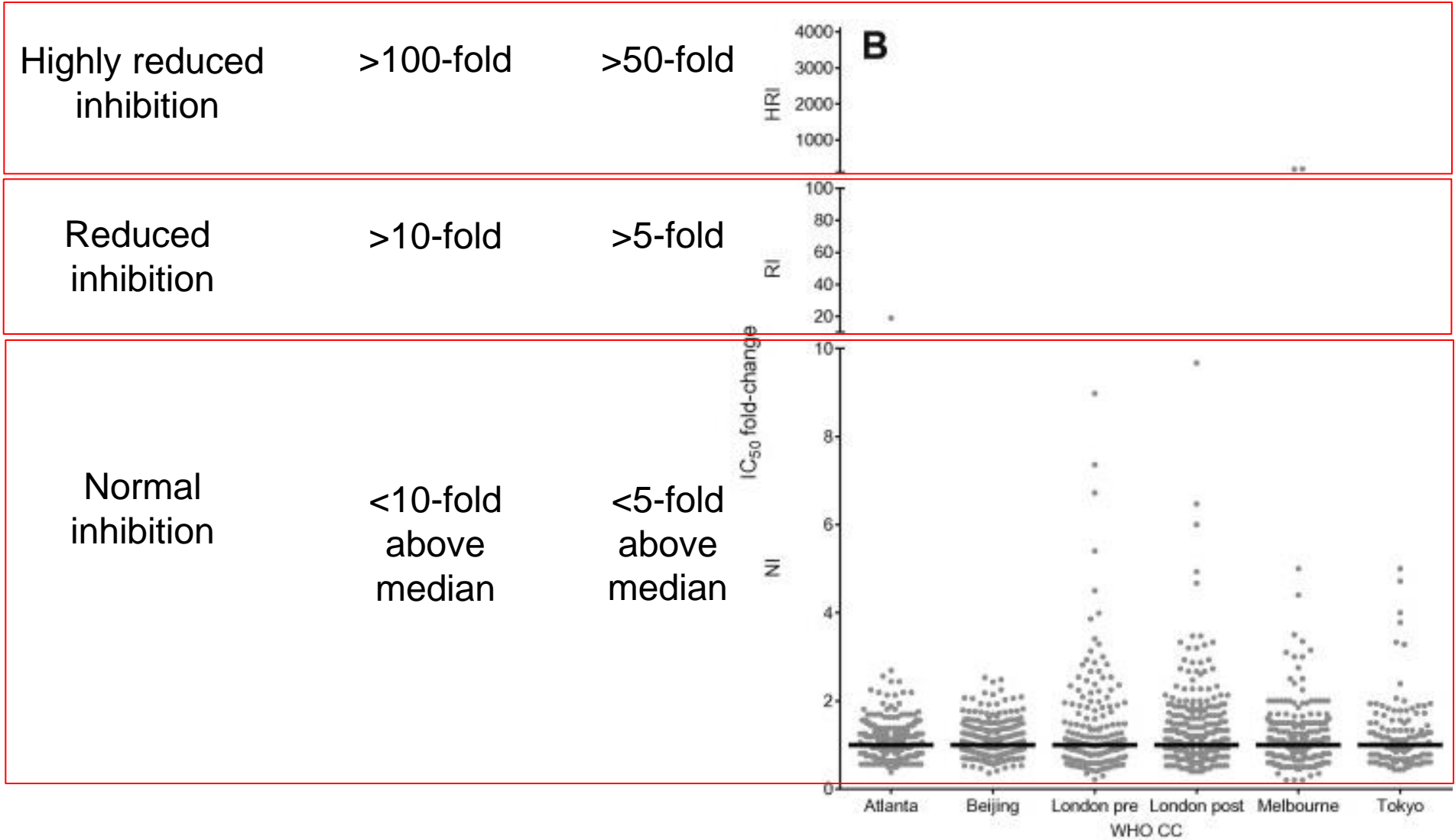
<5-fold above median

IC₅₀ fold-change

NI



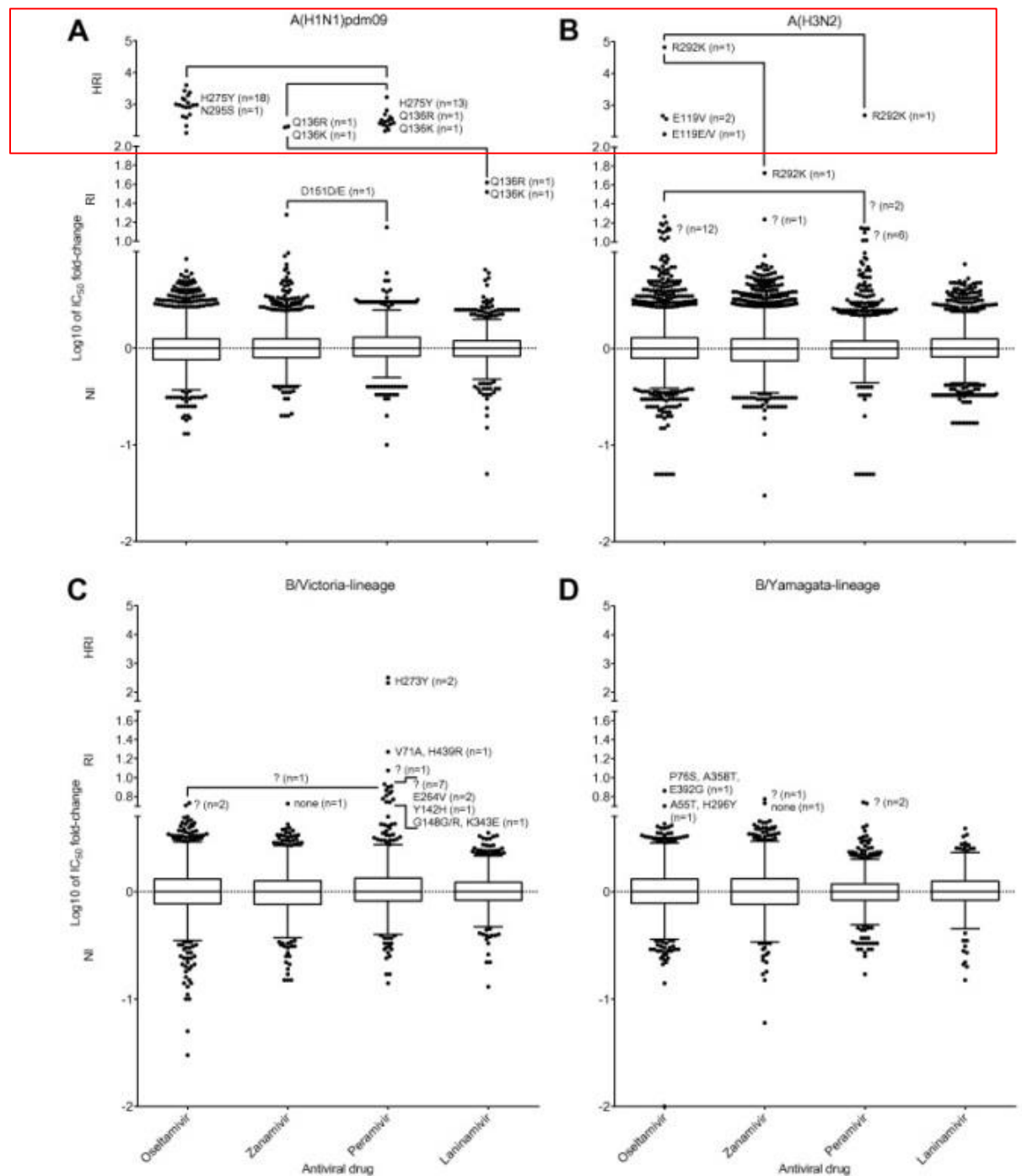
Atlanta Beijing London pre London post Melbourne Tokyo
WHO CC



2012/13

11,387 influenza viruses

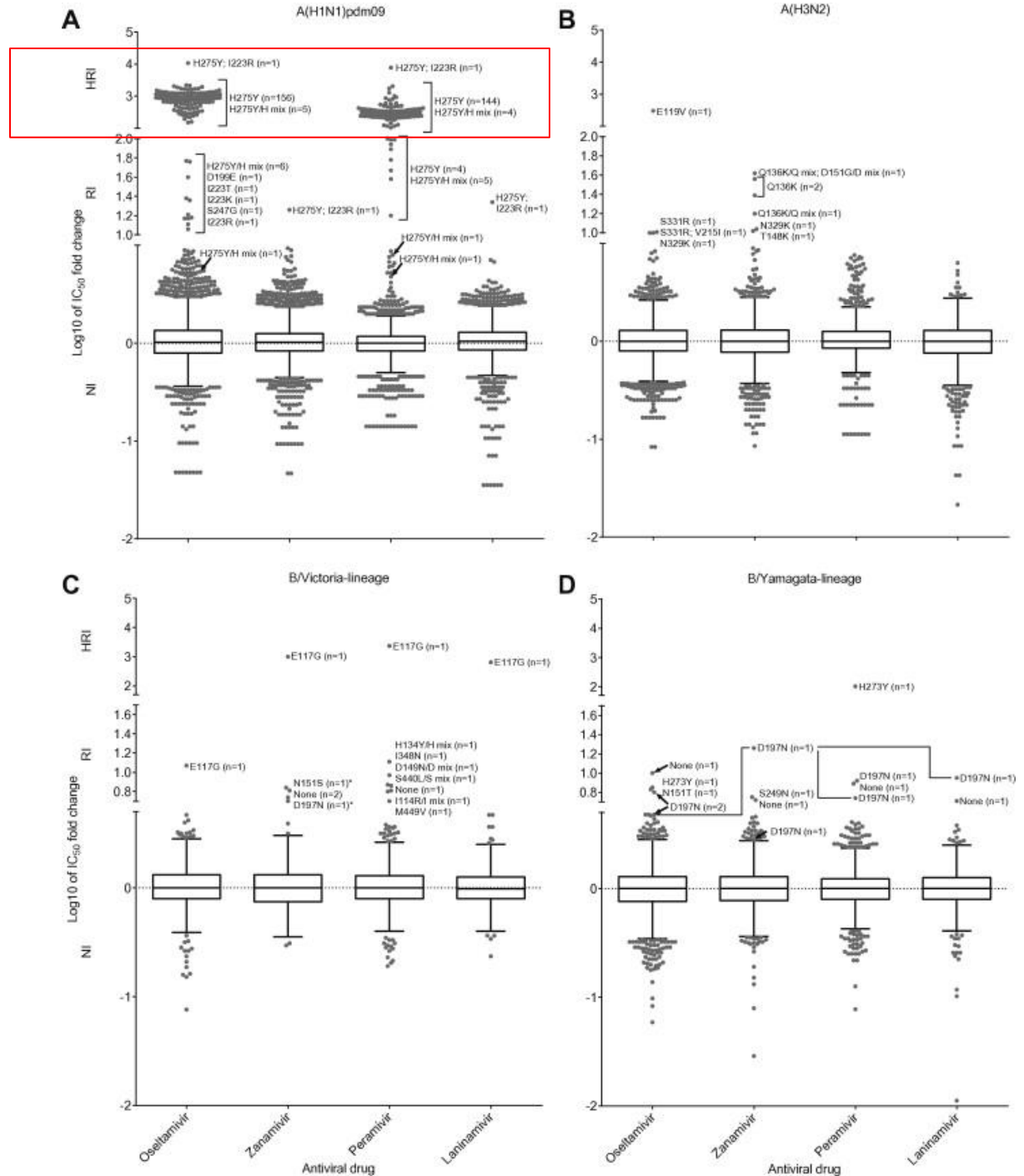
0.6% of influenza A and B viruses each showed reduced/highly reduced inhibition by at least one neuraminidase inhibitor.



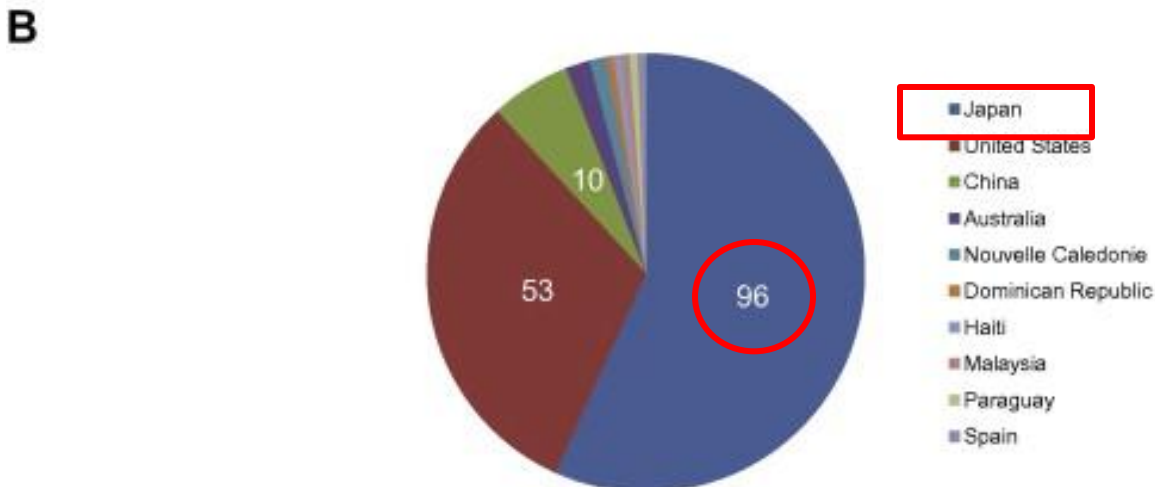
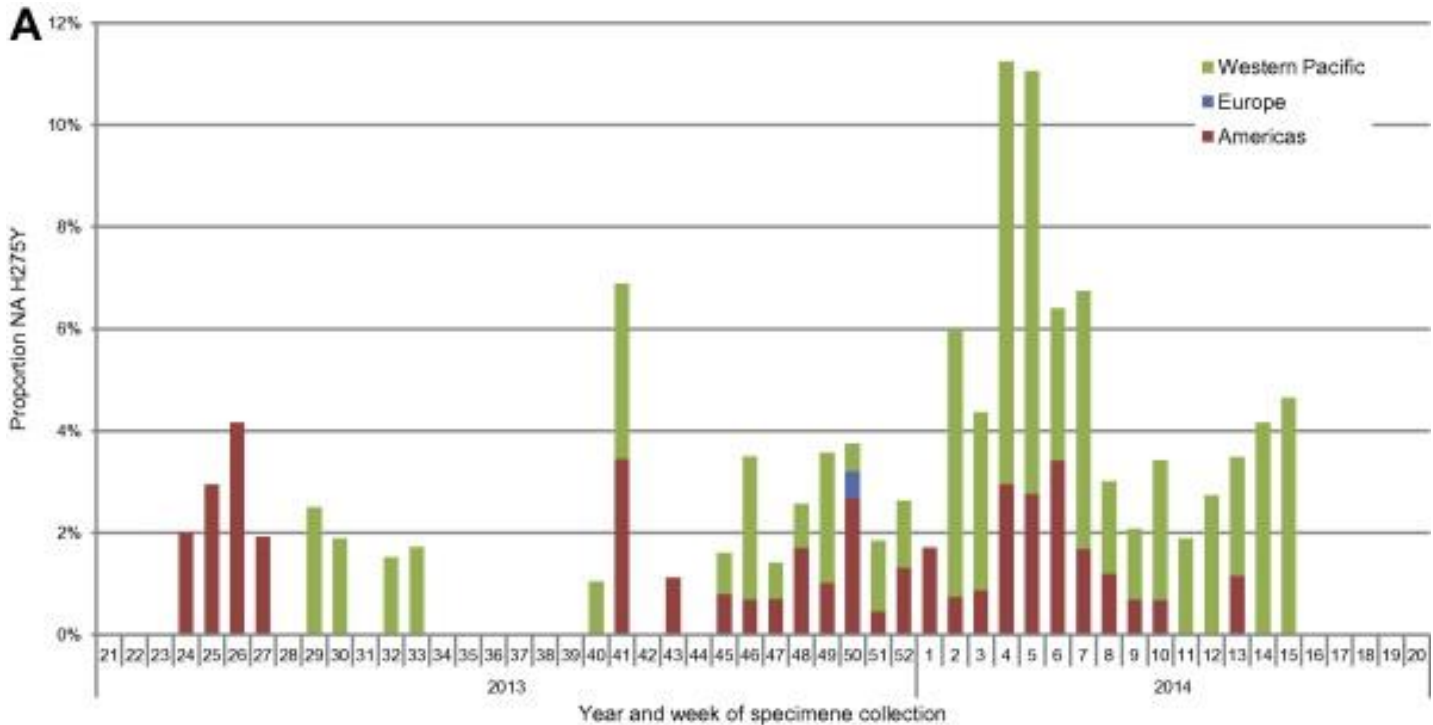
2013/14

10,641 influenza viruses

2% of influenza A and B viruses each showed reduced/highly reduced inhibition by at least one neuraminidase inhibitor.



Specimen collection timing and geographic distribution of 169 neuraminidase (NA) H275Y containing A(H1N1)pdm09 viruses.

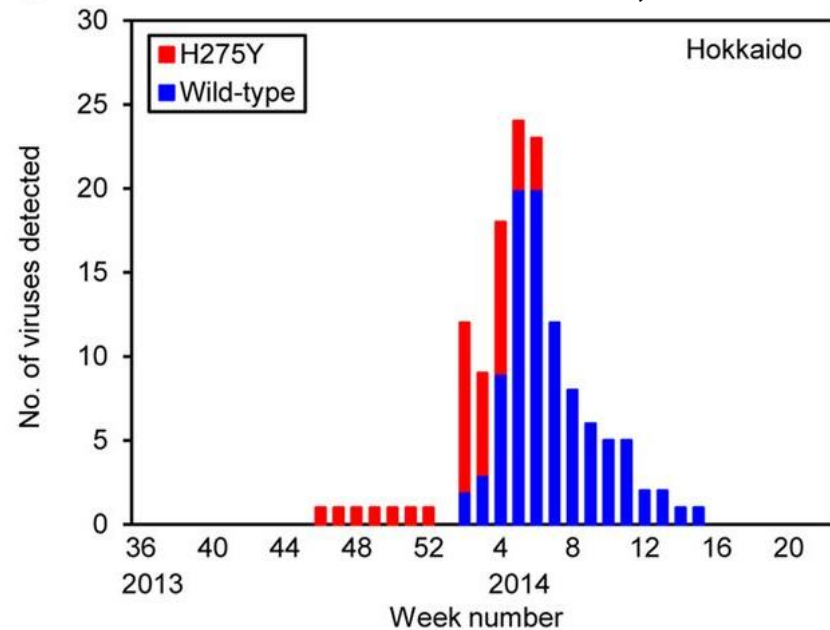


- Cluster of cases detected in Sapporo, Japan, early in 2013/14 season

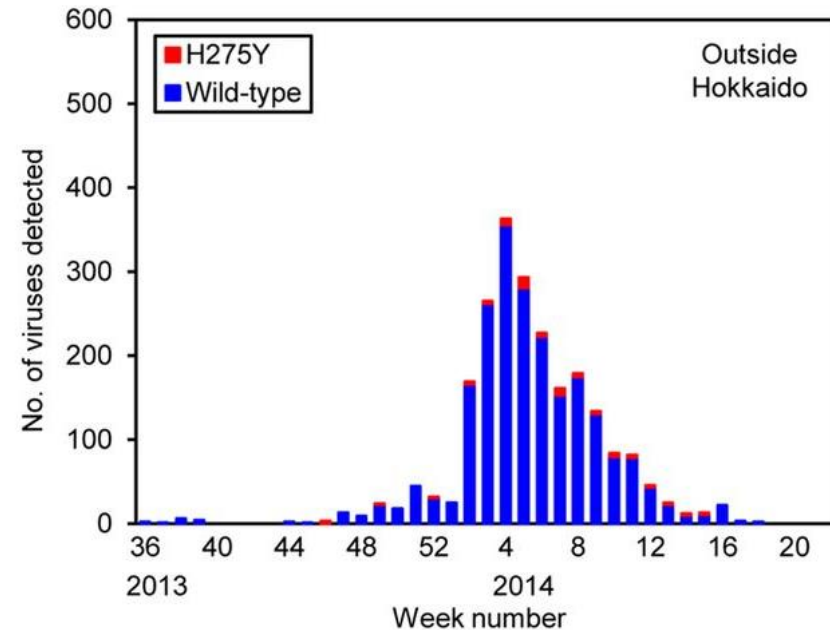
- Reports of similar viruses in China (pers comm. Dr Yuelong Shu, China WHO CC)

- Very similar to another cluster of oseltamivir-resistant H275Y A(H1N1)pdm09 cases detected in Newcastle, Australia in 2011

C

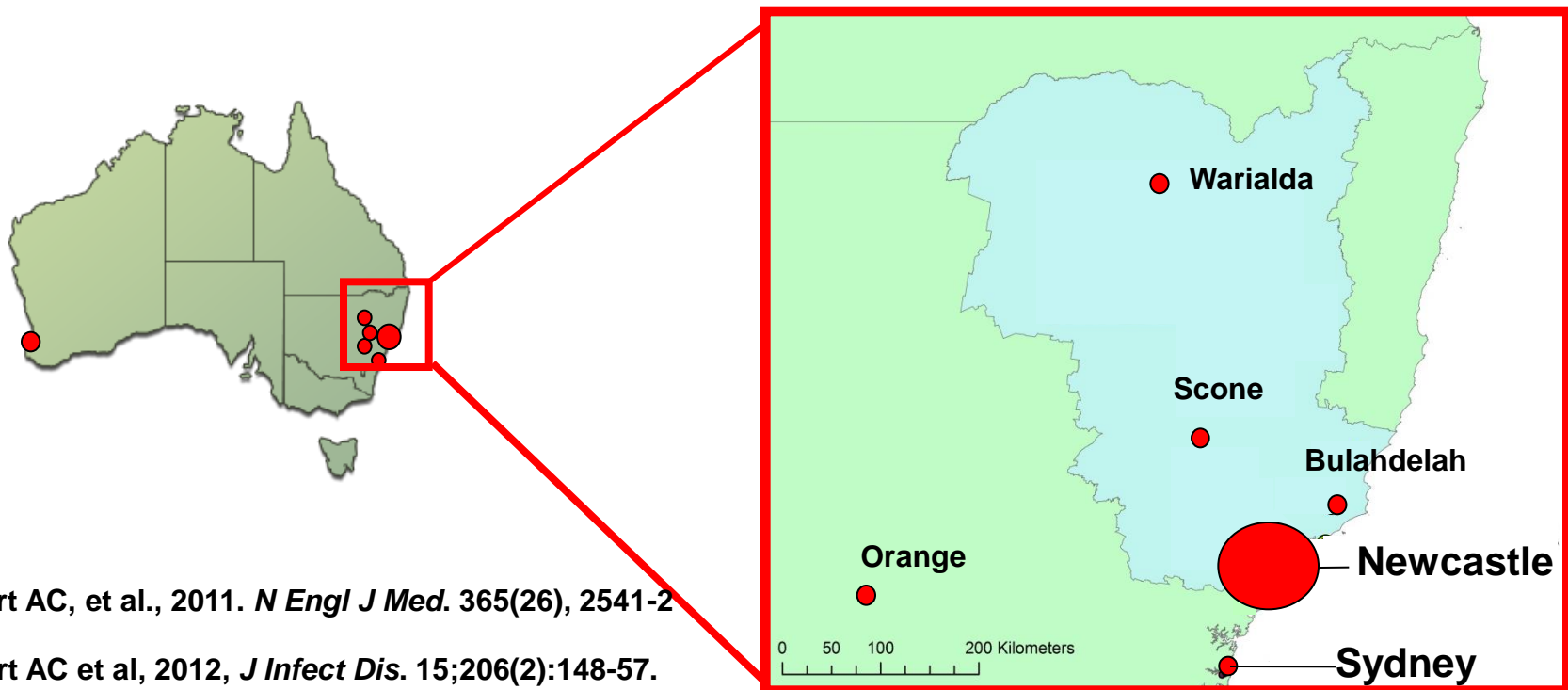


D



Earlier cluster of oseltamivir-resistant A(H1N1)pdm09 - Newcastle, NSW, Australia

- Between May and September, 2011 detected a large cluster of oseltamivir resistant A(H1N1)pdm09 H275Y variants in the community
- 32 cases detected, majority (n=26) were within 50 km of Newcastle
- At its peak in July 20/85 H1N1pdm09 viruses tested (24%) were resistant
- Only one case had undergone oseltamivir treatment (not the index case)
- Appeared to be a 'fit' H275Y variant circulating in the community

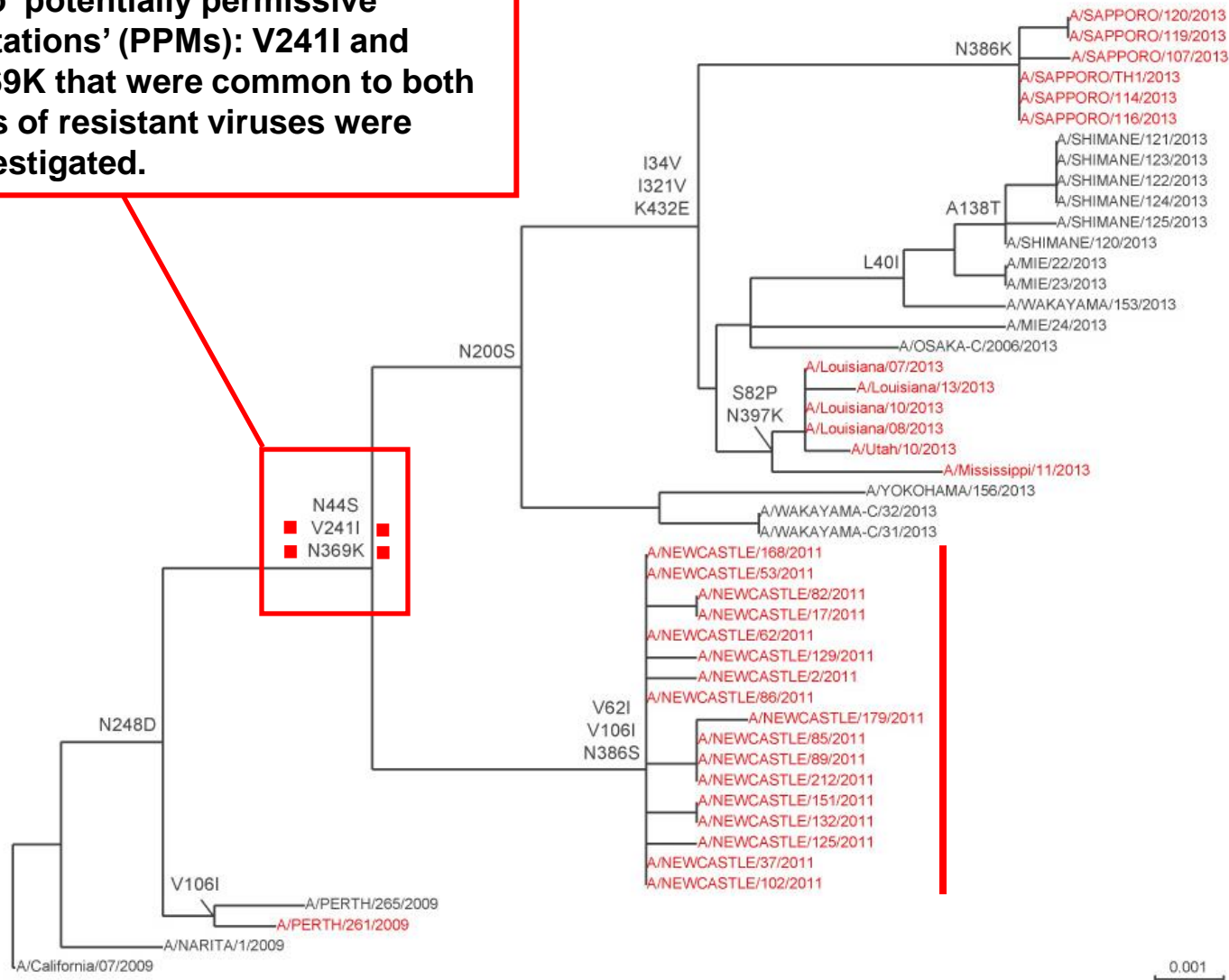


Hurt AC, et al., 2011. *N Engl J Med.* 365(26), 2541-2

Hurt AC et al, 2012, *J Infect Dis.* 15;206(2):148-57.

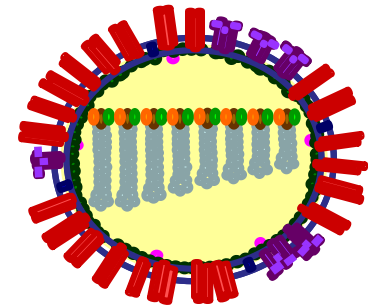
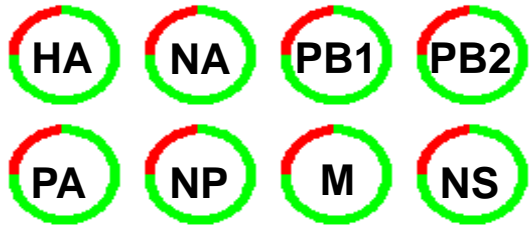
So what is it about the Newcastle or Hokkaido H275Y viruses that allowed them to spread ?

- Two 'potentially permissive mutations' (PPMs): V241I and N369K that were common to both sets of resistant viruses were investigated.



NA sequence phylogenetic tree

Used reverse genetics to create viruses with and without PPM's



H275Y virus from Newcastle cluster

369K
241I Vs. **369N**
241V

rgA/Newcastle/17/11 **rgA/Newcastle/17/11 – PPM's**

Will removal of PPM's decrease fitness of A/Newcastle/17/11 ?

H275Y virus from early in 2009 pandemic (before acquisition of PPMs)

369N
241V Vs. **369K**
241I

rgA/Perth/261/09 **rgA/Perth/261/09 + PPM's**

Will introduction of PPM's increase fitness of A/Perth/261/09 ?

Fitness of viruses in ferret studies

**Will removal of PPM's decrease
fitness of A/Newcastle/17/11 ?**

**Will introduction of PPM's increase
fitness of A/Perth/261/09 ?**

Various mixtures of the two viruses prepared: 20%:80%, 50%:50%, 80%:20%

Within-host fitness



Will one virus outgrow
the other within ferrets

Transmission fitness

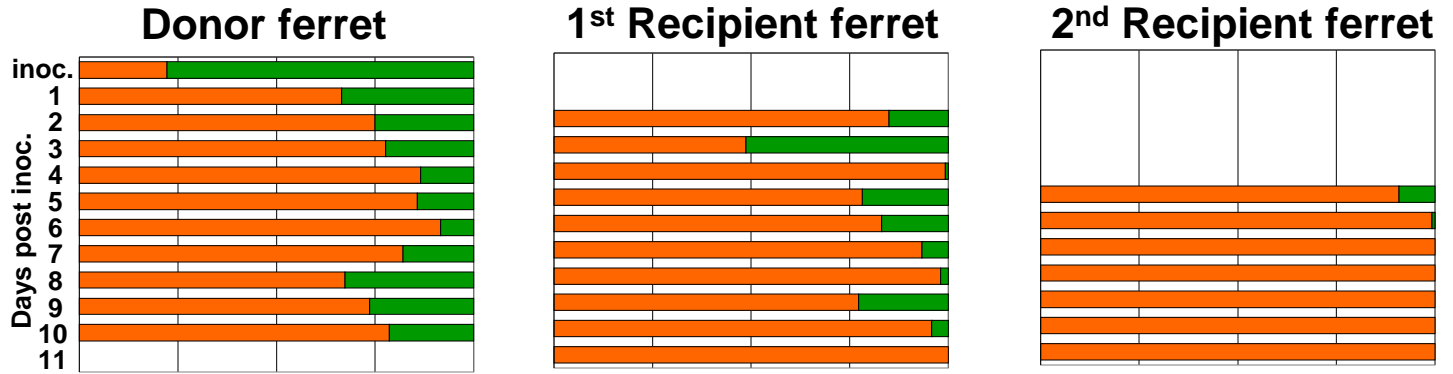


Will one virus transmit better
than other within ferrets

Removal of PPM's from Newcastle 2011 H275Y H1N1 viruses:

█ With PPM's
█ Without PPM's

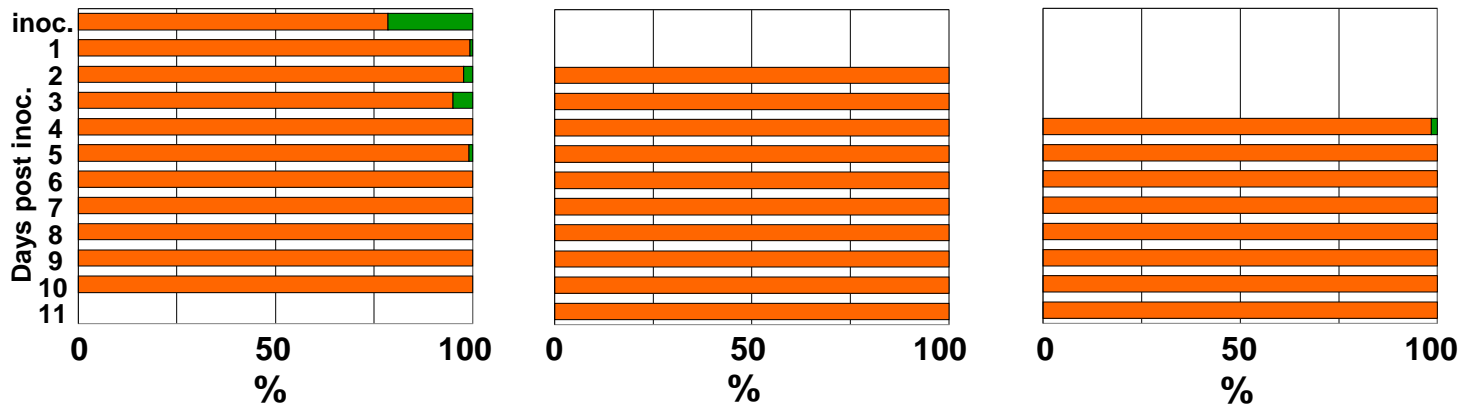
20% with PPM
80% without PPM



50% with PPM
50% without PPM

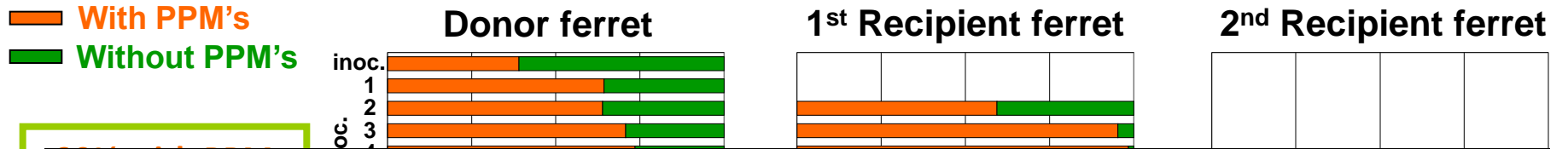


80% with PPM
20% without PPM



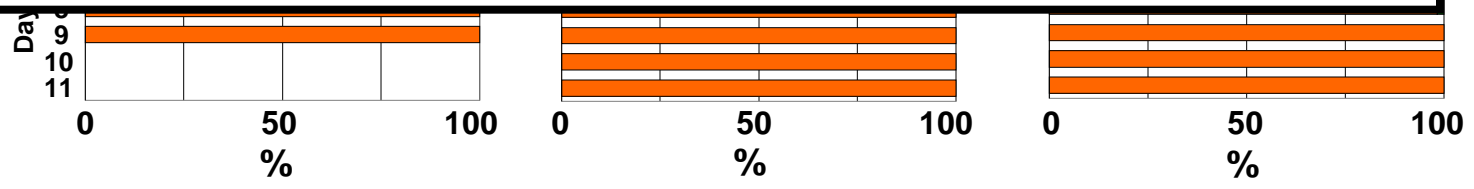
█ Removal of PPM's = ↓ fitness of Newcastle 2011 H275Y H1N1 viruses

Introduction of PPM's to early H275Y H1N1pdm09 virus



NA permissive mutations N369K + V241I improved the fitness H275Y variants

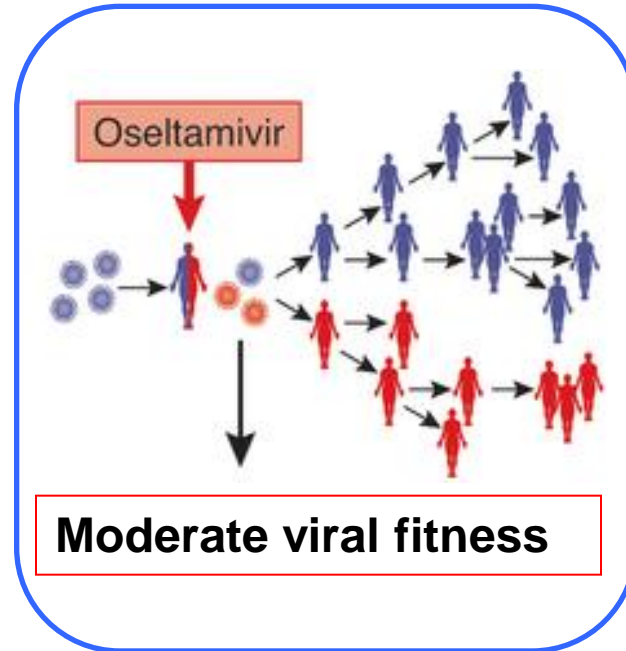
Importantly, these permissive mutations are now in all circulating A(H1N1)pdm09 viruses



▪ Addition of **PPM's** = ↑ fitness of other **H275Y** H1N1pdm09 viruses

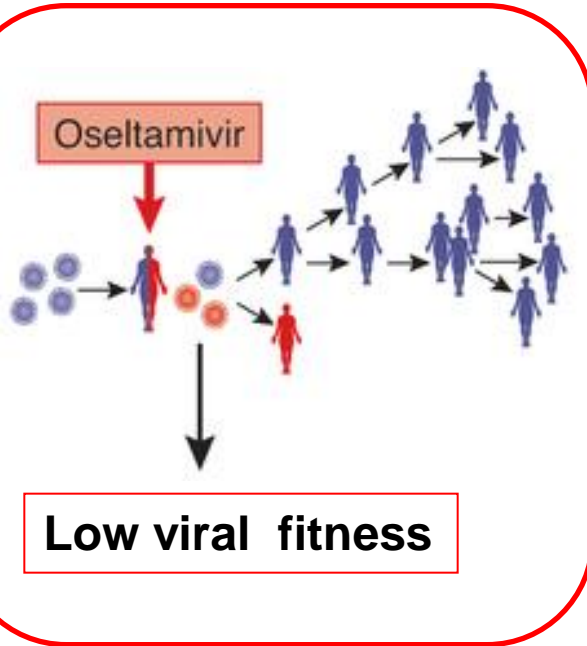
The fitness of H1N1pdm09 H275Y variants

2011-2015

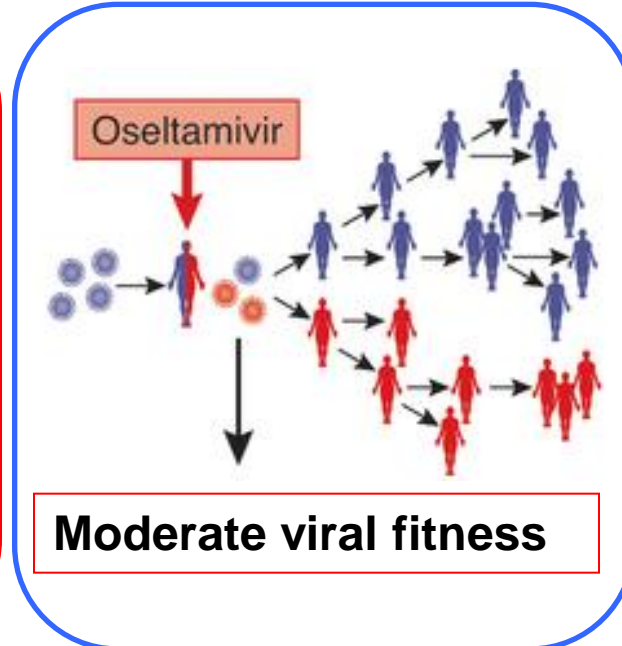


The fitness of H1N1pdm09 H275Y variants

2009-2010

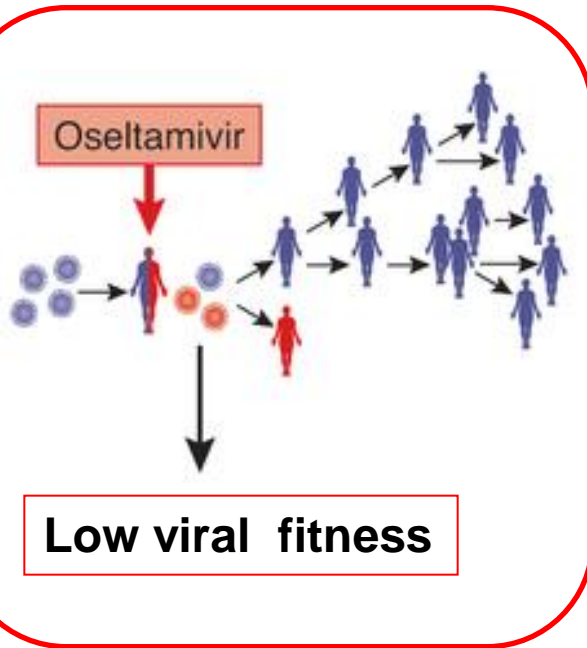


2011-2015

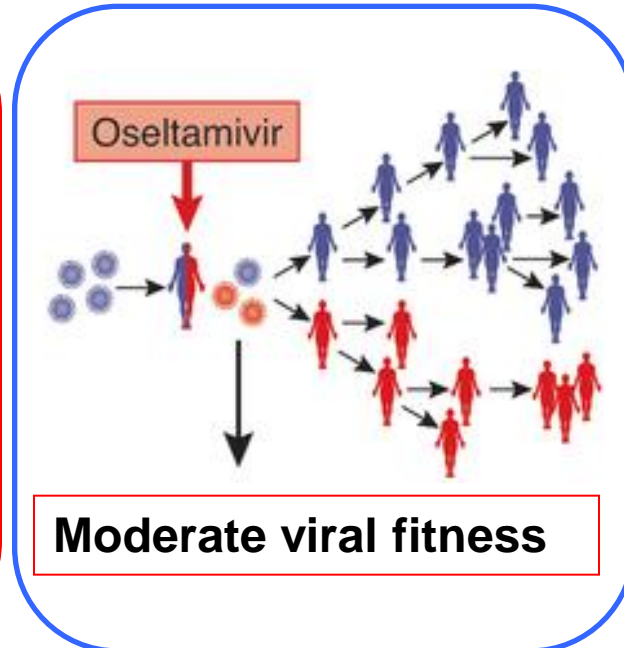


The fitness of H1N1pdm09 H275Y variants

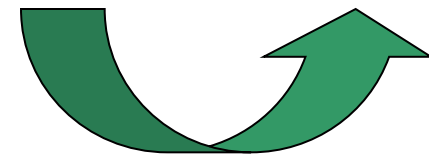
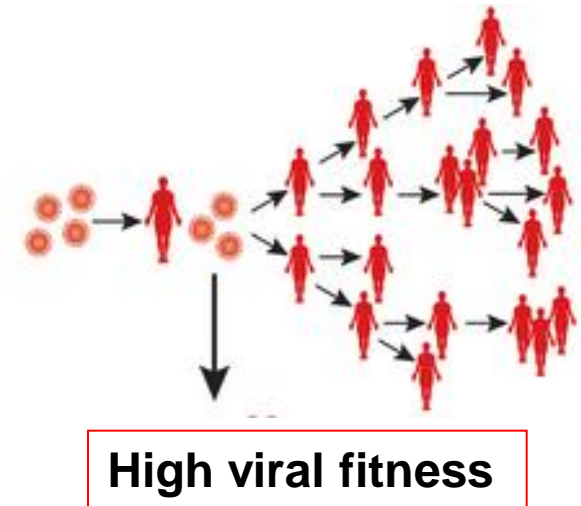
2009-2010



2011-2015



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What further changes in the virus are needed before we get to this???

Summary

- **NAIs remain the only antivirals available, with reliance on oseltamivir**
- **Global analysis provides useful insights into susceptibility of circulating viruses (mostly untreated cases)**
- **Oseltamivir resistance is low, but clusters of H275Y H1pdm09 are concerning given the experience of the spread of seasH1N1 H275Y**
- **Ferret studies suggest that ‘permissive’ mutations are currently present in circulating strains which enable H275Y viruses to be fit**
- **Further studies are underway to understand what additional permissive mutations might further improve fitness**
- **Many new antivirals in later phase clinical trials appear less prone to select resistance than oseltamivir**



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'Permissive mutation work'



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