

EU-CIS Seminar

New trends in Infectious Diseases

26 – 28 November 2008 / Lyon, France

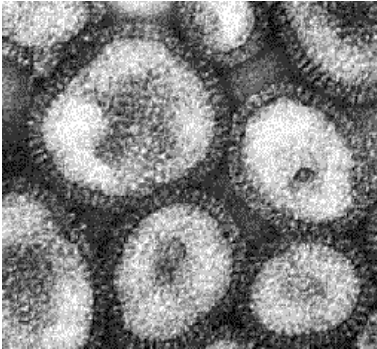
Flu, Avian Flu  
and emerging aspects  
(H1N1 resistance)

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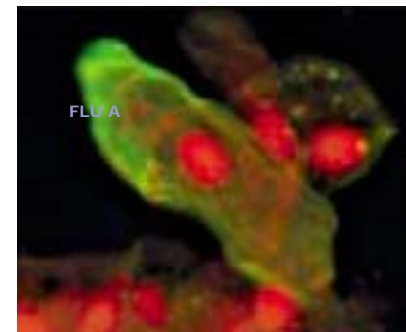
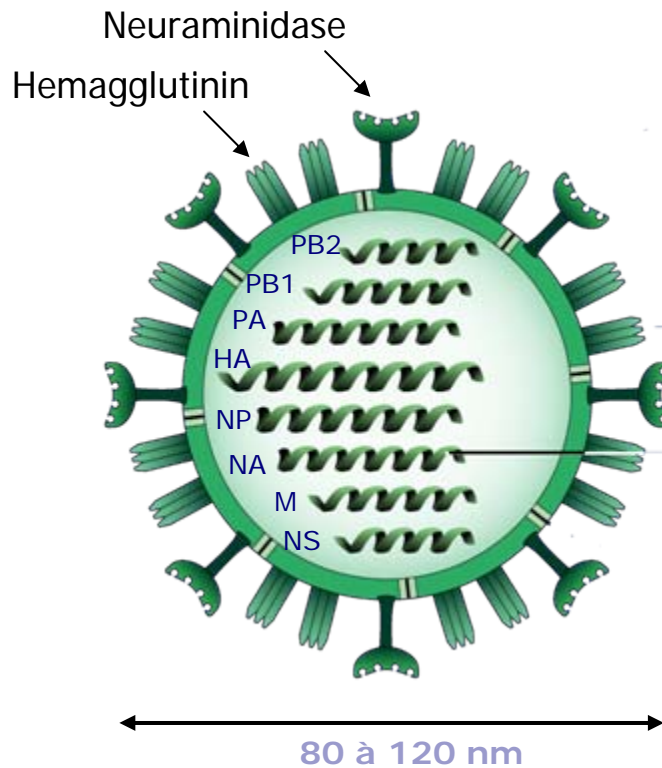
# Influenza virus



EM

## Genome :

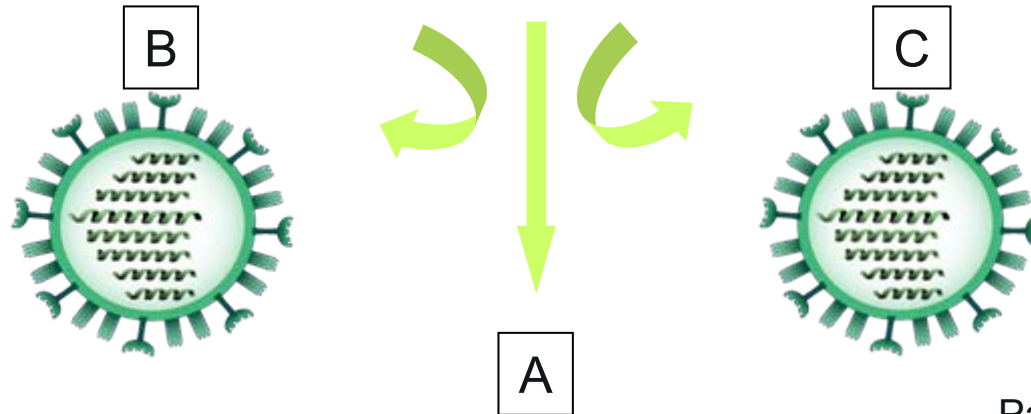
- Segmented
- Negative single strand RNA



IF

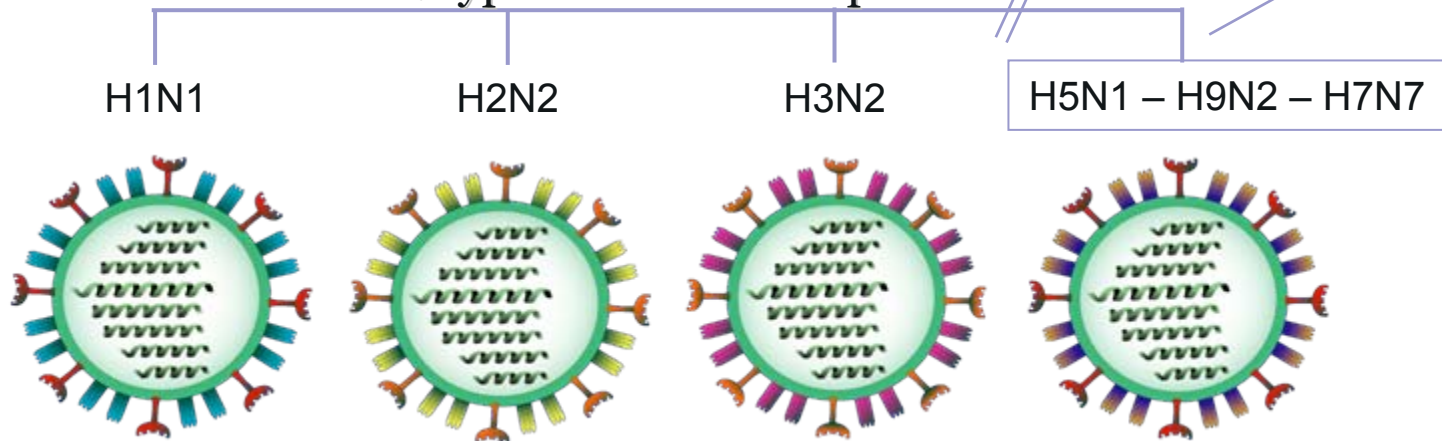
# Influenza virus

3 types : NP and M proteins



Pandemic virus HxNy ?

subtypes : HA and NA proteins



Since 1976 : seasonal flu with H1N1 and H3N2
































## Envelop glycoproteins : Hemagglutinin (HA) and Neuraminidase (NA)





















### Hemagglutinin :

16 types  $H_1$  to  $H_{16}$   
entry into cells

### Neuraminidase :

9 types  $N_1$  to  $N_9$   
liberation of virus  
(entry into cells)

H1				
H2				
H3				
H4				
H5				
H6				
H7				
H8				
H9				
H10				
H11				
H12				
H13				
H14				
H15				
H16				

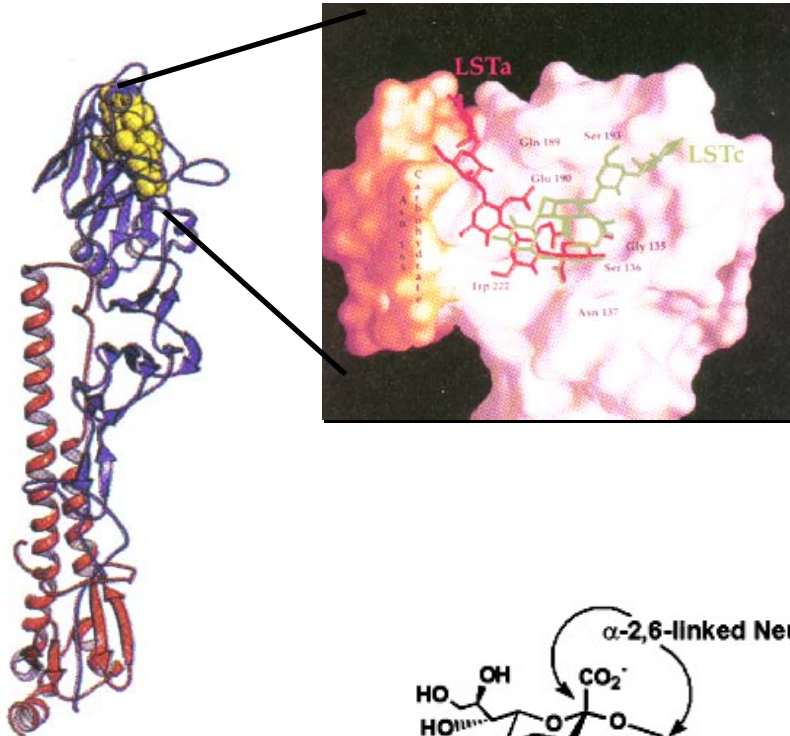
N1				
N2				
N3				
N4				
N5				
N6				
N7				
N8				
N9				

# Hemagglutinin

Viral HA

RBS  
Receptor Binding Site

Cell receptors

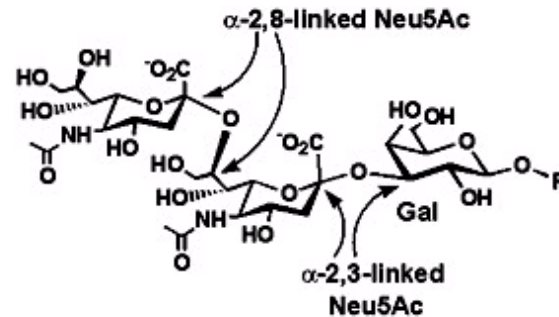
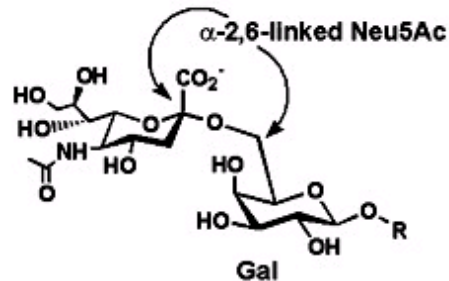


Sialic acids linked to galactose residues

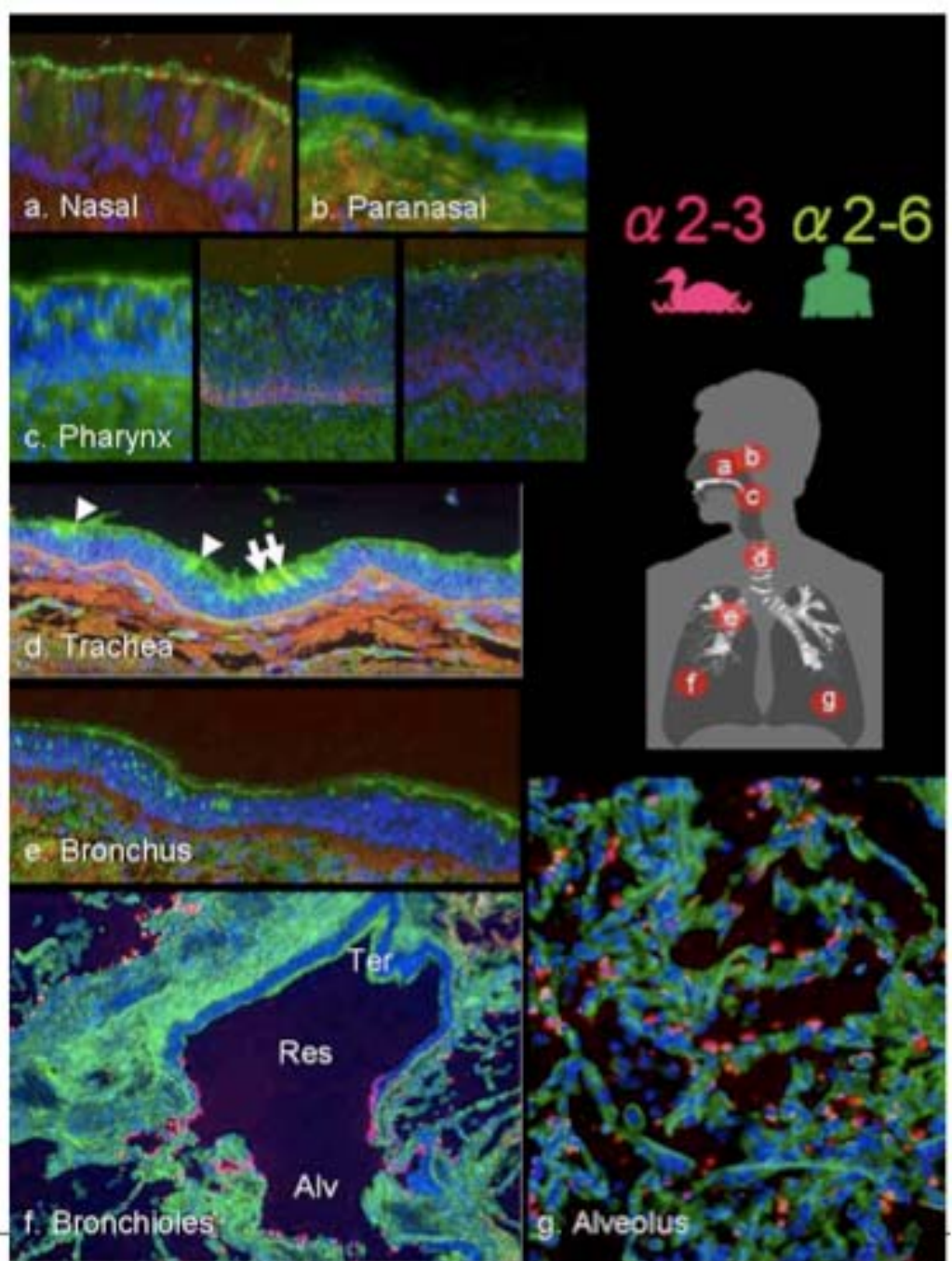
HA of human virus =  $\alpha$ 2-6 R

HA of avian virus =  $\alpha$  2-3 R

In pigs :  $\alpha$  2-3 and  $\alpha$  2-6 R



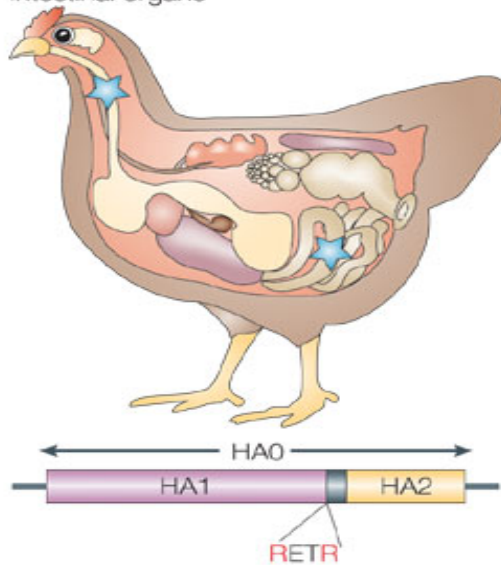
Distribution  
of  $\alpha 2-3$   
and  $\alpha 2-6$   
receptors  
in human  
respiratory  
tract



# Hemagglutinin

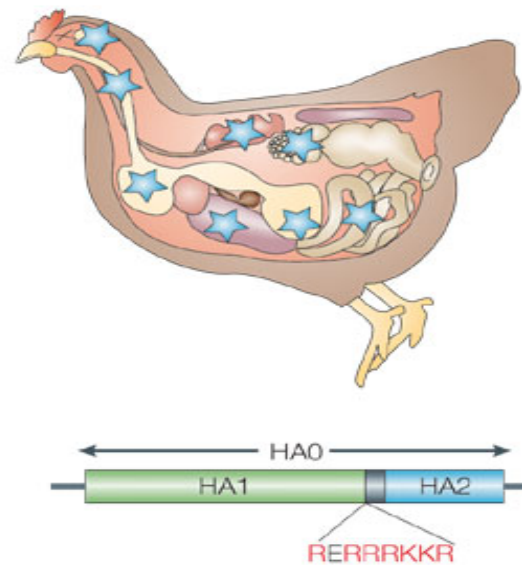
## LPAI

Proteases localized in respiratory and intestinal organs



## HPAI

Ubiquitous proteases



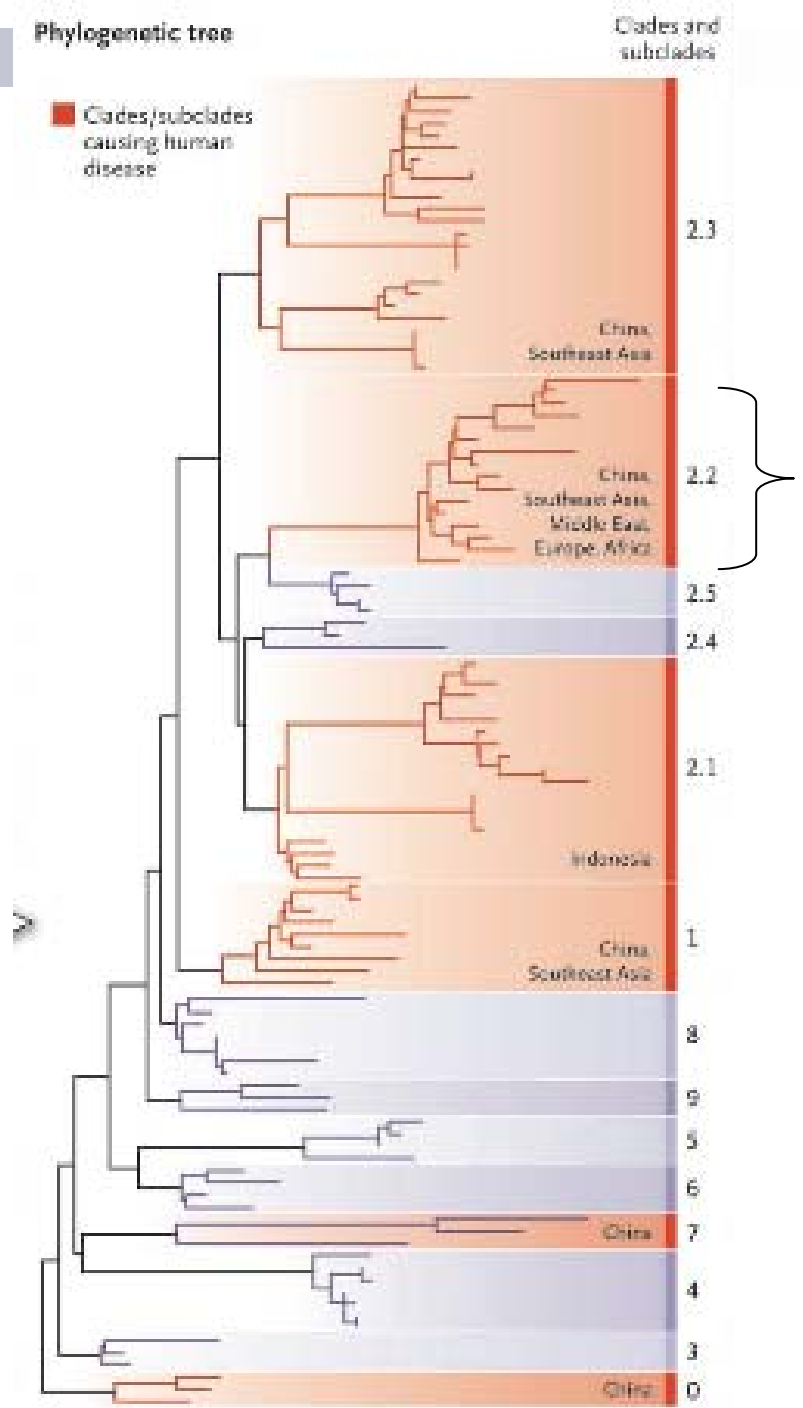
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Polybasic cleavage site :

H1 (1918), H5, H7

# H5N1 influenza virus

- Avian origin
- Circulation since 1997 in Asia
- Rapid genetic evolution :
  - 9 different clades (1 and 2 +++)
- Infections of different species
  - pigs
  - tigers
  - cats
  - Human
- Clade 2.2 :
  - higher pathogenicity (birds)
  - broader dissemination



# Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO

Country	2003		2004		2005		2006		2007		2008		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	8	5	0	0	0	0	8	5
Bangladesh	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Cambodia	0	0	0	0	4	4	2	2	1	1	0	0	7	7
China	1	1	0	0	8	5	13	8	5	3	3	3	30	20
Djibouti	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	25	9	7	3	50	22
Indonesia	0	0	0	0	20	13	55	45	42	37	20	17	137	112
Iraq	0	0	0	0	0	0	3	2	0	0	0	0	3	2
Lao People's Democratic Republic	0	0	0	0	0	0	0	0	2	2	0	0	2	2
Myanmar	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Nigeria	0	0	0	0	0	0	0	0	1	1	0	0	1	1
Pakistan	0	0	0	0	0	0	0	0	3	1	0	0	3	1
Thailand	0	0	17	12	5	2	3	3	0	0	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	0	0	12	4
Viet Nam	3	3	29	20	61	19	0	0	8	5	5	5	106	52
Total	4	4	46	32	98	43	115	79	88	59	36	28	387	245

# Clinical settings of H5N1 influenza virus infection

**Table 2. (Continued.)**

Variable	Vietnam, Thailand, Cambodia, 2004–2005, Clade 1 <sup>†</sup>	Indonesia, 2005–2006, Clade 2.1 <sup>‡</sup>	China, 2005–2006, Clade 2.3 <sup>§</sup>	Egypt, 2006–2007, Clade 2.2 <sup>¶</sup>	Turkey, Azerbaijan, 2006, Clade 2.2 <sup>  </sup>
Deaths — no./ total no. (%)	32/41 (78)	41/54 (76)	7/8 (88)	15/38 (39)	9/16 (56)
Time from onset of symptoms to death — days					
Median	8–12	9	9	11.5	10–13
Range	4–30	5–19	8–19	6–32	9–17

## Initial settings:

Flu infection +/- **respiratory and digestive symptoms**  
 Lymphopenia and thrombocytopenia  
 Followed by **pneumonia**

## Severe cases:

Evolution to **ARDS** (in  $\approx$  6 days)  
 Multi-organ failure  
 Death in 4 to 30 days (**Death rate = 60 to 80%**)

• NEJM, 358,3, 2008

Incidence of asymptomatic forms : unknown

# Specificities of Human infections by Avian H5N1 influenza virus

## **Viral replication :**


- Low in the upper respiratory tract (few  $\alpha$  2-3 R)  
=> low transmission to contacts
- High in the lower respiratory tract (alveola)  
=> pneumonia, ARDS

**Viremia** : long, high => dissemination to extra-respiratory localisations

- Brain (increased neurotropism)
- Digestive tube ( $\alpha$  2-3 R)

## **Associated symptoms :**

Diarrhea, vomiting, myalgia, convulsions



# Specificities of Human infections by Avian H5N1 influenza virus

Why a disseminated not controled infection ?

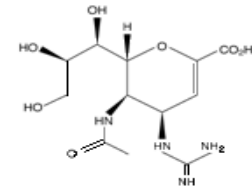
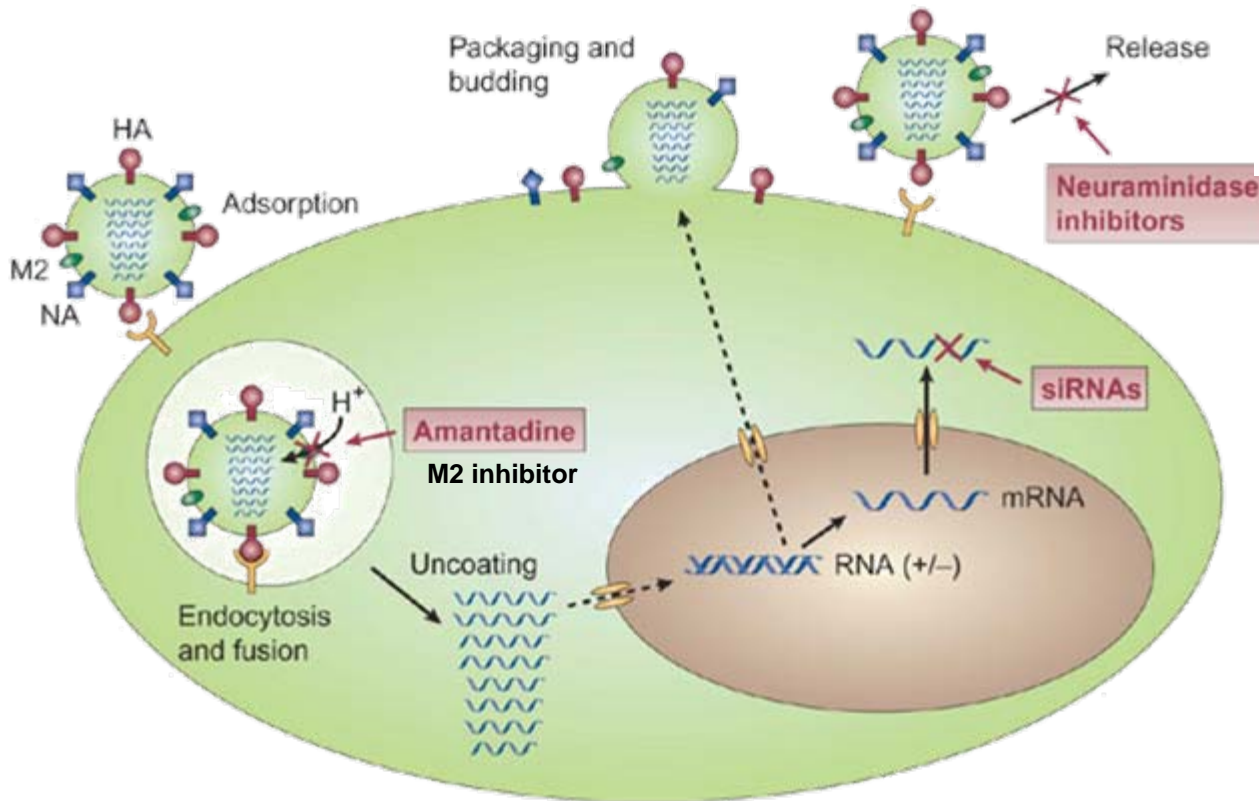
## Host factors :

- No pre-exisiting immunity
- Genetic susceptibility ? (familial clusters)

## Viral factors :

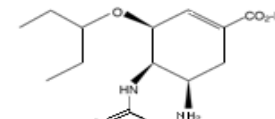
- Polybasic cleavage site
- Cytokines dysregulation

# Antiviral drugs



Zanamivir

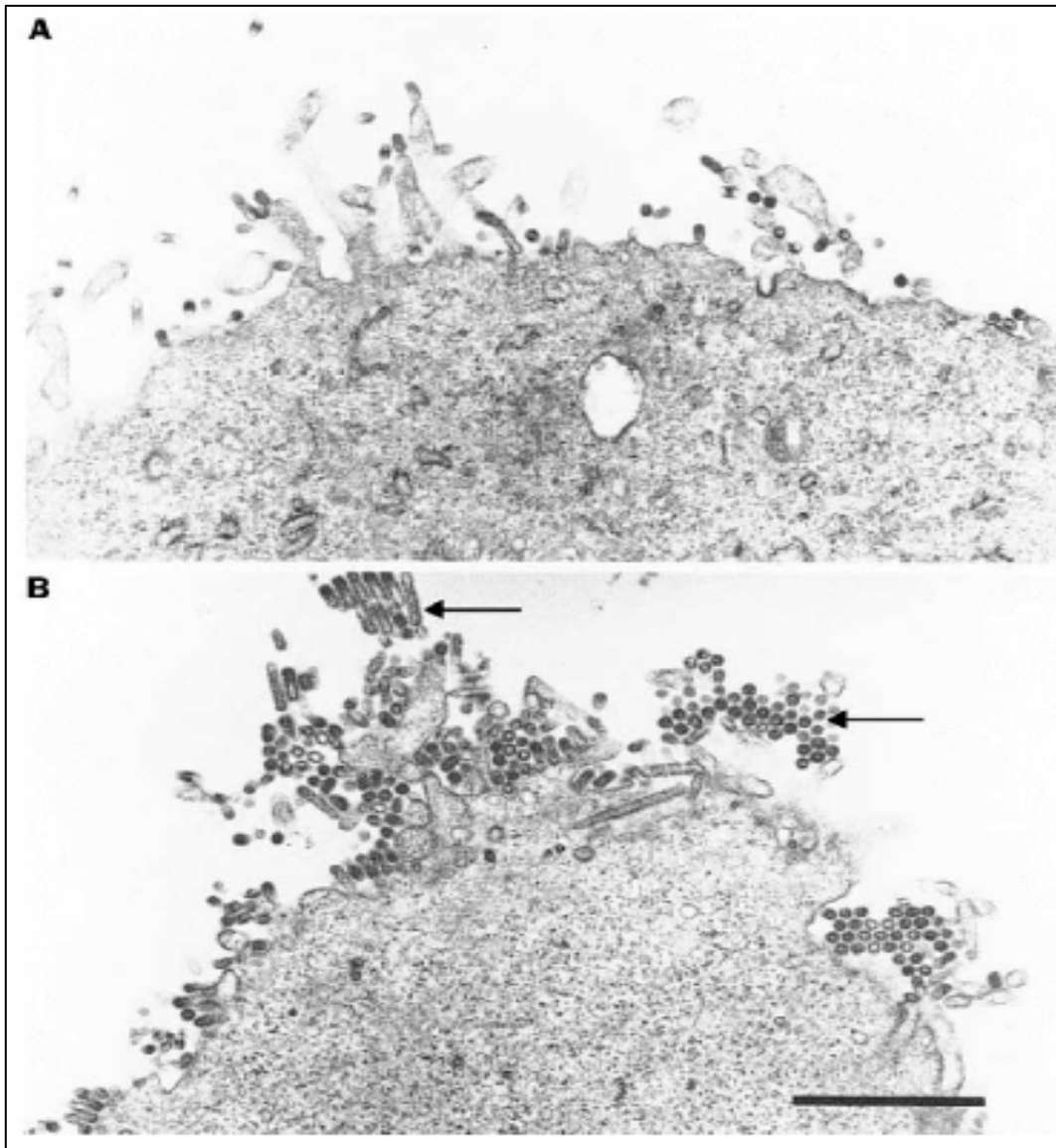
**Relenza®**  
Nasal spray



GS 4104 : RCH<sub>2</sub>CH<sub>3</sub>  
GS 4071 : R = H

Osetamivir

**Tamiflu®**  
Oral route



With NAI

**MDCK cells infected by Influenza A virus (TEM)**  
*(Gubareva et al., Lancet 2000)*

# Resistance to antiviral drugs

## Resistance to amantadine

H<sub>3</sub>N<sub>2</sub> : 50 to 80%

=> M2 mutation S<sub>31</sub>N

H<sub>5</sub>N<sub>1</sub> : Clade 1 : amantadine R

=> M2 mutation S<sub>31</sub>N

Clade 2 : amantadine S

# Resistance to Neuraminidase Inhibitors Until the end of 2007 : very low level

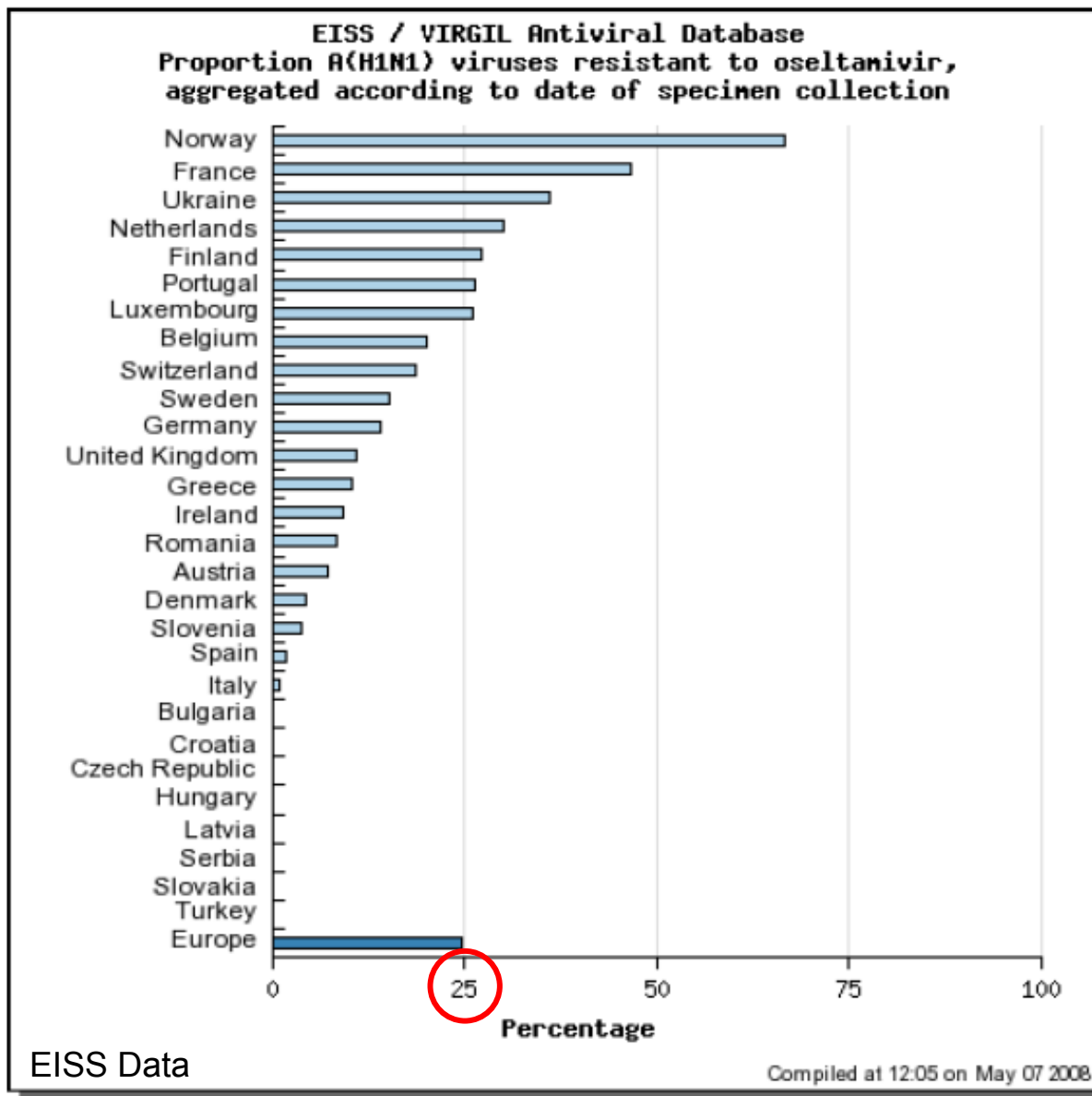
## ■ Systematic surveillance :

- No resistant strain before NAI introduction (1996-1999)  
(McKimm-Breschkin et al., AAC 2003)
- 0,35% after 3 years of NAI use (1999-2002)  
(Monto et al., AAC 2006)

## ■ In treated patients :

- Immunocompetent
  - Adults : 1 to 2 % (*Gubareva et al., JID 2001*)
  - Children :
    - 5 % (*Whitley et al., Pediatr Infect Dis J 2001*)
    - 16% to 18% (*Kiso et al., Lancet 2004; Ward et al., J Antimicrob Chemother 2005*)
- Immunocompromised : 3 cases (*Ison et al., JID 2006*)

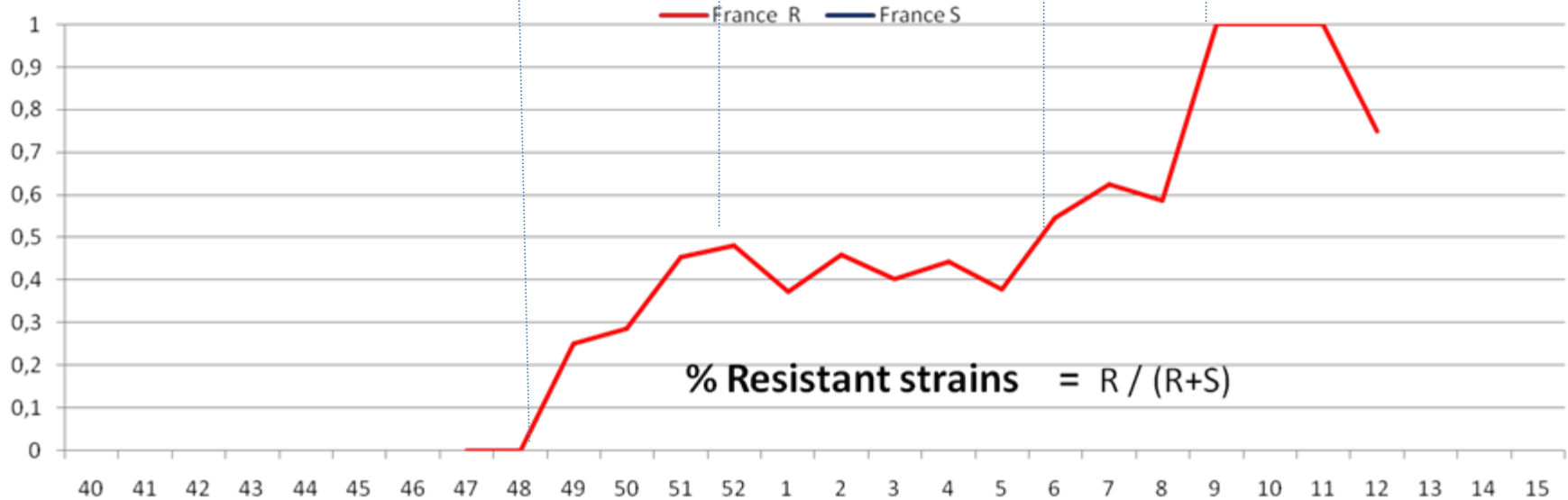
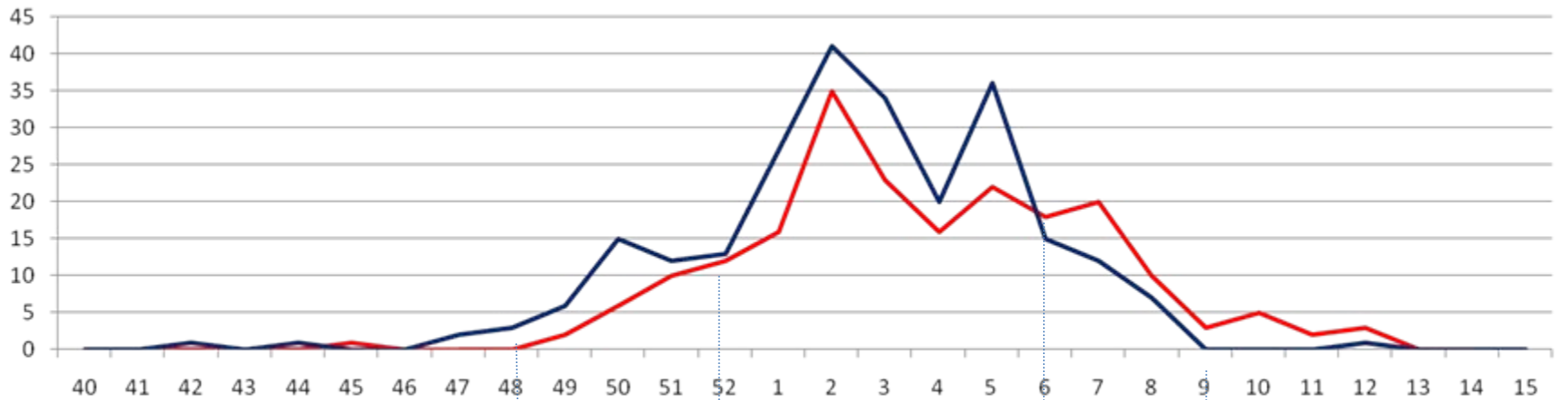
# H1N1 viruses resistant to NAI detected in Europe (winter 2007-2008)



3158 viruses tested

789 resistant

## Resistant strains (red) vs sensitive strains (blue)



France, winter 2007-2008

# Mutations associated with resistance to NAIs

function	position	virus	treatment	oseltamivir	zanamivir	Reference
Framework	E119V	H3N2	Oseltamivir	R	S	Baz et al., 2006
	D198N	B	Oseltamivir	R	R	Ison et al., 2006
	<b>H275Y</b>	<b>H1N1</b> H5N1	<b>Oseltamivir</b>	<b>R</b>	<b>S</b>	Wang et al., 2000 Le et al., 2005
	N295S	H3N2	Oseltamivir	Low R	Not tested	Kiso et al., 2004
Catalytic	R292K	H3N2	Oseltamivir	R	Not tested	Covington et al., 2000
	R152K	B	Zanamivir	R	R	Gubavera et al., 2004

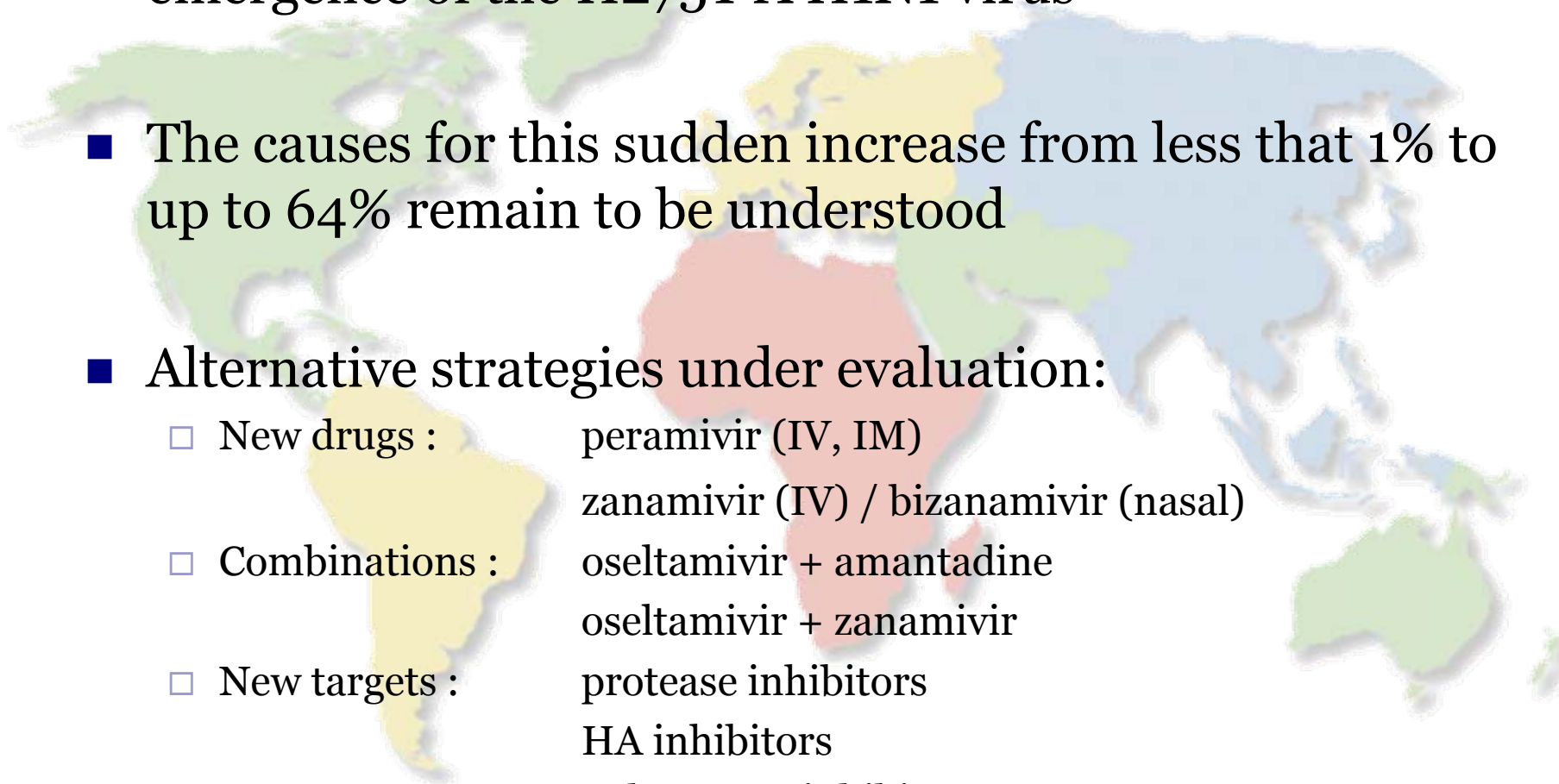
# Theoretical mechanisms leading to resistance and large diffusion of NAI resistant H1N1

- No selective pressure due to prior use of antivirals
  - Limited use of NAI in Europe
  - High use of NAI in Japan / no or very little resistance (3%)
  
- No obvious difference in clinical presentation, severity, transmissibility between H275 and Y275 strains
  
- Selection of a new variant :
  - A/New Caledonia/20/99
  - A/Brisbane/59/2007 (H1N1) : better NA enzymatic activity
  
- Compensatory mutations in NA, HA and/or other viral gene ?

# NAI resistant H5N1

- A(H5N1) 275Y after oseltamivir treatment
  - 3 cases reported in Vietnam  
*(De Jong et al., NEJM 2005; Le et al., Nature 2005)*
  
- Replicative capacity
  - Lower viral titers in ferrets infected by NAI resistant A(H5N1) resistants *(Le et al., Nature 2005)*
  - Recombinants virus : A/Vietnam/1203/2004 with H275Y and N295S mutations *(Yen et al., J Virol 2007)*
    - => Similar in vitro replication

# Conclusion

- Virgil network implemented in time to monitor the emergence of the H275Y A H1N1 virus
  - The causes for this sudden increase from less than 1% to up to 64% remain to be understood
  - Alternative strategies under evaluation:
    - New drugs :            peramivir (IV, IM)  
                              zanamivir (IV) / bizonamivir (nasal)
    - Combinations :        oseltamivir + amantadine  
                              oseltamivir + zanamivir
    - New targets :            protease inhibitors  
                                  HA inhibitors  
                                  polymerase inhibitors
- 

# Acknowledgements

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