

Human and animal surveillance in live bird markets in Cambodia demonstrating the intense circulation of several avian influenza subtypes including HPAI A(H5N1)

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Mutations on the HA associated to better adaptation to mammalian cells

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# Disclosure

- I am a GSK employee

# Areas with confirmed human cases for avian influenza A(H5N1) reported to WHO, 2013

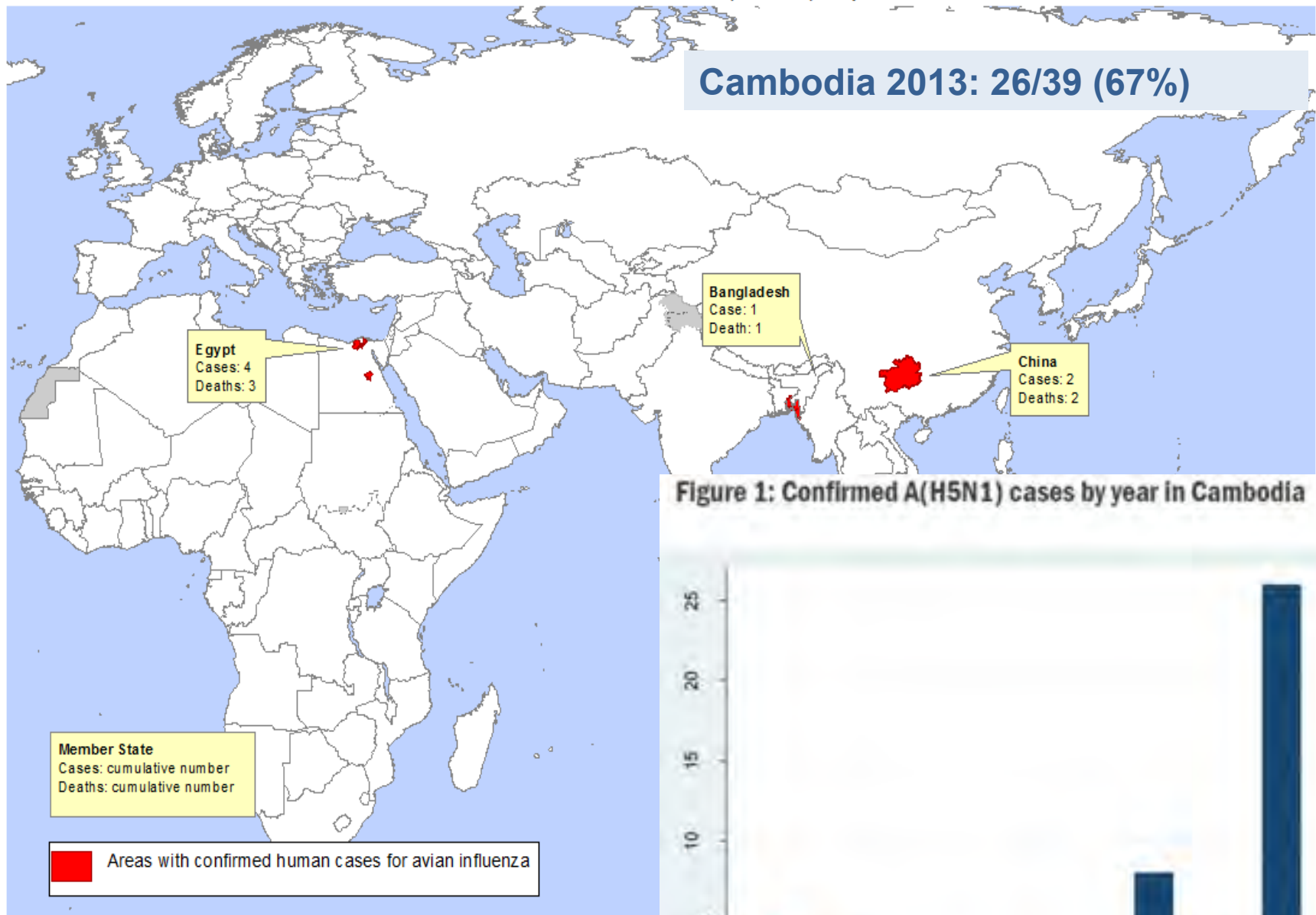
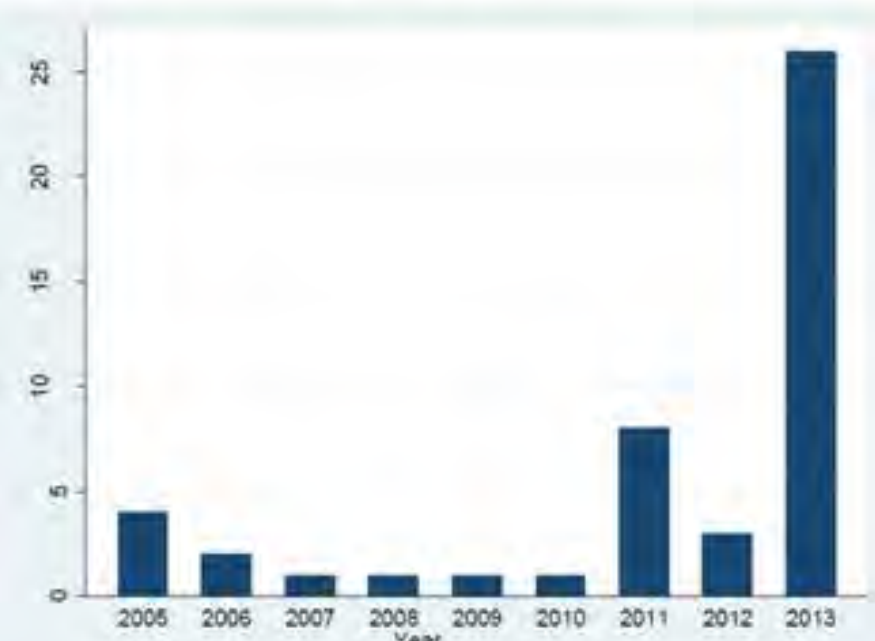


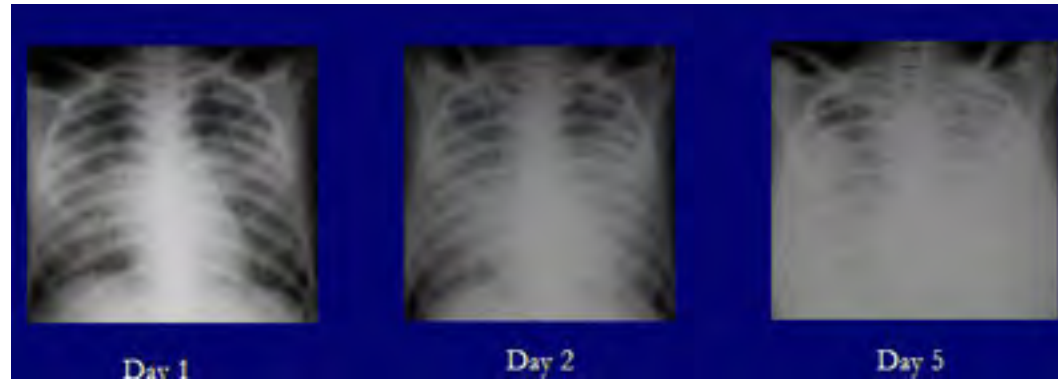
Figure 1: Confirmed A(H5N1) cases by year in Cambodia



\*All dates refer to onset of illness  
Data as of 10 December 2013  
Source: WHO/GIP

# Case fatality rate of detected cases

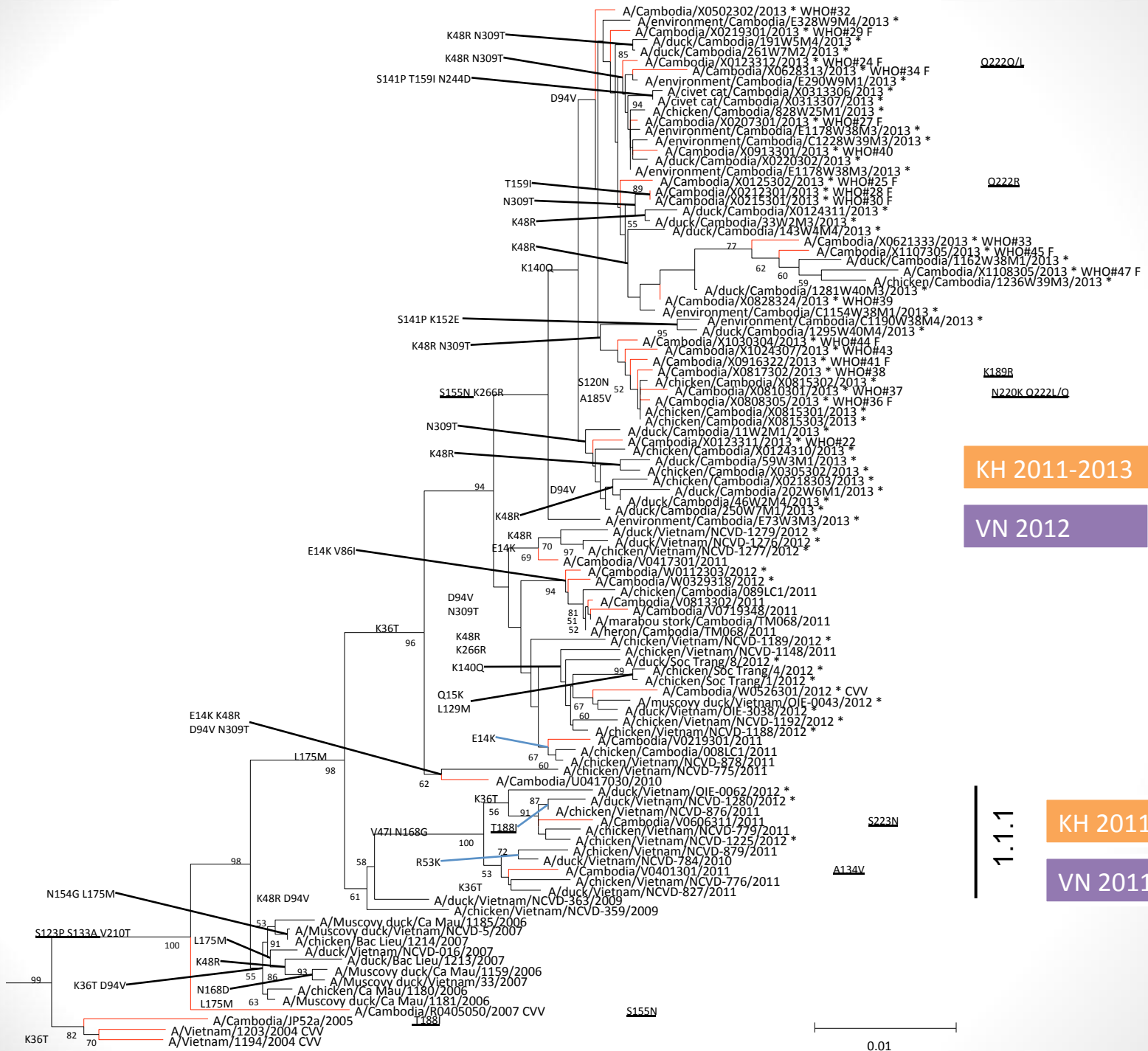
- Before 2013: **90%**
- In 2013: **54%**
- In 2014: **44%**



Source: [www.AuntMinnie.com](http://www.AuntMinnie.com)

- Better detection of human cases (including milder cases)?
- Better clinical management?
- Change in virulence?

HA



1.1.2

KH 2011-2013

VN 2012

1.1.1

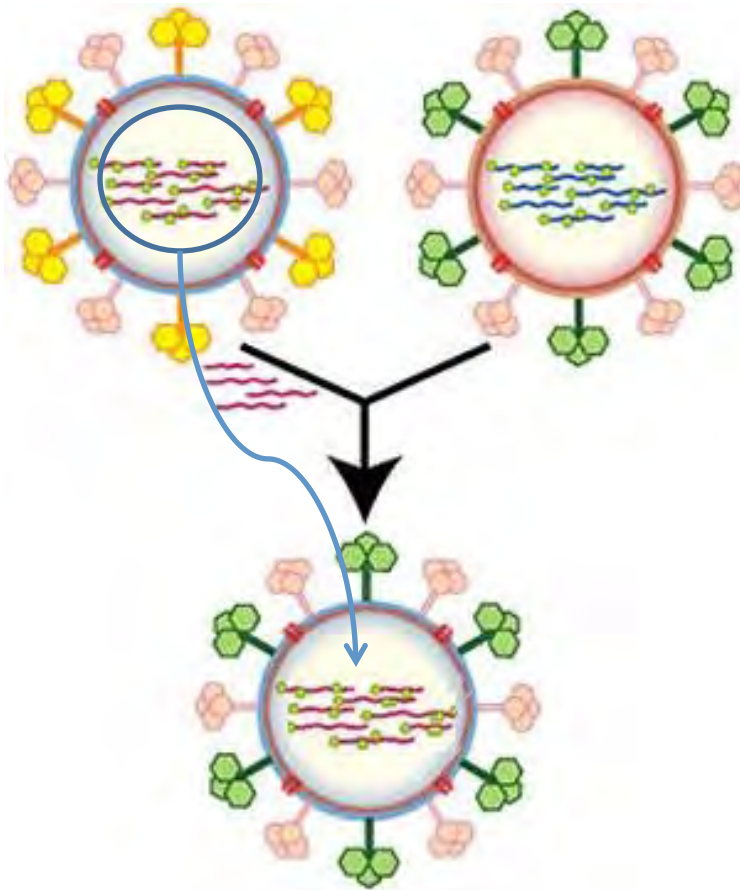
KH 2011

VN 2011-2012

0.01

# New genotype of clade 1.1.2 H5N1 virus in Cambodia 2013

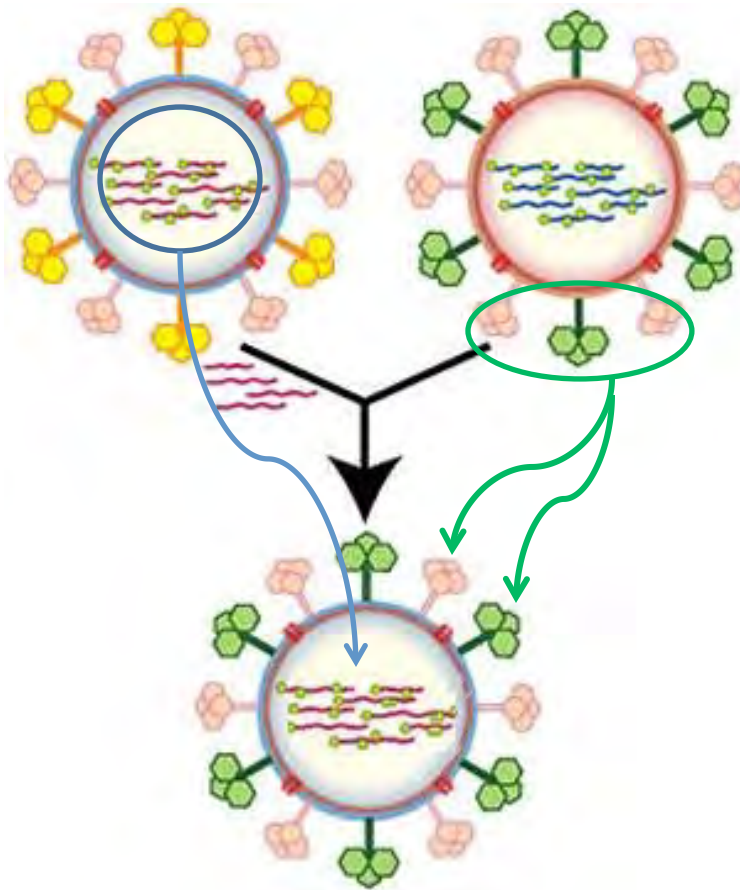
Clade 2.3.2.1



Clade 1.1.2 genotype Z

# New genotype of clade 1.1.2 H5N1 virus in Cambodia 2013

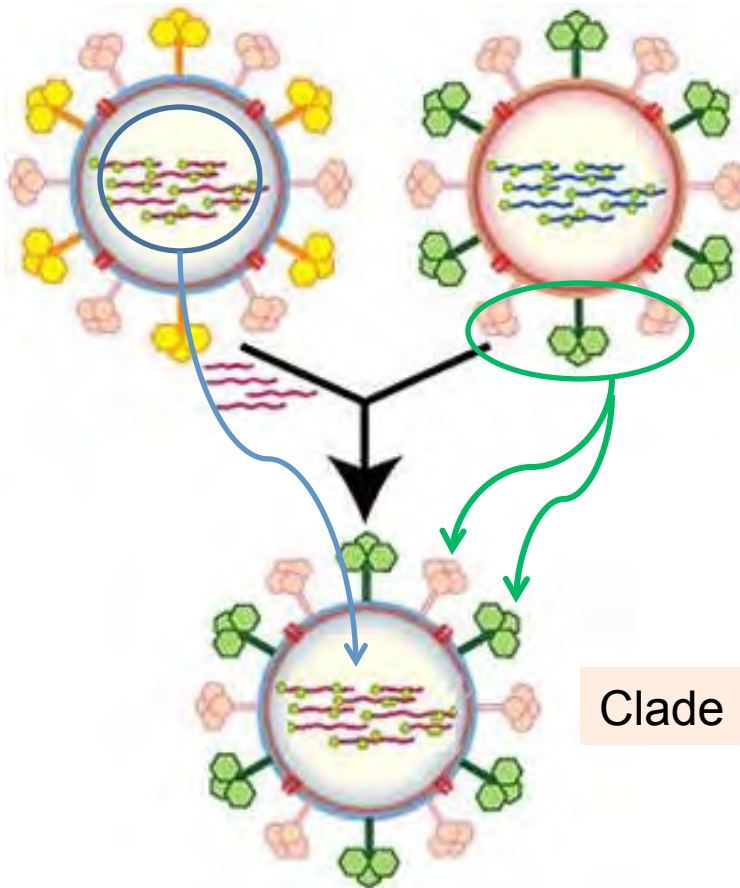
Clade 2.3.2.1



Clade 1.1.2 genotype Z

# New genotype of clade 1.1.2 H5N1 virus in Cambodia 2013

Clade 2.3.2.1



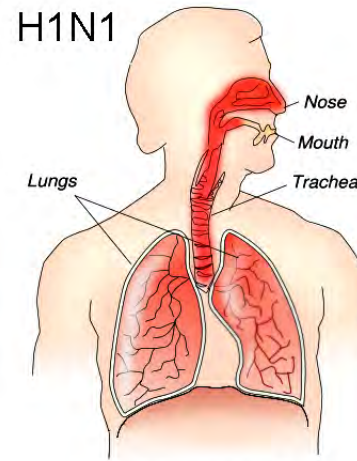
Clade 1.1.2 genotype Z

Clade 1.1.2 « **new genotype** »



- **Human influenza viruses**

- bind to  $\alpha 2-6$  receptors
- Epithelial cells of upper resp tract
- Usually mild disease

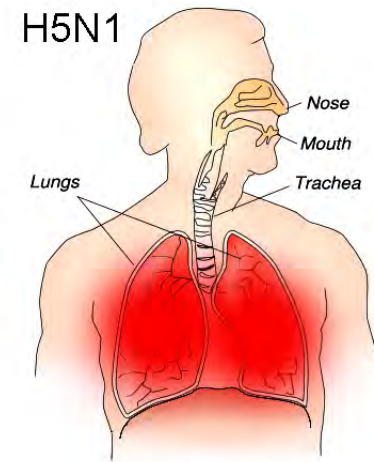


Easily spread  
Rarely fatal

*Chan RWY et al. Virus Res. 2013.*

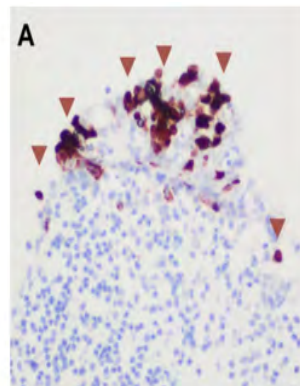
| Seasonal H1N1                 | Seasonal H3N2  | H7N7 (2003)          | H1N1pdm (2009)                |
|-------------------------------|--|----------------------|-------------------------------|
| No data                       | No data  | No data              | No data                       |
| Yes (ciliated > goblet cells) | Yes (ciliated > goblet cells)                          | No Data              | Yes (Ciliated > goblet cells) |
| Yes                           | Yes  | No data              | Yes                           |
| Yes                           | Yes  | No data              | No data                       |
| No                            | Minimal  | Yes                  | No data                       |
| Yes                           | Yes  | Yes                  | No data                       |
| van Riel et al. (2010, 2007)  | Chutinimitkul et al. (2010),<br>van Riel et al. (2007) | de Wit et al. (2010) | van Riel et al. (2010)        |

- **Avian influenza viruses**
  - bind to  **$\alpha 2-3$**  receptors
  - Type 2 pneumocytes in lung
  - Severe pulmonary disease

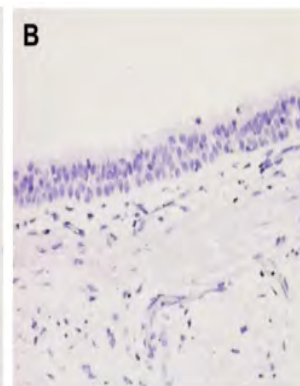


Spreads slowly  
Often fatal

Nasopharynx



Bronchiole



Alveolar epithelium

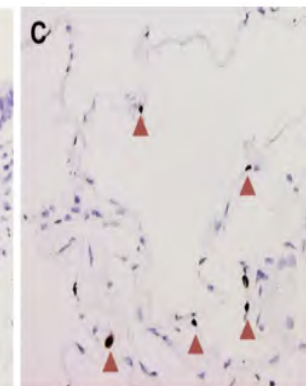


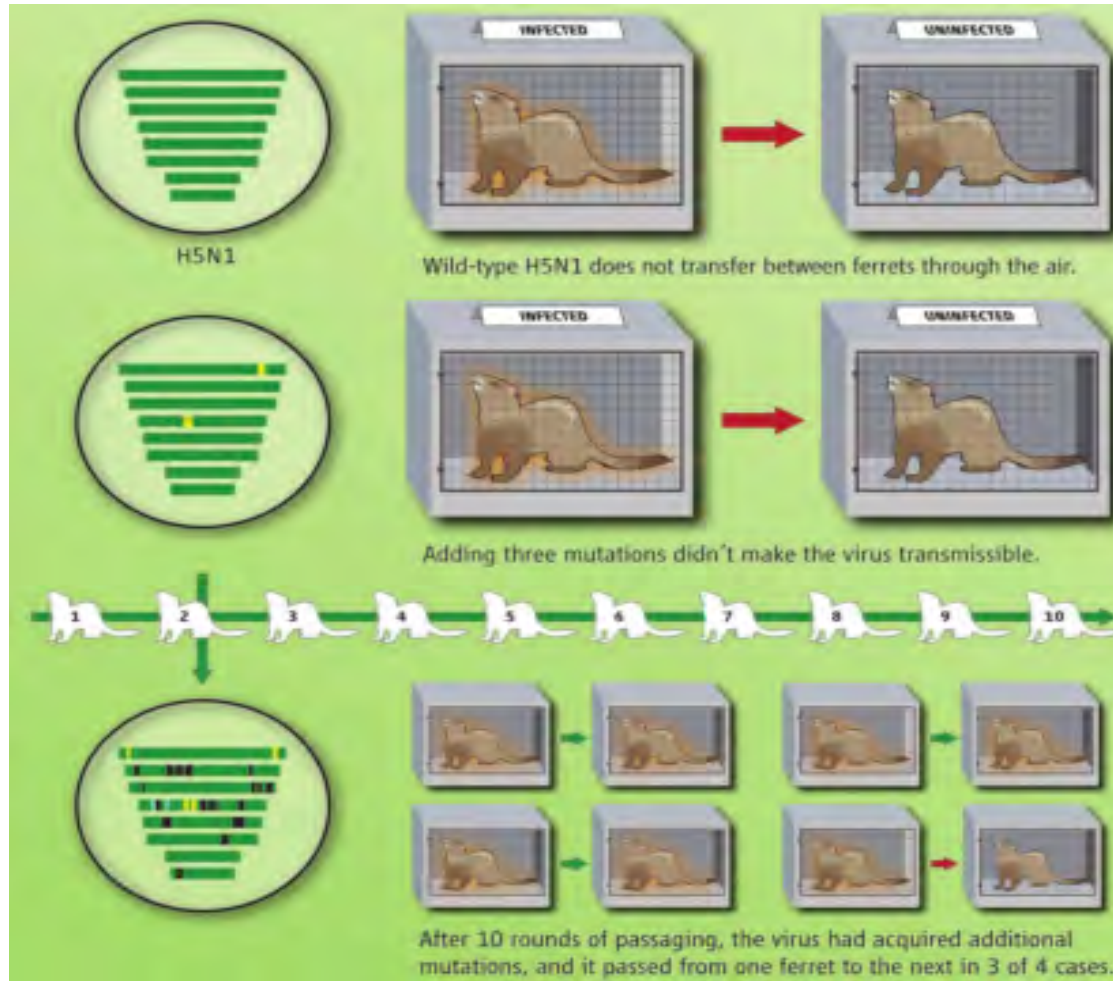
Fig 1. H5N1 virus infected *ex vivo* cultures of human (A) nasopharyngeal, (B) bronchial and (C) alveolar epithelium with red arrows indicating the infected cells stained positive with influenza viral antigen in reddish brown.

Chan RWY et al. *Virus Res.* 2013.

HPAV H5N1

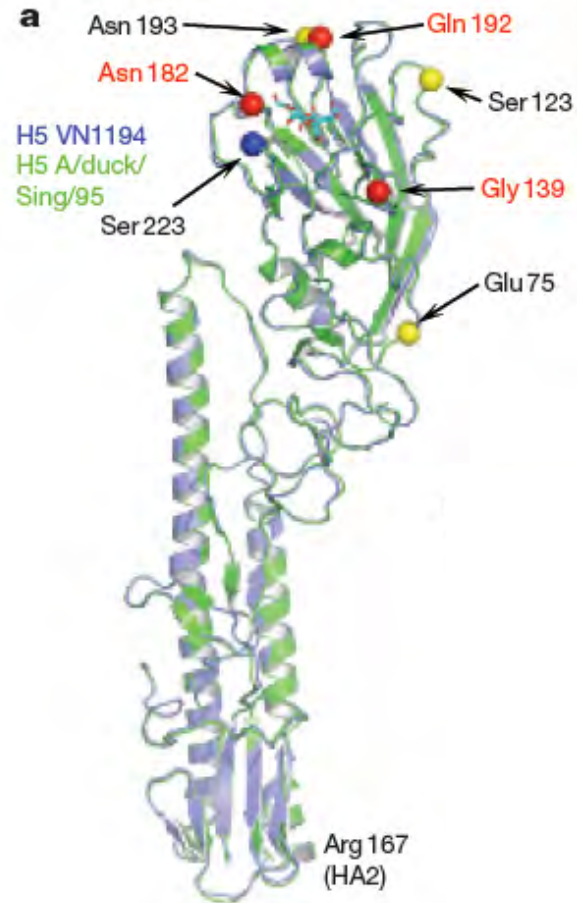
|                         |   |
|-------------------------|---|
| <i>Virus attachment</i> |   |
| Conjunctiva             | No data   |
| Nasal/Nasopharynx       | No (scant binding to nasopharynx and paranasal sinuses, ciliated cells) |
| Trachea                 | No  |
| Bronchus                | Minimal   |
| Bronchiole              | Yes   |
| Lung                    | Yes (Type II)   |
| References              | Chutinimitkul et al. (2010), van Riel et al. (2006, 2007)               |

# Mutations of associated with airborne transmission in ferrets



# Mutations in H5N1 strains isolated in Cambodia in 2013

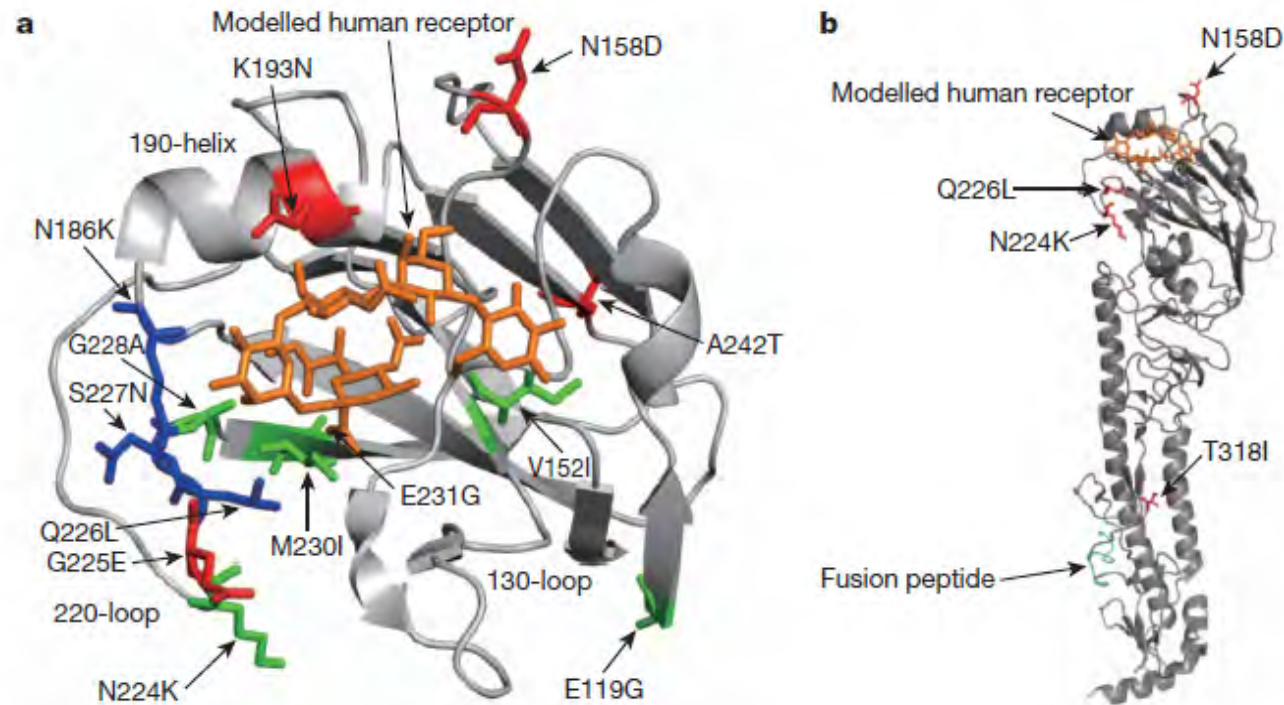
- Mutations present in all Cambodian strains already before 2013 and associated increased binding to human-type receptors
  - S123P (HA)
  - S133A (HA)
  - S155N (HA)



Yang *et al.*, EID 2007  
Wang *et al.*, J Virol 2010  
Yamada *et al.*, Nature 2006

Yamada *et al.*, Nature 2006

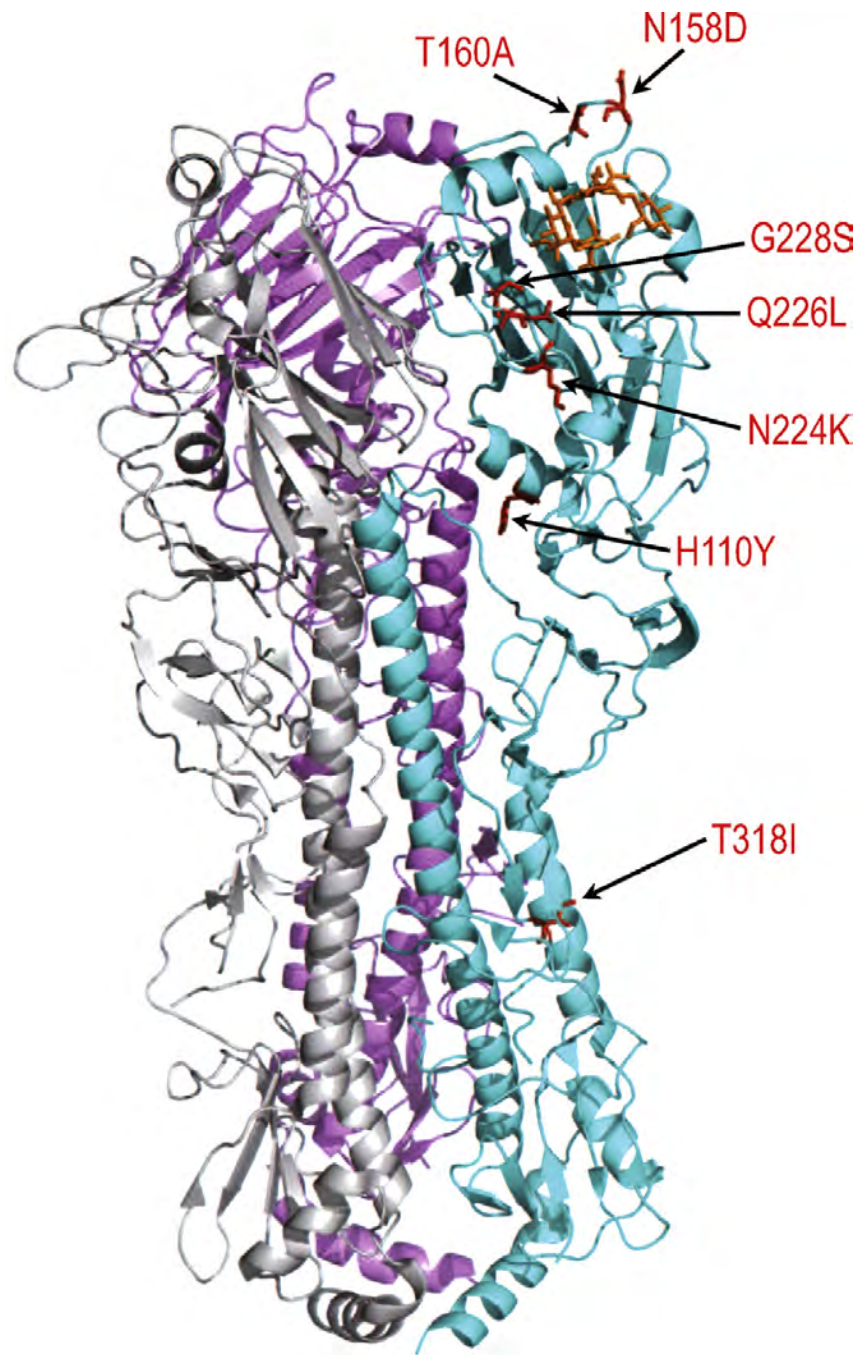
# Mutations of associated with airborne transmission in ferrets

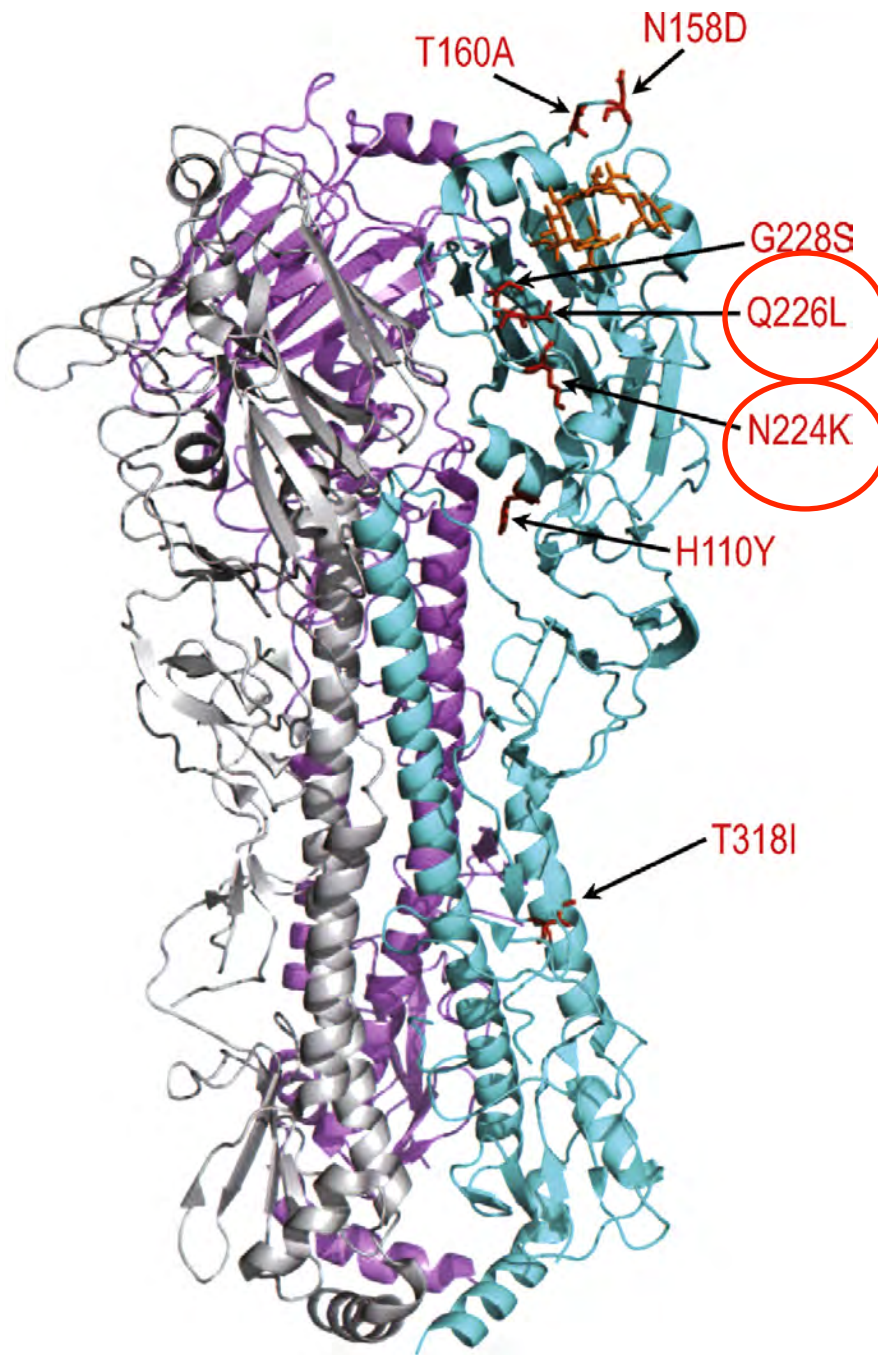


■ Mutations known to increase affinity to human-type receptors

■ Mutations not previously known to affect receptor binding

■ 4 mutations found in reassortant transmissible mutant






Patient n°37, 2013

# Mutations of adaptation to human-type receptors and/or airborne transmission

- Human case 38: S123P, S155N, K189R




Increased  
binding to  
 $\alpha$ 2-6 receptors



# Mutations of adaptation to human-type receptors and/or airborne transmission

- Human case 38 : S123P, S155N, **K189R**
- Human case 37: S123P, S155N, **N200K + Q222L**



**Enhanced  
respiratory drop  
transmission in  
ferret model**

# Mutations of adaptation to human-type receptors and/or airborne transmission

- Human case 38 : S123P, S155N, K189R
- Human case 37: S123P, S155N, N200K, Q222L
- Human case 24: S123P, S155N, Q222L/Q



Sub-populations



# Mutations of adaptation to human-type receptors and/or airborne transmission

- Human case 38 : S123P, S155N, K189R
- Human case 37: S123P, S155N, N200K, Q222L
- Human case 24: S123P, S155N, Q222L/Q
- Human case 25: S123P, S155N, Q222R/Q



Sub-populations

# Other gene segments analysis

- Human case 38 : S123P, S155N, K189R
- Human case 37: S123P, S155N, N200K, Q222L
- Human case 24: S123P, S155N, Q222L/Q
- Human case 25: S123P, S155N, Q222R/Q
  
- **NA:**
  - V129A (↓sens. Zanamivir) in all 2013 strains
  - I203T (↓sens. Oseltamivir) in case 37
- **PB2:**
  - R368Q (Polymerase activity & ↑ virulence in mice) in all 2013 strains
  - M28I, T339R (Polymerase activity & ↑ virulence in mice) in some
- **NS1**
  - P42S, L98F, I101M, N200S (↑ virulence in mice and ↓ antiviral response) in all 2013 strains

# Conclusion 1

- 1st identification in human avian influenza cases of the association of 2 of the 4 mutations described in the ferret-transmissible H5 mutant
  - Critical mutations on the RBS detected as subpopulations in several human cases but not in poultry
- **Mutations of adaptation in human developed during patient's infection**
- No evidence of human-to-human transmission

# Market surveillance

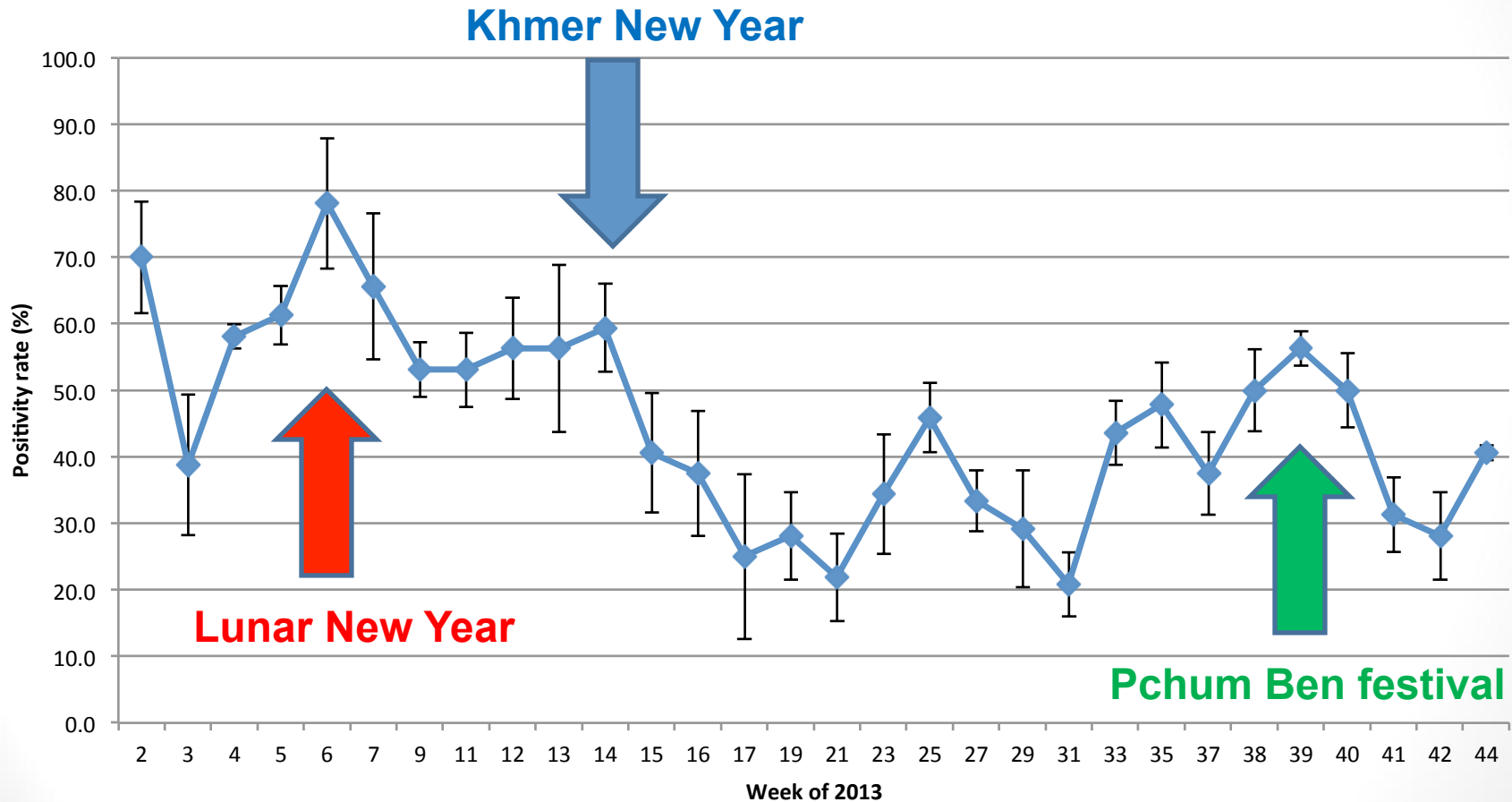


# Live bird market study

- 1st live bird market (LBMs) environment study conducted in 2011: Environment in LBMs in Cambodia is highly contaminated with H5N1 virus (18% viral RNA, 2% infectious virus) (Horm et al., EID 2013)
- 2nd study (conducted in 2013)
  - 4 sites (3 markets and 1 slaughterhouse)
  - **1048 environmental** and animal samples of LBMs
    - Water, mud/soil, discarded feathers
    - Oropharyngeal and cloacal swabs
  - Close monitoring of poultry workers
    - Blood samples from **125 poultry workers**, sellers and middlemen collected at 4 time points during the year

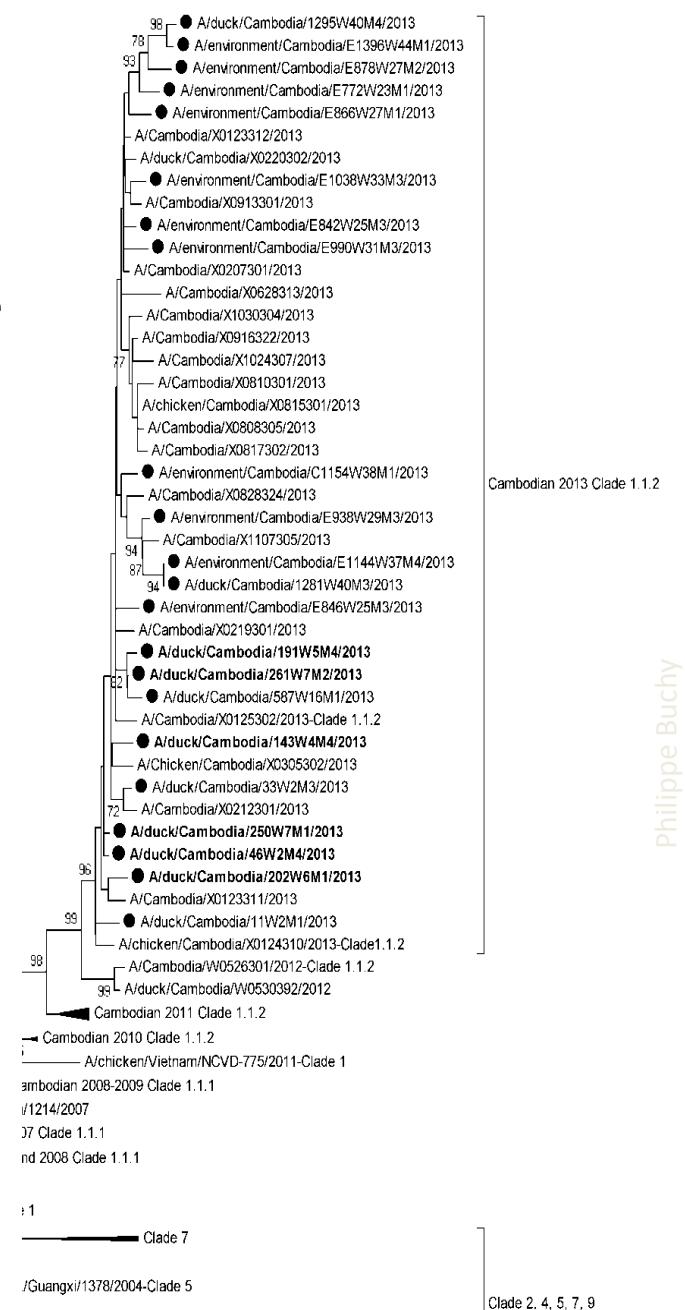


# Proportion of environment samples positive for Influenza A virus



# Influenza A typing

- Overall, **45%** of the samples tested positive for influenza A.
- H5N1 detected in **59%** of the environment samples (3.3 times more than in 2011) and in **28%** of the poultry swabs.
- During the Lunar New Year, the proportion of environment samples that tested positive reached **78%**.
- All H5N1 strains detected belonged to the **new genotype** (reassortant clade 1.1.2 / clade 2.3.2.1)
- Large variety of over avian influenza viruses also detected: HA1, HA2, HA3, HA4, HA6, HA7, HA9, HA10, and HA11.



Cambodian 2013 Clade 1.1.2

Clade 2, 4, 5, 7, 9

# Serosurvey results

- Serosurvey of **125 poultry workers** to monitor the risk of H5N1, H9N2 and H7N9 infection
- Acute H5N1 infection during the study – 2.4% (n=3)
- Acute H9N2 infection during the study – 1% (n=1)
- Recent infection with H5N1 -- 1% (n=1)
- Recent infection with H9N2 -- 1% (n=1)
- Possible recent or acute infection with H5N1 – 12% (n=15)
- Possible recent or acute infection with H9N2 – 6.4% (n=8)
- No antibodies to H7N9 detected
- **High exposure risk in LBM environment**

## Conclusion 2

- Complex & dynamic H5N1 situation in Cambodia in 2013
  - Reassortment, mutations, etc
  - Increased transmission of reassortant strains?
- Need improved surveillance in the poultry sector
  - Circulation of viruses
  - Introduction of new viruses
- Very high circulation of H5N1 and other avian influenza viruses in LBMs
  - Introduction of interventions?
- Importance of continuous **molecular surveillance** of avian influenza strains isolated from clinical specimens and to maintain/promote **research** on avian influenza viruses

# Acknowledgments

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WHO Collaborating Centre  
for Reference and  
Research on Influenza  
VIDRL



Centers for Disease Control and Prevention  
CDC 24/7: Saving Lives. Protecting People™



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# THANK YOU

