



# OFFLU contribution to the WHO vaccine selection meeting

***WHO Consultation on the Composition  
of Influenza Vaccines for the Southern  
Hemisphere 2012 (20-22 Feb 2012,  
Geneva)***

*Giovanni Cattoli*

*OFFLU Annual technical meeting  
4-5 April 2012 – Royal Holloway, London*



## Acknowledgements

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WHO

*With gratitude to all the contributing labs  
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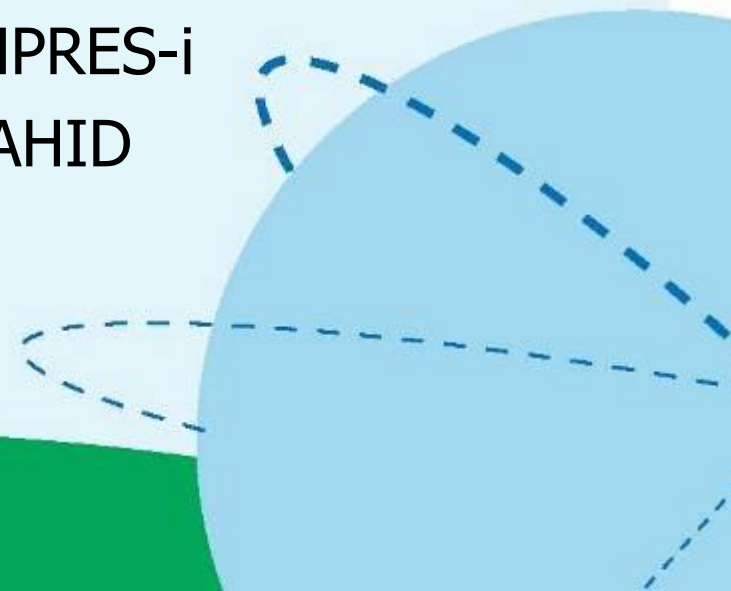
# OFFLU sources of information

## **Genetic information**

- OIE/FAO reference centers
- National laboratories
- FAO national and regional offices

## **Epidemiological info**

- EMPRES-i
- WAHID



# Distribution of H5 outbreaks:

## 1 Sept 2011 to 13 Feb 2012

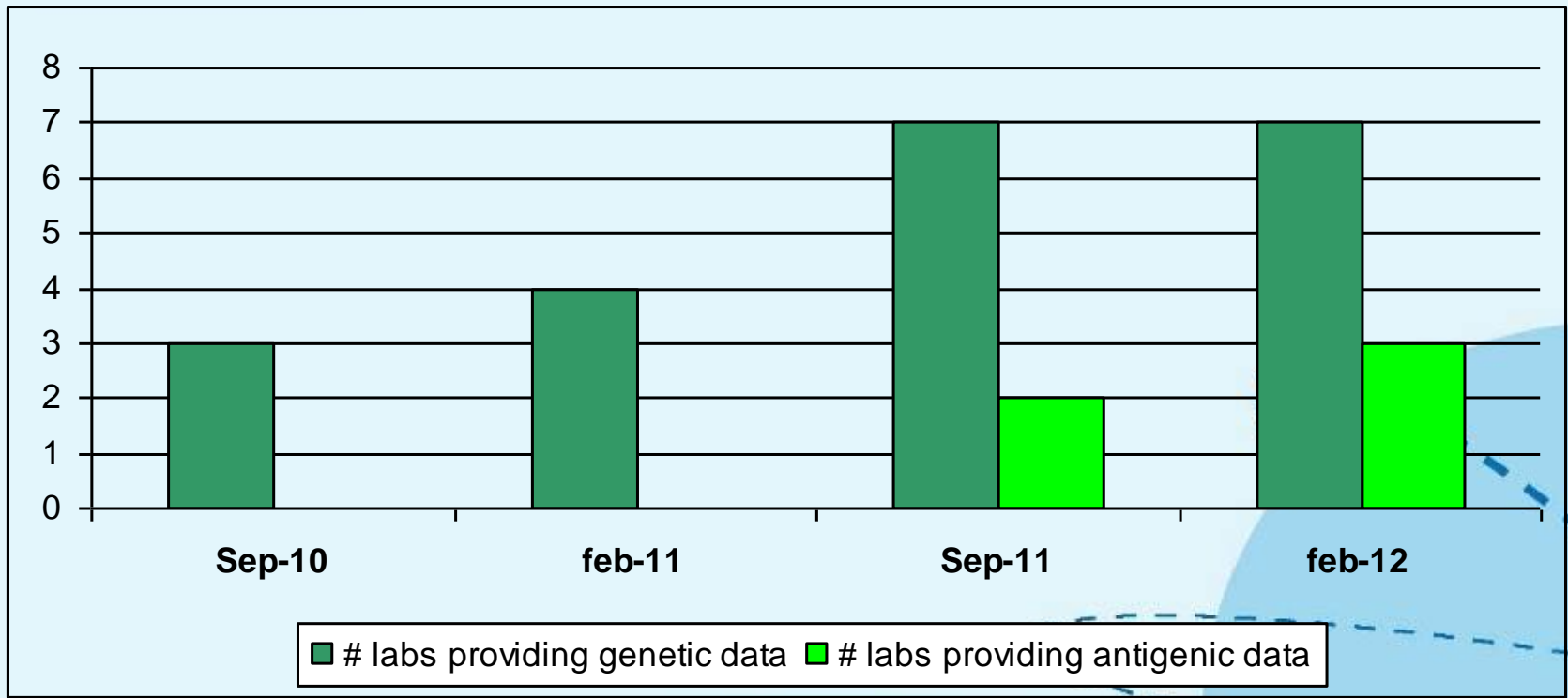
(source: EMPRES-i - includes WAHID entries)



Country	chicken / plus	duck/ geese	unspecified bird	wild	Grand Total
Bangladesh	10				10
Bhutan			2		2
Cambodia	1		1		2
China			3		3
China, Hong Kong SAR				2	2
Egypt	31	2	5		38
Hong Kong	1	1		4	6
India	2		4	1	7
Indonesia	113		13		126
Iran (Islamic Republic of)	1	2			3
Nepal	1				1
Viet Nam	2		4		6
<b>Grand Total</b>	<b>162</b>	<b>5</b>	<b>32</b>	<b>7</b>	<b>206</b>

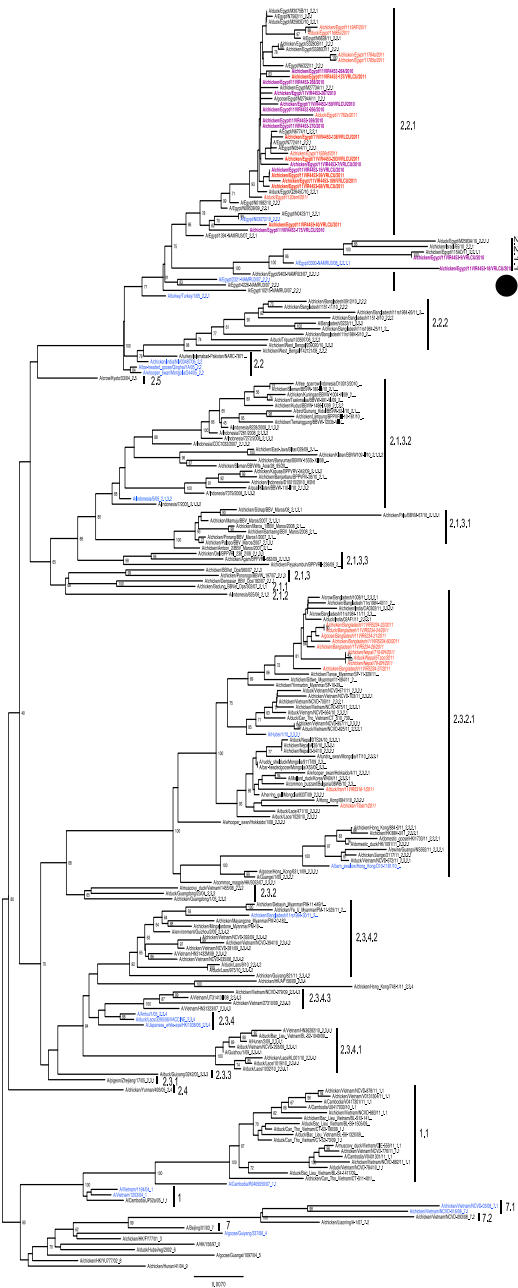


## Evolution of OFFLU contribution to VCM



# OFFLU H5 sequences: 1 Sept 2011 to 13 Feb 2012

- 39 Sequences from 3 active H5N1 clades
  - 2011: **RED ITALICS (non-public), RED BOLD (public)**
  - 2009-2010: **PURPLE ITALICS (non-public)**



Clade	Country	chicken - ducks	duck	goose	turkey	Grand Total
2.2.1	Egypt	22	2			24
	Israel				2	2
2.2.1 Total		22	2		2	26
2.2.1.1	Egypt	2				2
2.2.1.1 Total		2				2
2.3.2.1	Bangladesh	4	1		1	6
	Iran		1			1
	Nepal	2	1			3
	Tibet *	1				1
2.3.2.1 Total		7	3		1	11
<b>Grand Total</b>		<b>30</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>39</b>
* not reported in epidemiologic data						

**Table 1. Influenza A(H5N1) activity reported from 20 September 2011 to 22 February 2012**

Country, area or territory	Host	Genetic clade
Bangladesh	Poultry	2.2.2/2.3.2.1
	Wild birds	2.3.2.1
Bhutan	Poultry	unknown
Cambodia	Poultry	1.1
	Wild birds	1.1
	Human (1)*	1.1
China	Poultry	2.3.2.1
	Human (2)	2.3.2.1/2.3.4.2
China, Hong Kong SAR	Poultry	2.3.2.1
	Wild birds	2.3.2.1
Egypt	Poultry	2.2.1/2.2.1.1
	Humans (9)	2.2.1 [5] <sup>#</sup>
India	Poultry	unknown
	Wild bird	unknown
Indonesia	Poultry	unknown
	Humans (7)	2.1.3.2 [2]
Iran (Islamic Republic of)	Poultry	2.3.2.1
Nepal	Poultry	2.3.2.1
	Wild birds	2.3.2.1
Viet Nam	Poultry	1.1/2.3.2.1
	Humans (2)	1.1 [2]

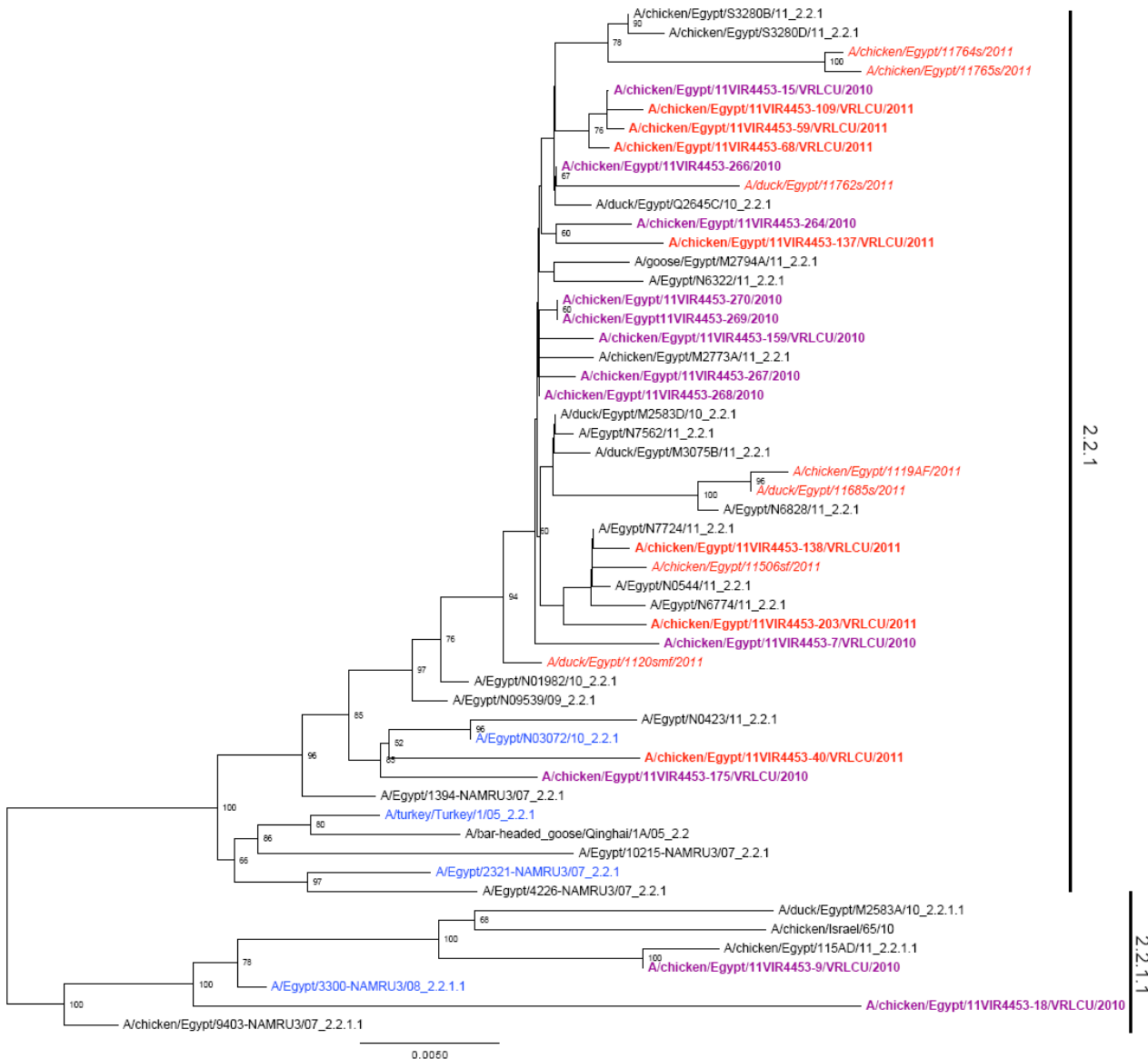
\*number in parentheses denotes number of reported cases during this period

<sup>#</sup>number in brackets denotes the number of viruses for which genetic information is available

# CLADE 2.2.1/2.2.1.1

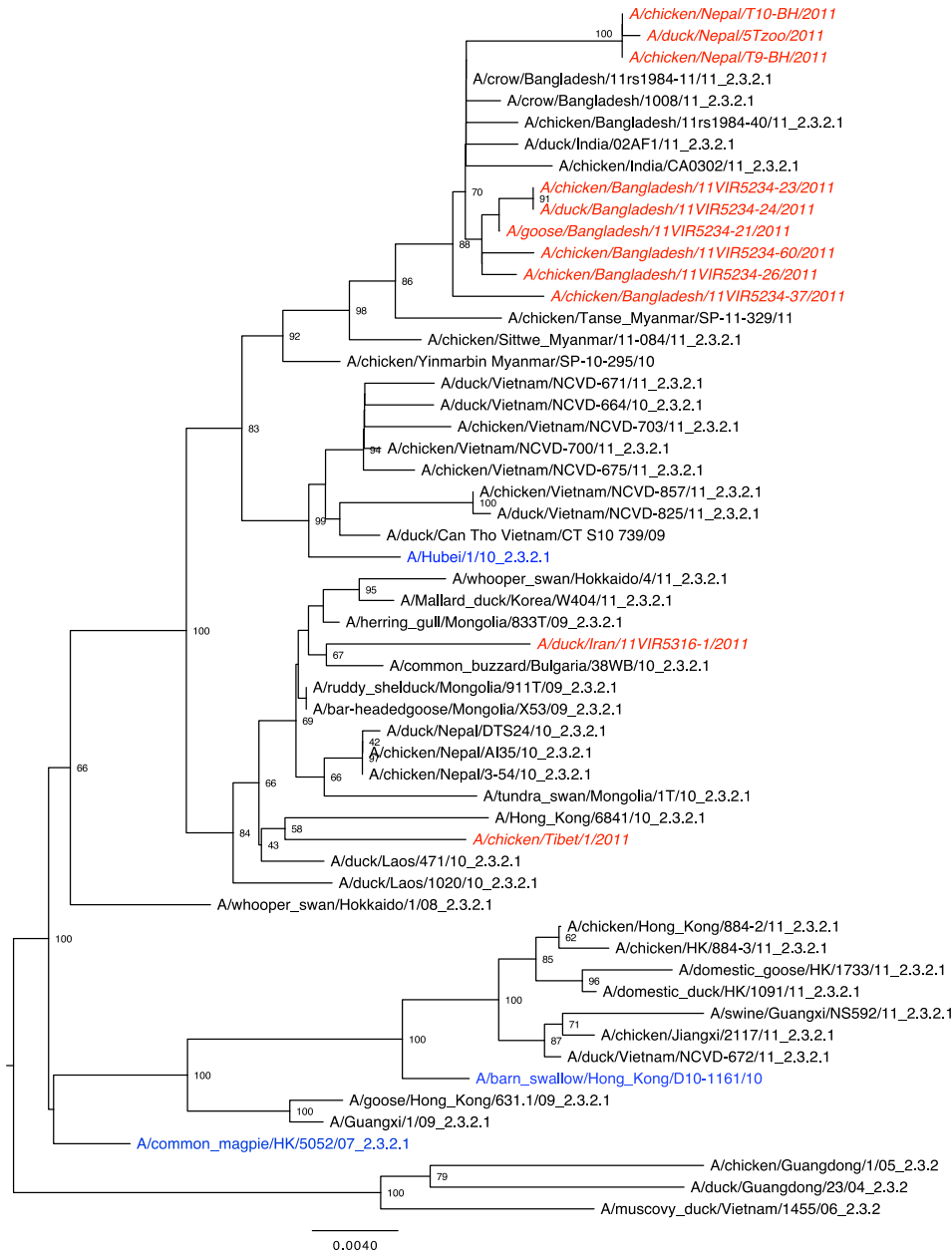
## Preliminary residue analysis

- All the novel Egyptian 2.2.1 viruses included have **129 deletion and substitution I151T (RBS)**
- AA changes in HA1
  - 2.2.1: 9-12 (compared to 321/07)
  - 2.2.1.1: 8-12 (compared to 3300-NAMRU/2008)





# CLADE 2.3.2.1



– Iran, Bangladesh, PR China, Nepal

➤ **Hubei-like viruses**

Residue analysis

- *No AA substitutions in potential RBS*
- *5-9 AA changes in HA1 compared to A/Hubei/1/10*

# Ferret antisera used to generate antigenic (HI) data

sera	clade	clade	antigen
Anti-rg-A/VN/1203/04	1	1	rg-A/VN/1203/04
Anti-rg-A/Duck/Hunan/795/2002	2.1	1	rg-A/Cambodia/R0H05050/2007 (no homolog)
Anti-rg-A/Bar-Headed goose/Qinghai/IA/05	2.2	2.1	rg-A/Duck/Hunan/795/2002
Anti-rg-A/Whooper Swan/Mongolia/244/05	2.2	2.2	rg-A/Bar-Headed goose/Qinghai/IA/05
Anti-A/turkey/Israel/364/2006 (no homolog)	2.2	2.2	rg-A/Whooper Swan/Mongolia/244/05
Anti-A/turkey/Islamabad-Pakistan/NARC-7871/2007 (no homolog)	2.2	2.2	rg-A/India/NIV-33487/06 (no homolog)
Anti-rg-A/Egypt/321/07	2.2.1	2.2.1	rg-A/Egypt/321-NAMRU3/07
Anti-rg-A/Common Magpie/Hong Kong/5052/2007	2.3.2	2.2.1	rg-A/Egypt/3300-NAMRU3/08 (no homolog)
Anti-A/common buzzard/Bulgaria/38WB/2010 (no homolog)	2.3.2	2.3.2	rg-A/Common Magpie/Hong Kong/5052/2007
Anti-A/Muscovy duck/Vietnam/33/2007 (no homolog)	2.3.2	2.3.4	rg-A/Japanese White Eye/HongKong/1038/06
Anti-A/barn swallow/HK/1161/2010 (no homolog)	2.3.2	2.3.4	rg-A/Ck/Hk/AP156/08
Anti-rg-A/Japanese White Eye/HongKong/1038/06	2.3.4	2.3.4	rg-A/Duck/Laos/3295/06
Anti-rg-A/Ck/Hk/AP156/08	2.3.4	2.3.4	rg-A/Anhui/01/2005 (no homolog)
Anti-rg-A/Duck/Laos/3295/06	2.3.4	4	rg-A/goose/Guiyan/337/06
Anti-rg-A/Goose/Guiyan/337/06	4	7	rg-A/chicken/Vietnam/NCVD-016/08 (no homolog)

# WHO ferret anti-sera panel

## Test antigens



### Antigenic data for WHO ferret panel | VCM FEBRUARY 2012

HI/chicken/sera treatment: 1:4 RDE; O/N 37°C; 56°CX30min; +PBS: final dilution 1:10

Lab	clade	Test antigens	clade 2.2.1	clade 2.3.2			
			Anti-rg- A/Egypt/321/07	A/Common Magpie/Hong Kong/5052/2007	A/common buzzard/Bulgaria/38WB/2010 (no	A/Muscovy duck/Vietnam/33/2007 (no homolog)	Anti-A/barn swallow/HK/1161/2010 (no homolog)
St Judes	2.2.1	<i>rg-A/Egypt/321-NAMRU3/07</i>	<b>160</b>	20	20	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-59/VRLCU/2011	80	<10	10	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-40/VRLCU/2011	40	40	<10	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-138/VRLCU/2011	80	<10	20	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-137/VRLCU/2011	80	<10	20	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-7/VRLCU/2010	10	<10	10	20	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-203/VRLCU/2011	20	<10	<10	<10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-270/2010	40	<10	20	<10	<10
IZSve	2.2.1 sub B	A/chicken/Egypt/11VIR4453-9/VRLCU/2010	40	<10	<10	<10	<10
IZSve	2.2.1 sub B	A/chicken/Egypt/11VIR4453-18/VRLCU/2010	<10	80	<10	<10	<10
IZSve	2.3.2	A/goose/Bangladesh/11Vir5234-21/2011	80	160	80	<10	<10
St Judes	2.3.2	<i>rg-A/Common Magpie/Hong Kong/5052/2007</i>	20	<b>160</b>	40	<10	<10
AHVLA	2.2.1	A/turkey/Israel/341/11	80	<10	n/d	n/d	<10
AHVLA	2.3.2	A/crow/Nepal/84/12	<10	40	n/d	n/d	<10
AHVLA	2.3.2	A/chicken/Nepal/1447/11	20	40	n/d	n/d	<10

# CLADE 2.2.1/2.2.1



## Antigenic data for WHO ferret panel | VCM FEBRUARY 2012

HI/chicken/sera treatment: 1:4 RDE; O/N 37°C; 56°CX30min; +PBS: final dilution 1:10

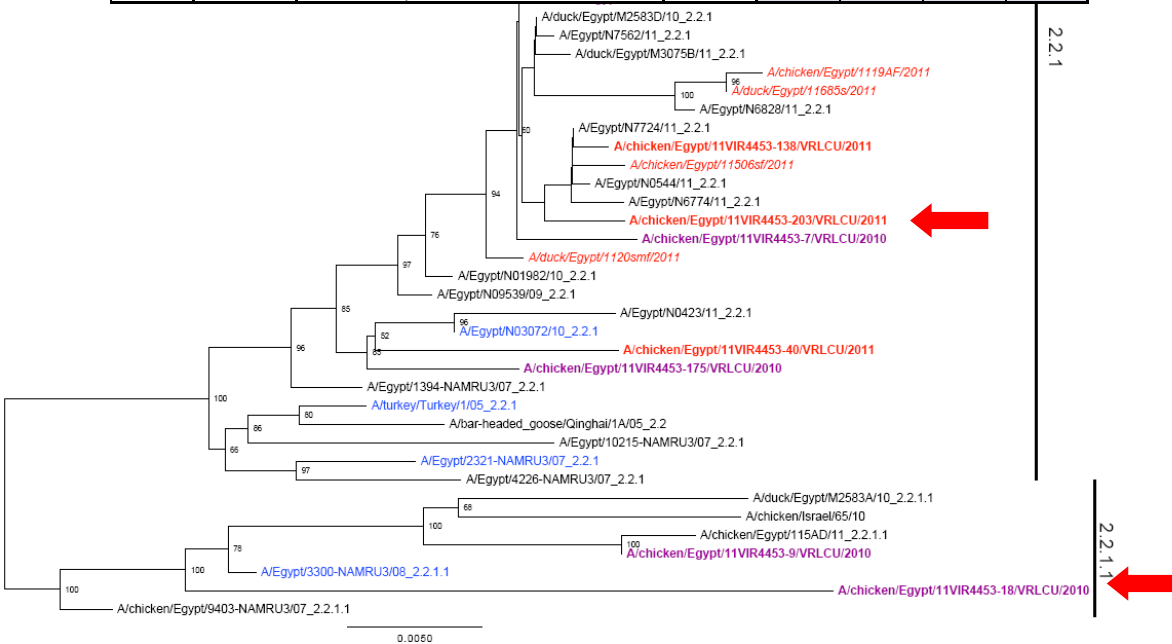
Lab	clade	Test antigens	clade 2.2.1	clade 2.3.2			
			Anti-rg- A/Egypt/321/07	A/Common Magpie/Hong Kong/5052/2007	A/common buzard/Bulg aria/38WB/2010 (no homolog)	A/Muscovy duck/Vietna m/33/2007 (no homolog)	Anti-A/barn swallow/HK/ 1161/2010 (no homolog)
St Judes	2.2.1	rg-A/Egypt/321-NAMRU3/07	160	20	20	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-59/VRLCU/2011	80	<10	10	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-40/VRLCU/2011	40	40	<10	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-138/VRLCU/2011	80	<10	20	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-137/VRLCU/2011	80	<10	20	10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-7/VRLCU/2010	10	<10	10	20	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-203/VRLCU/2011	20	<10	<10	<10	<10
IZSve	2.2.1 sub A	A/chicken/Egypt/11VIR4453-270/2010	40	<10	20	<10	<10
IZSve	2.2.1 sub B	A/chicken/Egypt/11VIR4453-9/VRLCU/2010	40	<10	<10	<10	<10
IZSve	2.2.1 sub B	A/chicken/Egypt/11VIR4453-18/VRLCU/2010	<10	80	<10	<10	<10
IZSve	2.3.2	A/goose/Bangladesh/11Vir5234-21/2011	80	160	80	<10	<10
St Judes	2.3.2	rg-A/Common Magpie/Hong Kong/5052/2007	20	160	40	<10	<10
AHVLA	2.2.1	A/turkey/Israel/341/11	80	<10	n/d	n/d	<10
AHVLA	2.3.2	A/crow/Nepal/84/12	<10	40	n/d	n/d	<10
AHVLA	2.3.2	A/chicken/Nepal/1447/11	20	40	n/d	n/d	<10

- AA substitutions compared to the other novel viruses

- **4453-203**  
N/D155A Antigenic site A

- **4453-7**  
D/S120N Antigenic site A  
R189K Antigenic site B

- **4453-18**  
S120N Antigenic site A  
T151I Antigenic site B  
K152E Antigenic site B  
I190L Antigenic site B



# OFFLU Laboratory ring trial

- **3 OFFLU laboratories involved** (IZSVE-Italy; AHVLA-UK; AAHL-AUS).
- **Main purpose** - evaluate the level of standardization and harmonization among the OFFLU laboratories of the HI methodology applied on ferret sera and avian influenza antigens according to the WHO standards.
- **Mat&Meth** - A panel of 15 ferret sera and H5N1 antigens belonging to different clades was provided by WHO CC -St Jude to all the 3 laboratories together with the protocol to be applied.

# Ferret antisera used to generate HI data and included in the ring trial



sera	clade	clade	antigen
Anti-rg-A/VN/1203/04	1	1	rg-A/VN/1203/04
Anti-rg-A/Duck/Hunan/795/2002	2.1	1	rg-A/Cambodia/R0H05050/2007 (no homolog)
Anti-rg-A/Bar-Headed goose/Qinghai/IA/05	2.2	2.1	rg-A/Duck/Hunan/795/2002
Anti-rg-A/Whooper Swan/Mongolia/244/05	2.2	2.2	rg-A/Bar-Headed goose/Qinghai/IA/05
Anti-A/turkey/Israel/364/2006 (no homolog)	2.2	2.2	rg-A/Whooper Swan/Mongolia/244/05
Anti-A/turkey/Islamabad-Pakistan/NARC-7871/2007 (no homolog)	2.2	2.2	rg-A/India/NIV-33487/06 (no homolog)
Anti-rg-A/Egypt/321/07	2.2.1	2.2.1	rg-A/Egypt/321-NAMRU3/07
Anti-rg-A/Common Magpie/Hong Kong/5052/2007	2.3.2	2.2.1	rg-A/Egypt/3300-NAMRU3/08 (no homolog)
Anti-A/common buzzard/Bulgaria/38WB/2010 (no homolog)	2.3.2	2.3.2	rg-A/Common Magpie/Hong Kong/5052/2007
Anti-A/Muscovy duck/Vietnam/33/2007 (no homolog)	2.3.2	2.3.4	rg-A/Japanese White Eye/HongKong/1038/06
Anti-A/barn swallow/HK/1161/2010 (no homolog)	2.3.2	2.3.4	rg-A/Ck/Hk/AP156/08
Anti-rg-A/Japanese White Eye/HongKong/1038/06	2.3.4	2.3.4	rg-A/Duck/Laos/3295/06
Anti-rg-A/Ck/Hk/AP156/08	2.3.4	2.3.4	rg-A/Anhui/01/2005 (no homolog)
Anti-rg-A/Duck/Laos/3295/06	2.3.4	4	rg-A/goose/Guiyan/337/06
Anti-rg-A/Goose/Guiyan/337/06	4	7	rg-A/chicken/Vietnam/NCVD-016/08 (no homolog)

# Ring trial outcomes

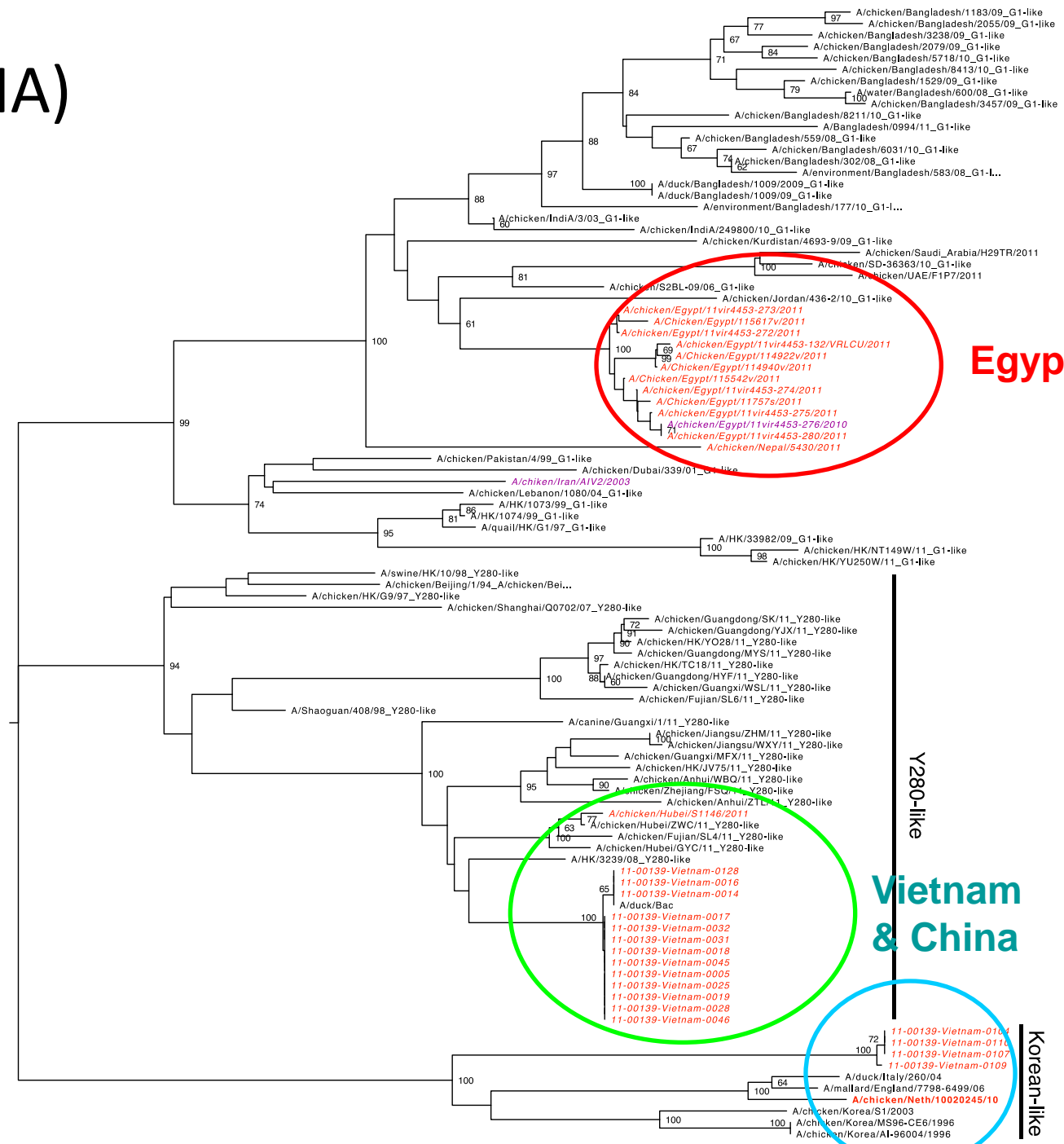
- overall the results are consistent between the 3 laboratories with variations in the HI titres limited to  $\pm \log_2$  dilution in the vast majority of cases.
- Basic statistical analysis performed on the results of the 3 labs (Kendall's coefficient of Concordance)

# OFFLU H9N2 sequences: 1 Sept 2011 to 13 Feb 2012

Year	Country	chicken	duck	quail	turkey	Grand Total
2003	Iran	1				1
2003 Total		1				1
2009	Viet Nam		17			17
2009 Total			17			17
2010	Egypt	1				1
	Netherlands	1				1
2010 Total		2				2
2011	China	1				1
	Egypt	13		1		14
	Nepal	1				1
	Netherlands				1	1
	Saudi Arabia	1				1
	UAE	1				1
2011 Total		17		1	1	19
<b>Grand Total</b>		<b>20</b>	<b>17</b>	<b>1</b>	<b>1</b>	<b>39</b>



# H9N2 (HA)



G1-like

Egypt

Y280-like

Vietnam & China

Korean-like

Vietnam & Europe

0.01

# Future activities

- Standardized panel of reference antigens and ferret sera to be distributed among OFFLU Labs
- WHO CC-OFFLU labs harmonized phylogenetic tree backbone / residue analysis
- Antigenic data on H9N2
- Candidate vaccine strains from one OFFLU lab shipped to WHO-CC (CDC) – PIP framework