# H5N1 in Egypt: Situation, OIE twinning, and escape mutants

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*Institute of Diagnostic Virology*

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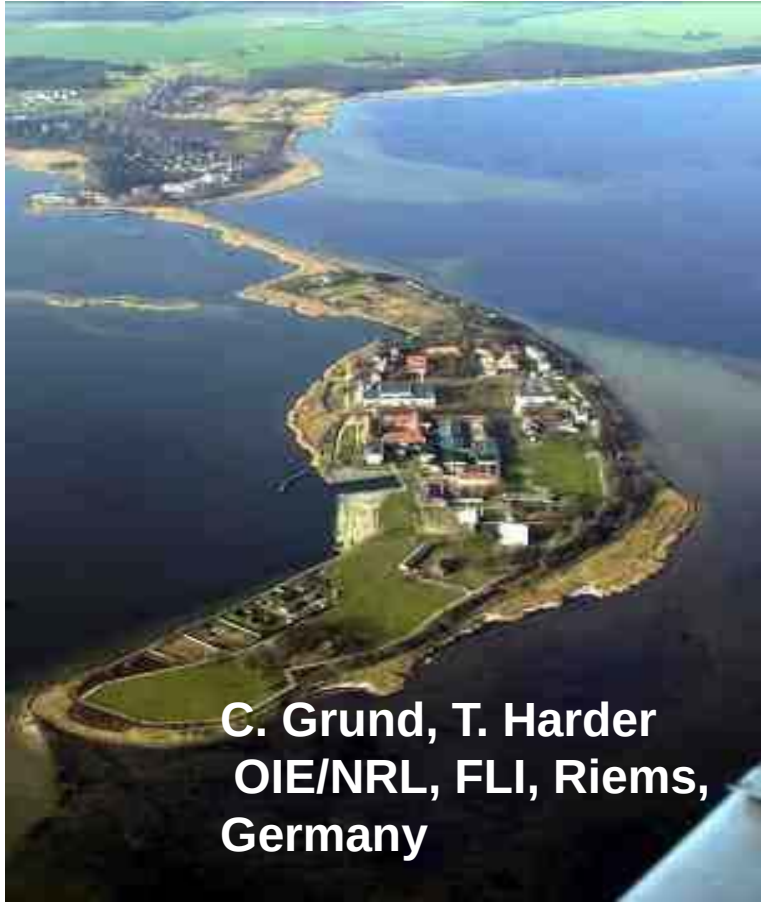
FRIEDRICH-LOEFFLER-INSTITUT

FLI

Bundesforschungsinstitut für Tiergesundheit

Federal Research Institute for Animal Health



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# Praxis of O.I.E. Laboratory Twinning

**Reference laboratories for avian influenza viruses**
**NLQP (Egypt) and FLI (Germany)**

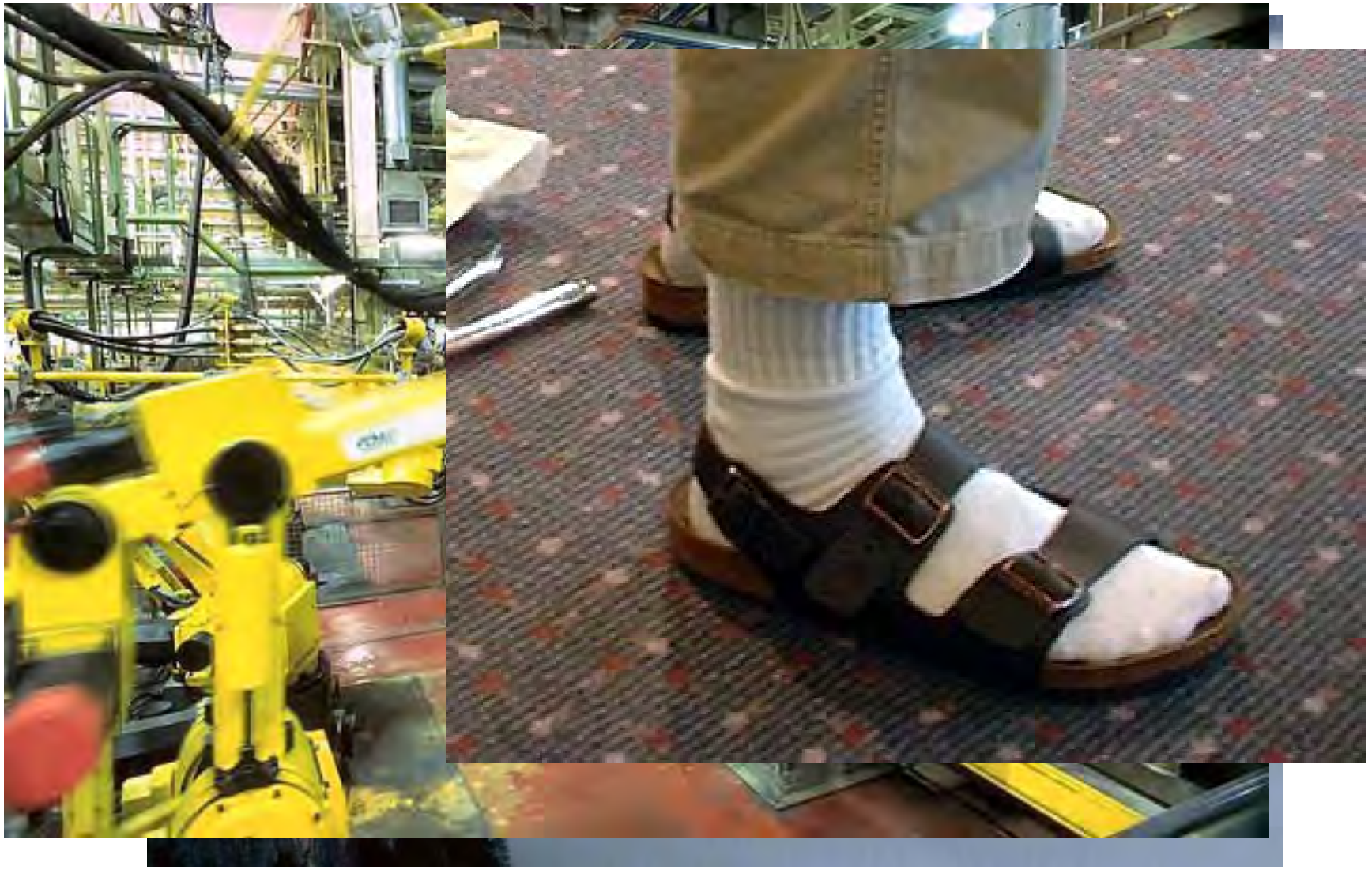
# Putatively matching twins to re-inforce AI diagnostic and management powers in Northern Africa and the Near East

**Twin-1.** Animal Health Research Institute, National Laboratory for Quality Control of Poultry Production (NLQP), Giza, Egypt

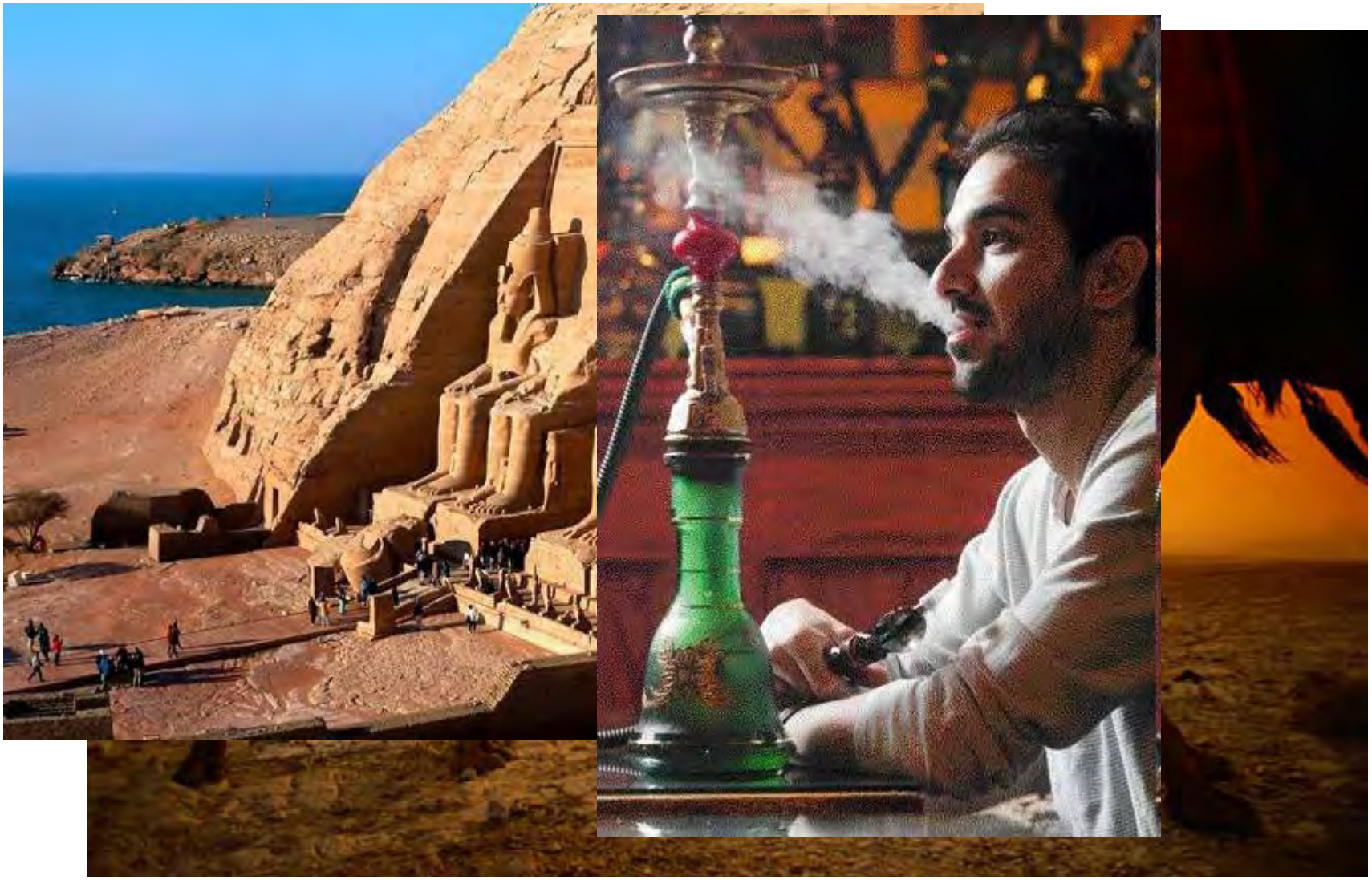
**Twin-2.** Friedrich-Loeffler-Institute, O.I.E. and National Reference Laboratories for Avian Influenza and Newcastle Disease, Isle of Riems, Germany

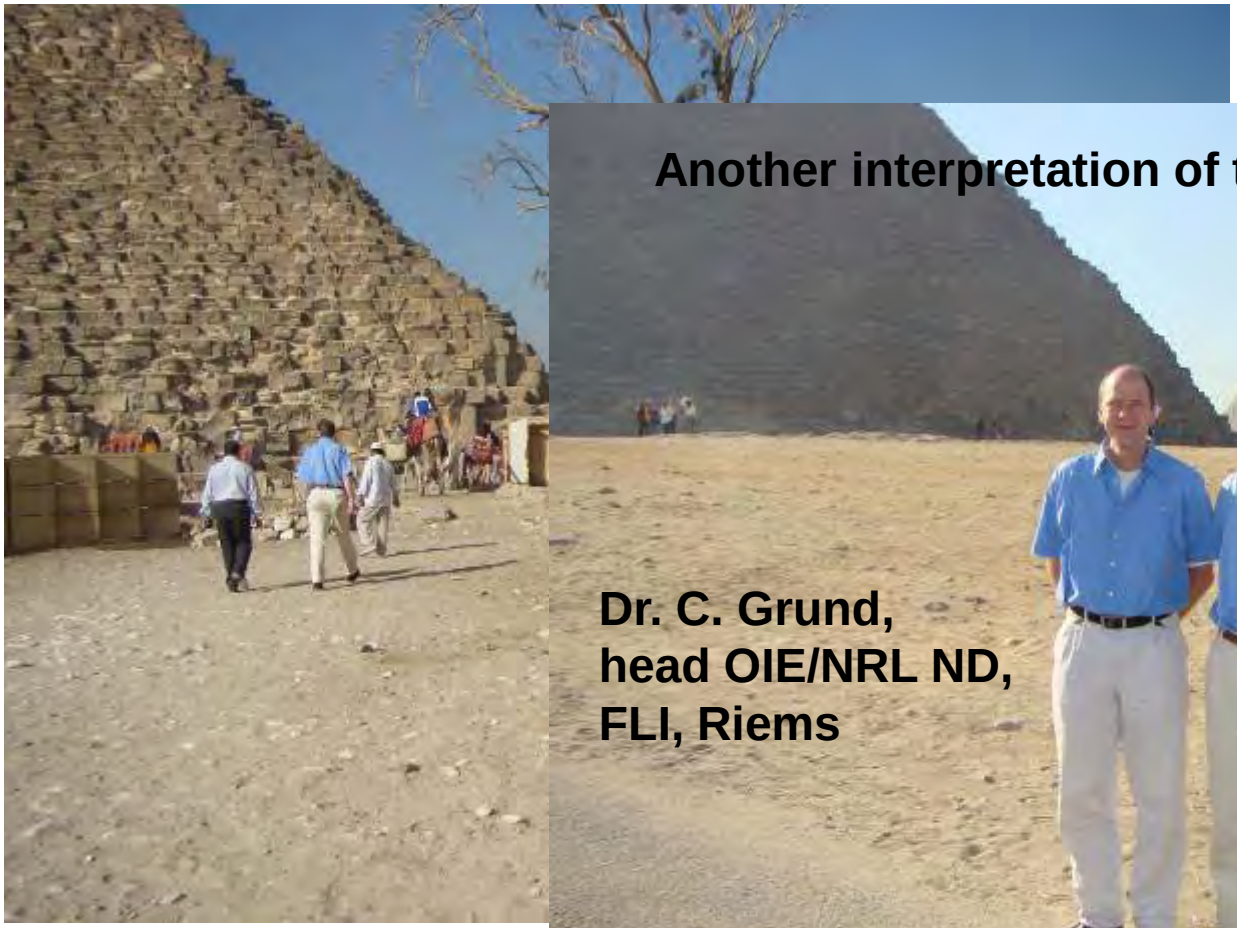
# Starting the first meeting

# Overcoming prejudices and clichés



# Overcoming prejudices and clichés



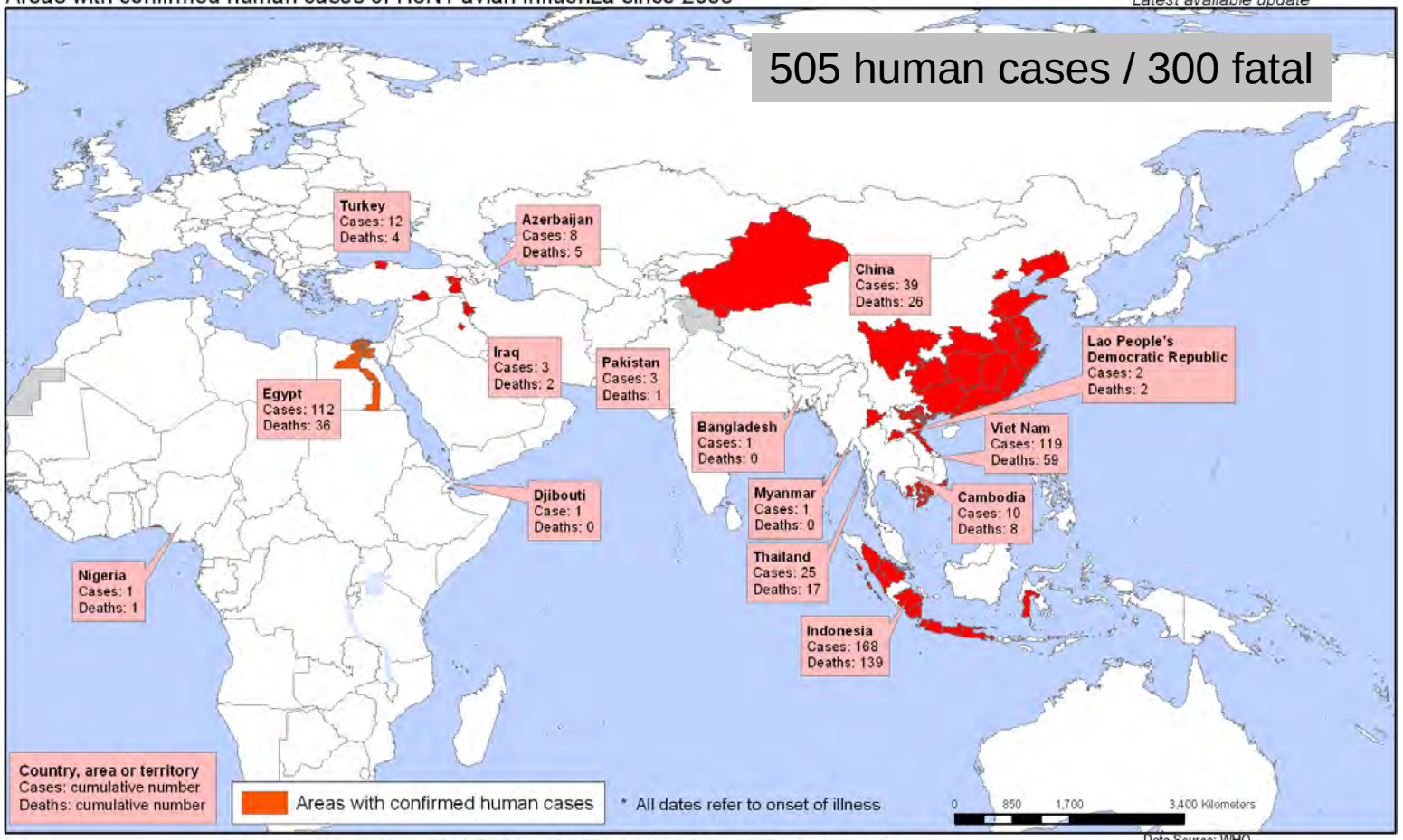


## Another interpretation of twinning?

**Dr. C. Grund,**
**head OIE/NRL ND,**
**FLI, Riems**

Areas with confirmed human cases of H5N1 avian influenza since 2003 *

*Status as of 31 August 2010*
*Latest available update*

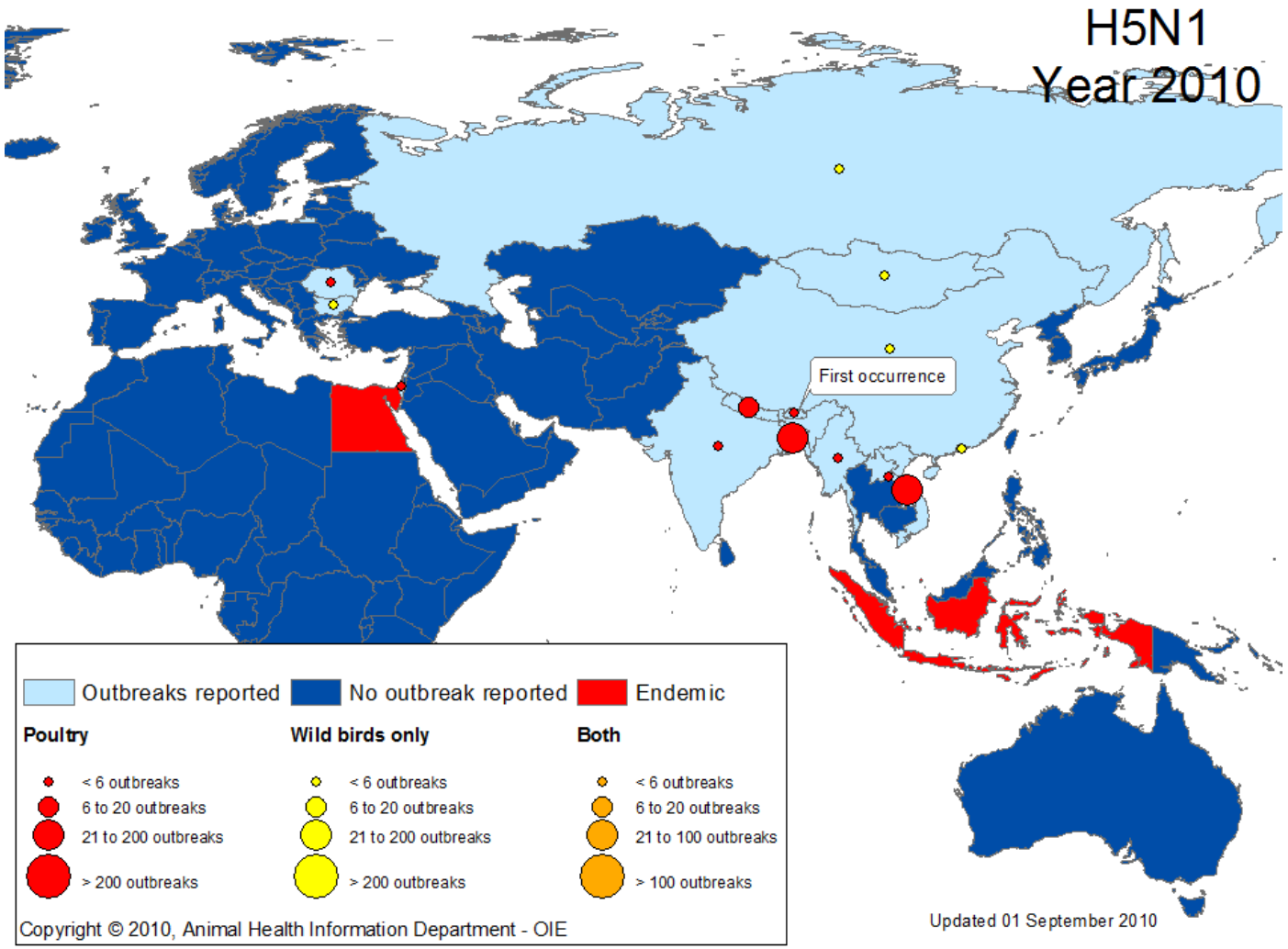



World Health Organization

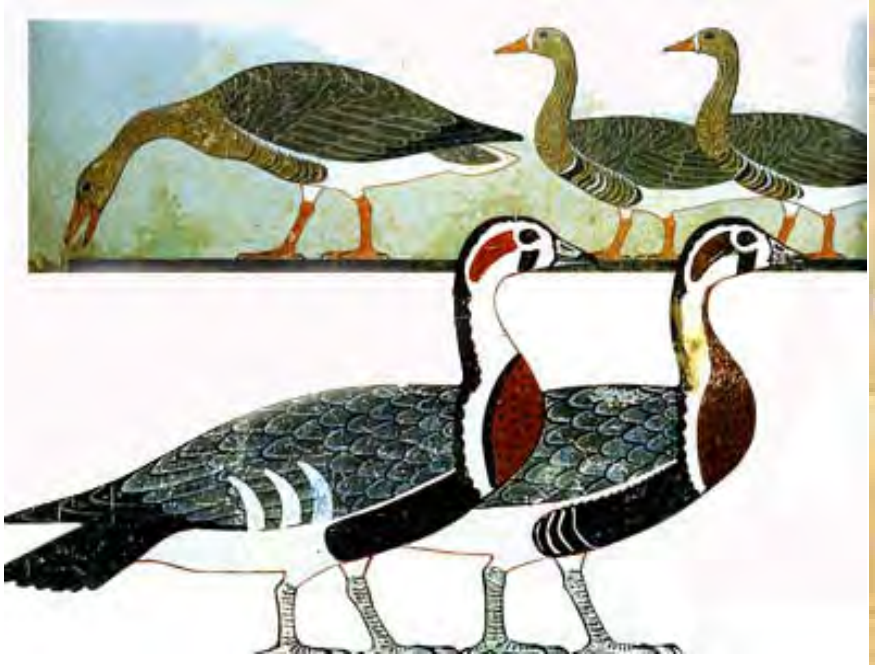


Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization

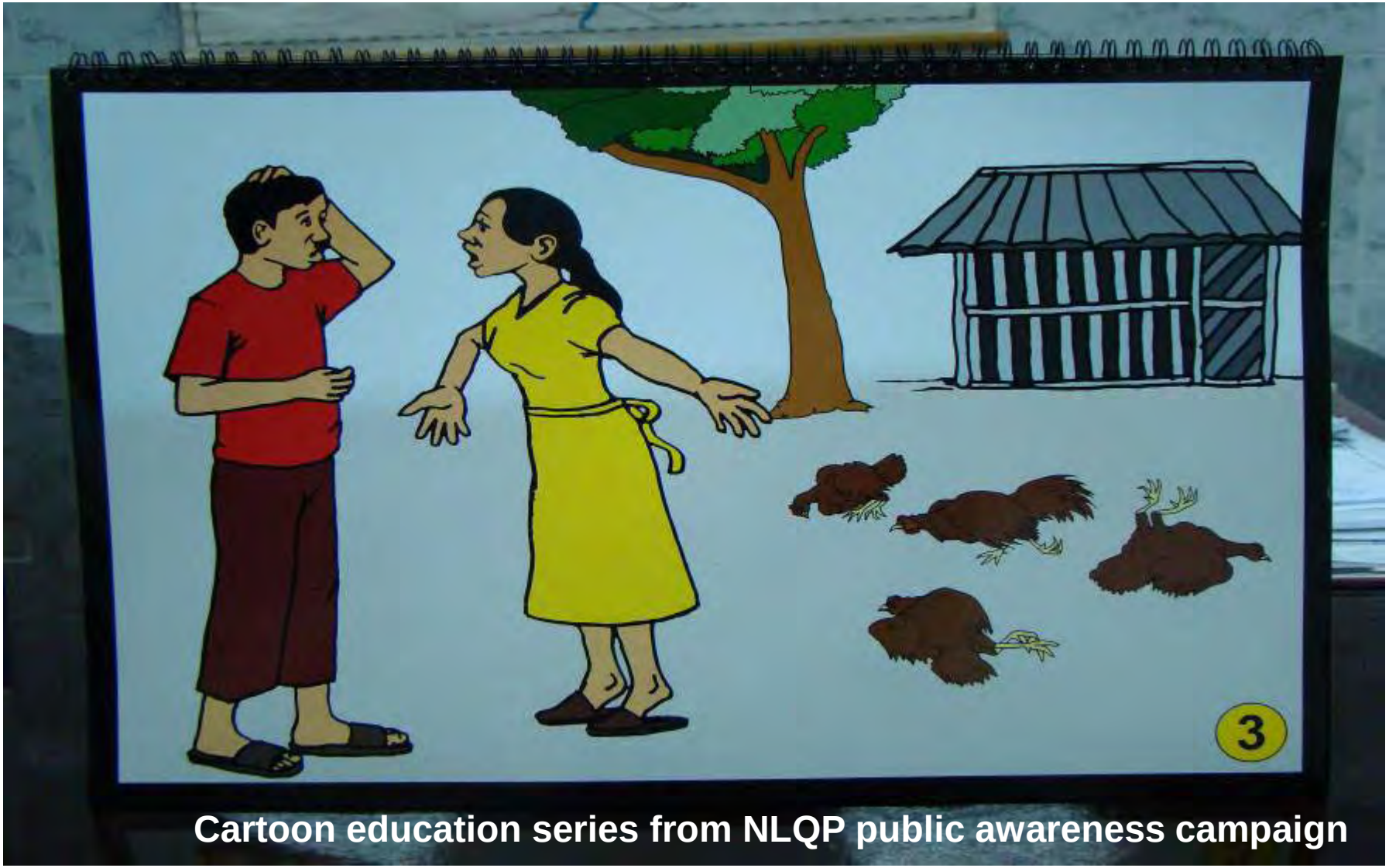
# Avian Influenza - 2010 (OIE)



# The Egyptian tree of life: It s all about birds



# Egypt today: It s all about HPAIV H5N1 infected birds




Cartoon education series from NLQP public awareness campaign

# Egypt today: It s all about HPAIV H5N1 infected birds

- Introduction of HPAIV H5N1 clade 2.2 into Egyptian poultry in 2006
- Introduction into Northern Africa by migratory birds (late 2005)
- Rapid spread along the Nile valley
- Highly fissured poultry-human interface leads to spill-over infections into humans (pandemic risks)
- Serious and frequent outbreaks in poultry on-going despite massive intervention by cull/control and vaccination campaigns
- Virus established endemicity
- Vaccine escape mutants emerged and started to circulate

# Impact of AI on Egyptian poultry industry

Poultry industry in Egypt

- Commercial sectors ~850 Mio birds,
- Backyard sectors ~ 250 Mio birds
- around 15,892 retail shops / Live bird markets

Abdelwhab et al., 2010

- estimated loss after the first emergence of HPAI H5N1 in February 2006 was 1 billion US$
- affected income of 1.5 million people whose livelihoods depended on poultry (Meleigy, 2007).
- About 30 million birds were culled or depopulated in Egypt in the first wave of 2006.

Hafez et al., 2010

# HPAIV H5N1 detection on live bird markets

| Month | No. positive LBMs | % positive LBMs/total tested | Positive LBMs/ total positive (%)[A] |
|---|---|---|---|
| January | 7 | 14 | 7/71 (9.9%) |
| February | 29 | 15 | 29/71 (40.8%) |
| March | 23 | 12 | 23/71 (32.4%) |
| April | 12 | 8 | 12/71 (16.9%) |
| Total | 71 | 12.4 | 100% |

Egyptian Natioinal Laboratory for Veterinary Quality Control on Poultry Production

Abdelwhab et al., 2010

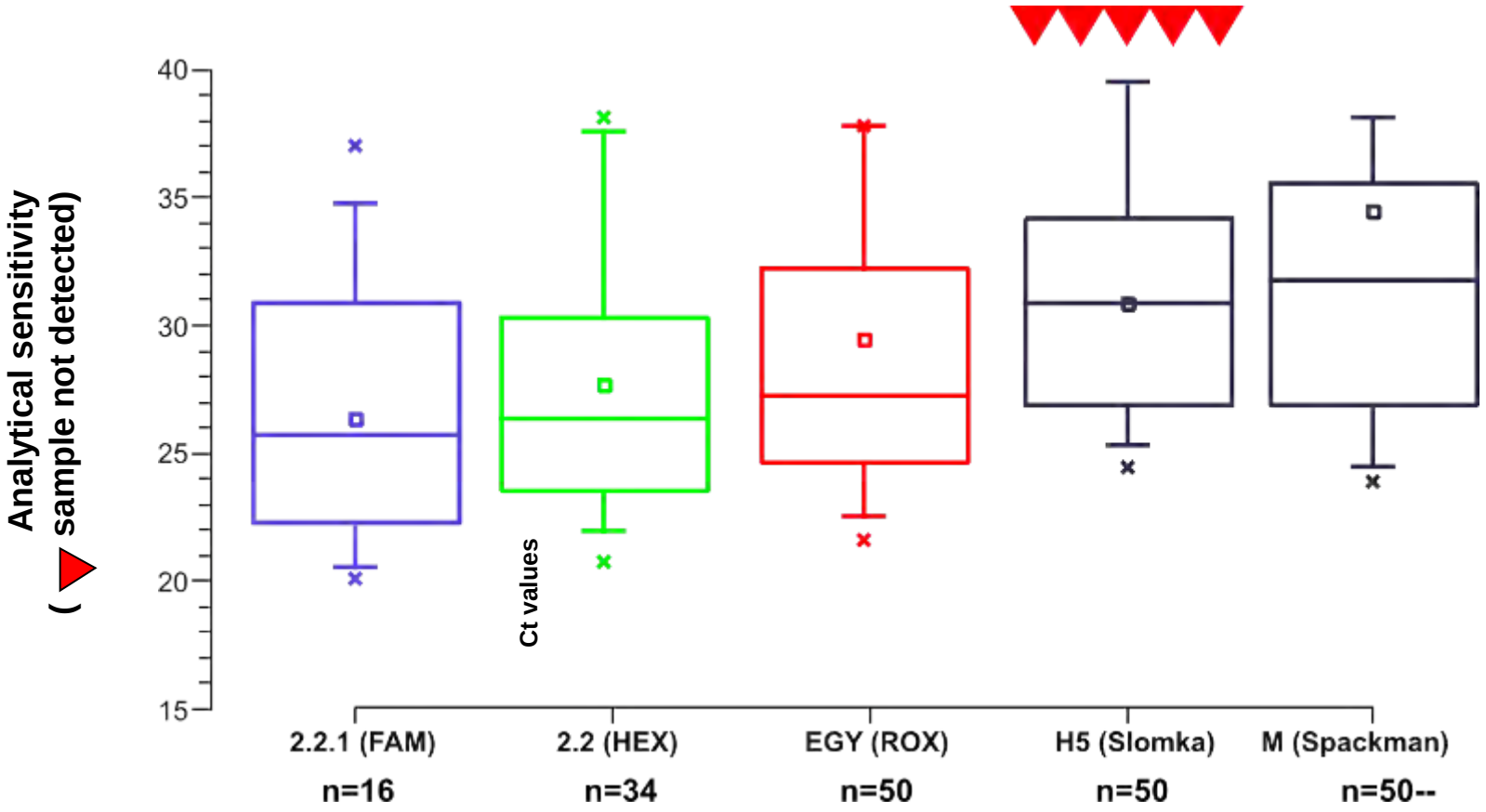
# HPAIV H5N1 detection in Egypt

| | 2008 | |
|---|---|---|
| Season[1] | Commercial farms | Backyard |
| Winter | 20/2,989 (0.7%) | 66/832 (7.9%) |
| Spring | 6/1,785 (0.3%) | 11/421 (2.6%) |
| Summer | 1/1,932 (0.05%) | 6/103 (5.8%) |
| Autumn | 0/1,976 (0%) | 6/367 (1.6%) |
| Total | 27/8,682 (0.31%) | 89/1,723 (5.2%) |

Egyptian Natioinal Laboratory for Veterinary Quality Control on Poultry Production

Hafez et al., 2010

# Evidence for improved sensitivity and clade-specific differentiation of Egyptian H5N1 by multiplex H5 RT-qPCR



Live bird market – Giza, Pyramids Road


Market is ready
for customers


Fresh poultry must be kept fresh


Butchering on the spot(s)


Gulp!

# Biosecurity measures: Practical training in overpressurized suits (BSL3+)

# AI vaccination strategy in household poultry

- 2 campaigns per year, each one lasting 3 months
- Door-to-door vaccination protocol
- one dose of vaccine is administered for all birds (no
- booster)
- Chicks and ducklings are vaccinated
- In some Governorates, double or more volume dose of vaccine
- is given to ducks and geese

# AIV H5N1 detection in vaccinated flocks

Vaccines used

- A/Goose/Guangdong/1/1996 H5N1
- A/chicken/Mexiko/232/CPA/1994 H5N2, etc.

| | Backyard flocks, n (%) | | |
|---|---|---|---|
| Year | Vaccinated[1] | Mixed[2] | Total |
| 2007 | 32 (94.1) | 2 (5.9) | 34 (100) |
| 2008 | 34 (97.1) | 1 (2.9) | 35 (100) |

Egyptian Natioinal Laboratory for Veterinary Quality Control on Poultry Production

Hafez et al., 2010

# AIV H5N1 detection in vaccinated flocks

Vaccines used

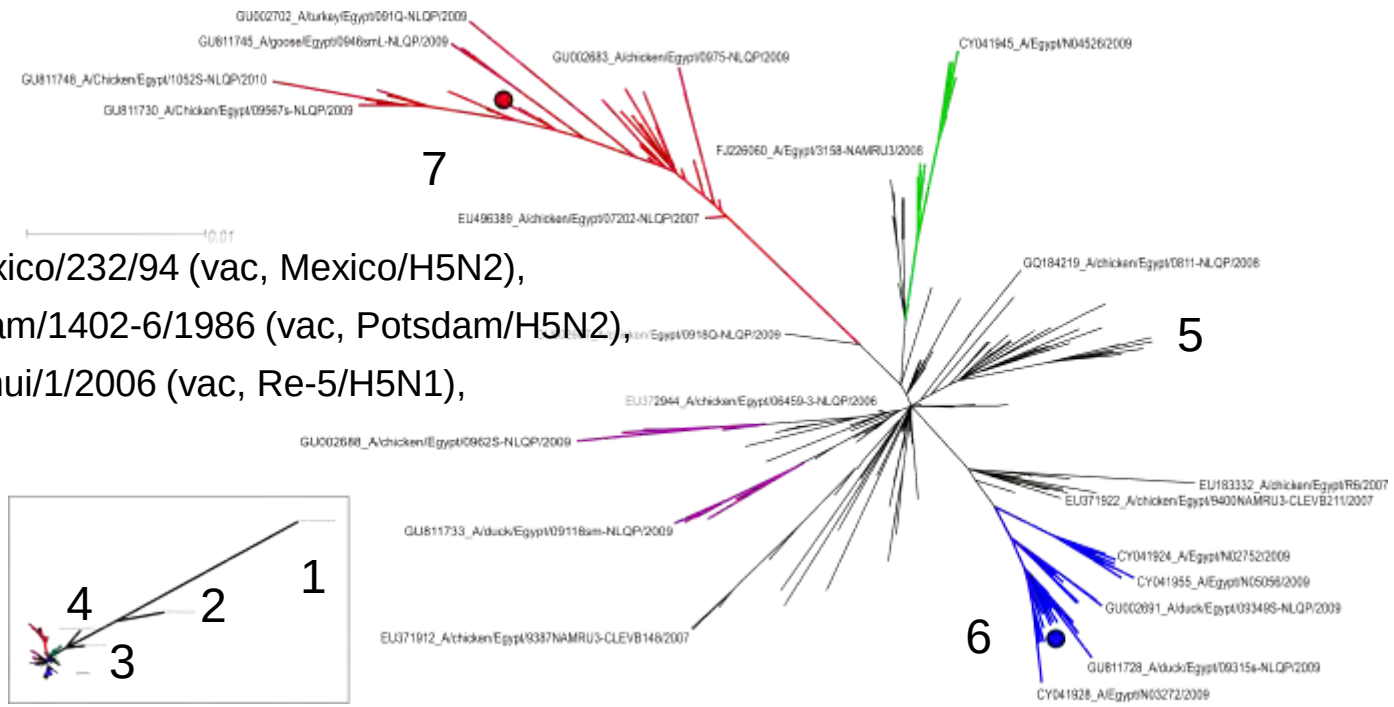
- A/Goose/Guangdong/1/1996 H5N1
- A/chicken/Mexiko/232/CPA/1994 H5N2, etc.

| | Commercial farms, n (%) | | | | |
|---|---|---|---|---|---|
| Year | Grandparent | Breeders | Layers | Broilers | Total |
| 2007 | 1 (2.8)[3] | 5 (14.3) | 15 (42.9) | 14 (40) | 35 (100) |
| 2008 | 0 (0) | 0 (0) | 10 (37) | 17 (63) | 27 (100) |

Egyptian Natioinal Laboratory for Veterinary Quality Control on Poultry Production

Hafez et al., 2010

# Phylogenetic analysis of Egyptian HPAIV H5N1 viruses




1 – A/chicken/Mexico/232/94 (vac, Mexico/H5N2),

2 – A/duck/Potsdam/1402-6/1986 (vac, Potsdam/H5N2),

3 – A/chicken/Anhui/1/2006 (vac, Re-5/H5N1),

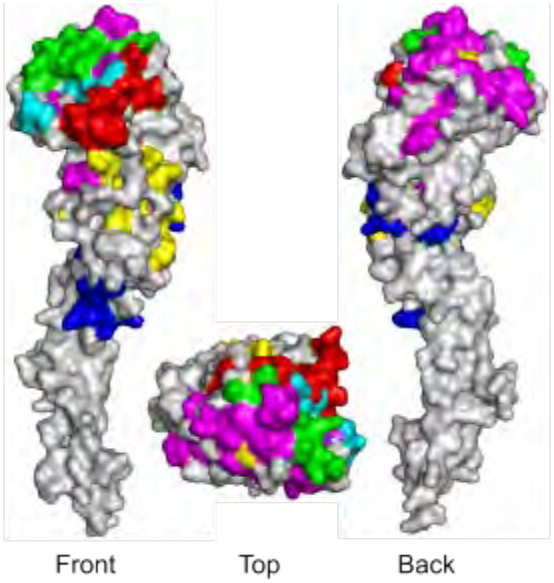
4 – rec A/Vietnam/1194/2004(H5N1) (NIBRG-14),

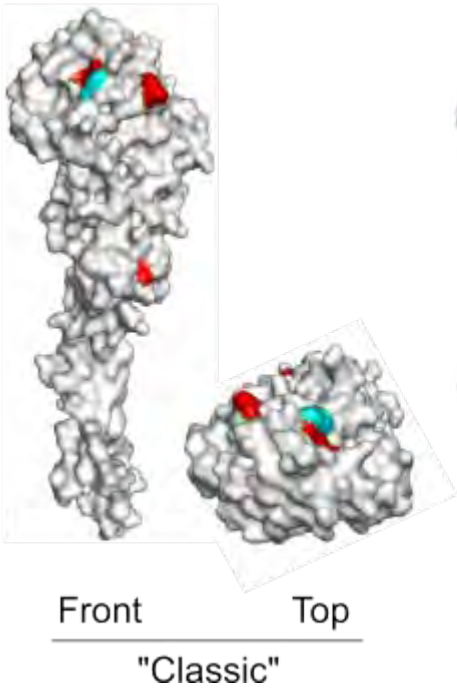
5 – A/duck/Egypt/NLQP-0827/2009 (***EGYext***;HI),

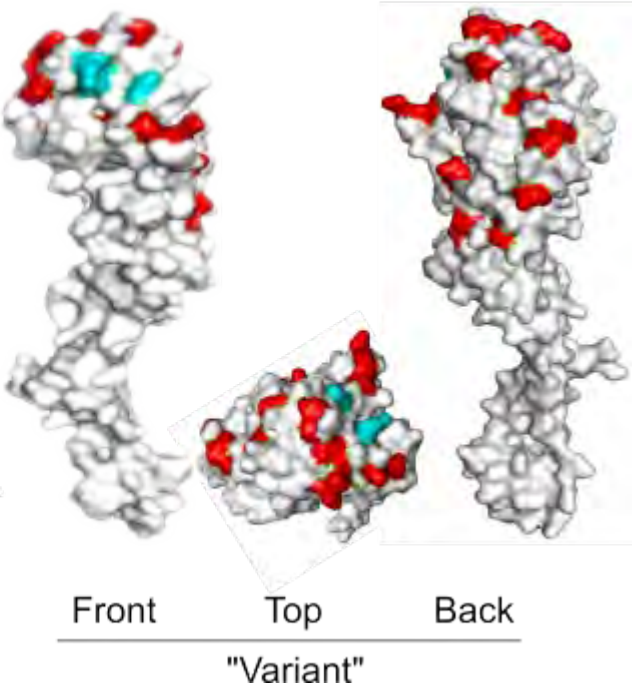
6 – A/chicken/Egypt/NLQP-0918/2009 (chg, ***EGYcls/H5N1***),

7 – A/chicken/Egypt/0879/2008 (chg, ***EGYvar*/H5N1**).

# 3D prediction model of the hemagglutinin




antigenic sites (Duvvuri et al. 2009)
A – red,
B – green
C – blue
D – magenta
E – yellow)
receptor binding domain (cyan)

mutations in relation to the 2006 index virus
3 23

# Cross reactivity of HPAIV H5N1 and vaccine strains

- Sera from chickens vaccinated once with inactivated adjuvanted subtype H5 vaccines, three weeks post vaccination
- Tested by hemagglutination inhibition test (HI)

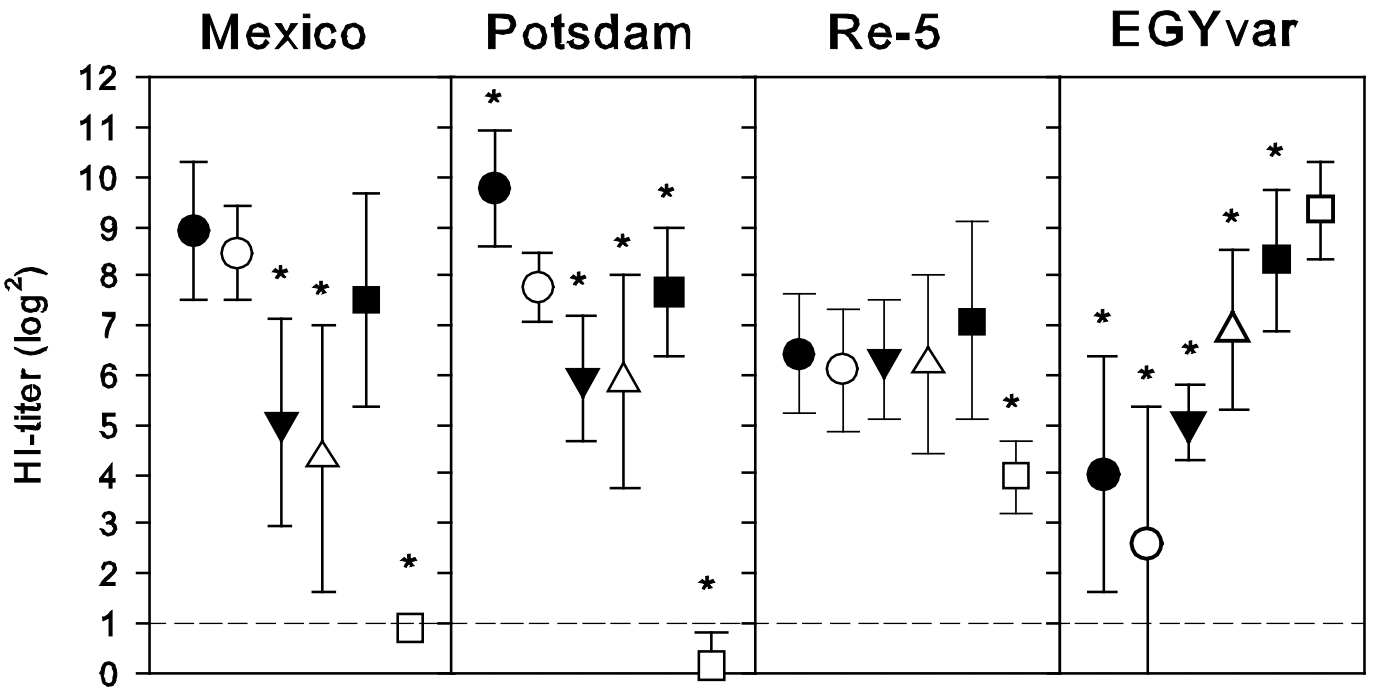
| Vaccine | Serum | Antigen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Potsdam/ H5N2 | Mexico/ H5N2 | Vietnam/ H5N1 | A/ck/EGY/0918/09 [1] | A/dk/EGY/0897/08 [1] | A/dk/EGY/0827/08 [1] | A/ck/EGY/083/08 [1] | A/ck/EGY/0879/08 [1] | A/ck/EGY/0815/08 [1] | A/ck/EGY/g3h4/08 [1] |
| Potsdam/ H5N2 | #1 | **9** | 9 | 7 | 9 | 10 | 7 | 3 | <1 | 2 | <1 |
| | #2 | **8** | 8 | 6 | 9 | 10 | 6 | <1 | <1 | <1 | <1 |
| | #3 | **8** | 7 | 8 | 9 | 10 | 6 | 4 | <1 | 3 | <1 |
| Mexico/ H5N2 | #1 | 7 | **9** | 6 | 7 | 9 | <3 | <3 | <1 | <3 | <3 |
| | #2 | 7 | **9** | 6 | 9 | 8 | 4 | <3 | <1 | <3 | <3 |
| | #3 | 6 | **7** | 5 | 9 | 9 | 5 | <3 | <1 | <3 | 5 |
| EGYvar | #1 | 6 | <1 | 5 | 8 | 10 | 8 | 5 | **11** | 4 | 5 |
| A/ck/EGY/0879/08 [1] | #2 | <1 | <1 | 5 | 8 | 10 | 8 | 7 | **8** | 4 | 6 |
| | #3 | 2 | <1 | 4 | 9 | 5 | 7 | 8 | **10** | <1 | <1 |

bold: homologous antiserum / antigen

[1]: HP AIV /H5N1-isolates from NLQP

"classic" (2.2. proper) extinkt clade "variant" (clade 2.2.1)

# HI antibody responses of vaccinated chickens



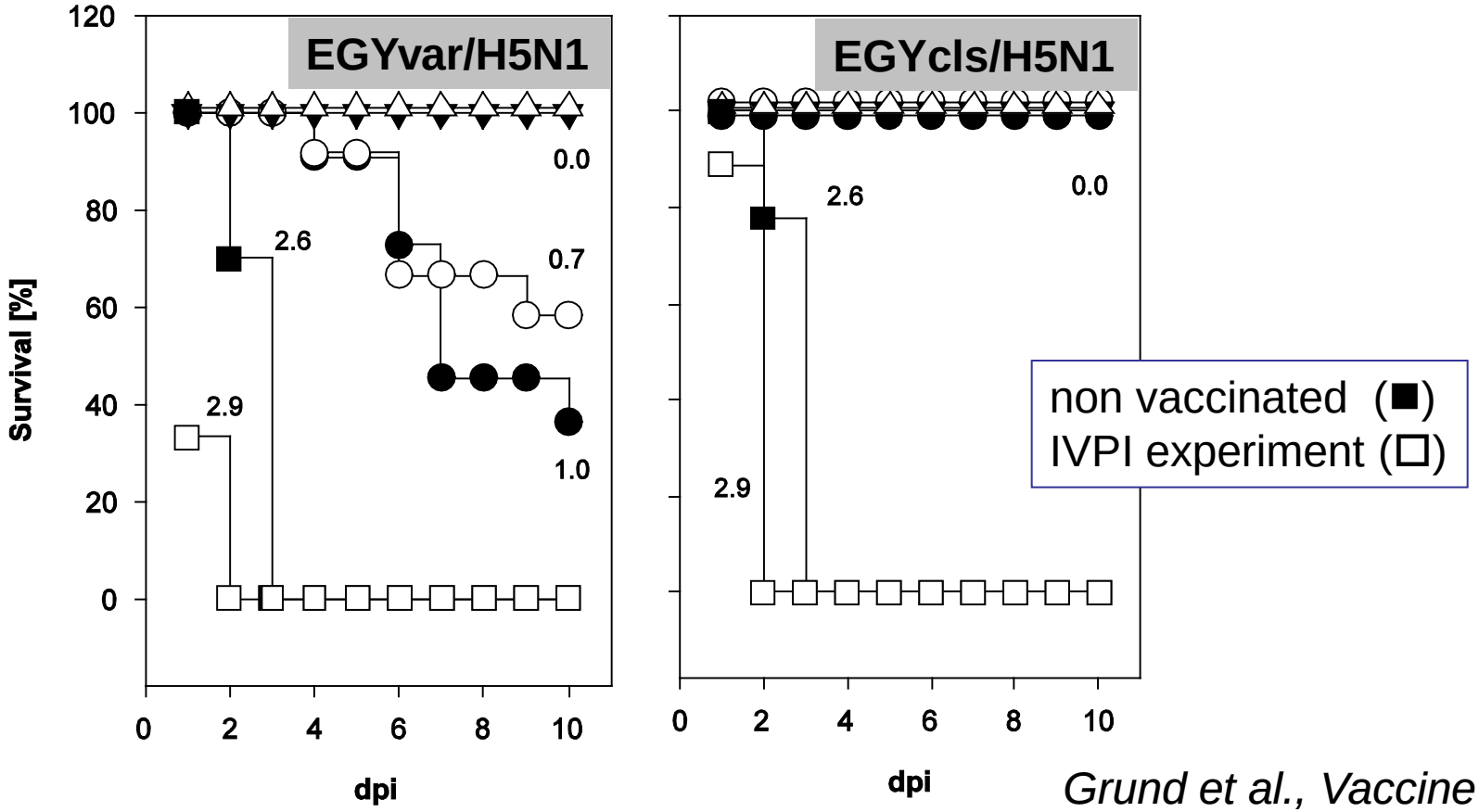

**Antigens**

Mexico/H5N2 (●)
NIBRG-14/H5N1 (▼)
EGYcls/H5N1 (■)
Postsdam/H5N2 (❍)
EGYext/H5N1 (△)
EGYvar/H5N1 (□)

*Grund et al., Vaccine 2011*

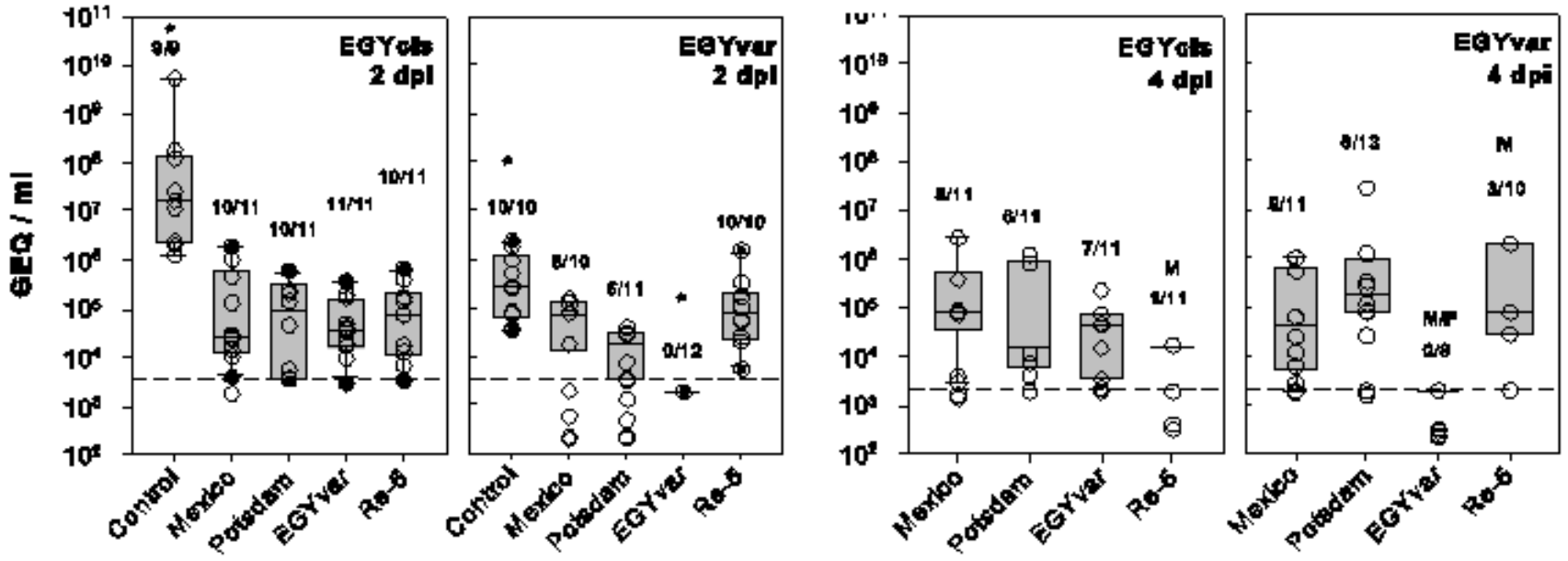
# Survival after challenge

Potsdam/H5N2 (❍), Mexico/H5N2 (●), EGYvar/H5N1 (▼) or Re-5/H5N1 (△)




*Grund et al., Vaccine 2011*

# Virus shedding after challenge



# Summary

- Available inactivated commercial H5N2 virus vaccines induce protection against “classic” (2.2. proper) but not “variant” (clade 2.2.1) HPAIV H5N1
- H5N1 vaccines, representing antigenic sites of “variant” (clade 2.2.1) HPAIV H5N1, recognized by HI (R5 and EGYvar) are inducing protection against “classic” and “variant” HPAIV H5N1
- Indication that antigenic drift appears to be vaccine driven (cls <->var)

# Conclusion

- In case of ongoing vaccination, surveillance of the circulating HPAIV H5N1 viruses in Egypt and their antigenic characterization is needed
- AIV vaccines for poultry have to be optimised for the current / local situation
- Vaccination of poultry has to be paralleled by concerted efforts for efficient AI control


Thank you for your attention!
Prolonged until 11/2011!!

- more than 80% (24 million USD) of the available budget for HPAI control has been devoted to vaccination since the programme was launched (GOVS, 2009).

# Thank you ……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Martin Beer

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Greifswald-Insel Riems

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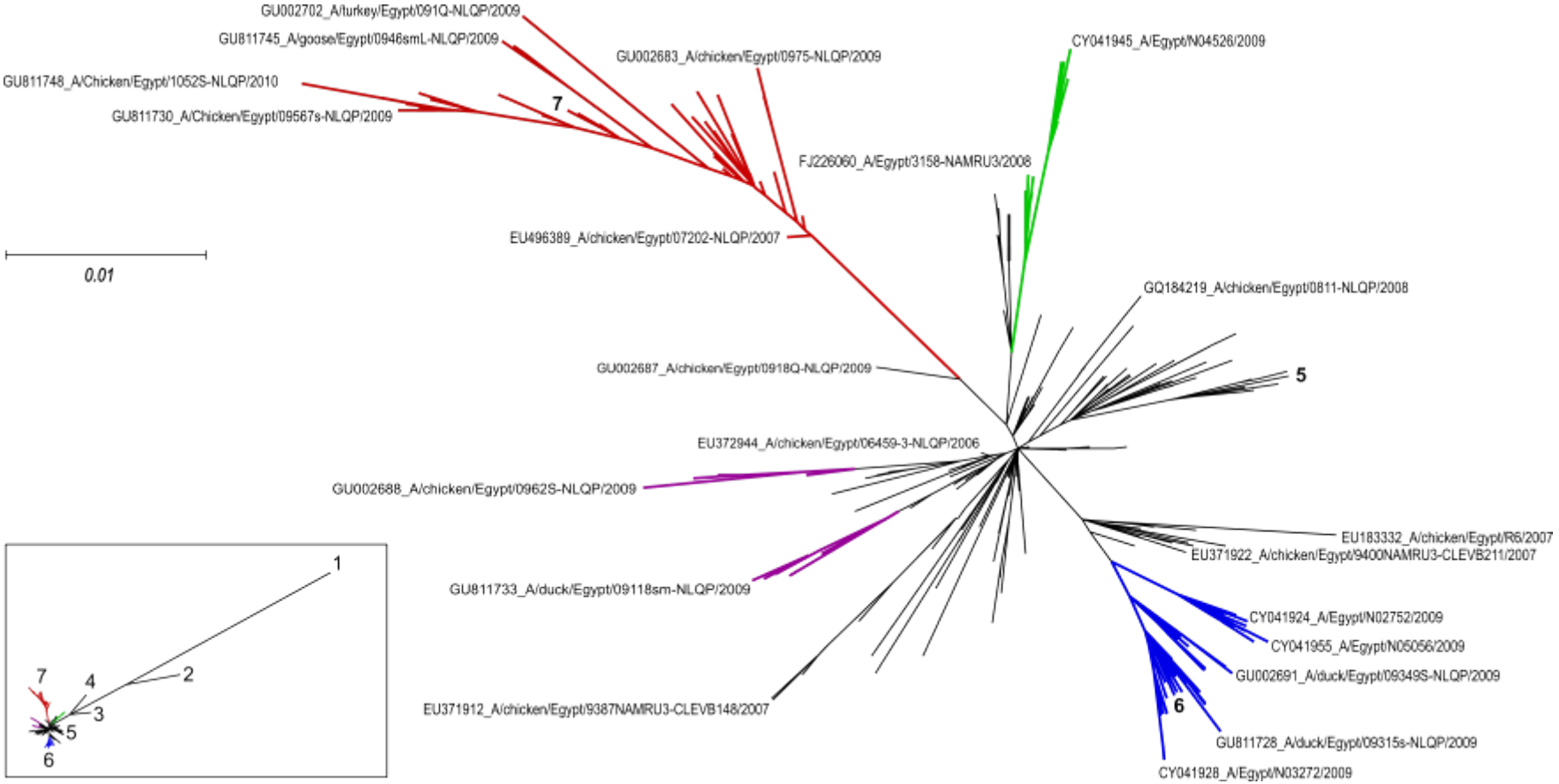
# Diversifying evolution of of Egyptian H5N1

## Lineage 2.2.1



# Amino acid changes in the hemagglutinin

| Amino acid position | Virus strains | | | | Epitope[5] | RBD[6] | N-glyco-silation |
|---|---|---|---|---|---|---|---|
| | "Parent"[1] | "Classic"[2] | Study (30)[3] | "Variant"[4] | | | |
| 43 | D | N | | | E | | |
| 71 | L | | | P | E | | |
| 74 | P | | S | S | e | | |
| 97 | D | | N | N | | | |
| 110 | H | | R | R | A | | |
| 120 | S | N | | | a | | |
| 123 | S | | P | P | a | | |
| 129 | S | | | L | A | + | |
| 140 | R | | G | G | B | | |
| 141 | S | | P | P | B | | |
| 144 | E | | Y | Y | B | | |
| 151 | I | T | | | D | | |
| 154 | D | | | N | D | | + |
| 156 | A | | | T | D | | + |
| 162 | R | | K | E | d | | |
| 165 | N | | H | H | | | - |
| 169 | Q | | | P | b | | |
| 183 | D | | | N | b | | |
| 184 | A | | E | E | d | | |
| 189 | R | | | S | | + | |
| 190 | L | | | I | | + | |
| 192 | Q | | | K | D | | |
| 194 | P | | | S | D | | |
| 195 | T | | | N | D | | |
| 226 | M | | V | V | D | | |
| 238 | A | | | T | | | |

# Aim of the vaccination study

- Are available inactivated commercial and experimental whole H5 virus vaccines able to protect against HP AIV H5N1 viruses which are currently co-circulating in Egypt?

- Testing two antigenically widely distinct HPAIV H5N1 “variant” (clade 2.2.1) and “classic” (2.2. proper) lineages

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