OFFLU Avian Influenza virus characterisation meeting
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Egypt experience with HPAI virus evolution and vaccination

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Structure of the poultry industry in Egypt

- Egypt produces about 850 K Ton live poultry and about 8 billion eggs yearly. About 8 million people are working in this industry.

- Commercial sector (Grandparents, breeders, layers and broilers) produces about 85% of chickens of foreign breeds.

- While rural poultry is about 15% mainly depends on local native breeds.

- Ducks, quails and turkeys are produced in medium scale commercial production and more than 70% of ducks, geese, turkeys and pigeons are reared in rural areas.

H5N1 in Egypt

- Total number for H5N1 outbreaks detected from the 1st of January till the 31th of December 2016 were 172 Outbreaks distributed as follow:

  89 Backyard

  24 Commercial farm

  59 L.B.M.

- 74 case recorded during 2017 till now

Human cases of Avian influenza H5N1 in Egypt (2006- 2017)

Year	Suspected	Confirmed Numbers	Deaths	CFR
2006	1991	18	10	55.60%
2007	1829	25	9	36.00%
2008	1907	8	4	50.00%
2009	5583	39	4	10.30%
2010	662	28	12	42.90%
2011	455	40	16	40.00%
2012	324	11	5	45.50%
2013	251	4	3	75.00%
2014	1090	29	13	44.80%
2015	3373	144	41	28%
2016	1980	10	4	40%
2017	166	3	1	33%
Total	19611	359	122	34%

H9N2 in Egypt

- Total number for H9N2 outbreaks detected from the 1st of January till the 31th of December 2016 were 574 Outbreaks distributed as follow:

  32 Backyard

  430 Commercial farm

  106 L.B.M

  6 Poultry shops.

- 93 cases recorded during 2017 till now

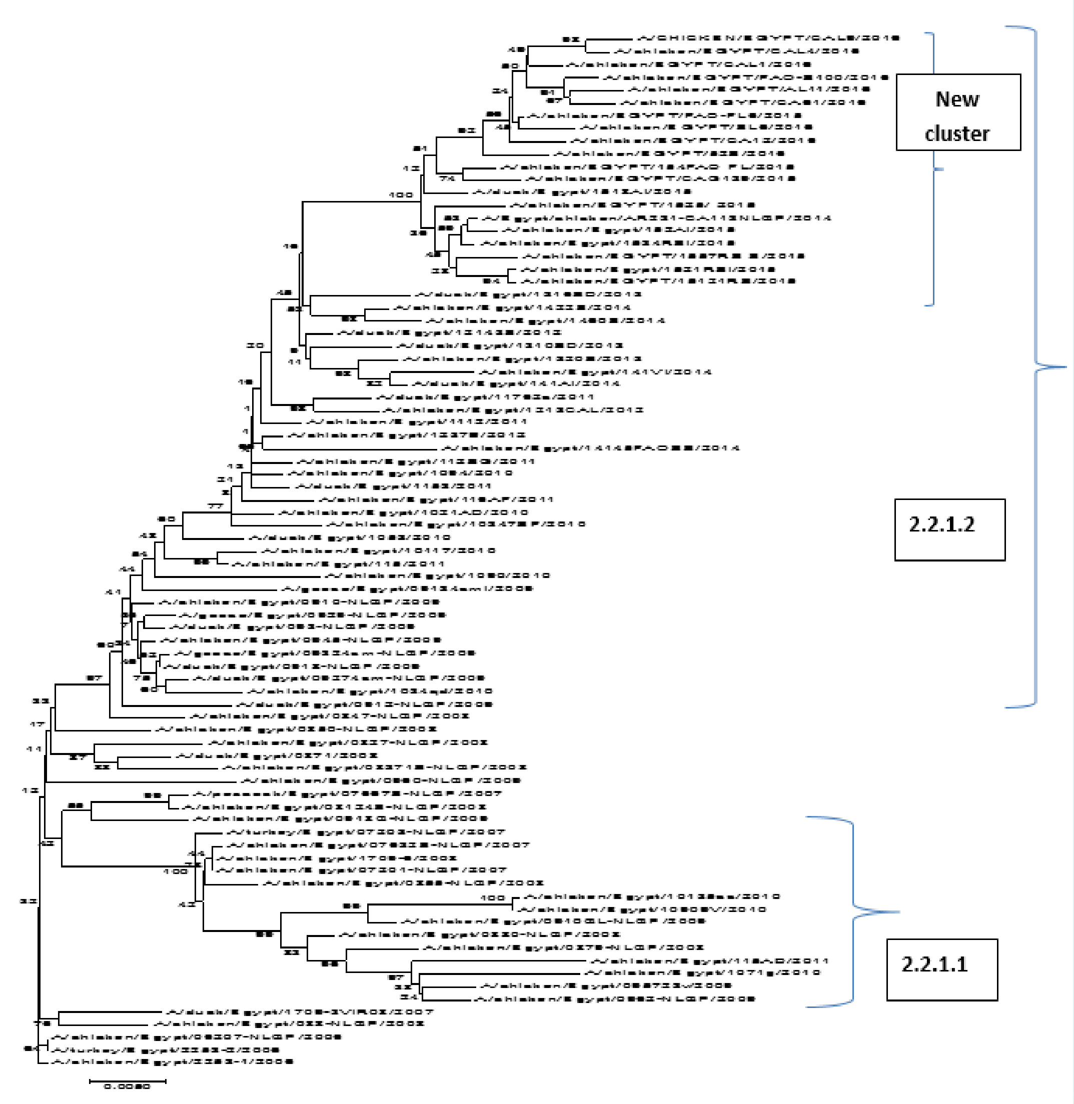
H5N1 genetic analysis

- There were 60 sequenced viruses for HA gene from Lower and Upper Egypt governorates during 2016/17

- The recently sequenced H5N1 Egyptian viruses are classified in one group with the same viruses of clade 2.2.1.2 indicating presence of one group of viruses in 2016 related to the 2.2.1.2 clade that still the predominant sense 2011.

H5N1 phylogenetic analysis

The recent Egyptian viruses are clustered with clade 2.2.1.2



Changes in the Antigenic sites

- The viruses isolated in 2016/17 carry mutations in the antigenic sites (at positions 120, 151, 154 and 162)

- That indicating progressive virus evolution in comparison to original 2006 viruses and antigenic variation.

Changes in the cleavage site

- Most Egyptian 2016 viruses have a common cleavage site of "**PQGEKRRKKR/GLF**"

indicate high pathogenic nature of those viruses

NA Mutation Analysis

- There was no any Tamiflu resistance markers **(H274 or**
- **N294)** in Egyptian viruses.

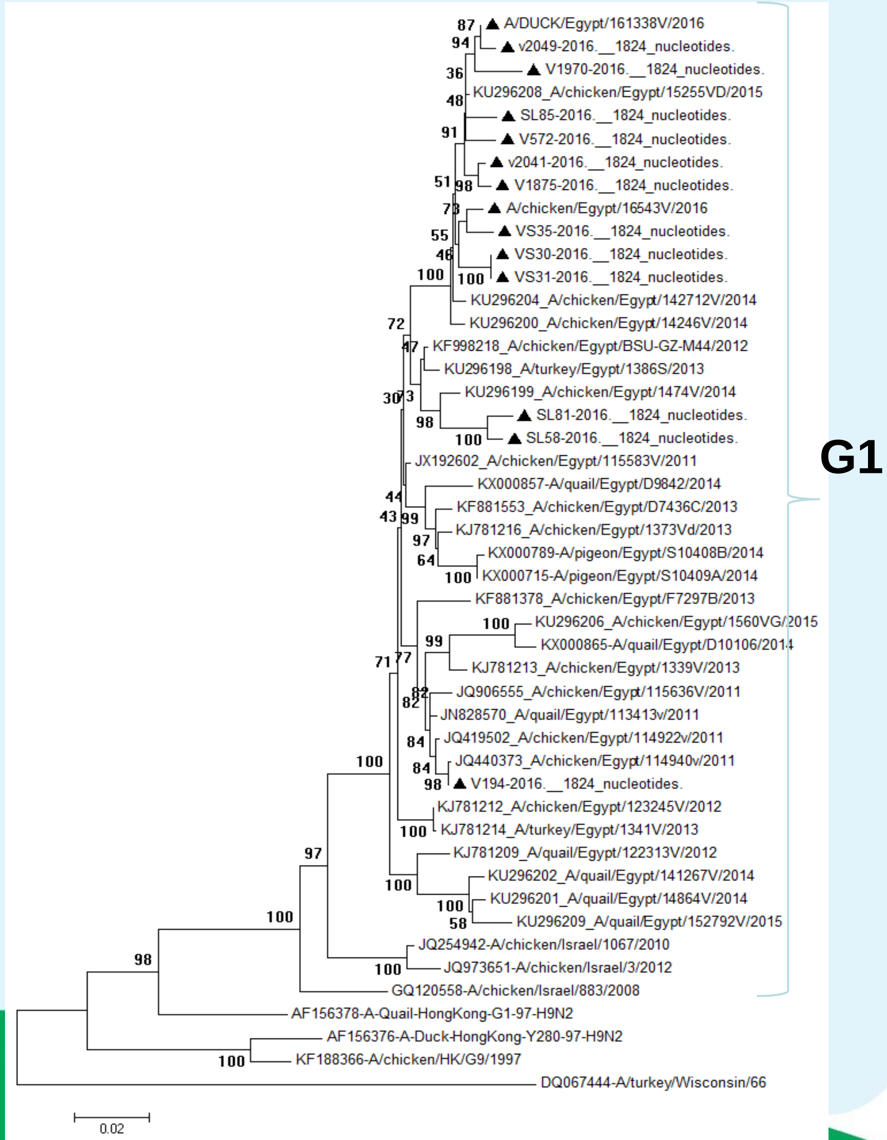
- At least 12 AA substitutions were characterized in the recent viruses from the new cluster of 2.2.1.2 of 2016 viruses in the NA gene.

Gene analysis of H9N2

- Phylogenetic analysis of the HA gene showed that the isolates from Egypt are grouped in Quail/Hong Kong/G1/97 lineage - strains similar to the one circulating in the Middle East (about 92% homology)
- Viruses of 2016 fall into one group.

phylogenetic analysis of H9N2

Viruses of 2016 fall
into one group
within G1-lineage



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HPAI H5N8

- Samples from 2 dead **migratory birds (common coot duck from Dumyat** governorate in north Egypt) were collected during surveillance in live bird markets.

- First case of H5N8 in Egypt confirmed in wild bird **using real time RT-PCR on** 26/11/2016 (2 different sets of primer and probes were used for confirmation).

- 13 cases were reported in 2017

Gene analysis of H5N8

- **Sequence of the first confirmed case characterized as highly pathogenic avian influenza subtype H5N8 clade 2.3.4.4b resembling the current circulating Europian countries.**

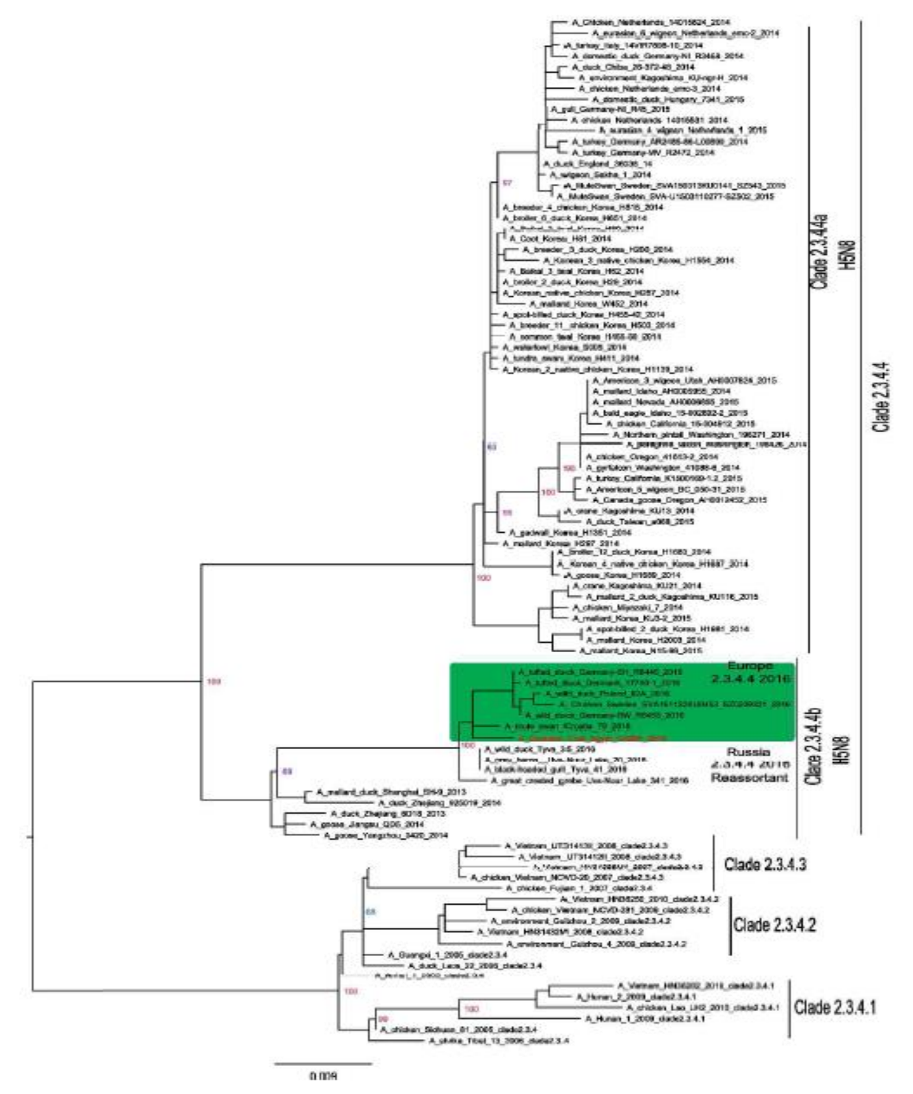
Gene analysis of H5N8

- Three new substitution mutations were observed (R22K, E268G and D487Y) distinguishing the HA protein of the Egyptian virus from the recent European H5N8 virus of clade 2.3.4.4b.

- Genetic analysis of NA gene confirm N8 subtype with 4 substitution mutations (V8A, V31L,G126E and I407T) that distinguish the NA protein of the Egyptian virus from the recent European H5N8 viruses.

phylogenetic analysis of H5N8

Egyptian H5N8 is grouped with recent viruses from clade 2.3.4.4b



Vaccination against avian influenza in Egypt

Currently used vaccines against H5N1 in Egypt

- There are 2 sources of vaccines in Egypt
- Imported (China, Europe, Mexico)
- Locally produced (1 governmental and 1 private)
- And 2 types of vaccines (Inactivated and recombinant)
- The vaccines are licensed by GOVs after passing the challenge with Egyptian virus (current circulating 2015 strain) in Central Laboratory for evaluation of veterinary biologics (CLEVB).

Current vaccination strategy

Geographical coverage of vaccination

- Vaccination almost covers no more than 70% of commercial sector, rural birds are not vaccinated
- commercial birds are vaccinated (chickens, turkeys, ducks and quails)
- Vaccination still the choice of producer in commercial sector, while it done by the government in infected village after case record in rural birds.

Vaccine selection

- The commercial vaccine products and their strains are selected based on passing the higher vaccine committee evaluation first then passing the challenge test using 2015 strain in CLEVB.

- vaccine matching with circulating strains is based on cross HI test and sequence comparison of antigenic sites of HA gene in NLQP, while challenge test is done in CLEVB

Recent vaccine evaluation experiment

Protective efficacy of two locally produced H5N1 and H5ND vaccines against HPAI H5N8 clade 2.3.4.4b

The two tested vaccines showed 90% protection. The two tested vaccines were able to minimize virus shedding (10%) from 3rd dpc. There was no virus shedding at 6th dpc.

These results indicate that the two tested vaccines were able to provide adequate protection against HPAI H5N8 clade 2.3.4.4b virus.

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Conclusion

- Analysis of Egyptian H5N1 HA genes indicates continuous evolution of Egyptian viruses in domestic birds.
- H5N1 still avian-not human type viruses.
- There was a frequent incidence of H9N2 LPAI subtype in chickens with co-infection with H5N1
- First detection and start to spread of H5N8 subtype in Egypt

Recommendations

- Continuously revise and update the virological surveillance plans.
- Continuous monitoring of the circulating strains and sequencing of HA, NA and other internal genes for complete virus characterization and vaccine selection.
- Information and sample sharing with private veterinarians, research centers and MOH.
- Efficacy of H5N1 vaccine against H5N8 strain study
- Monitor and validate matching between the vaccine strains and currently circulating viruses (cartographic analysis – genetic markers).

Thank You

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