# Occurrence in avian H5N1 strains isolated in Africa and in the Middle East (2006 -2008) of mutations with potential human health implications

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## Background

- The A/H5N1 HPAI virus emerged in poultry for the first time in Africa in 2006 in the north of Nigeria (Joannis et al, 2006; De Benedictis et al., 2007) and rapidly spread in 10 countries; namely Nigeria, Niger, Burkina Faso, Ivory Coast, Egypt, Sudan, Cameroon, Togo, Benin
- In Africa, the vast majority of the outbreaks have been notified in poultry (chickens, ducks, guinea fowl) and only few sporadic reports indicated the detection of A/H5N1 in wild birds found dead (Egypt – Saad et al., 2007, Burkina Faso- Ducatez et al.,2007, Cameroon?- Njouom et al., 2007).
- 52 human infections have been reported in this continent (September, 2008). Of these, 23 were fatal. 50/52 occurred in Egypt, 1 in Djibouti, 1 in Nigeria.

## Background

- In the Middle East, H5N1 HPAI virus was first reported in January 2006 in Turkey (wildlife), then in Iraq (poultry), Iran and Azerbaijan (wildlife)
- Subsequently (February 2006-March 2007), virus was detected in poultry and/or wild birds in Kuwait, Jordan, Afghanistan and Saudi Arabia.

23 human infections have been reported in the Middle East (September, 2008). Of these, 11 were fatal.

12/23 occurred in Turkey, 8/23 in Azerbaijan, 3/23 in Iraq.

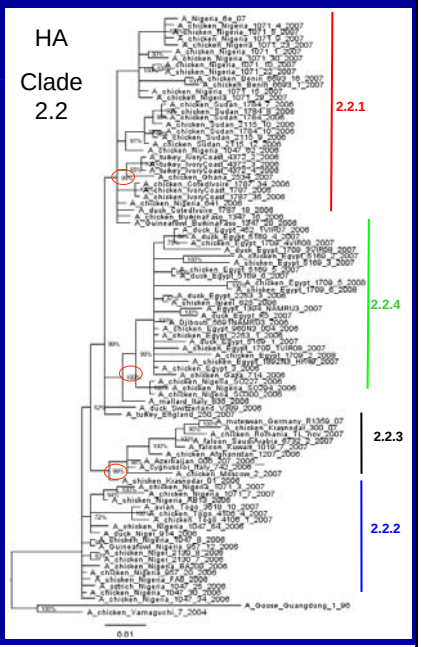


## A/H5N1 in Africa/Middle East - Phylogeny

- **At the IZSVe OIE/FAO Reference Laboratory in Italy, over 100 H5N1 isolates from Africa and the Middle East have been genetically characterized (HA, NA genes) and the whole genome of the majority of them was sequenced, analyzed and compared to the sequences deposited in public databases (2006-2008).**
- **For Africa, the sequences generated and deposited represent @ 60% of the sequences. For the internal genes, this value increases to 74% (*manuscript submitted*)**
- **According to the current WHO nomenclature, isolates from Africa and Middle East are included in clade 2.2 (corresponding to the previously described clade EMA – Salzberg et al., 2007 – also generically identified as "Qinghai-like" – Smith et al., 2006).**

## A/H5N1 in Africa - Phylogeny

- According to previous publications, in clade 2.2 distinct sublineages could be identified (EMA1, EMA2, EMA3; Salzberg et al 2007; A,B, C; Ducatez et al., 2007; 2.2.1, 2.2.2, 2.2.3; Starick et al., 2008, Monne et al., 2008);
- For consistency with the latest publications, here we adopted the 2.2 nomenclature:
  - 2.2.1 (EMA1 or C)
  - 2.2.2 (EMA2 or A)
  - 2.2.3 (EMA3)
  - 2.2.4 (B – previously included in EMA1)
- In Africa, from 2006 to early 2008, evidence of circulation of 3/4 sublineages (2.2.1-2.2.2-2.2.4).
- 2.2.3, circulating in Europe and Middle East in 2006 and 2007, was not detected in Africa until July 2008.






## Typical amino acid signature of human influenza viruses

- Molecular markers that discriminate human influenza viruses from avian influenza viruses (highly preserved in human isolates over time).
- Markers are located almost esclusively in PB2, NP, PA, NS and M gene (Chen et al., 2006; Finkelstein et al., 2007)
- Four mutations are 100% conserved in human pandemic isolates (3 in PB2; 1 in PA – Finkelstein et al., 2007)
- Of these, one (PB2 E627K) is present in all African and Middle Eastern strains analysed.
- 2 Egyptian avian strains contain 2 of these mutations (PB2 E627K & PB2 A199S)
- None H5N1 viruses contain more than 2 out of 4 of these mutations
- PB1-F2 N66S mutation has been detected in one avian isolate in Africa (A/duck/Nigeria/2008)

## Typical amino acid signature of human influenza viruses / Africa

| Protein | aa | Predicted aa | | Reference | Strains | Mutation |
|---|---|---|---|---|---|---|
| | | Avian | Human | | | |
| PB2 | 661 | A | T | Shaw *et al.*, 2002 | A/ck/Ghana/2534/07 | A661T |
| | 199 | A | S | Shaw *et al.*, 2002<br>Chen, *et al.*, 2006 | A/ck/Egypt/5169-5/07; A/dk/Egypt/5169-6/07 | A199S |
| PB1-F2 | 73 | K | R | Chen, *et al.*, 2006 | All strains analysed in this study belonging to 2.2.4 sublineage | K73E |
| | 82 | L | S | Chen, *et al.*, 2006 | 5 A/avian/Egypt//06 and 07; A/Egypt/902786/06 | L82S |
| | 79 | R | Q | Chen, *et al.*, 2006 | A/ck/Egypt/5169-3/07 | R79Q |
| | 66 | N | S | Conenello et al., 2007 | A/duck/Nigeria/3724-2/2008 | N66S |
| PA | 100 | V | A | Shaw *et al.*, 2002<br>Finkelstein *et al.*, 2007 | A/ck/Nigeria/1071-3/07; A/ck/Nigeria/1071-7/07<br>A/ck/Nigeria/AB13/06; A/ck/Nigeria/AB14/06 | V100A |
| | 400 | Q/T/S | L | Shaw *et al.*, 2002 | A/dk/Egypt/1709-3VIR08/07; A/ck/Egypt/2628-2/07 | S400L |
| | 356 | K | R | Chen, *et al.*, 2006 | A/ck/Burkina Faso/1347-16/06 | K356R |
| NP | 33 | V | I | Shaw *et al.*, 2002<br>Chen, *et al.*, 2006 | 78/81 viruses | V33I |
| | 109 | I | V | Chen, *et al.*, 2006 | 2 A/ck/Egypt/07; A/ck/Nigeria/1047-8/06 | I109V |
| M2 | 55 | L | F | Shaw *et al.*, 2002 | All 6 A/ck/Sudan/06;<br>A/hooded vulture/BurkinaFaso/2/06 | L55F |
| NS1 | 227 | E | R o K (H1N1) | Chen, *et al.*, 2006<br>Finkelstein *et al.*, 2007 | A/ck/Nigeria/FA4/06; A/ck/Nigeria/FA7/06 | E227G |
| NS2 | 70 | S | G | Chen, *et al.*, 2006 | A/ck/Nigeria/FA4/06; A/ck/Nigeria/FA7/06 | S70G |



## Typical amino acid signature of human influenza viruses / Africa & Middle East

| Position | Residue | Substitution associated with enhanced replication | Reference | Strains | Mutation |
|---|---|---|---|---|---|
| PB2 627 | E | K | Subbarao *et al.* 1993<br>Chen et al., 2006 | All strains analyzed in this study | E627K |

| Protein and Position | Residue | Substitution associated | Reference | Strains | Mutation |
|---|---|---|---|---|---|
| NS1 227 | E | R or K | Chen *et al.* 2006<br>Finkelstein *et al.*, 2007 | 6 A/poultry/Saudi Arabia/07 | E227K |
| PB1 327 | R | K | Chen *et al.* 2006 | 6 A/poultry/Saudi Arabia/07 | R327K |
| NP 33 | V | I | Chen et al., 2006 | A/ck/Saudi Arabia/6732-4/07<br>A/Houbara/Saudi Arabia/6732-1/07<br>A/falcon/Saudi Arabia/6732-2/07<br>A/ostrich/Saudi Arabia/6732-3/07 | V33I |

## Molecular changes in the HA glycoprotein (2006-2008)

| Virus strains | Position*1 | Mutations at RBS or adjacent to it |
|---|---|---|
| 10 Egyptian isolates, 2007 | 133<br>155 | Deletion<br>I155T |
| 2 Nigerian isolates, 2007;<br>6 Egyptian isolates 2007, | 133 | S133L |
| 1 Egyptian isolate 2007 | 186 | N186S |
| 1 Egyptian isolate 2006 | 227 | S227N |
| 32 Egyptian isolates | 230 | M230I |
| 11 Egyptian isolates | 230 | M230V |
| **Virus strains** | **Position*2** | **Additional glycosylation site** |
| 1 Egyptian isolate 2007 | 70 | NCS |
| 11 Egyptian isolates 2007-2008 | 88 | NVS |
| 2 Egyptian isolates 2007 | 252 | NDT or NDS |
| 6 Egyptian isolates 2007 | 135 | NSS |



## Molecular changes associated to antiviral drugs resistance

- Resistance to antiviral drugs (adamantanes and neuraminidase inhibitors) is increasingly detected in human influenza isolates.
- Adamantanes derivates-resistance (H3N2) range from 0.8-2.2% (USA, 1997-2004) to 11% (USA, 2004-2005).
- In 2005-2006, 92.3% of H3N2 strains did possess a resistant genotype (S31N in M2) (Bright et al., 2008)
- In 2007-2008, 10.8% and 14% of H1N1 strains showed reststance to Oseltamivir in USA and Europe, respectively
- In 2000-2006 the occurence of adamantane-resistance in avian H5N1 strains in SouthEast Asia varied according to different papers (Ilyushina et al., 2005; Cheung et al., 2006; Hurt et al., 2007) and to the geographical area (e.g.6.3% Indonesia; 100% Thailand)

## Molecular changes associated to antiviral drugs resistance

- Genotypes associated with resistance to antiviral drugs (adamantanes and neuraminidase inhibitors) were detected in African/Middle Eastern (H5N1) avian influenza isolates.
- Adamantanes derivates resistance associated genotypes (H5N1) were detected in 14/132 isolates (10.6%)
- M2 S31N mutation was detected in 10/132 (7.6%)
- Genotypes associated to resistance to neuraminidase inhibitors (e.g. oseltamivir) were revealed in Egypt (Saad et al., 2007) and detected in 2/154 H5N1 (human) isolates (1.3%).
- None of the avian isolates revealed neuraminidase inhibitors associated genotypes



## Molecular changes associated to antiviral drugs resistance

| Susceptibility of avian influenza viruses to neuraminidase inhibitors | | | | | |
|---|---|---|---|---|---|
| **Position** | **Residue in NA** | **Substitution associated with resistance** | **Reference** | **Strains** | **Mutation** |
| 294* | N | S | Saad *et al.* 2007 | A/Egypt/14725-NAMRU3/06<br>A/Egypt/14724-NAMRU3/06 | N294S |
| **Susceptibility of avian influenza viruses to adamantanes** | | | | | |
| **Position** | **Residue in M2** | **Substitution associated with resistance** | **Reference** | **Strains** | **Mutation** |
| 27 | V | A or T<br>V or A or I | Suzuki, *et al.* 2003<br>Cheung *et al.*, 2006 | A/ck/Burkina Faso/13.1/06 | V27I |
| 30 | A | T or V<br>S | Suzuki, *et al.* 2003<br>Cheung *et al.*, 2006 | A/avian/Togo/3618-10/07<br>A/ck/Togo/4106-1/07<br>A/ck/Togo/4106-4/07 | A30S |
| 31 | S | N | Cheung *et al.*, 2006<br>Hurt *et al.*, 2007 | A/ck/Egypt/1709-5/08<br>A/ck/Egypt/1709-6/08<br>A/ck/Egypt/1709-8VIR08/07<br>A/tk/Egypt/1709-9VIR08/07<br>6 A/poultry/KSA/2007 | S31N |

## Discussion

- Phylogeny confirms that distinct lineages are circulating in Africa and Middle East, reflecting different introductions.
- Typical amino acid substitutions in the HA molecule suggest that the H5N1 viruses are developing specific molecular characteristics related to geografical origin.
- Amino acid substitutions associated to antiviral (adamantanes) drug-resistance have been detected in avian viruses belonging to different sublineages in Africa and Middle East.



## Discussion

- The identification of specific amino acid substitutions which are believed to modify the efficacy of the replication in non-avian species or associated to antiviral drug-resistance, highlights how whole genome analysis is instrumental to recognize rapidly any adaptive change to human host.



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Thank you
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