# Epidemiological Findings of Outbreaks of Disease Caused by Highly Pathogenic H5N1 Avian Influenza Virus in Poultry in Egypt During 2006

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Received 31 October 2007; Accepted and published ahead of print 1 February 2008

## SUMMARY:

This paper describes the first threats of H5N1 avian influenza outbreaks in Egypt recorded from February to December 2006 in commercial and domestic poultry from different species and summarizes the major characteristics of the outbreak. There were 1024 cases from different poultry species (rural and commercial chickens of different breeding types, turkeys, ducks, geese, and quail) either in commercial breeding or in backyards from different locations in Egypt. All tested positive for the H5N1 subtype. From these cases only 12 avian influenza A viruses were isolated and characterized from samples collected during outbreaks. All isolates were characterized, and the data confirmed that the isolated viruses belong to highly pathogenic avian influenza of subtype H5N1. Full hemagglutinin (HA) gene (segment 4) sequencing was also done, and the sequences of these isolates were compared with other strains from Russia, Africa, and the Middle East. The data revealed that all Egyptian strains were very closely related and belonging to subclade 2.2 of the H5N1 virus of Eurasian origin, the same one circulating in the Middle East region and introduced into Africa at the beginning of 2006. This study showed evidence of the wide spread of H5N1 virus infection in domestic poultry in Egypt within a short time. The most obvious features of these outbreaks were severe clinical signs and high mortalities as well as very rapid and widespread occurrence within the country in a very short time. The possible causes of its rapid spread and prospects of disease control are discussed.

**RESUMEN**. Hallazgos epidemiolo´gicos de brotes causados por virus de Influenza Aviar H5N1 de alta patogenicidad en aves comerciales en Egipto durante 2006.

Este art´ıculo describe las primeras amenazas del virus H5N1 de influenza aviar en Egipto en brotes registrados desde Febrero hasta Diciembre del an˜ o 2006 en aves comerciales y dome´sticas de diferentes especies y resume las principales caracter´ısticas del brote. Se registraron 1024 casos de diferentes especies de aves (pollos rurales y comerciales, con diferentes me´todos de crianza,

pavos, patos, gansos, y codornices) tanto en cr´ıa comercial como en aves de traspatio de diferentes lugares en Egipto. Todas las pruebas realizadas fueron positivas para el subtipo H5N1. De estos casos solamente 12 virus de influenza tipo A fueron aislados y caracterizados a partir de las muestras tomadas durante los brotes. Todos los aislamientos fueron caracterizados y los datos confirmaron que los virus aislados pertenec´ıan al subtipo H5N1 del virus de influenza aviar de alta patogenicidad. Tambie´n se realizo´ la secuenciacio´n completa del segmento 4 del gen de la prote´ına hemoaglutinina; las secuencias de estos aislamientos fueron comparados con otros virus de Rusia, Africa y el Medio Oriente. Los datos revelaron que todos los virus de Egipto estaban estrechamente relacionados y pertenec´ıan al subgrupo 2.2 del virus H5N1 originado en Eurasia, el mismo que circula en la regio´ n del Medio Oriente e introducido en Africa en los inicios del an˜ o 2006. Este estudio mostro´ que la diseminacio´n de la infeccio´ n del virus H5N1 en aves dome´sticas en Egipto sucedio´ en un corto tiempo. Las caracter´ısticas ma´s obvias de estos brotes fueron signos cl´ınicos severos y la presentacio´ n de alta mortalidad as´ı como la ra´pida diseminacio´n y ocurrencia dentro del pa´ıs en un tiempo muy corto. Tambie´n se discuten las posibles causas de la ra´pida diseminacio´n y las perspectivas de control de la enfermedad.

**Key words**: highly pathogenic avian influenza, subtype H5N1, Egypt, rural and commercial poultry, genetic characterization of HA gene, epidemiological findings, laboratory diagnostic methods

**Abbreviations**: AGID = agar gel immunodiffusion; HA = hemagglutinin; HPAI = highly pathogenic avian influenza; LPAI = low-pathogenic avian influenza; NA = neuraminidase; NDV = Newcastle disease virus; OIE = World Organisation for Animal Health; PCR = polymerase chain reaction; PMV2 = paramyxovirus-2; PMV3 = paramyxovirus-3; RRT = real-time reverse transcription.

Influenza viruses are segmented negative sense ssRNA, belong to the family Orthomyxoviridae. This family is classified into influenza viruses type A, type B, type C, Thogotovirus, and Isavirus (4). Only influenza type A viruses infect poultry, and they are subdivided into subtypes based on the antigenic relationships of the surface glycoprotein hemagglutinin (HA) and neuraminidase (NA). There are 16 H (H1–H16) and 9 N (N1–N9) (1,16). Also avian influenza viruses can be classified according to pathogenicity into two categories, low pathogenic (LPAI) and highly pathogenic (HPAI) (2). Only the viruses of H5 and H7 subtypes, which contain multiple basic amino acids at the cleavage site of the hemagglutinin molecule have been shown to cause HPAI in susceptible species (13).

However, not all H5 and H7 viruses are highly pathogenic. It has been proven that HPAI viruses emerge in domestic poultry from LPAI progenitors of the H5 and H7 subtypes (17,26,29). HPAI causes systemic disease in chickens and turkeys, and many outbreaks have been reported from 1959 till 2004 (5,33). The evolution of H5N1 in Hong Kong in 1996 and its spread worldwide in Asia, Europe, and Africa with interspecies transmission and many human deaths have been recorded (33,37). Most of the recent outbreaks of HPAI of subtype H5N1 infection in Asia have occurred in domestic chickens. However; Korea reported nine H5N1 cases in duck farms (20). The currently circulating H5N1 appears to be virulent for a variety

of wild bird species (10,21,25). According to Chen et al. (9), a variant strain of H5N1 virus emerged by mid-2005 that killed large numbers of migratory birds in Qinghai, China, and subsequently in Mongolia and southern Siberia and caused infection for wide range of migratory birds and poultry in Europe, the Middle East, and Africa. Clade 2 of H5N1 viruses appears to be most diversified, and members of subclade 2.2 have been shown responsible for the westward spread of H5N1. Meanwhile, within subclade 2.2 three further subtypes have been distinguished and were designated with respect to their geographic origin, European–Middle East–African (28). Also, discrimination of wild ducks in introduc- tion of the disease to Egypt through wild migratory ducks was reported (27).

Alexander (3) suggested that two isolated incursions of HPAI H5N1 virus into Europe occurred in 2004 and 2005 to analyze the potential spread of avian influenza viruses. The first was detected when eagles smuggled from Thailand to Belgium were shown to be infected with H5N1 virus genetically similar to those isolated in Thailand (36). The second was when investigations of deaths in captive caged birds held in quarantine in England, presumably from Taiwan, showed the deaths to be as a result of HPAI H5N1 infection (11); the virus was genetically closest to viruses isolated in China. Isolates from dead swans were obtained in Croatia in October 2005 (24). These infected swans were a forerunner of the spread of HPAI H5N1. During January to April 2006 wild mute swans were shown to be infected in Azerbaijan, Iran, Kazakhstan, Georgia, and many European, African, and Middle East countries. Thirty-one countries from Asia, Europe, and Africa including Egypt had reported HPAI caused by H5N1 virus to the World Organization for Animal Health (OIE) in the first three months of 2006 and by early April 2006 (24).

Although in several outbreaks it was not possible to trace the primary source of infection, movement of birds (through dealers and live-bird markets), rearing of mixed populations, and contact with migratory waterfowl represent important

risk factors for this infection (8,30). Limited epidemiological data and late recognition of early cases preclude analysis of these outbreaks to determine their source with certainty.

Exposure of humans to H5N1 viruses has probably increased considerably in recent months, especially in Asia and Africa. This exposure may increase the probability of H5N1 re-assortment with human or other influenza viruses, although this does not necessarily change the pandemic potential of H5N1 viruses (12). Recent HPAI outbreaks in Europe and particularly the ongoing H5N1 outbreaks have necessitated the development of control and management strategies in an unprecedented eco-epidemiological situation (6).

Use of vaccines as a tool for control of avian influenza was successful in different parts of the world (7). Avian influenza H5N1 vaccine was adopted in Southeast Asia, and other countries such as Mexico and the United States used the H5N2 vaccine for eradication of avian influenza (18). Currently there are different types of seed strains used in production of commercial vaccines: H5N1 (Eurasian lineage), H5N2 and H5N9 (American lineage), as well as recombinant fowl pox virus vaccine.

Vaccination was adopted in Egypt 1 month after the introduction of the disease to help in control efforts. There were different types of avian influenza vaccines introduced into Egypt during 2006 as H5N1 reverse genetic vaccine (Chinese origin), H5N2 (European and American origin), and H5N9 (European origin) dead vaccines sourced from different suppliers, for example, China, Mexico, and Germany. These vaccines were applied in the field by different dosage schemes and age of vaccination and intervals between vaccinations, but usually H5N1 vaccine was adopted during this period at 1-day-old using a full dose, and H5N2 vaccines were applied by mid-2006 usually according to the manufacturer's instructions. Vaccination was applied to all commercial sectors, but there was no vaccination for

backyards and household poultry during this period.

The present paper describes the virological epidemiological investigations and geographic distribution of H5N1 outbreaks in poultry in Egypt from February to December 2006 and discusses the possible causes of this serious outbreak and sheds light on the genetic characterization of Egyptian strains isolated during the course of this outbreak.

## MATERIAL AND METHODS:

Sample collection and preparation. Oropharyngeal and cloacal swabs were collected from the affected 1024 commercial flocks and rural cases from five species of domestic poultry (rural and commercial chickens of different breeding types, rural and commercial turkeys, rural and commercial ducks, rural geese, and commercial quail) during the outbreaks recorded from February to December 2006. Samples were collected in accordance with standard methods (23).

Virus isolation and identification. The first five cases of the outbreaks and further seven selected samples were processed for virus isolation. The clarified supernatant fluid was used to inoculate 10- day-old embryonated SPF eggs into the allantoic cavity. The inoculated eggs were held at 37 C and candled daily for 5 days. The allantoic fluid was collected from dead embryos and examined for hemagglutination activity.

Further identifications were carried out using hemagglutination inhibition (HI) tests by using a panel of reference antisera against 15 HA subtypes. The test was done using 4 hemagglutinating units (HAUs) of virus and 0.5% chicken blood cells. In addition, the agar gel immunodiffusion (AGID) test was conducted for detection of common matrix (M) protein and nucleoprotein of avian influenza isolates; and this test was used to exclude the paramyxoviruses: Newcastle disease virus (NDV), paramyxovirus-2 (PMV2), and paramyxovirus-3 (PMV3) using reference antisera. The reference antisera used were obtained from the OIE

Reference Laboratory (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy).

The molecular identification of the M gene of isolated viruses was carried out by using the real-time reverse transcription (RRT) AIV polymerase chain reaction text (PCR) for the M gene of avian influenza (PG-Biotech, Qiagen, Valencia, CA) as described by the manufacturer. For molecular detection of H5 and H7 genes of AIV, the isolates were tested by using reverse transcription-PCR screening kits for subtyping H5 and H7 (Sacace, Caserta, Italy).

All isolates were tested by RT-PCR for the H5 and N1 gene; the test was conducted using AIV H5 RT-PCR (Qiagen) and H5N1 (Roche, Mannheim, Germany) kits as described by the manufacturers. Genetic analysis of HA gene. This procedure was conducted to investigate the nucleotide sequence of the H5 gene in order to identify the pathogenicity of the 12 isolated strains. Briefly, 7 out of the 12 selected isolates were submitted to either the U.S. Naval Medical Research Unit, NAMRU-3, Cairo, Egypt (n = 4), or to OIE reference laboratory (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy) (n = 3), and 5 isolates were sequenced in the National Laboratory for Veterinary Quality Control on Poultry Production, Giza, Egypt, where the PCR products of the whole H5 gene were directly sequenced. The HA subtypes were identified by a nucleotide BLAST search on viral nucleotide sequences that were available from the National Center for Biotechnology Information, Bethesda, Maryland (http:// www. ncbi. nlm.nih.gov/BLAST/).

Sequencing was performed with a Ready Reaction BigDye Terminator Cycle Sequencing Kit, v3.1 (Applied Biosystems, ABI, Foster City, CA) according to the kit manual and run on an ABI 3130 automated sequencer. The nucleotide sequences were compared with the Megalign program (DNASTAR, Lasergene, Madison, WI), using the Clustal W alignment algorithm. Pairwise sequence alignments

and phylogenetic comparisons of the aligned sequences for the HA gene were also performed with the Megalign program to determine nucleotide and amino acid sequence similarities and relationships. The sequence of the A/wild duck/ Omsk/103-01/05 Russian strain was obtained from GenBank.

Direct detection of H5N1 virus using RRT-PCR. For the epidemiological investigations the received samples from suspected commercial flocks or rural cases were routinely tested by RRT-PCR. Briefly, RNA was extracted from pools of cloacal, oropharyngeal specimens using either the virus RNA Extraction Kit (Qiagen) or MagNA Pure LC Total Nucleic Acid Extraction Kit and MagNA Pure LC Instrument (Roche). Samples were amplified using a one- step Qiagen RRT-PCR kit (Qiagen) for influenza A virus. Positive type A samples were further tested by RRT-PCR for H5N1 (Roche).

Epidemiological investigation. The outbreaks occurred during 2006 were divided into three phases. Phase I: the early introduction of the disease without any vaccination or early after vaccination, the period from February to May. Phase II: sharp drop in reported cases due to vaccination and summer season during June to October. Phase III: reoccurrence and increase in the number of reported cases during November and December 2006 in the early winter season. The field veterinarians collected data for epidemiological investiga- tion that were helpful in studying the risk factors for the spread of infection and the incidence in poultry production types in different governorates. From the epidemiological point of view, the study considered the village house as an epidemiological unit and recorded the type of birds raised in the house, which was either chickens, ducks, geese, turkeys, or mixed species raised together.

**Table (1)**: Record of the confirmed Egyptian H5N1 isolates and their Genbank accession numbers:

| Virus Name | Host/ Province | collection date | Type of breeding | Genbank Accession No.(HA) [a] |
|---|---|---|---|---|
| A/chicken/Egypt/960N3/004/2006(H5N1) | Chicken/ Giza | 13/2/2006 | House-hold | DQ447199 |
| A/chicken/Egypt/2253-1/2006(H5N1) | Chicken/ Giza | 12/2/2006 | House-hold | DQ862001 |
| A/turkey/Egypt/2253-2/2006(H5N1) | Turkey/ Menia | 13/2/2006 | House-hold | CY020653 |
| A/duck/Egypt/2253-3/2006(H5N1) | Duck/ Dakahlia | 2/5/2006 | Farm | DQ862002 |
| A/chicken/Egypt/1078-NAMRU3/2006(H5N1) | Chicken/ Menofia | 12/2006 | House-hold | EF441276 |
| A/chicken/Egypt/1080-NAMRU3/2006(H5N1) | Chicken/ Damietta | 28/9/2006 | House-hold | EF441278 |
| A/chicken/Egypt/1081-NAMRU3/2006(H5N1) | Chicken/ Gharbiya | 22/11/2006 | House-hold | EF441279 |
| A/Chicken/Egypt/06207-NLQP/2006(H5N1) | Chicken/ Sharkia | 19/2/2006 | Farm | EU372943 |
| A/Chicken/Egypt/06459-3-NLQP/2006(H5N1) | Chicken/ Menofia | 13/3/2006 | House-hold | EU372944 |
| A/Chicken/Egypt/06495-NLQP/2006(H5N1) | Chicken/ Sharkia | 17/3/2006 | Farm | EU372945 |
| A/Chicken/Egypt/06541-NLQP/2006(H5N1) | Chicken/ Cairo | 20/3/2006 | House-hold | EU372946 |
| A/Chicken/Egypt/06959-NLQP/2006(H5N1) | Chicken/ Gharbiya | 27/11/2006 | House-hold | EU372947 |

[a] Sequence analysis of HA cleavage site of these isolates indicated that all isolates were belonging to Highly Pathogenic Avian Influenza (HPAI) of H5N1 subtype.

**RESULTS:**

Clinical signs and post mortem lesions. Infected chickens and turkeys usually showed cyanosis of comb and wattles (or snout), facial edema, conjunctivitis, subcutaneous hemorrhages in the shank, and diarrhea; nervous signs such as lack of coordination, head shaking, abnormal gait, loss of balance, and recumbency were also recorded. Mortality and morbidity were varied and commonly reached 100% within a few days, especially in layer flocks. Clinical signs in other species, especially in rural ducks, were less severe and usually accompanied with nervous signs and sudden death. Usually no clinical signs were seen in geese except sudden death. The only reported case from commercial quail was from meat of slaughtered birds from a slaughter house, so the data on clinical signs were not reported. Sometimes there was no prominent lesion at post mortem because the birds died quickly before development of gross lesions. However, in most cases a variety of congestion and hemorrhages were noticed. The internal organs (liver, spleen, kidneys, intestine) were hemorrhagic. Mild to severe hemorrhagic tracheitis was also recorded, and hemorrhages in the proventriculus and caecal tonsils were observed.

Isolation and antigenic characterization of avian influenza viruses. The inoculated chicken embryos with the 12 selected samples died usually within 24–72 hr. HA titer of the 12 isolated viruses ranged from 5 to 9 $\log_2$. All samples were positive for HI using H5 antisera only, while they were negative using other tested avian influenza subtypes of HA antisera. All samples also reacted positive in the AGID test using avian influenza reference antiserum, while they were negative for other tested viruses as NDV, PMV2, and PMV3.

The 12 isolates were confirmed to be of subtype H5 using RT- PCR for H5/H7 avian influenza typing (Sacace). The result was positive for detection of subtype H5 (256 bp) and negative for H7. In addition, RT-PCR for H5 and H5N1 avian influenza revealed positive results.

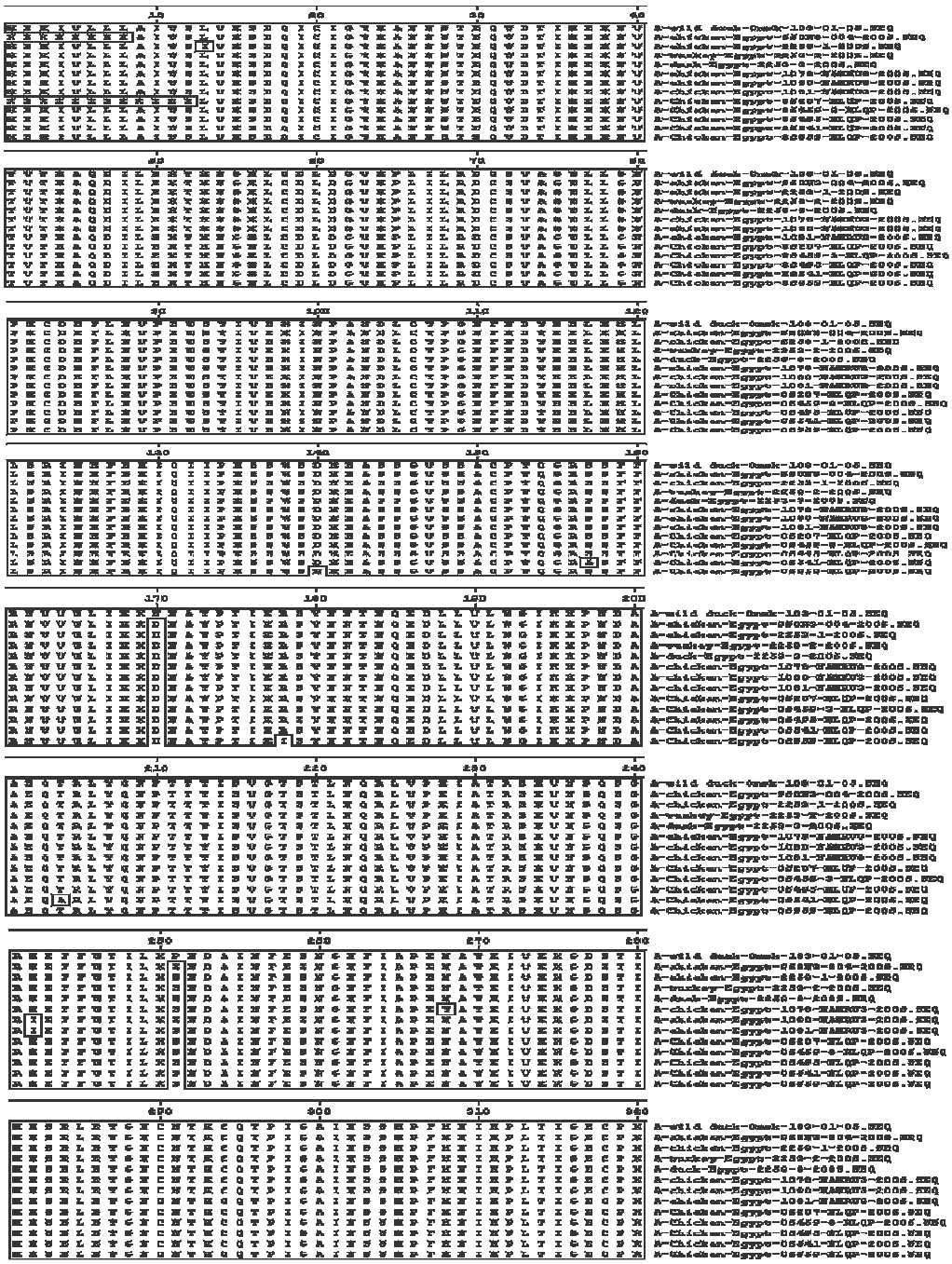

**Fig. 1.** Alignment of amino acids sequences of HA gene for the Egyptian strains.

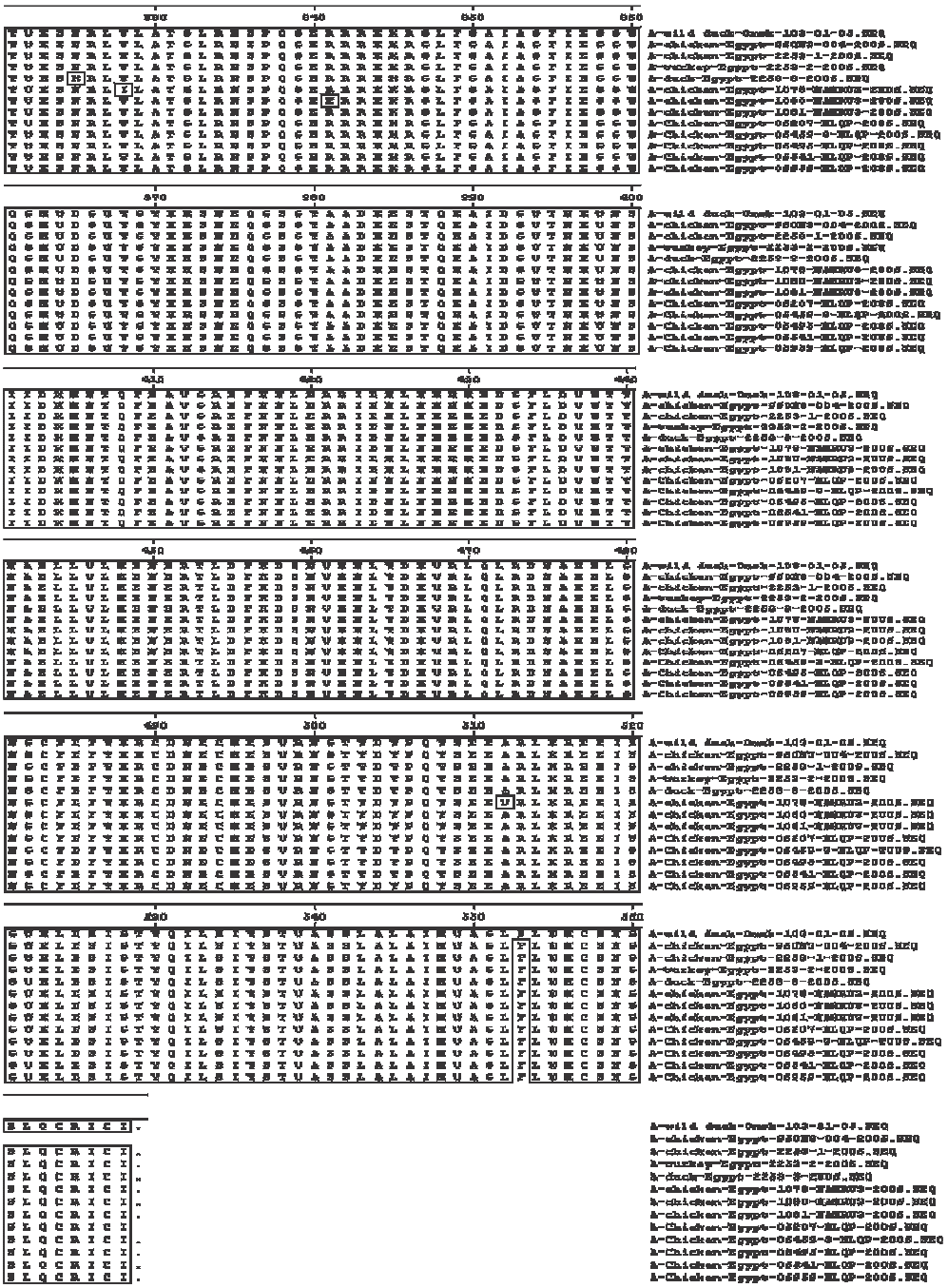

**Fig. 1.** Continued

For interlaboratory confirmation, 7 out of the 12 isolates were sent to either the U.S. Naval Medical Research Unit, NAMRU-3, Cairo, Egypt, or to the OIE reference laboratory (Padova, Italy) for verification and further analysis. Results of isolation, identification, and typing were identical and confirmed that these isolates are the H5N1 subtype. The genetic analysis of the HA cleavage site (PQGERKKRGLFGAIA) identified the H5N1 viruses isolated from domestic poultry as highly pathogenic avian influenza (HP AIV) (Table 1). Analyses of the amino acid sequence of HA gene for the 12 isolates and comparison with the strain from Russia (A/wild duck/Omsk/103-01/05) revealed that the 2006 Egyptian strains were very closely related with similarity percentage of more than 99%. Many amino acid substitution mutations appeared to be fixed in Egyptian strains (N158D, P239S, and S541F), one mutation was recorded in two isolates (M230I), and 10 mutations were recorded once along the entire HA of the 12 sequenced isolates. All Egyptian H5N1 strains studied had glutamine at position 226, which is the receptor binding site specific to avian species.

Phylogenetic analyses of the amino acid sequence of the HA gene for the 12 isolates were done by comparison with the strains from Russia, Africa, and the Middle East (Figs. 1, 2). The data revealed that the Egyptian strains are clustered within the cluster of isolates from Africa and the Middle East and belong to subclade 2.2 of H5N1 Eurasian strains.

For the epidemiological investigations, direct detection of viral RNA of subtype H5N1 was carried out using RRT-PCRs. All tested 1024 pool samples during the period from February to December 2006 from infected flocks and cases tested positive for H5N1 (Table 2).

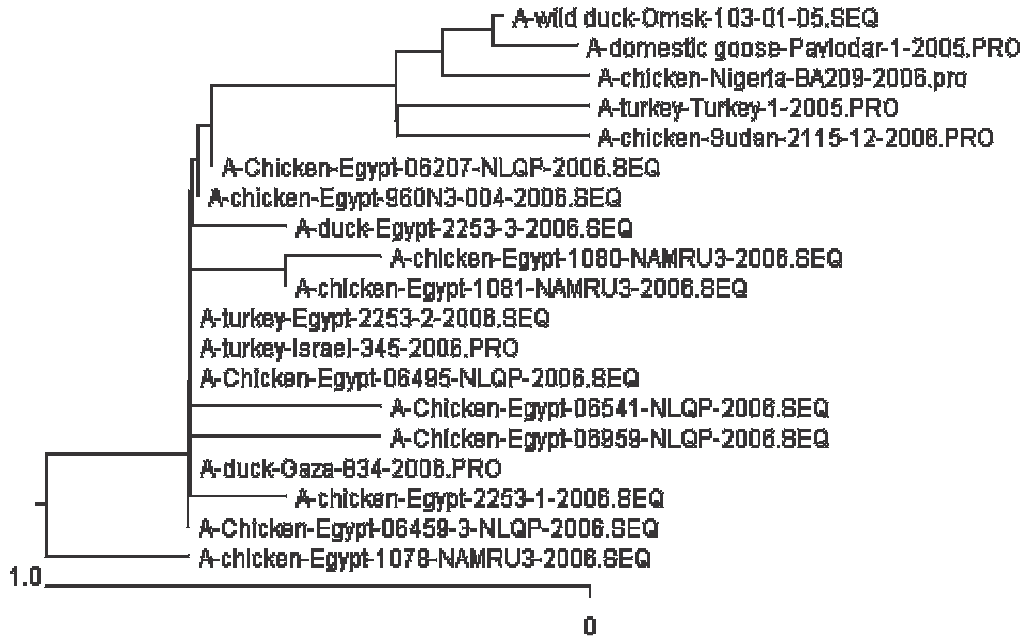


**Fig. 2:** Phylogenetic tree of HA gene for the Egyptian strains

Epidemiological investigation. Geographic distribution and incidence of H5N1 cases reported from February to December 2006 are summarized in Tables 2, 3, 4a,b, and Fig. 3. The disease is shown to be distributed all over Egypt. There were 20 provinces affected out of the 26 Egyptian provinces during 2006. The total positive cases from the Nile Delta region were the highest recorded, 554 cases, whereas greater Cairo (Cairo, Kalyobia, and Giza) had 338 cases, upper Egypt recorded 126 cases, and the Canal region recorded 6 cases. Also there were 963 cases recorded during phase I of the outbreaks, which was the first introduction of the disease into Egypt, while there were 23 and 38 cases recorded during phases II and III, respectively.

**Table (2):** H5N1 outbreaks in different types of poultry species recorded from February to December, 2006:

| Bird species | Rearing type | No. of confirmed flocks/cases [a] | No. of Provinces |
|---|---|---|---|
| Chickens | Rural chickens | 76 | 11 |
| | Broiler grandparents | 1 | 1 |
| | Broiler breeders | 67 | 10 |
| | Broilers | 366 | 17 |
| | Layers | 332 | 16 |
| Turkeys | Rural | 4 | 2 |
| | Commercial | 31 | 10 |
| Ducks | Rural | 19 | 14 |
| | Commercial | 22 | 11 |
| Geese | Rural | 2 | 2 |
| Quail | Commercial | 1 | 1 |
| Mixed popul. | Rural | 103 | 12 |
| **Total** | | **1024** | **20[b]** |

[a] = Number of infected commercial flocks and infected rural cases.
[b] = Total number of provinces had positive H5N1 (20 out of all the 26 Egyptian Provinces) either from commercial flocks or rural cases.

## DISCUSSION:

The results of virus isolation, identification, and molecular investigation using RRT-PCR as well as genetic analysis of the HA gene confirmed that the observed clinical signs, lesions, and mortalities were due to HPAI of subtype H5N1. The HA genes of the Egyptian H5N1 viruses possessed multiple basic amino acid motifs at the cleavage site and were thus characterized as HP AIV. RRT-PCR for common M and H5N1 genes of avian influenza used in molecular diagnosis or typing of the virus had successful results. This test was able to detect few copies of the virus in the samples (19,31). RRT-PCR for H5 and H5N1 avian influenza results revealed that this test is a useful rapid test for diagnosis, typing, and epidemiological investigations of avian influenza viruses.

Egypt is considered to be one big village as people live in a densely populated area along the Nile River in close contact with poultry. There are four sectors of poultry production in Egypt: sectors 1 and 2 include the grandparent and parent commercial production where there are good hygienic and biosecurity measures; sector 3 includes nonregulated, nonregistered small to medium-scale commercial activities; and sector 4 includes backyard rural, in-house, and rooftop-raised poultry. Production practices in sectors 3 and 4 as well as live bird markets mainly bring infected poultry into close contact with humans. The outbreaks in Egypt involved both commercial and backyard flocks, with considerable impact on socioeconomic aspect and food security. It is probable that a large sector of citizens in Egypt are at risk of H5N1 exposure.

Currently it was not possible to trace the primary source and the means of introduction of the infection into Egypt. Since 2003 the H5N1 avian influenza virus has spread easily between various wild and domestic bird species in many locations worldwide. The wide spread of the H5N1 virus from the East in wild birds in 2006 including nonmigratory species in many countries and infection of some domestic poultry has also been recorded (15,37). The currently circulating H5N1 appears to be virulent for a variety of wild bird species (9,21). However, there is an assumption that migratory birds are playing a significant role in transmission, since Egypt is in the flyway of migratory birds (14). In addition movement of live poultry is likely to have an important role. Free-ranging backyard chickens and illegal transportation of domestic birds also have been shown to contribute to spread of the virus as reported in Thailand (34).

From the epidemiological data collected during the period from February to December 2006, it is obvious that phase I (early outbreaks from February to May) recorded the highest incidence (963 flocks/cases), while in phase II (summer season from June to October) 23 cases were reported, and in phase III (early winter during November and December) 38 cases were

found. This illustrates that the climatic conditions are critical for increasing incidence of cases reported during the winter season. It is obvious that the geographic region, time of year, and the environment have a role in the incidence and distribution of the disease (33).

In Egypt, transmission and widespread occurrence of the disease among different poultry backyard species has been shown to be due to raising of chicken, turkey, ducks, and geese together. Interspecies transmission usually occurs especially between closely related host species in the same taxonomic family (22,32).

This study illustrates that the highest incidence on the rural level was recorded in mixed poultry populations (mostly chickens, ducks, geese, and usually with turkeys reared together); 103 household poultry cases were recorded. Field observations indicated that highest outbreak phases I, II, and III as shown in parts A, B, and C, respectively. The arrows refer to the recorded cases that were distributed over the four regions of Egypt (Delta, Cairo, Canal, and Upper Egypt). mortalities have been recorded mostly in chickens and turkeys; however, mortalities were also noticed in some ducks and geese, therefore further studies are required to investigate the susceptibility of different breeds of water fowls to infection with HPAI-H5N1 (35). Under village conditions, it is very difficult to separate different species of birds such as chickens, ducks, geese, turkeys, and pigeons.

**Table (3):** Geographic distribution of H5N1 cases reported from February to December 2006

| Month / Governorate | Phase I | | | | Phase II | | | | | Phase III | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feb | Mar | Apr | May | Jun | Jul | Aug | sep | Oct | Nov | Dec | |
| **1-Domiata** | 8 | 21 | 1 | | | | | 3 | | | 1 | 34 |
| **2-Sharkia** | 8 | 219 | 48 | 2 | | | | | | | 1 | 278 |
| **3-Gharbia** | 1 | 34 | 15 | 4 | 1 | | | | | 2 | 4 | 61 |
| **4-Monofia** | 2 | 35 | 5 | 1 | 1 | | | | | 3 | 17 | 64 |
| **5-Dakahlia** | 3 | 40 | 12 | 2 | | | | | | | | 57 |
| **6-Behera** | 1 | 4 | 18 | 2 | | | | | | 1 | | 26 |
| **7-Kafr-El-Shikh** | 1 | 11 | 11 | | | | | | | | | 23 |
| **8-Alexandria** | | 4 | 3 | 1 | | | | | 1 | 2 | | 11 |
| **Delta Region** | 24 | 368 | 113 | 12 | 2 | | | 3 | 1 | 8 | 23 | 554 |
| **9-Cairo** | 19 | 15 | 2 | | | | 3 | | | | | 39 |
| **10-Giza** | 58 | 41 | 5 | | | 1 | | 3 | | | 4 | 112 |
| **11-Kalyobia** | 26 | 134 | 26 | 1 | | | | | | | | 187 |
| **Great Cairo** | 103 | 190 | 33 | 1 | | 1 | 3 | 3 | | | 4 | 338 |
| **12-Ismailia** | | 6 | | | | | | | | | | 6 |
| **Canal Region** | | 6 | | | | | | | | | | 6 |
| **13-Menia** | 3 | 19 | 20 | 1 | 4 | | | | | | 1 | 48 |
| **14-Fayoum** | 2 | 12 | 12 | 1 | | | | | | | | 27 |
| **15-Beni-Suif** | 2 | 8 | 3 | | | | | | 1 | | | 14 |
| **16-Sohag** | 7 | 4 | 9 | 2 | 2 | | 1 | 1 | | 1 | | 27 |
| **17-Quena** | 1 | 1 | | | | | | | | | | 2 |
| **18-Loxor** | 1 | 1 | | | | | | | | 1 | | 3 |
| **19-Assuit** | | 1 | 2 | | | | | | | | | 3 |

| Month / Governorate | Phase I | | | | Phase II | | | | | Phase III | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feb | Mar | Apr | May | Jun | Jul | Aug | sep | Oct | Nov | Dec | |
| **20-Aswan** | | 1 | | | | | | 1 | | | | 2 |
| **Upper Egypt** | 16 | 47 | 46 | 4 | 6 | | 1 | 2 | 1 | 2 | 1 | 126 |
| **Total*** | **143** | **611** | **192** | **17** | **8** | **1** | **4** | **8** | **2** | **10** | **28** | **1024** |
| | **963** | | | | **23** | | | | | **38** | | |

**[A] Total cases recorded were 1024 from 20 governorates**

**Table (4a):** Type of infected species recorded in backyards during February to December 2006

| Month<br>Species | Feb | Mar | Apr | May | Jun | Jul | Aug | sep | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chickens** | 50 | 9 | 7 | - | 1 | | 2 | 2 | 2 | 1 | 2 | 76 |
| **Ducks** | 8 | 3 | - | - | | | 2 | | | | 6 | 19 |
| **Turkeys** | 4 | - | - | - | | | | | | | | 4 |
| **Geese** | 1 | 1 | - | - | | | | | | | | 2 |
| **Mixed*** | 20 | 24 | 14 | 4 | 5 | 1 | | 6 | | 9 | 20 | 103 |
| **Total** | **83** | **37** | **21** | **4** | **6** | **1** | **4** | **8** | **2** | **10** | **28** | **204** |

***** mixed two or more species reared together

**Table (4b):** Type of infected species recorded in Farms during February to December 2006

| | Feb | Mar | Apr | May | Jun | Jul- Dec | Total |
|---|---|---|---|---|---|---|---|
| **Broiler Chickens** | 20 | 268 | 72 | 4 | 2 | 0 | 366 |
| **Broiler breeder Chickens** | 12 | 42 | 11 | 2 | | 0 | 67 |
| **Layer chickens** | 22 | 243 | 63 | 4 | | 0 | 332 |
| **Broiler grandparent Chickens** | - | 1 | - | - | | 0 | 1 |
| **Ducks** | 4 | 12 | 5 | 1 | | 0 | 22 |
| **Turkeys** | 2 | 8 | 19 | 2 | | 0 | 31 |
| **Quail** | - | - | 1 | - | | 0 | 1 |
| **Total** | **60** | **574** | **171** | **13** | **2** | **0** | **820** |

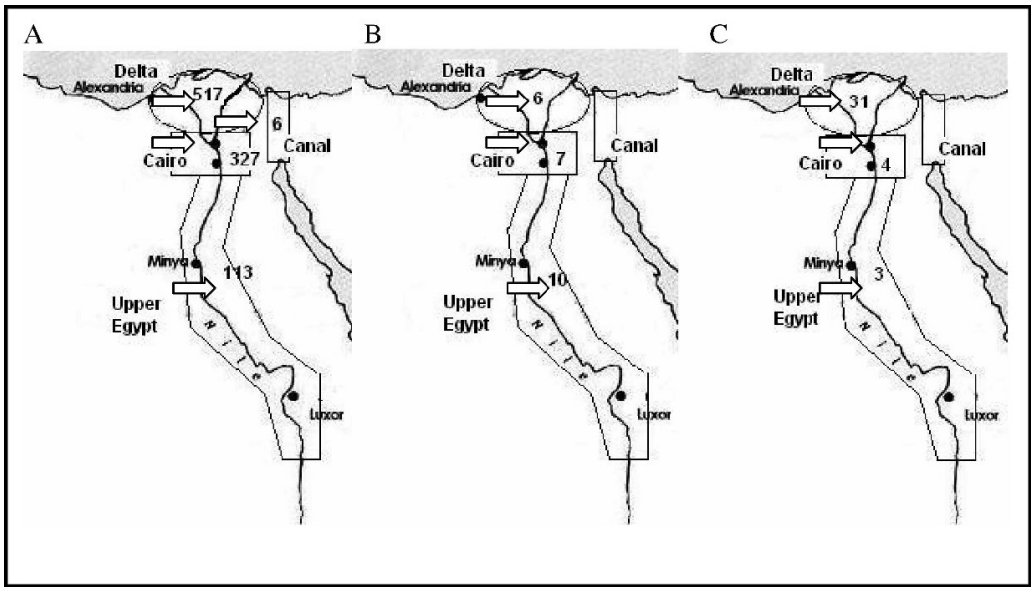


**Fig. 3**: Geographic distribution of H5N1 cases reported from February to December 2006. Distribution of positive cases recorded during

Such situations will complicate the efforts for control. One of the most important observations of this study is that H5N1 cases were reported only in backyard flocks during July to December, and no cases were recorded in commercial flocks during this period. This may reflect that vaccination against

H5N1 in commercial flocks in combination with quarantine and depopulation of infected and contact farms significantly control the further spread of the disease in the industrial poultry production sectors. This showed that vaccination should be considered as a successful tool in controlling and minimizing this serious outbreak in Egypt during 2006.

Vaccination is used as a mean of increasing resistance of susceptible birds, to reduce the risk of introduction from the reservoir host, and to reduce secondary spread in densely populated poultry areas. Many vaccine manufacturers are introducing different inactivated avian influenza vaccines into the Egyptian field; these vaccines are apparently sourced from different suppliers.

Despite the efficacy of vaccines used, inadequate biosecurity or vaccination practices can lead to transmission between flocks and selection of variants that exhibit antigenic drift. The antigenic drift of H5N2 viruses belonging to the Mexican lineage resulting in less homology in the vaccine strain has been described recently (18).

Until now there has been no universal solution to avian influenza control and prevention given the multifaceted characteristics of the poultry industry in developed and developing countries (7).

Biosecurity must always be improved in the face of an avian influenza threat, and it may be used in conjunction with vaccination under certain circumstances.

In conclusion, this study showed evidence of the wide spread of H5N1 virus infection in domestic poultry in Egypt. The 1024 cases from different poultry species (rural and commercial chickens, turkeys, ducks, geese, and quail) either in commercial breeding or in backyards from different locations in Egypt tested positive for H5N1 subtype. RRT-PCR for common Mand H5N1 genes of avian influenza was the main test used

for epidemiological investigation and molecular diagnosis or for typing the virus and had rapid and successful results. Mortality and morbidity in the affected flocks or household cases were varied and commonly reached 100% within a few days. Under field conditions it was easily to detect clinically affected flocks or birds by severe signs, especially cyanosis of the comb and wattles and subcutaneous hemorrhages in the shank. There was no prominent lesion at post mortem except for various types of congestion and hemorrhages. Prospects for successful control are difficult because of insufficient measures and facilities to restrict the movement of poultry from infected areas to noninfected ones. Also, other risk factors can explain this rapid and widespread occurrence, such as a high density of poultry population raised in the crowded Nile Delta, live bird markets, random and unorganized rural poultry productions, backyards and rooftop rearing as well as fast and randomized movement of poultry, by-products, manure and humans. Moreover, insufficient human awareness and knowledge about the disease are additional factors. All these factors shared in the spread of the disease within a very short period, and so dedicated efforts are needed to eradicate the disease.

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## ACKNOWLEDGMENTS:

We would like to thank Dr. Marchalle Monteville and Dr. Magdy Darwish from the U.S. Naval Medical Research Unit (NAMRU-3), Cairo, Egypt, as well as Dr. Ilaria Capua and Dr. Giovanni Cattoli from the World Organization for Animal Health (OIE) Reference Laboratory (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy) for their cooperation. Highly pathogenic H5N1 avian influenza virus in poultry in Egypt 277

# دراسات وبائية للمرض المسبب بفيروس أنفلونزا الطيور شديد الضراوة H5N1 في الدواجن في مصر عام 2006

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## الملخص

يصف هذا البحث تسجيل أول حالات لوباء فيروس أنفلونزا الطيور المسبب بالنوع H5N1 في مصر من فبراير إلى ديسمبر 2006 في قطعان الدواجن المختلفة التجاري والتربية المنزلية من مختلف أنواع الدواجن ويلخص البحث أهم خصائص هذا الوباء.

سجلت في هذا البحث حدوث 1024 حالة من مختلف أنواع الدواجن مثل دجاج المنازل والدجاج التجاري من مختلف قطاعات التربية وكذلك الرومي والبط والاوز والسمان من جميع الأنحاء في مصر وكانت كل الحالات التي سجلت من النوع H5N1. ومن هذه الحالات تم عزل 12 فيروس لأنفلونزا الطيور وتم تصنيفه من العينات التي جمعت أثناء الوباء.

وكانت نتائج التصنيف تشير إلى أن جميع المعزولات من نوع H5N1 شديد الضراوة وكذلك تم عمل التحليل الجيني لجين التلزن الدموي (HA) وتمت المقارنة مع الفيروسات الأخرى من روسيا وأفريقيا والشرق الأوسط وكانت النتائج تشير إلى أن المعزولات المصرية جميعها متشابهة فيما بينها وتتبع تحت مجموعة 2.2 للفيروسات H5N1 الاورواسيوية في التصنيف وهو نفس النوع الموجود في منطقة الشرق الأوسط والذي انتقل إلي أفريقيا في بداية عام 2006.

تشير نتائج هذه الدراسة إلى انتشار فيروس H5N1 في قطعان الدواجن في مصر خلال فترة قصيرة. وكانت أهم السمات المميزة لهذا الوباء هي شدة الاعراض الاكلينيكية وارتفاع نسبة النفوق وسرعة انتشار الوباء في مختلف المحافظات في وقت قصير.

وفي هذا البحث تمت مناقشة الأسباب المحتملة لهذا الانتشار السريع وطرق التحكم والسيطرة علي المرض.

# Epidemiological findings of outbreaks of disease caused by highly pathogenic H5N1 avian influenza virus in poultry in Egypt during 2006

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## SUMMARY:

This paper describes the first threats of H5N1 avian influenza outbreaks in Egypt recorded from February to December 2006 in commercial and domestic poultry from different species and summarizes the major characteristics of the outbreak.

There were 1024 cases from different poultry species (rural and commercial chickens of different breeding types, turkeys, ducks, geese, and quail) either in commercial breeding or backyards from different locations in Egypt and all revealed positive for H5N1 subtype. From these cases only twelve avian influenza A viruses were isolated and

characterized from samples collected during outbreaks. All isolates were characterized and the data confirmed that the isolated viruses are belonging to Highly Pathogenic Avian Influenza (HPAI) of subtype H5N1. This study showed evidence of widespread of H5N1 virus infection in domestic poultry in Egypt within a short time. The most obvious features of these outbreaks were severe clinical signs and high mortalities as well as very rapid and wide spread occurrence within the country in very short time. The possible causes of rapid spread and prospects of disease control were discussed.