

## WHO Global Influenza Program Surveillance and Network, 2005; ([www.oie.int](http://www.oie.int)).

### Outbreak of highly pathogenic avian influenza (AI) H5N1 in poultry in Egypt 2006

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Avian influenza outbreak was firstly appeared in poultry reared in backyards or roofs of homes in Qalubia & Giza governorates as many notifications were received by the General Organization of Veterinary Services (GOVSs) in February 2006. The disease was spread and recorded in chickens (foreign breeds , Balady , and hybrids ) , turkeys, ducks , geese and wild birds (from Giza Zoo) in 20 out of 26 (76.9 %) of the Egyptian governorates within few weeks. The number of confirmed farm cases was 840 farms (layers 327, Broilers 164, Balady 117, Saso 86, parents 84, turkeys 32, ducks 29 and grand parents 1) and those of rural birds was 129 (home birds 119, wild birds 5, abattoir 3, shops sales live birds 1, migratory bird 1) from February to June 2006. The clinical picture of the disease were observed in infected birds that reared in farms and those of backyards (most of domestic birds) , or sold in shops (sells broilers or parrots ). The number of dead and culled birds in this outbreak was 34.4 millions with economical losses 2-3 billion dollars.

#### Key Words:

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## Possible Causes Of Re-Emerging

The observed clinical signs in chicken were typical for avian influenza. Contact domestic pigeons did not show any clinical observation and H5N1 AI virus did not isolated from contact pigeon. Oropharyngeal & cloacal swabs were collected, for virus isolation and identification, from infected chicken located at El Kanater El Khiria-center, Qaluobia governorate. The causative agent was isolated on SPF embryonated chicken eggs and identified as H5 AI virus in WHO National Influenza Center (WHONIC), VACSERA, Agouza, Egypt using WHO influenza Kit. The identified influenza strains were sequenced at WHO Calibrating Center (WHOCC) at London, UK. The nucleotide sequences analysis of HA and NA genes were obtained and compared with other world AI isolated strains. The obtained results revealed that the Egyptian strains that isolated in 2006 from El-Qanater El- Khiria Center, Qaluobia (h5ckeegypt 106 ha , h5ckeegypt 206 ha , h5ckeegypt 306 ha / h5ckeegypt 106 n , h5ckeegypt 106 n . h5ckeegypt 106 n1), according to their heamagglutinin and neuaminidase sequencing, are closely related to H5N1 strain isolated from turkeys located in Turkey at 2005 (A/ty/Turkey/1/05) influenza strain and to H5N1 strain isolated from human cases in Viet Nam at 2004 (A/Vietnam/1194/04) .

## INTRODUCTION

Avian influenza is caused by specified viruses related to the family Orthomyxoviridae, genus influenza A virus. Many species of birds have been shown to be susceptible to infection with IAV. These viruses have antigenically related nucleocapside & matrix proteins, but are classified into subtypes on the basis of their hemagglutinin (H) and neuraminidase (N) antigens (15). At present, 16 H subtypes (H1-H16) and 9N subtypes (N1-N9) are recognized. To date the highly virulent IAV that produce clinical disease in chickens and turkeys have been associated only with the H5 and H7 subtypes (with the exception of two H10 subtypes that

would also have fulfilled the definition of highly pathogenic notifiable avian influenza although the reasons for this are not clear). In spite that, many viruses of H5 and H7 subtypes isolated from birds have been of low virulence, becoming virulent by mutation, all H5 and H7 viruses have been identified as notifiable avian influenza viruses (16). The highly pathogenic avian influenza induces severe mortality may be reached to 100% in highly susceptible birds with sever economic losses in domesticated chickens and turkeys. The most important issue of influenza type A viruses is their genetic instability. Several AI strains emerged from other strains by reassortment process (genetic shift) or point mutation (genetic

drift) and this may be explaining their perpetuation in nature. Full genome sequences of 7 influenza (H5N1) viruses isolated from poultry in Western Siberia and the European part of the Russian Federation during July 2005-February 2006 showed high homology to Qinghai-like influenza (H5N1) viruses. Phylogenetic analysis not only showed a close genetic relationship between the H5N1 strains isolated from poultry and wild migratory waterfowls but also suggested genetic reassortment among the analyzed isolates(10). Comparison between more than 3000 RNA sequences of segment 8 of type A influenza viruses a unique single nucleotide substitution typically associated with recent H5N1 strains was found and this explain the aggressive recent outbreaks of H5N1 strains(12). Phylogenetic analyses of H6N2 chicken viruses indicated that the H6N2 most likely arose from a reassortment between two South African LPAI ostrich isolates: an H9N2 virus isolated in 1995 and an H6N8 virus isolated in 1998. It is probable that the ostrich H6N8 and H9N2 progenitors of the chicken H6N2 viruses were introduced to ostriches by wild birds. Ostriches, in which AI infections are often sub-clinical, may serve as mixing vessels for LPAI strains that

occasionally spill over into other poultry (1, 2). The H9N2 strain that was isolated from quails and H5N1 subtype viruses isolated from chicken have also exchanged gene segments to generate currently circulating reassortants of both subtypes that have pandemic potential (20). The Italian HPAI viruses arose from low pathogenicity strains, and that a deletion in the NA stalk followed by the acquisition of additional glycosylation near the receptor binding site of HA1 may be an adaptation of H7 viruses to a new host species i.e. domestic poultry (3). The highest homology between the internal genes of H9N2, H6N1, and the H5N1 isolates indicates that these subtypes are able to exchange their internal genes and are therefore a potential source of new pathogenic influenza virus strains(8). Thus, genetic reassortment between avian and human influenza strains does occur in the emergence of pandemic and inter-pandemic influenza A viruses (13). The present study aimed to isolate and identify the circulated virus that inducing severe losses in poultry industry in our country followed by genotyping of the isolated strains in comparison with other worldwide HPAI strains.

## MATERIAL AND METHODS

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### Sample collection

In Egypt, at 10/2/2006, typical signs of AI were observed on affected chickens and turkeys located at different backyards and roofs of houses at large number of villages in El Qanater El khiria centre, Qaluobia governorate and, all observed birds were died within 72 hrs. Oropharyngeal and cloacal swabs were collected in February 2006, from affected chicken and contact pigeons; in viral transport media (HBSS with isotonic pH 7-7.2 contained penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50µg/ml) and mycostatin (1000 units/ml) for oropharyngeal swabs but at five-fold higher concentrations for faeces and cloacal swabs. Swab suspensions were processed and stored in ice box, then transported to WHONIC for diagnosis of influenza viruses Egypt, (within 24 hours) and preserved at -70 till used.

Virus isolation and identification were done according to the protocol of OIE manual 2005 following standard bio-safety guidelines of WHO (16), the virus was isolated from oropharyngeal and cloacal swabs immersed in HBSS, the swabs were centrifuged at 3000 rpm and 0.2 ml were inoculated in each of SPF embryonated chicken eggs (ECE),

inoculated SPF eggs were incubated at 35-37°C and all eggs remaining at the end of the incubation period, were chilled to 4°C, the allantoic fluid was aseptically collected and tested for haemagglutination (HA) activity using 0.5% Chicken RBCs. The isolated viruses identified by using WHO Influenza Kit.

Antigenic analysis: the isolated and identified viruses in WHONIC in Egypt were sent to WHOCC influenza reference Lab., London, UK, for antigenic analysis by HI influenza Kit contained reference influenza type A H5N1 antisera against human and avian influenza virus strains.

Phylogenetic analysis :- the identified virus was sent to WHOCC influenza reference Lab., at London for phylogenetic analysis.

Data collection: data were collected from the General Organization of Veterinary Services (GOVSS) during the outbreak

### RESULTS AND DISCUSSION

**Clinical signs** :-the affected chicken showed depression, off food, ruffled feather, in-coordination, cyanosis of comb and wattle, swelled head, nasal discharge, heavy stringy

salivation , coughing , sneezing , swollen of the eye lids , closed eyes , diarrhea with heavy ureates (photo 1 & 2) .Before the appearance of clinical symptoms ,the chicken were of good health with best condition of egg production , after the appearance of symptoms cessation of egg production was observed The morbidity rate 100% and the mortality rate reached 100% within 72 hours .The contact pigeons didn't show any clinical signs.

### DISCUSSION

Highly pathogenic avian influenza H5N1 is still circulate since 1997 in South East of Asia as the first case of spread from a bird to a human was seen during an outbreak of bird flu in poultry in Hong Kong in 1997(17). However, the Outbreaks of influenza H5N1 occurred among poultry in eight countries in this area (Cambodia, China, Indonesia, Japan, Laos , South Korea , Thailand . and Vietnam) during late 2003 and early 2004. At this time. more than 100 million birds in the affected countries either died from the

disease or were killed in order to control the outbreak. By March 2004, the outbreak was reported to be under control. Beginning in late June 2004, however, new outbreaks of influenza H5N1 among poultry were reported by several countries in Asia (Cambodia, China Tibet, Indonesia, Kazakhstan, Malaysia, Mongolia, Russia [Siberia], Thailand, and Vietnam). It is believed that these outbreaks are ongoing then transmitted to Europe at the end of 2005 (Romania , Turkey , Ukraine , Croatia ) and still circulate in Europe from the beginning of 2006 (Bulgaria , Slovenia , Slovakia . Germany . France , Austria , Greece , Italy . Bosnia and Herzegovina ,Switzerland , Serbia , Hungary . Poland , Albania , Denmark . Sweden , Czech Republic . United kingdom & Spain (7). The disease appeared in Africa at the beginning of 2005 In Burkina Faso and at 2006 in Nigeria , Egypt , Gazza strip, Niger . Cameroun . Sudan , Cote d Ivoire & Djibouti. Human infections of influenza A (H5N1) have been reported in most of affected countries (5, 11, 12, 18, 19)

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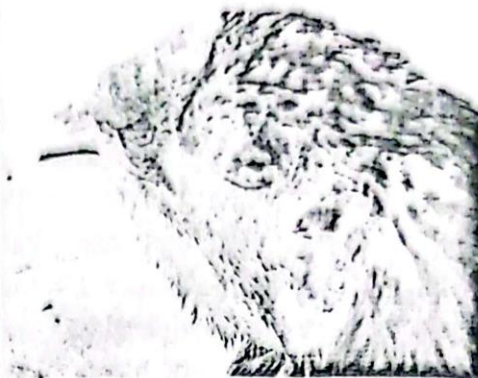


Photo (1) diarrhea with heavy ureates  
head,  
eye lids, closed eyes

Photo (2) cyanosis & swelled  
swollen of the

Table (1) Antigenic analysis of avian influenza viruses by (HI) in  
WHONIC Egypt

tested	Reference control positive anti influenza viruses type A sera		
	H1N1* A/NC/20/99	H3N2** A/Panama/2007/96	H5N1***
Reference Control positive antigens			
A/H1*	5120	160	40
A/Panama/2007/96*	0	5120	0
A/H5*	0	0	640
Tested sample 2006			
ck/Egypt/1/06 N	0	0	320
ck/Egypt/2/06 N	0	0	320
ck/Egypt/3/06 N	0	0	320
ck/Egypt/1/06 C	0	0	640 ▼
ck/Egypt/2/06 C	0	0	640 ▼
Pigeon pharyngeal swabs	0	0	0
Pigeon cloacal swabs □	0	0	0

\*A/H1 (H1N1) A/New Caledonia) human strain

\*\*A/H3 (H3N2) A/Panama/2007/96\*: human strain

\*\*\* A/H5(H5N1): chicken strain

▼ The HA activity of cloacal swabs differed one log<sub>2</sub> HI titer from the oropharyngeal swabs

□ H5N1 AI virus did not isolated neither from oropharyngeal nor from cloaca of contact pigeons.

Table (2) Antigenic analysis of avian and human influenza viruses (HI)

Type of samples	Haemagglutination inhibition titre										
Reference positive antigens	CK/Scot/59 NIBSC Sh	A/HK/156/97 NIBSC Sh382	A/HK/213/03 RGW Goat 358	RG-14 (Viel/194) NIBSC (Sh 440)	Qu/Cirebon/B B/05 CDC F61/05	A/Ind/5/05 CDC F89/5	Ty/Turkey/1/ 05 WIC fC6/05				
CK/Scotland/59	1280	320	80	640	40	80	80				80
A/hong Kong/156/97	320	640	80	320	80	40	20				20
A/hong Kong/213/03	640	160	160	640	80	<40	<20				<20
A/Vietnam/1194/04	1280	40	80	640	40	<40	<20				<20
Qu/Cirebon/B/BVet/05	320	80	40	160	640	160	<20				<20
A/Indonesia/5/05	160	40	40	160	640	320	<20				<20
Ty/Turkey/1/05	320	80	80	320	80	80	320				320
Tested samples											
ck/Egypt/1/06 N	160	80	80	320	80	80	320				320
ck/Egypt/2/06 N	160	40	40	320	40	40	320				320
ck/Egypt/3/06 N	160	40	40	160	40	40	320				320
ck/Egypt/1/06 C	160	40	40	160	40	40	160				160
ck/Egypt/2/06 C	160	40	40	320	40	80	320				320

Data presented in this table revealed that Egyptian isolates at 2006 are closely related to AI H5N1 strain isolated from turkeys in Turkey (Ty/Turkey/1/05) and to human strain isolated from human cases in Viet Nam (RG-14 (Viel/194))

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Table (3) WHO Human Avian Influenza confirmed cases

Country	Cumulative Number of cases* : December 28, 2007									
	Cases**					Deaths				
	2003	2004	2005	2006	2007	2003	2004	2005	2006	2007
Azerbaijan	0	0	0	8	0	0	0	0	5	0
Cambodia	0	0	4	2	1	0	0	4	2	1
China	1	0	8	13	5	1	0	5	8	3
Djibouti	0	0	0	1	0	0	0	0	0	0
Egypt	0	0	0	18	23	0	0	0	10	6
Indonesia	0	0	20	55	41	0	0	13	45	36
Iraq	0	0	0	3	0	0	0	0	2	0
Laos	0	0	0	0	2	0	0	0	0	2
Myanmar	0	0	0	0	1	0	0	0	0	0
Nigeria	0	0	0	0	1	0	0	0	0	1
Pakistan	0	0	0	0	1	0	0	0	0	1
Thailand	0	17	5	3	0	0	12	2	3	0
Turkey	0	0	0	12	0	0	0	0	4	0
Viet Nam	3	29	61	0	8	3	20	19	0	5
Total by year	4	46	98	115	83	4	32	43	79	55
Total	346					213				

\*This chart represents WHO confirmed human cases of H5N1 . WHO reports only laboratory-confirmed cases. All dates refer to onset of illness

\*\*The total number of cases includes number of deaths



Table (4) Recorded cases of AI in Egypt from 12/2-24/6/2006†

Governorate	Farms	Backyards	Total
Sharqiya	277	0	277
Qaluobia	181	6	187
Giza	106	33	139
Daqahliya	43	14	57
Gharbiya	50	0	50
Meniya	30	16	46
Menofiya	34	5	39
Fayoum	25	1	26
Behyra	25	0	25
Cairo	14	11	25
Kafr El Sheikh	17	7	24
Sohag	10	14	24
Demiatta	10	5	15
Beni-Suef	2	12	14
Alexandriya	5	2	7
Ismailiya	6	0	6
Assuit	3	0	3
Qena	1	1	2
Luxor	1	1	2
Aswan	0	1	1
Total	840	129*	969

† Source of these data is the GOVSS

\* One reported case from shop sells live birds (broilers) and other from shop sells parrots

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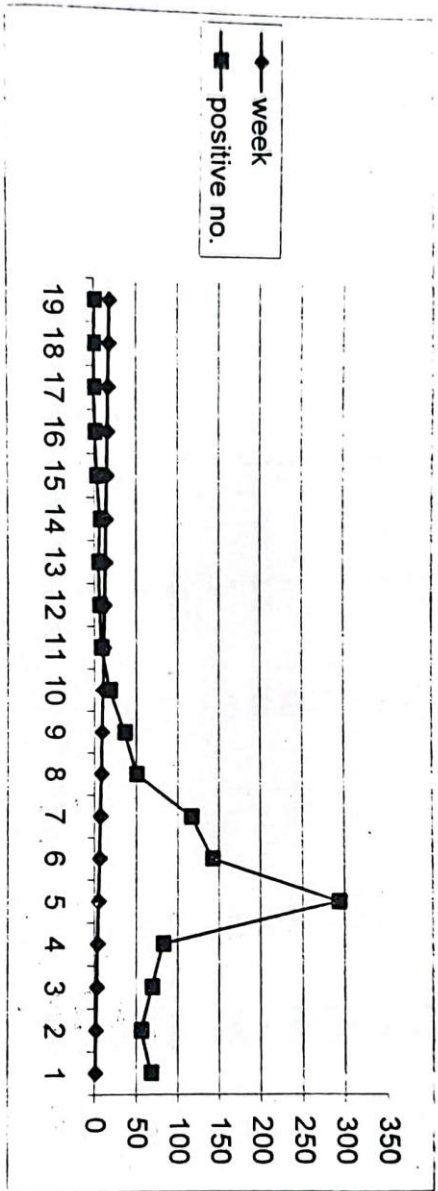
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ty/Turkey/1/05	320	80	80	320	80	80	320				320
Tested samples											
ck/Egypt/1/07 N	160	80	80	320	80	80	80				80
ck/Egypt/2/07 C	160	40	40	320	40	40	40				40

Source of these data Dr/A.Hay WHOCC AI reference lab., London, UK.  
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Fig. 1 Duration and peak of AI outbreak in Egypt 2006



from table (5) and figure (1) it is very clear that the peak of the outbreak, in intensive poultry farms, within the 4,5,6, and 7 weeks of the infection (10 March-6 April/2006) then the intensity of the outbreak decreased gradually with no reported cases at the 22nd week of the outbreak

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Table (6) Types and numbers of recorded poultry cases affected by AI virus from 12/2- 24/6/2006†

Governor ate	Types of birds in the farms										Backyards				Total
	Grant parent.	parent layer	Broiler	Saso	Balady	Duck	Turkey	Backyards			Home bird	Migrato ry. Bird*			
								Wild*** bird	Slaughter house**	Shop					
Sharqiya	01	18	132	47	65	11	2	01	-	-	-	-	-	-	277
Qaluobia	-	09	095	26	10	33	4	04	-	-	-	-	-	06	187
Giza	-	28	029	18	01	23	5	02	5	3	-	-	24	1	139
Daqahliya	-	01	015	13	07	06	-	01	-	-	-	-	14	-	057
Gharbiya	-	09	014	10	01	12	4	-	-	-	-	-	-	-	050
Meniya	-	-	002	07	-	01	2	18	-	-	-	-	16	-	046
Menofiya	-	04	016	11	-	03	-	-	-	-	-	-	05	-	039
Fayoum	-	-	005	06	-	14	-	-	-	-	-	-	01	-	026
Behyra	-	06	005	06	-	04	3	01	-	-	-	-	-	-	025
Cairo	-	04	-	04	-	01	4	01	-	-	-	-	11	-	025
Kafr El Sheikh	-	02	004	04	01	05	1	-	-	-	-	-	07	-	024
Sohag	-	-	001	04	-	03	2	-	-	-	-	-	14	-	024
Demiatta	-	02	001	05	-	-	1	01	-	-	-	-	05	-	015
Beni-Suef	-	-	001	-	-	-	-	01	-	-	-	-	12	-	014
Alexandrya	-	-	003	-	-	-	1	01	-	-	-	-	02	-	007
Ismailiya	-	01	004	-	01	-	-	-	-	-	-	-	-	-	006
Assuit	-	-	-	03	-	-	-	-	-	-	-	-	-	-	003
Qena	-	-	-	-	-	-	-	01	-	-	-	-	01	-	002
Luxor	-	-	-	-	-	01	-	-	-	-	-	-	-	-	002
Aswan	-	-	-	-	-	-	-	-	-	-	-	-	01	-	001
Total	1	84	327	164	86	117	29	32	5	3	1	1	119	1	969

† Source of these data is GOVSS

\*\*The only recorded migratory bird at 17/2/2006

\*\*Reported cases in poultry abattoir in freshly , frozen , and carcass processed chicken

\*\*\*Reported in different wild birds located at Giza Zoo

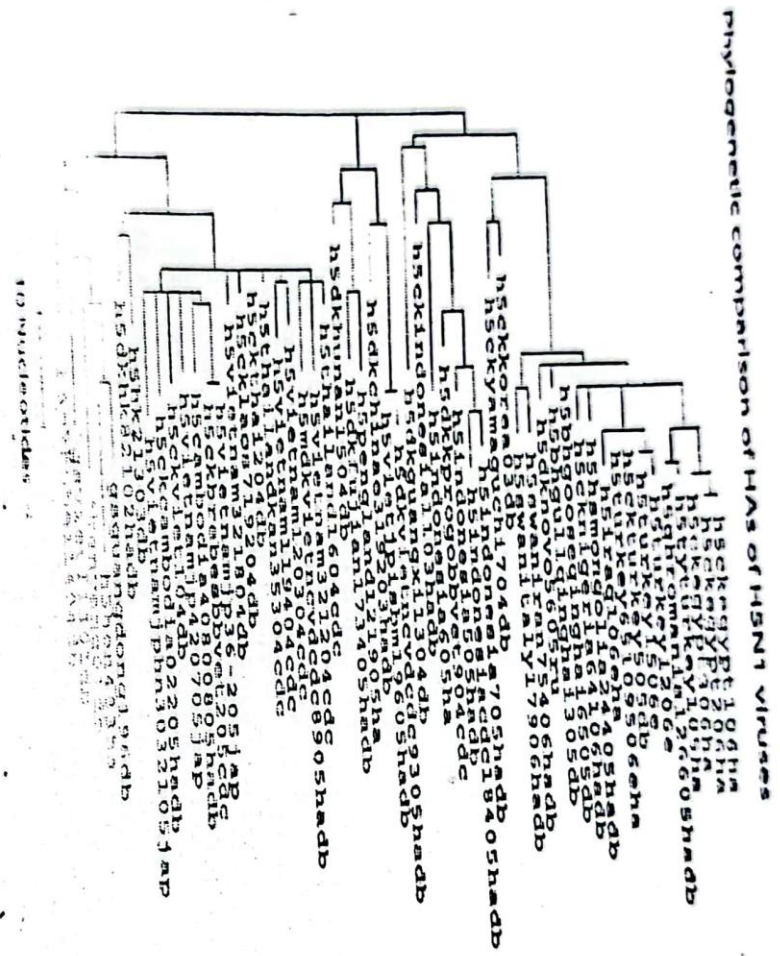


Fig. (2) Phylogenetic comparison of H5 HA genes of outbreaks 2006

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Phylogenetic comparison of NAs of H5N1 viruses

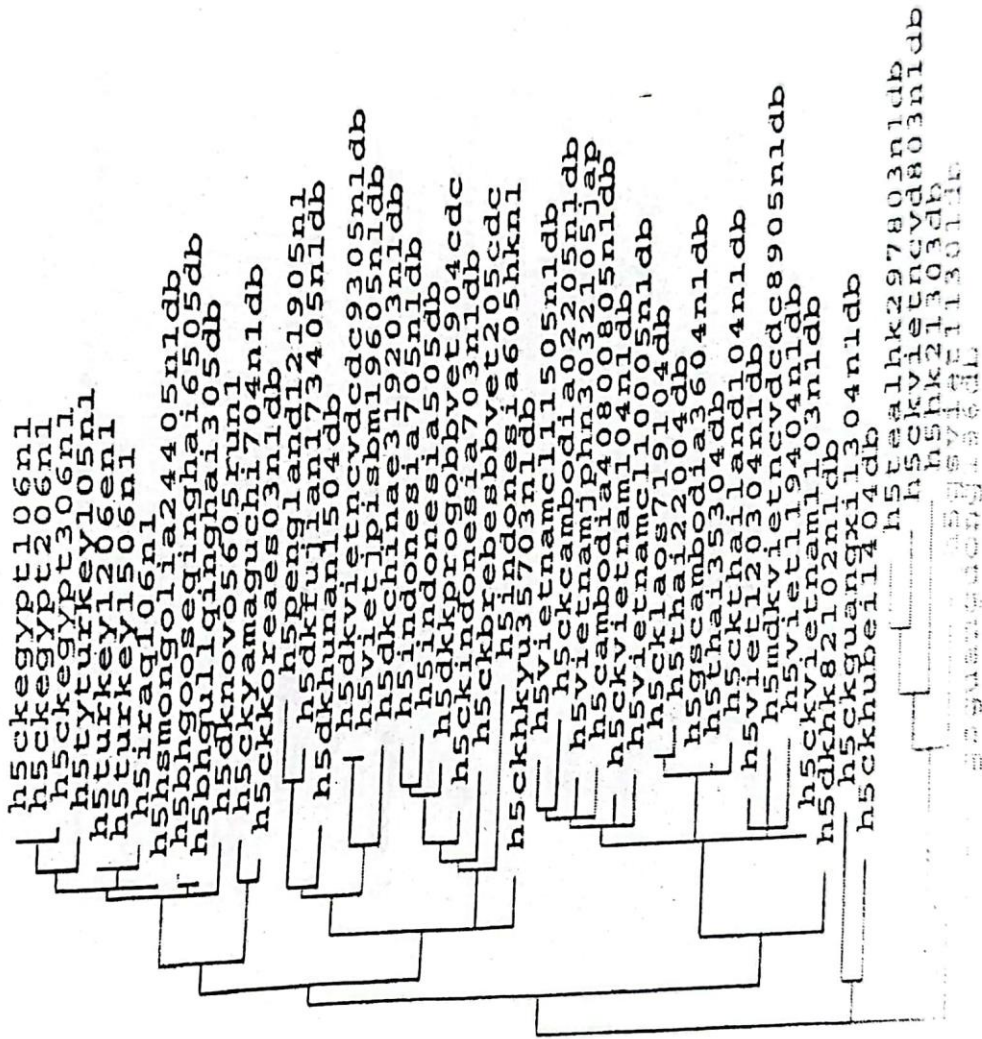


Fig. (2) Phylogenetic comparison of H5 NA genes of outbreaks 2006

## DISCUSSION

Highly pathogenic avian influenza H5N1 is still circulate since 1997 in South East of Asia as the first case of spread from a bird to a human was seen during an outbreak of bird flu in poultry in Hong Kong in 1997(17). However, the Outbreaks of influenza H5N1 occurred among poultry in eight countries in this area (Cambodia, China, Indonesia, Japan, Laos , South Korea , Thailand , and Vietnam) during late 2003 and early 2004. At this time, more than 100 million birds in the affected countries either died from the disease or were killed in order to control the outbreak. By March 2004, the outbreak was reported to be under control. Beginning in late June 2004, however, new outbreaks of influenza H5N1 among poultry were reported by several countries in Asia (Cambodia, China Tibet, Indonesia, Kazakhstan, Malaysia, Mongolia, Russia [Siberia], Thailand, and Vietnam). It is believed that these outbreaks are ongoing then transmitted to Europe at the end of 2005 (Romania , Turkey , Ukraine , Croatia ) and still circulate in Europe from the beginning of 2006 (Bulgaria .Slovenia , Slovakia , Germany , France , Austria , Greece , Italy , Bosnia and Herzegovina

,Switzerland , Serbia , Hungary , Poland , Albania , Denmark . Sweden , Czech Republic , United kingdom & Spain (7). The disease appeared in Africa at the beginning of 2005 In Burkina Faso and at 2006 in Nigeria , Egypt . Gazza strip, Niger , Cameroun . Sudan , Cote d Ivoire & Djibouti. Human infections of influenza A (H5N1) have been reported in most of affected countries (5, 11, 12, 18, 19)

In Egypt, the disease in its clinical form was observed firstly in several backyards containing chickens, turkeys, geese, ducks, breeders, broilers, and layers, in Qaluobia and Giza governorates one week before the declaration of the authorities at 17/2/2006 .Pigeon in contact with affected birds didn't show any clinical signs. Oropharyngeal and cloacal samples were collected from affected chicken, and contact pigeons, sent to the (WHONIC) Egypt, for isolation and identification of influenza viruses. The isolated viruses from chicken (but not from contact pigeons) were identified as H5N1 AI by using WHO kit (table 1). The isolated viruses were tested, in WHONIC- Egypt, by III test using different types of human type A influenza antiserum (H1N1 New Caledonia strain isolated in 1999 and H3N2 Panama strain

isolated in 1996 beside antiserum of H5N1 recent isolate of chicken strain. The isolated viruses were reacted with H5N1 antiserum but not with New Caledonia or Panama strain (table 1). Moreover, the identified influenza type A H5N1 viruses of chicken were sent to WHOCC influenza reference Lab., at London, United Kingdom for antigenic and phylogenetic analysis. In this reference Lab., the identified H5N1 Type A influenza Egyptian strains were tested against four human influenza type A H5N1 antisera (A/hong Kong/156/97, A/hong Kong/213/03, A/Vietnam/1194/04 and A/Indonesia/5/05); one chicken (Ck/Scotland/59); one quail (Qu/Cirebon/BBVet/05); and one turkeys (ty/Turkey/1/05) influenza type A H5N1 antiserum by HI and test. The obtained results from this reference Lab., revealed that the Egyptian strains that isolated from El-Qanater El- Khiria Center, Qaluobia (ck/Egypt/1/06 N, ck/Egypt/2/06 N, ck/Egypt/3/06 N, ck/Egypt/1/06 C, ck/Egypt/2/06 C) are similar to influenza type A H5N1 strain isolated from turkeys in Turkey in 2005 (ty/Turkey/1/05) and human influenza type A H5N1 of Vietnamese (A/Vietnam/1194/04) (table 2) and this explain the resemble of high mortalities and case fatality in human cases in Egypt (~5.5% during 2006) to the Vietnamese cases (69% during 2004) in the year of the onset of illness in both countries (table 3). Furthermore, the disease was spread in-between poultry flocks in 20 out of 26 (76.9%) Egyptian governorates within few weeks (table 4) and the peak of the outbreak, in intensive poultry farms, within the 4, 5, 6, and 7 weeks of the infection (10 March - 6 April/2006) were recorded in 82, 293, 142, and 116 farms that confirmed AI H5N1 in these weeks respectively (table 5). From 12 February - 24 June 2006 the disease was recorded in 840 poultry farms and 129 backyards (table 6). The affected poultry species includes broilers, Balady, layers, Saso, parent, grand parent, ducks, geese, turkeys besides some species of wild birds located at Giza Zoo and one migratory bird (table 6). Chickens were the mostly affected species of birds. Mostly, the affected chicken farms were layers (327), followed by broilers (164), Balady (117), Saso (86), parents (84), grand parent (1). Other species of poultry like turkeys (32) and ducks (29) farms were affected. The majority of affected farms were located in Sharqiya (277), Qaluobia (181) and Giza (106) governorates. In this outbreak, about 34.4 million birds had been culled with economic losses 3 billion dollars (14). The



most affected governorates are those of the Mid and Lower Egypt. Many reasons lead to this severe affection in these areas among them: the majority of poultry farms all over the country were located in these areas due to the good climate; near the capital Cairo where Cairo is considered a good market for poultry and their products. Another forced reason raised at the beginning of the outbreak by the owners themselves who transmitted the infected birds from any place in the country to the Animal Health Research Institute (AHRI) at Doqi, Giza, in a manner lead to spread of the infection during their voyage from the infected farm to the AHRI. They doesn't aware the precautions for sample transmission and aimed to diagnosis the disease and gaining the proposed compensation offered by the government. On the other hand, the restriction of diagnosis of AI in the AHRI only (without involving the regional diagnostic Labs located in other governorates and related to the AHRI) gave a chance for rapid spreading by this manner. In contrast, the governorates that located in Upper Egypt (Beni-Suef, Meniya, Assuit, Sohag, Qena, Luxor and Aswan) only 23 chicken farm and 19 turkey farms were affected where higher temperature is found and the number of poultry farm in this area

is few in comparison with those located in Mid and Lower Egypt and to some extent some of these governorate are far from Cairo where no intensive rearing of poultry in this area. After thoroughly application of the planned control program including precaution for samples collection and transmission, the recorded affected farms decreased gradually until the 17th, and 18th weeks were no affected farm was recorded but only one farm was recorded in the 19th week of the outbreak. From the 20th to 25th week of the outbreak no cases were recorded. The recorded case in the 19th week was observed in unvaccinated farm. Although all domestic poultry showed clinical symptoms of AI, the domestic pigeon did not show any clinical symptoms and failure of isolation of H5N1 from contact pigeon may be due to the resistance of pigeon to this AI H5N1 strains and this result is parallel with those obtained by (9) who revealed that domestic pigeons are only partially susceptible to influenza A viruses of the haemagglutinin subtype H7 and less susceptible to infection with influenza A viruses of the H5 subtype. Consequently, pigeons may play only a minor role in the epidemiology of H5 influenza viruses by mechanical transmission (9).

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The phylogenetic analysis of HA and NA genes of Egyptian strains isolated in 2006 (h5ckegypt 106 ha, h5ckegypt 206 ha, h5ckegypt 306 ha / h5ckegypt 106 n1, h5ckegypt 206 n1, h5ckegypt 106 n1) as declared in Fig. (2&3) denotes that all these strains are the same and did not differ from each other and closely related to influenza type A H5N1 strain isolated from turkeys in Turkey in 2005 (ty/Turkey/1/05) and put in Eurasia-African lineage when compared with worldwide strains and differed from China, Indonesia, and South-Eastern Asian (Vet Nam, Thailand & Cambodia) avian lineage strains (Fig. 2, 3).

The decrease of recorded cases of AI in Egypt from 15/6/2006 till the end of August 2006 depends mainly on the effective policy planned by the Supreme National Committee to Combat Bird Flu and applied by the cooperation between the Ministry of Agriculture, Ministry of Health, Ministry of Environment and the Police of Aqua Areas. This policy includes: quarantine around the focus of any recorded farm with a circle 3 km diameter, condemnation of birds in the restricted quarantined area, thorough disinfection of the affected farm, awareness about the risk of the disease and its zoonotic

nature, prevention of transportation of birds from and in-between centers in any given governorates or from governorate to another, surveillances in a circle of 10 km diameter from the focus of infection, prevention of selling live birds in shops, vaccination of all species and breeds of birds reared in farms with H5N1 vaccine, free charged vaccination of backyard and rural birds with the same vaccine offered by the veterinarians of General Organization of Veterinary Services (GOVS), studying needs for building new abattoirs according to the capacity of reared birds in each governorate to avoid buying live birds, re-evaluation and re-stocking of present farms on the bases of sanitation and bio-security. The reactivation of the obtained decisions for combating AI in our country is the best way for controlling the disease specially those concerning prevention of buying live birds, building of new abattoirs in each governorate according to the capacity needs and re-evaluation and re-stocking of farms according to the sanitation and bio-security. Moreover, in our opinion an effective future plan is needed on the national and international levels for controlling this disease. The national plan includes the followings, besides the decisions obtained by the Supreme

National Committee to Combat Bird Flu: (a) Renews the diagnostic labs, in each governorate and this achieved by providing these governorates labs with recent equipments, devices, instruments and biological materials with a good qualified persons for correct and rapid diagnosis ; or building 4 specified regional diagnostic labs, representing governorates in the four directions of our country (Upper, Lower, West, and East ) of the country (b) continuous obligatory booster vaccination of rural birds in all backyards with consequently checking for presence of AI virus antibodies in them (c) Avoidness of building poultry farms in culture lands specially near people houses and re-looking on the present situation of poultry farms in culture lands (d) Avoidness of addition of animal concentrates to poultry rations specially those prepared from poultry origin (e) Increasing the awareness about the risk , economic impact and the zoonotic nature of the disease throughout all culture media . The future plan on the international level should be based on the decisions obtained by the WHO & OIE (6, 18) besides the followings: (A) Each country enforced to prepare effective AI vaccine from the locally circulating strain to vaccinate all species and

breeds of birds all over the country (B) Prevention of selling live birds in shops or opened markets (C) Increasing the awareness about the economic impact and the zoonotic nature of the disease (D) Re-evaluation and re-stocking of farms according to the sanitation and bio-security international systems. The very important question now how HPAI introduced into Egypt. To answer this question we looked for studies analyzed the appearance of AI in Africa (4, 5) which revealed that, the strains from Burkina Faso seemed to originate from northern Nigeria. At least two of the sub lineages also circulated in Europe in 2006 as seen in Germany, and the sub lineages had been already emerged outside of Africa and seemed to have followed the east African/west Asian and Black Sea /Mediterranean flyways of migratory birds (6). Genetic analysis of highly pathogenic avian influenza (H5N1) viruses from poultry and hooded vultures in Burkina Faso shows that these viruses belong to 1 of 3 sub lineages initially found in Nigeria and later in other African countries (5). Molecularly characterized hemagglutinin (HA) and neuraminidase (NA) genes of eleven H5N1 viruses isolated from domestic poultry in Gaza in March-April 2006 revealed that the

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HA and NA genes of the Gazian viruses were closely related to viruses isolated in Egypt in 2006 on the basis phylogenetic analysis (11). On looking for the phylogenetic analysis data of HPAI-H5N1 circulated during the outbreak in Northern Europe in Spring 2006, it demonstrated that two distinct sub-lineages. Sub-lineage I viruses fell into the same clade as viruses found in Denmark and Germany and formed a sub-clade which also included viruses isolated in the Russian Federation in late 2005. Sub-lineage II viruses formed a sub-clade closely related to European, Middle Eastern and African isolates reported in 2006 (10, 19).

While the spread of H5N1 viruses seems to be mainly due to the movement of poultry and poultry products to areas free from infection, recent sublineages of outbreaks in sub-Saharan Africa, Egypt and Europe seem to suggest that the virus may have been introduced by wild birds. Therefore, the highly pathogenic avian influenza strains introduced into Egypt in February 2006 may be from Turkey strain emerged from Nigeria or European strains and across the Mediterranean Sea. Indeed, the source of these viruses and the exact time of introduction remain obscure.

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