A surveillance for Detection Of Avian Influenza Virus From Broiler Flocks At Delta, Egypt

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ABSTRACT

A total of 100 broiler farms reprsenting 765 500 chickens from 5 different Egyptian governorates at Delta, including ; Al-Dakahlia (56), Dametta (20), Al-Sharkia (6), Kafer Elsheakh (8), Al-Gharbia (10) farms respectively; were examined with both clinical and seroexamination. Sample for rapid test contains pool from 5 tracheal swabs were collected randomly from birds showed clinical symptoms of AI, samples for PCR contains pool from 5 tracheal and 5 colacal swabs. Samples were collected during period from 2/1/2010 to 28/12/2010. Results of rapid test showed 39.2%, 50%, 60% , 75% , 40% also , results of RT PCR test showed 33.9% , 30% , 33.3% , 50% and 30% for governorates of Al-Dakahlia, Dametta, Al-Sharkia, Kafer Elsheakh and Al-Gharbia, respectively.

INTRODUCTION

Avian influenza is the most important and dangerous disease in birds, so the rapid and accurate diagnosis is a main target to control and eliminate the disease *(1)*.

The poultry industry in Egypt depends on the broiler chickens, also the nature of the Egyptian customers prevlage is purchasing fresh chicken meat. The Delta region is considered one of most higher regions in poultry density and productivity *(2)*.

Emergence of the highly pathogenic avian influenza (HPAI) H5N1 virus in Egypt in mid-February 2006 caused significant losses for the poultry industry and constituted a potential threat to public health. Since late 2007, there has been increasing evidence that stable lineages of H5N1 viruses are being established in chickens and humans in Egypt. Most of the outbreaks in poultry and humans occurred in the highly populated Nile delta *(3)*.

The Egyptian government initiated a poultry stamping out program that appeared to limit the 2006 outbreak; however, H5N1 viruses reemerged in a series of avianepidemics in several governorates in 2007 and 2008. Poor hygiene, lack of awareness, and uncontrollable, random raising of domestic poultry, especially in the rural areas of Egypt, increases the chances of virus transmission. From 2006 till 2012, there have been 2578 outbreaks of H5N1 among poultry in Egypt *(3)*.

The aim of this study was to detection of avian influenza virus from broiler flocks at Delta, Egypt.

MATERIAL AND METHODS

Material

Samples

A total of 100 broiler farms from 5 different Egyptian governorates at Delta, including Al-Dakahlia (56), Dametta (20), Al-Sharkia (6), Kafer Elsheakh (8), Al-Gharbia (10) were examined. Sample for rapid test contains pool from 5 tracheal swabs were collected randomly from birds showed clinical symptoms of AI, sample for PCR contains pool from 5 tracheal and 5 colacal swabs. Samples collected during period from 2/1/2010 to 28/12/2010. Detailed data of samples were shown in Table 1.

Table 1. History of samples collected from different governorate farms

Egyptian governorate	Date of collection	breed	State of vaccination	Total number of birds	Daily mortality
	2/1/2010	Hubbard	vaccinated	20000	1740
	2/1/2010	cobb	vaccinated	10000	1120
	3/1/2010	cobb	vaccinated	10000	870
	3/1/2010	Ross	vaccinated	10000	900
	3/1/2010	Ross	vaccinated	20000	300
	5/1/2010	Cobb	vaccinated	10000	110
	6/1/2010	Arboreccurs	vaccinated	10000	590
	6/1/2010	Hubbard	vaccinated	10000	415
	6/1/2010	Cobb	vaccinated	20000	330
	10/1/2010	Arboreccurs	vaccinated	10000	560
	10/1/2010	Ross	vaccinated	5000	320
	14/1/2010	Sasso	vaccinated	4500	120
	14/1/2010	Cobb	vaccinated	10000	660
Al-Dakahlia	14/1/2010	Hubbard	vaccinated	20000	760
	2/2/2010	Cobb	vaccinated	10000	370
	2/2/2010	Avian 48	vaccinated	14000	510
	15/2/2010	Sasso	vaccinated	21000	1320
	15/2/2010	Hubbard	vaccinated	9000	870
	25/2/2010	Hubbard	vaccinated	13000	317
	27/2/2010	Cobb	vaccinated	22000	220
	27/2/2010	Hubbard	vaccinated	19000	990
	27/2/2010	Cobb	vaccinated	10000	570
	27/2/2010	Hubberd	vaccinated	15000	1350
	1/3/2010	Sasso	vaccinated	10000	615
	1/3/2010	Hubbard	vaccinated	22000	1820
	1/3/2010	Cobb	vaccinated	16000	920
	5/3/2010	Sasso	vaccinated	10000	711
	5/3/2010	Hubbard	vaccinated	10000	1870
	7/3/2010	Ross	vaccinated	20000	1950
	7/3/2010	Cobb	vaccinated	10000	500
	8/3/2010	Arboreccurs	vaccinated	10000	400
	10/3/2010	Sasso	vaccinated	20000	1700
	12/3/2010	Hubbard	vaccinated	10000	1250
	5/4/2010	Hubbard	vaccinated	10000	370
	7/4/2010	Cobb	vaccinated	10000	600
	15/4/2010	Hubbard	vaccinated	20000	1650
	17/4/2010	Cobb	vaccinated	10000	1400
	15/5/2010	Ross	vaccinated	5000	250
	18/5/2010	Sasso	vaccinated	4500	50
	25/5/2010	Cobb	vaccinated	10000	380
	2/6/2010	Hubbard	vaccinated	20000	268
	2/6/2010	Cobb	vaccinated	10000	467
	15/6/2010	Hubbard	vaccinated	10000	530
	20/6/2010	Cobb	vaccinated	10000	444
	20/6/2010	Hubbard	vaccinated	20000	760
	25/6/2010	Cobb	vaccinated	10000	130

	28/6/2010	Hubbard	vaccinated	10000	170
	1/7/2010	Cobb	vaccinated	10000	123
	5/7/2010	Ross	vaccinated	20000	855
	2/8/2010	Cobb	vaccinated	10000	255
	15/8/2010	Hubbard	vaccinated	5000	250
	20/8/2010	Cobb	vaccinated	10000	700
	2/9/2010	Hubbard	vaccinated	20000	570
	15/10/2010	Cobb	vaccinated	10000	270
	20/11/2010	Hubbard	vaccinated	10000	350
	20/12/2010	Cobb	vaccinated	10000	400
Total					
Dammetta	2/1/2010	Hubbard	vaccinated	10000	470
	6/1/2010	Arboreccurs	vaccinated	20000	2200
	5/2/2010	Ross	vaccinated	10000	380
	13/2/2010	Hubbard	vaccinated	5000	310
	27/2/2010	Sasso	vaccinated	13000	420
	27/2/2010	Hubbard	vaccinated	13000	130
	27/2/2010	Avain 48	vaccinated	14000	1300
	27/2/2010	Cobb	vaccinated	5000	370
	3/3/2010	Sasso	vaccinated	10000	390
	5/3/2010	Avain 48	vaccinated	15000	790
	15/5/2010	Hubbard	vaccinated	20000	1850
	20/5/2010	Arboreccurs	vaccinated	10000	750
	5/6/2010	Ross	vaccinated	10000	555
	15/6/2010	Avain 48	vaccinated	10000	700
	7/7/2010	Cobb	vaccinated	20000	1500
	2/8/2010	Sasso	vaccinated	10000	780
	1/9/2010	Cobb	vaccinated	5000	250
	5/10/2010	Hubbard	vaccinated	10000	300
	25/10/2010	Cobb	vaccinated	10000	650
	28/12/2010	Hubbard	vaccinated	5000	253
Total					
Al-Sharkia	20/1/2010	Cobb	vaccinated	10000	920
	18/2/2010	Hubbard	vaccinated	15000	430
	3/3/2010	Cobb	vaccinated	18000	790
	5/4/2010	Avain 48	vaccinated	5000	300
	1/8/2010	Cobb	vaccinated	10000	430
	11/11/2010	Sasso	vaccinated	10000	310
Total					
Kafr-El Sheakh	7/2/2010	Hubbard	vaccinated	16000	660
	19/2/2010	Hubbard	vaccinated	13000	530
	19/2/2010	Sasso	vaccinated	22000	2320
	3/3/2010	Avain 48	vaccinated	5000	380
	5/4/2010	Avain 48	vaccinated	15000	1250
	5/5/2010	Cobb	vaccinated	18000	650
	2/6/2010	Sasso	vaccinated	5000	210
	1/11/2010	Cobb	vaccinated	10000	130
Total					
Al-Gharbia	5/1/2010	Sasso	vaccinated	6000	690
	7/1/2010	Sasso	vaccinated	13000	460

	15/1/2010	Cobb	vaccinated	12000	210
	15/2/2010	Cobb	vaccinated	19000	820
	3/3/2010	Hubbard	vaccinated	5000	310
	1/4/2010	Sasso	vaccinated	20000	250
	1/5/2010	Avain 48	vaccinated	9000	150
	2/6/2010	Hubbard	vaccinated	12000	780
	10/8/2010	Arboreccurs	vaccinated	9000	140
	15/11/2010	Ross	vaccinated	15000	890
Total				765500	

Materials used for viral transporting medium

Viral transport medium contains both Phosphate buffer saline (PBS) and antibiotics.

The PBS was prepared according to *(3)*

Antibiotics were added in the following quantities

Penicillin G- Sodium *(Misr Co. Egypt)*	($2x10^6$ IU/liter),
Streptomycin *(Alnil Co. Egypt)*	200 mg/liter.,
Mycostatin *(Squibb. USA)*	$0.5x10^6$ IU/liter,
Gentamycin *(Alexandria Co. Egypt)*	50 µg/ml

NB. Regarding cloacal swabs, five-fold higher concentrations of antibiotics were used. The samples were kept at -80°C until used.

Materials used for AI antigen detection (Rapid Test)

Synbiotics influenza type A antigen test kit "Synbiotics Flu detect TM test Strip"

Synbiotic commercial kit Influenza type A antigen test kit (Synbiotic, USA) The kit consists of:

- 1 vial containing 20 test strips
- 1dropper bottle containing extraction buffer (6ml)
- 20 swabs
- 20 test tubes
- 20 caps for test tubes
- 1 test tube rack (for 5 tubes)
- Instructions for use

Materials used for Reverse Transcriptase-Polymerase Chain Reaction.

Total RNA extraction Silicon Nucleic Acid Binding Column (Qiampviral RNA mini Kit-Cat no 52904. Qiagen USA):

- Nuclease-free water.
- Nuclease-free plastic ware (i.e., micro centrifuge tubes)
- Qiagen RNeasy Mini Kit (Qiagen, Inc.) which contain RNeasy Mini spin columns, collection tubes (2ml), RNA carrier, Buffer ALT, Buffer AW1, Buffer AW2 and AVE
- 100% Ethanol.
- Unichanell pipette (1000ul, 100ul and 10ul-Biohite-Finland).
- Filter tips.
- Centrifuge (sigma. Germany)
- Vortex

Detection and Identification of the type A and sub type H5 Influenza Virus by Real-Time RT-PCR.

- Real-time PCR instrument (Strategen. 3005P).
- Reagent-grade H2O (nuclease-free).
- TE buffer (for probe and primers reconstitution).
- Quantitect RT-PCR Kit reaction buffer (Qiagen. 204443- USA).
- Positive control RNA.
- Centrifuge (sigma. Germany)
- Optical tubes, optical caps (Applied- USA).

Primers and probes

These were Primers of the M, H5, H7, H9 and N1 genes which manufactured by Metabion (Germany), and delivered in a lyophilized form. Reconstitution of the primers

was carried out in nuclease free water buffer to prepare concentrated stocks. Working solutions were prepared by individual dilution of the primer stocks in nuclease free water (Table 2).

Methods

Clinical and postmortem examination

Clinical and postmortem examination were carried out. Also, clinical and gross lesions were recorded. Specimens from affected birds showed suspected AIV infection were collected under complete aseptic condition for virus detection procedures.

Collection and preparation of samples

It was done according to previous described study *(3)*.

Tracheal swabs

The trachea of live or freshly dead birds was swabbed by inserting dry cotton or polyester swab into the trachea and gently swabbing the wall then the swabs were placed in 1-2 ml of transport medium.

Cloacal swabs

The cloacae of live or freshly dead birds were swabbed by inserting a swab deeply into the vent and vigorously swabbing the wall and then placed in 1-2 ml of transport medium.

Storage and transportation of collected samples

Each sample constitutes a pool of 10 tracheal swabs and 5 cloacal swabs. Swabs were placed in ice box and transported promptly to the laboratory. If swabs were transported to the laboratory within 2 days, they should be kept at 0 to 4°C; otherwise they were frozen at or kept below -70°C until transported to the laboratory.

Rapid chromatography AI detection test

Synbiotics influenza type A antigen test kit "Synbiotics Flu detect™ test Strip"

Method of RRT-PCR

Method of viral RNA extraction using "Silicon Nucleic Acid Binding kit, QIAamp, Qiagen, Germany" according to the manufacturer's instructions of the kit.

- All buffers were prepared and reagents in accordance with the RNA extraction kit protocol (Qiagen RNeasy Mini Kit)

Type A and sub type H5 Influenza Virus Detection by Real-Time RT-PCR according to manufacture of Quantitect kit.

Table 2. Different primers and probes used in amplification of different gene segments of AIVs

Primer	Target gene	Type	Sequence (5′ - 3′)	References
M24	M gene	Forward	AGA TGA GTC TTC TAA CCG AGG TCG	
M25	M gene	Reverse	TGC AAA AAC ATC TTC AAG TCT CTG	
Probe M	M gene	Probe	6-FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA	
H5LH1:	H5 gene	Forward	ACG TAT GAC TAC CCG CAG TAT TCA	
H5RH1:	H5 gene	Reverse	AGA CCA GCC ACC ATG ATT GC	
H5 Probe	H5 gene	Probe	6 -FAM-TCW ACA GTG GCG AGT TCC CTA GCA- TAMRA	
LH6H7	H7 gene	Forward	GGCCAGTATTAGAAACAACACCTATGA	
RH4H7	H7 gene	Reverse	GCCCCGAAGCTAAACCAAAGTAT	
Probe H7	H7 gene	Probe	6-FAM-CCGCTGCTTAGTTTGACTGGGTCAATCT-BHQ1a	
H9 -F0R	H9 gene	Forward	ATGGGGTTTGCTGCC	
H9 REV	H9 gene	Reverse	TTATATACAAATGTTGCAC(T)CTG	
H9-Probe	H9 gene	Probe	FAM-TTCTGGGCCATGTCCAATGG-TAMRA	
N1-For-474-502v2	N1 gene	Forward	TAYAACTCAAGGTTTGAGTCTGTYGCTTG	
N1-Rev-603-631v2	N1 gene	Reverse	ATGTTRTTCCTCCAACTCTTGATRGTGTC	
N1-Probe-501-525v3	N1 gene	Probe	FAM-TCAGCRAGTGCYTGCCATGATGGCA-MGB	
HGGT	H5 gene	Forward	CTC TTC GAG CAA AAG CAG GGG T	
H5-KH3	H5 gene	Reverse	TAC CAA CCG TCT ACC ATK CCY TG	
H5F4	H5 gene	Forward	AGT AAT GGA AAT TTC ATT GCT CCA GAA	
Bm-NS 890R(HR)	H5 gene	Reverse	ATA TCG TCT CGT ATT AGT AGG AAA CAA GGG TGT TTT	
N1F1-15	N1 gene	Forward	AGC AAA AGC AGG AGA TTG AAA TGA ATC CAA	
N1R2-865	N1 gene	Reverse	GGC ATC AGG ATA ACA GGA GCA CTC C	
N1F2-449	N1 gene	Forward	TTG CTG AAT GAC AAG CAC TCC AAT G	
BA-NA 1413	N1 gene	Reverse	ATA TGG TCT CGT ATT AGT AGA AAC AAG GAG TTT TTT	

(*Spackman et al., 2002*). (*Monne et al., (WHO, 2007) 2008*) Veterinary laboratory agency available at WWW.Defra.gov.UK/VLA.

RESULTS

Clinical and postmortem examination

The common clinical signs observed in examined birds were cyanosis of comb and wattle, facial swelling , oedematous wattles and necrosis of comb, conjunctivitis, congestion of the shank and some times haemorrrhagic spots on shank with elevated body temp, greenish diarrhea, nervous signs (torticollis), sneezing, nasal discharge. PM lesions the birds was showed apicture of septicemia, gelatinous exudate in submandibule, congested B.V of skin, congested lung and some times presence of frothy exudate, liver, trachea, intestine, necrotic and congested pancrease, congested kidney and presence of ecchymotic haem, ecchymotic haem on spleen, petechial haem on coronary fat and on inner aspect of keel bone and also at junction between proventriculus and gizzard and on papillae of proventriculus,

Table 3. Results of total samples tested by rapid test

Egyptian governorate	Total No. of samples	No. of positive samples	Percentage of positive sample
El-Dakahlia	56	22	39.2%
Dametta	20	10	50%
El-Sharkia	6	4	60%
Kafr El-Sheik	8	6	75%
El-Gharbia	10	4	40%
Total	100	46	46%

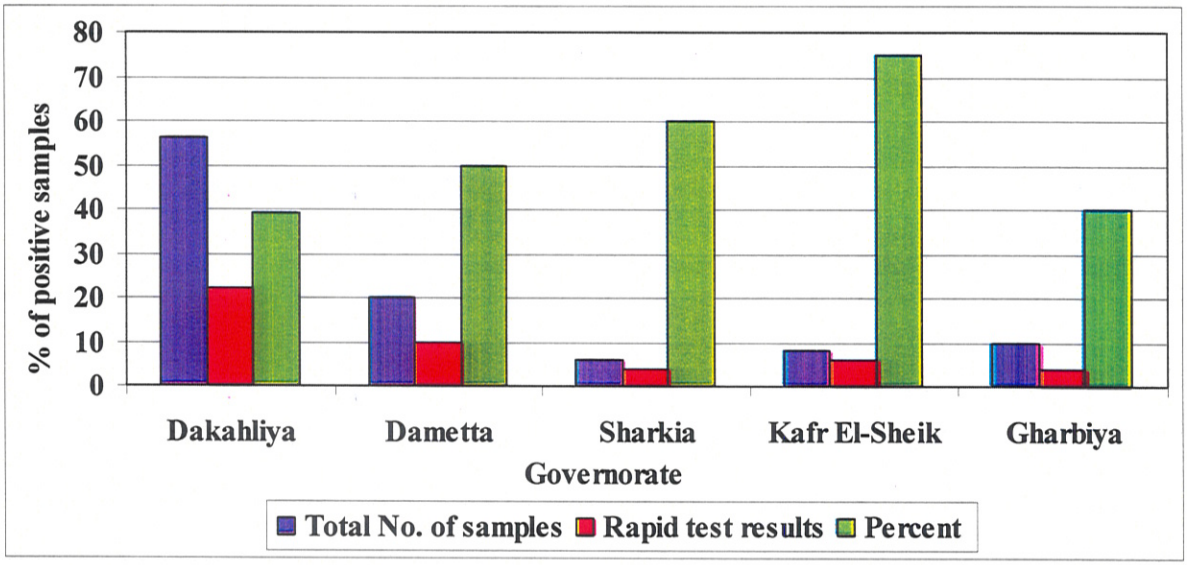


Fig. 1. Results of total samples tested by rapid test

Table 4. Results of Total Samples Tested by RRT-PCR

Egyptian governorate	Total No. of samples	RRT – PCR		Percenrte of positive samples
		M1,H5, N1	H7,H9	
El-Dakahlia	56	19	-ve	33.9%
Dametta	20	6	-ve	30%
El-Sharkia	6	2	-ve	33.3%
Kafr El-Sheik	8	4	-ve	50%
El-Gharbia	10	3	-ve	30%
Total	100	34	-	34%

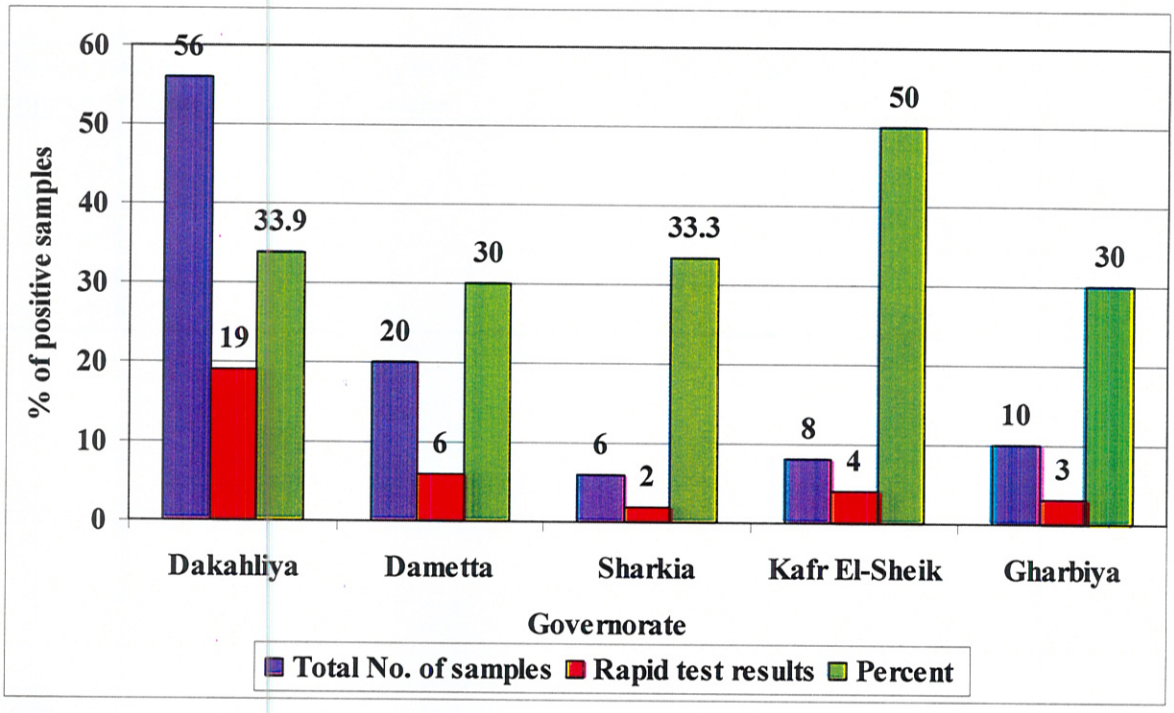


Fig. 2. Results of total samples tested by RRT-PCR

Table 5. Results of RRT-PCR for M, H5, H7, H9, N1 genes

Sample code number	Matrix Gene (M) RRT-PCR		H5 Gene RRT-PCR		H7 Gene RRT-PCR		H9 Gene RRT-PCR		N1 Gene RRT-PCR		Conclusion
	CT Value	Result	CT Value	Result	CT Value	Result	CT Value	Result	CT Value	Result	
1	19.72	Pos.	35.79	Pos.	No Ct	Neg.	No Ct	Neg.	18.68	Pos.	Positive-H5N1
2	24.57	Pos.	37.26	Pos.	No Ct	Neg.	No Ct	Neg.	21.18	Pos.	Positive-H5N1
3	18.06	Pos.	35.36	Pos.	No Ct	Neg.	No Ct	Neg.	20.80	Pos.	Positive-H5N1
4	24.95	Pos.	37.98	Pos.	No Ct	Neg.	No Ct	Neg.	17.45	Pos.	Positive-H5N1
5	21.74	Pos.	37.95	Pos.	No Ct	Neg.	No Ct	Neg.	16.13	Pos.	Positive-H5N1
6	26.32	Pos.	34.69	Pos.	No Ct	Neg.	No Ct	Neg.	33.08	Pos.	Positive-H5N1
7	19.91	Pos.	33.61	Pos.	No Ct	Neg.	No Ct	Neg.	24.48	Pos.	Positive-H5N1
8	21.41	Pos.	35.97	Pos.	No Ct	Neg.	No Ct	Neg.	23.26	Pos.	Positive-H5N1
9	29.84	Pos.	35.69	Pos.	No Ct	Neg.	No Ct	Neg.	17.69	Pos.	Positive-H5N1
10	23.70	Pos.	29.17	Pos.	No Ct	Neg.	No Ct	Neg.	18.39	Pos.	Positive-H5N1
11	18.43	Pos.	28.04	Pos.	No Ct	Neg.	No Ct	Neg.	17.95	Pos.	Positive-H5N1
12	26.04	Pos.	33.14	Pos.	No Ct	Neg.	No Ct	Neg.	18.15	Pos.	Positive-H5N1
13	22.50	Pos.	35.65	Pos.	No Ct	Neg.	No Ct	Neg.	15.61	Pos.	Positive-H5N1
14	22.54	Pos.	33.67	Pos.	No Ct	Neg.	No Ct	Neg.	32.30	Pos.	Positive-H5N1
15	24.26	Pos.	31.30	Pos.	No Ct	Neg.	No Ct	Neg.	17.82	Pos.	Positive-H5N1
16	19.38	Pos.	30.38	Pos.	No Ct	Neg.	No Ct	Neg.	20.02	Pos.	Positive-H5N1
17	23.29	Pos.	23.71	Pos.	No Ct	Neg.	No Ct	Neg.	23.90	Pos.	Positive-H5N1
18	18.85	Pos.	28.75	Pos.	No Ct	Neg.	No Ct	Neg.	25.20	Pos.	Positive-H5N1
19	24.45	Pos.	29.95	Pos.	No Ct	Neg.	No Ct	Neg.	25.16	Pos.	Positive-H5N1
20	30.64	Pos.	34.24	Pos.	No Ct	Neg.	No Ct	Neg.	21.22	Pos.	Positive-H5N1
21	31.87	Pos.	34.79	Pos.	No Ct	Neg.	No Ct	Neg.	18.84	Pos.	Positive-H5N1
22	25.84	Pos.	33.03	Pos.	No Ct	Neg.	No Ct	Neg.	15.48	Pos.	Positive-H5N1
23	25.28	Pos.	30.41	Pos.	No Ct	Neg.	No Ct	Neg.	18.57	Pos.	Positive-H5N1
24	27.10	Pos.	29.46	Pos.	No Ct	Neg.	No Ct	Neg.	28.62	Pos.	Positive-H5N1
25	32.30	Pos.	35.08	Pos.	No Ct	Neg.	No Ct	Neg.	31.76	Pos.	Positive-H5N1
26	25.60	Pos.	34.83	Pos.	No Ct	Neg.	No Ct	Neg.	28.10	Pos.	Positive-H5N1
27	28.50	Pos.	32.64	Pos.	No Ct	Neg.	No Ct	Neg.	28.67	Pos.	Positive-H5N1
28	24.44	Pos.	31.60	Pos.	No Ct	Neg.	No Ct	Neg.	28.20	Pos.	Positive-H5N1
29	25.52	Pos.	24.93	Pos.	No Ct	Neg.	No Ct	Neg.	31.15	Pos.	Positive-H5N1
30	24.74	Pos.	27.45	Pos.	No Ct	Neg.	No Ct	Neg.	31.63	Pos.	Positive-H5N1
31	21.90	Pos.	24.48	Pos.	No Ct	Neg.	No Ct	Neg.	29.14	Pos.	Positive-H5N1
32	29.89	Pos.	29.92	Pos.	No Ct	Neg.	No Ct	Neg.	32.19	Pos.	Positive-H5N1
33	21.51	Pos.	26.82	Pos.	No Ct	Neg.	No Ct	Neg.	32.78	Pos.	Positive-H5N1
34	22.42	Pos.	26.49	Pos.	No Ct	Neg.	No Ct	Neg.	30.29	Pos.	Positive-H5N1

CT: Threshold cycle
Pos.: Positive **Neg.:** Negative

Fig. 3. Amplification curve for AIV Matrix gene RRT-PCR

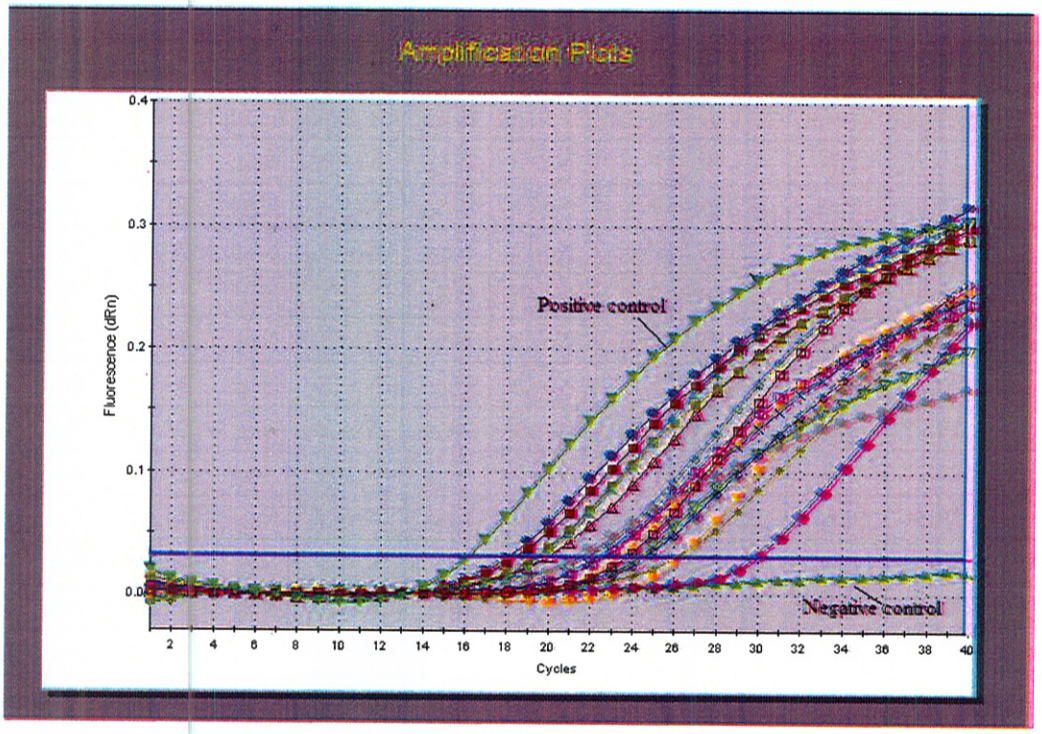


Fig.3.Continued

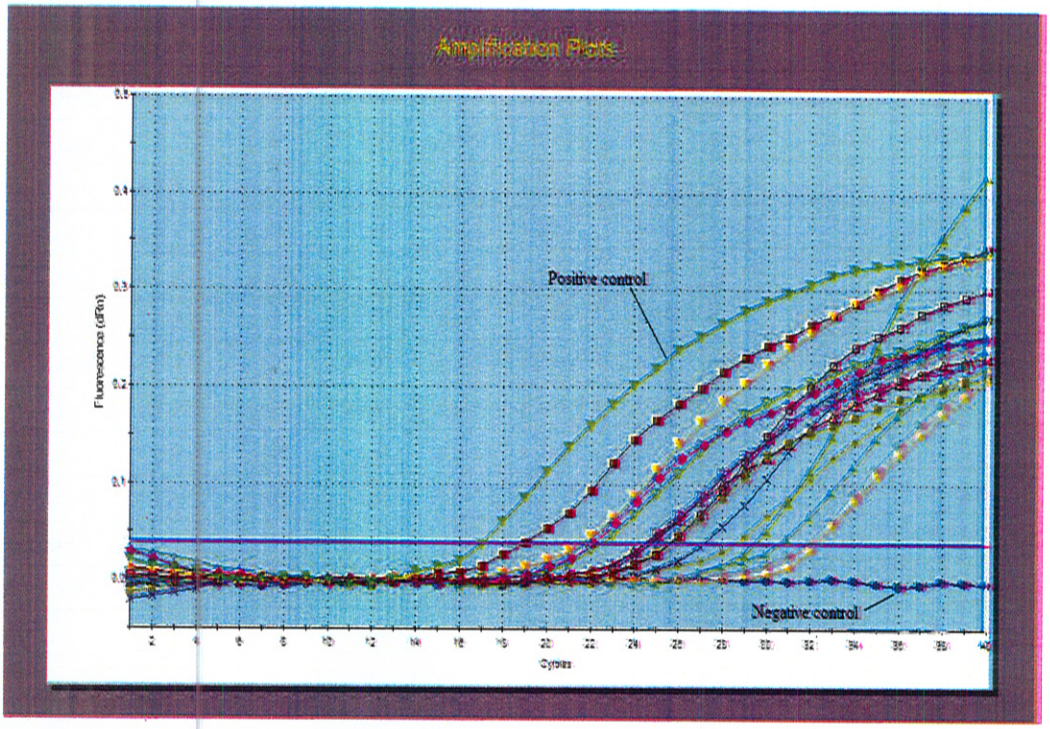


Fig. 4. Amplification curve for AIV H5 gene RRT-PCR

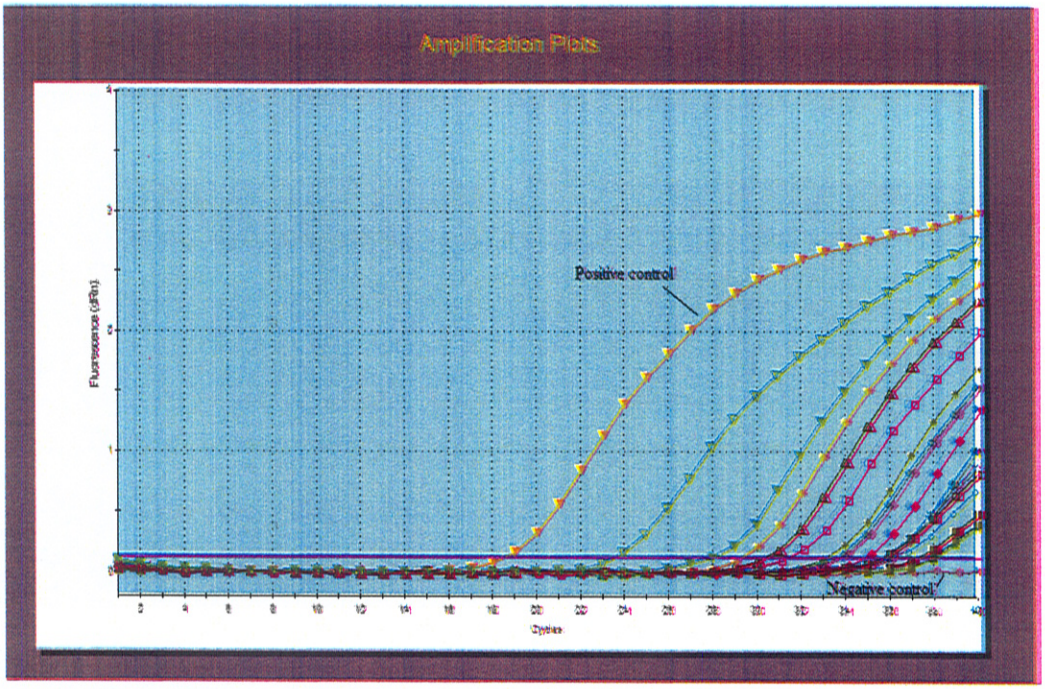


Fig. 4. Continued

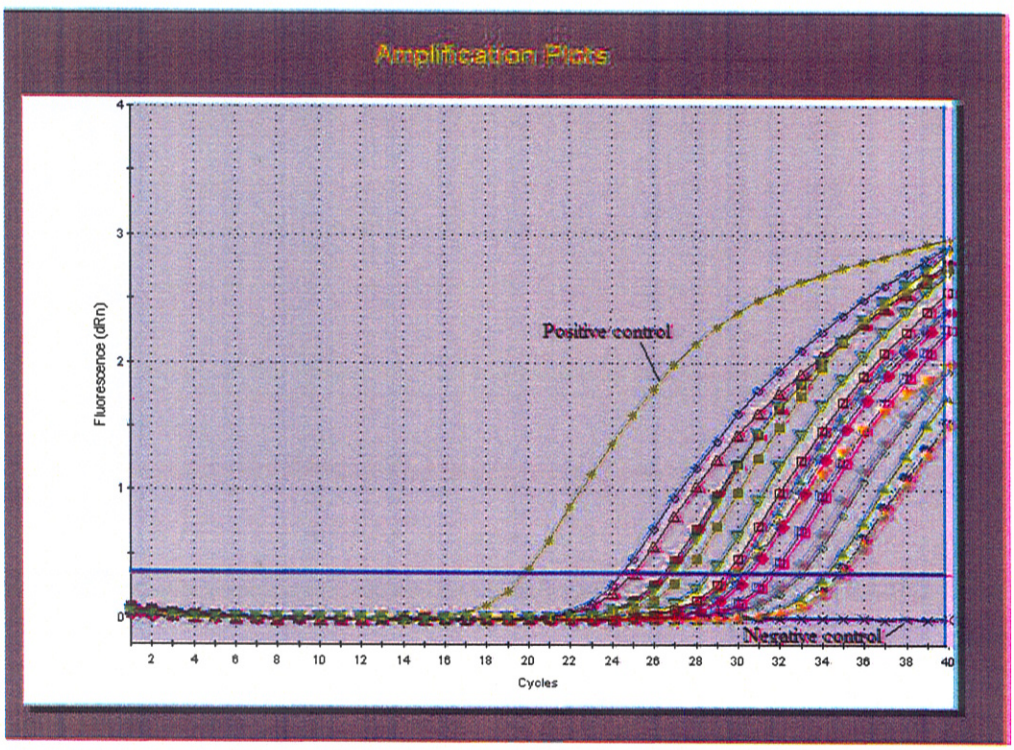


Fig. 5. Amplification curve for AIV H7 gene RRT-PCR

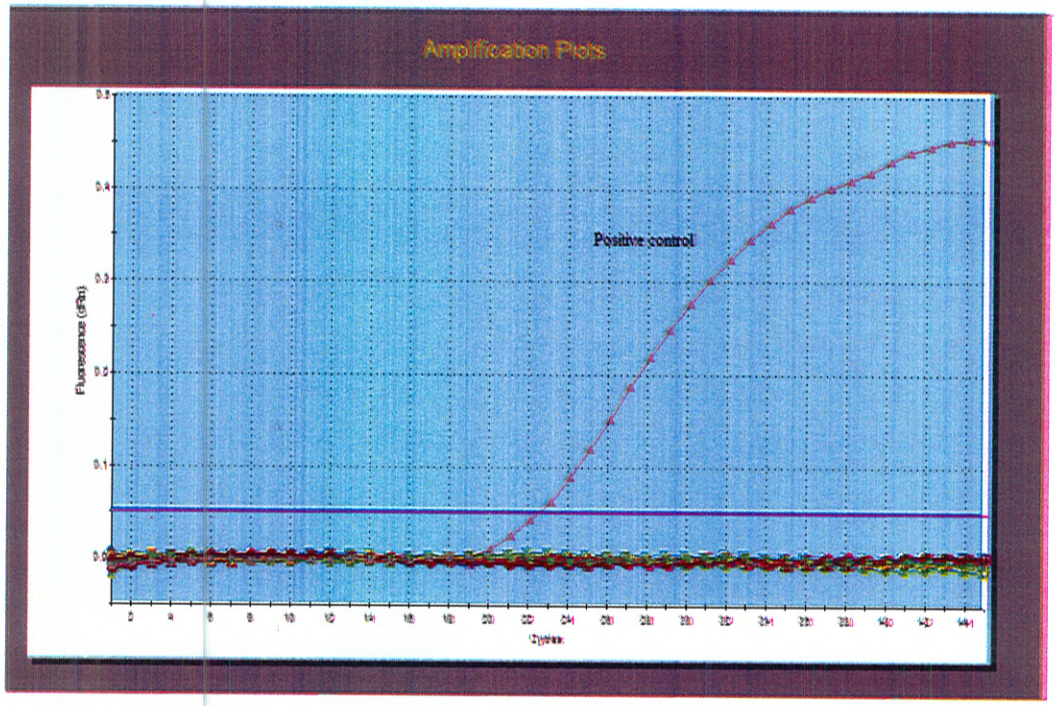


Fig. 6. Amplification curve for AIV H9 gene RRT-PCR

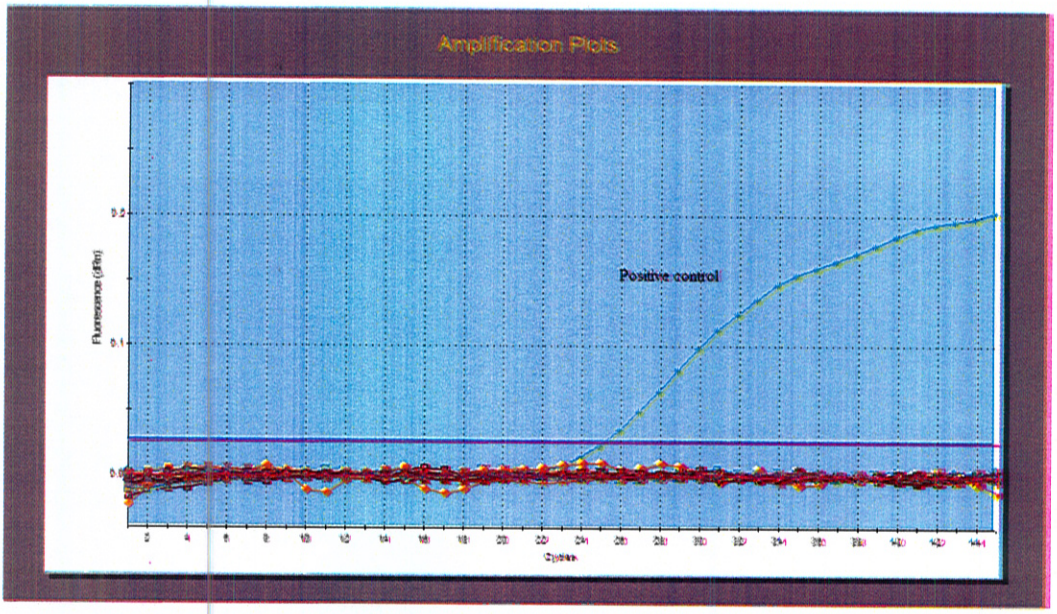
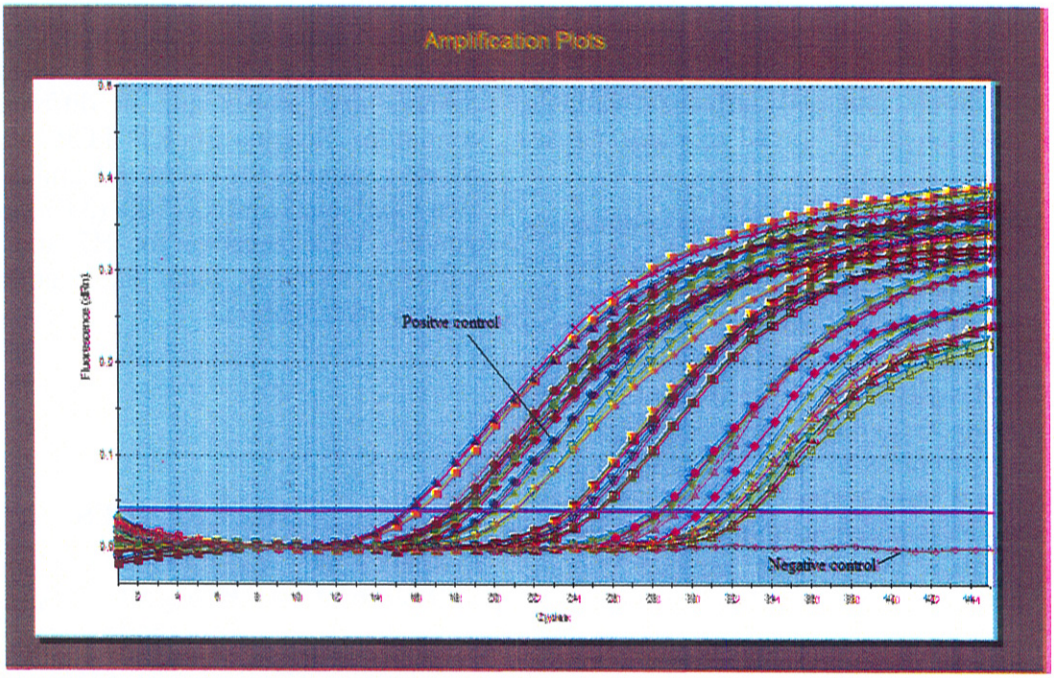


Fig. 7. Amplification curve for AIV N1 gene RRT-PCR



DISCUSSION

The influenza viruses are medium-sized, enveloped, negative sense ssRNA viruses with a segmented genome. Taxonomically, they belong to family *Orthomyxoviridae*. There are three genetically and antigenically distinct types of influenza viruses, A, B, and C ***(4)***. Type A viruses are further divided into subtypes according to the combination of two main envelope glycoproteins the hemagglutinin (HA) and neuraminidase (NA). To date, 16 HA subtypes (H1–H16) and 9 NA subtypes (N1–N9) have been found and almost all subtype combinations ***(5).***

In Egypt other than H5N1 subtypes were isolated as H3N1, H4N1 ***(6)***, H7N1 ***(2),*** and H7N7 ***(1).*** HPAIV H5N1 causes massive menace to the poultry industry. Furthermore, HPAIV H5N1 infections in poultry constitute a threat to humans. Till 21 April 2010, 495 human cases of H5N1 infections with 292 deaths were reported worldwide and in Egypt alone, a total of 109 cases with 34 deaths have been recorded ***(WHO 7).***

Extensive surveillance and genetic studies revealed that H5N1 viruses had become endemic in poultry in many countries since 2003 ***(8)*** and from 2007 this was also true for Egypt. Although the promoters of endemicity in Egypt have not yet been clarified, silently infected free ranging ducks and geese as well as mixed species backyard holdings are suspected to play essential role in the region. These domestic bird holdings are difficult to control. Culturally and socially coined poultry rearing as well as the predominance of live bird trading habits and economically related hesitant responses to public programs trying to raise awareness towards the potential dangers are at the basis of the endemic status of HPAIV H5N1.

The long-term endemic influenza virus infections in poultry broiler farms increases exposure risks to humans and in turn, creates opportunities for the emergence of human-adapted strains with subsequent pandemic

potential *(9)*.Continuing viral genome sequence comparisons and phylogenetic analyses of current HPAIV H5N1 are necessary to recognize newly emerged influenza variants, and to monitor global spread of these viruses *(10)*.

Molecular diagnostic tests are commonly used to diagnose avian influenza virus because they are sensitive and can be performed rapidly, with high throughput, and at a moderate cost *(11)*. Polymerase chain reaction (PCR) methods have been described that is up to 100 fold more sensitive than virus isolation procedures. This technology promises to revolutionize influenza diagnosis and monitoring *(12,13)*. Also it was found that PCR-based methods are of higher sensitivity than commercial antigen capture enzyme immunoassay (AC-EIA) in detection of AI *(14)*. Also the sensitivity of reverse transcriptase-PCR (RT-PCR) has been reported to be in the range of 90% to 100% when compared with cell culture; however, several researchers have reported significantly higher numbers of total positive specimens with RT-PCR, possibly reflecting its ability to detect nonviable virions *(15)*.

Quantitation and competitive replication study using RRT-PCR of H5 and H7 subtypes *(16)* and H9 subtype *(17)*, was specific, sensitive, reliable, easy to perform and rapid test than the conventional methods and could be useful in virological, pathogenesis and protection studies.

In the present study, A total of 100 broiler farms from 5 different Egyptian governorates at Delta, including Al-Dakahlia (56), Dametta (20), Al-sharkia (6), Kafer elsheakh (8), Al-Gharbia (10) were examined.

Samples were taken from broiler farms suspected to be infected by AI . The collected samples were tested for avian influenza using Rapid test and RRT-PCR. Rapid test for samples collected from 100 broiler farms revealed (46)positive while ,RT PCR results for the same samples revealed (34)positive . Our results clarify that PCR-based methods are of higher sensitivity than commercial antigen capture enzyme immunoassay (AC-EIA) in detection of AI. The samples were tested firstly for the M gene then tested for H5, H7, H9 and N1 in the recommended cascade using different primers and probes and in different thermal condition. Results of RT PCR revealed 34 positive samples for the M, H5 and N1 genes of avian influenza out of 100 (34%). All tested samples were negative for H7 and H9 avian influenza subtypes under the condition of the present investigation.

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الملخص العربى

تقصى لفيرس أنفلونزا الطيور فى قطعان بدارى التسمين فى دلتا مصر

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فى هذه الدراسة تم عمل تقصى حقلى لدراسة مدى انتشار فيروس انفلونزا الطيور فى محافظات الدلتا (محافظة الدقهلية – دمياط – الشرقية – كفر الشيخ – الغربية) تم فحص ١٠٠ عينة من ١٠٠ مزرعة مختلفة خلال الفترة من ٢٠١٠/١/٢ م حتى ٢٠١٠/١٢/٢٨م تم أختبار تلك العينات باستخدام الاختبار السريع لتشخيص انفلونزا الطيور وكذلك بأستخدام RT- PCR واظهرت النتائج انتشار فيروس انفلونزا الطيور بنسب ٣٩,٢% ، ٥٠% ، ٦٠% ، ٧٥%، ٤٠% من المحافظات (الدقهلية – دمياط – الشرقية – كفر الشيخ – الغربية) على التوالى بأستخدام الاخبتار السريع وأيضا اظهرت نتيجة RT- PCR انتشار فيروس انفلونزا الطيور بنسب ٣٣,٩% ، ٣٠% ، ٣٣,٣%، ٥٠% ، ٣٠% من المحافظات (الدقهلية – دمياط – الشرقية – كفر الشيخ – الغربية) على التوالى