

Avian Influenza H7 surveillance on backyard birds around El-Abassa Lake in Egypt, 2007

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Since Egypt is the main fly-way of migratory birds, so there is permanent risk of the transmission of emerging transboundary diseases, in particular Avian Influenza, from European and Asian countries to Egypt. Moreover, absence of biosecurity measures in backyard birds and possibility of continuous contact with wild migratory birds make the vulnerability of backyards to avian influenza much easier than commercial poultry farms. Avian Influenza subtype H7 was isolated from wild migratory ducks in El-Abassa Lake at El-Sharkia Governorate in 2007. To avoid repeating the scenario of H5N1 introduction to Egypt through wild migratory ducks to Egyptian poultry sectors, this prompted us to survey avian influenza in Egyptian wetlands, especially El-Abassa Lake and neighboring areas. Eleven villages around the lake were included in the surveillance. Serological examination using Avian Influenza type (A) antibody ELISA revealed 4/73 positive samples of chicken sera, while HI test against AI-H7 revealed negative results for all examined species. Molecular examination of swab samples by RRT-PCR revealed negative results for Avian Influenza H7. The current paper documents absence of AI-H7 in backyard birds around El-Abassa Lake in Egypt during the surveillance in October, 2007. Also this study highlights the need for continuous active surveillance of poultry especially in areas with high risk of exposure to migratory birds and wetlands and to monitor the presence of avian influenza viruses especially H5 and H7 which are known to threaten the domestic poultry.

Key words: Avian influenza H7, backyard, Egypt, RRT-PCR; ELISA, HI

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INTRODUCTION

Existence of Egypt in the main fly-way of migratory birds, jeopardize the transmission of emerging transboundary diseases, in particular Avian Influenza, from European and Asian countries to Egypt. Moreover, absence of biosecurity measures in backyard birds and continuous contact with wild migratory and/or feral birds make the vulnerability of backyards to Avian Influenza much easier than poultry farms.

Egypt had recorded highly pathogenic Avian Influenza H5N1 in February 2006 (Aly *et al.*, 2006 a, b). The disease was firstly described in backyard birds (Kilany, 2006 and Safwat, 2006). Discrimination of wild ducks in introduction of the disease to Egypt through wild migratory ducks was reported (Saad *et al.*, 2007). Backyard bird usually are moving or grazing through streets, roads or fields (Gilbert *et al.*, 2006). These birds are in close contact with either local feral birds (as sparrows,) or wild migratory birds (Kou *et al.*, 2006). Different species (chicken, duck, turkey, geese, and pigeon) are mostly reared together. Moreover, viral

circulation is facilitated by the interactions of the integrated aquaculture, animal raising, agroecosystem, and by the connections with the live-bird market system. The interactions of these factors determine the complex system in which wild birds interact with domestic birds and in which people are constantly exposed to sources of infection, leading to the association between poverty and AI infection in humans Cristalli and Capua (2007).

In August, (2007) the Egyptian Government reported the isolation of Avian Influenza subtype H7 from wild migratory ducks in El-Abassa Lake, El-Sharkia province which considered one of the largest wetland experienced with migratory birds specially ducks. Fearfulness of repeating the scenario of H5N1 introduction to Egypt through wild migratory ducks to Egyptian poultry sectors, incited us to plan and implement large scale surveillance on avian influenza in Egyptian wetlands, where El-Abassa Lake was the first one. Therefore, the present paper describes the results of AI-H7 surveillance in villages surrounding Lake Ponds.

MATERIAL AND METHODS

Active surveillance

The surveillance was conducted in October, 2007 for three days, where 45 houses from 11 villages were included as shown in figure (1).

Samples

Serum samples: 207 serum samples were collected from chickens (73), ducks (67), geese (52), pigeons (6) and turkey (9).

Tracheal and cloacal swabs: 200 tracheal swabs were collected from (66) chickens, (64) ducks, (47) geese, (12) pigeons, and (11) turkey and 200 cloacal swabs were collected from chickens (66), ducks (64), geese (47), pigeons (12), and turkey (11). As shown in table (1)

The birds were of different ages. The vaccination status against H5N1 virus was doubtful as told by the villagers, where two confirmed villages only were vaccinated at this time.

Avian Influenza Virus detection

Cloacal and tracheal swabs were collected from chickens, ducks, geese, pigeons and turkey. The swabs were immersed in 1- 2ml viral transport media containing

2000000 I.U. penicillin, 2 mg streptomycin and 1000 I.U. mycostatin/ml. Briefly, five tracheal or Cloacal swabs were pooled together. RNA was extracted by using virus RNA Extraction Kit (QIAGEN, Valencia, Calif., USA).

The RNA were amplified using One-Step Real-time reverse transcription-PCR (QRT-PCR) kit (PG-Biotech; QIAGEN, Valencia, Calif., USA) for detection of the matrix gene of influenza type A viruses.

Serology

Avian Influenza antibody ELISA test

Seventy-three collected chicken serum samples were examined for detection of AI antibodies using Avian Influenza antibody test kit, (Biochek B.V. Holland). The test was conducted according to the manufacturer instructions.

Hemagglutination Inhibition (HI) test

Procedures were performed according to OIE Manual, (2004) for detection of AI antibodies. 4 HAU of antigen were used. The validity of results were assessed against a negative control serum, which should not give a titer $>1/4$, and a positive control serum for

which the titer should be within one dilution of the known titer. Inactivated Avian Influenza H7 antigen was supplied by Instituto Zooprofilattico delle Venezie, OIE reference laboratory (Italy). While the H5 antigen was supplied by

Harbin Veterinary Research Institute (HVRI), China.

RESULTS

Figure (1)
Geographical distribution of the surveillance zone around El-Abassa Lake As appeared in Google earth

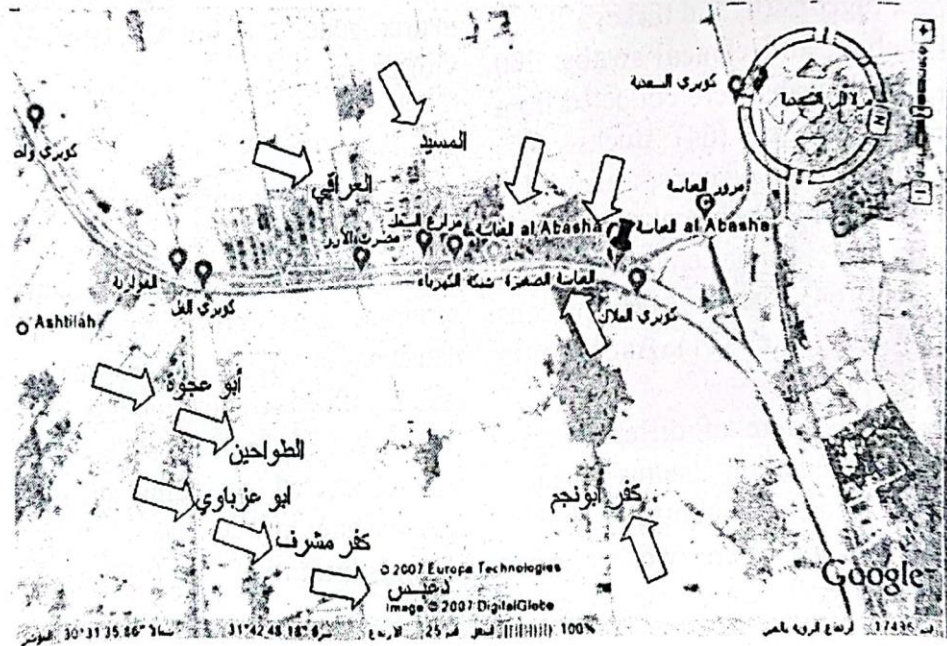


Table (1): Total examined samples per species in the surveillance

Species/ Samples	Chickens	Ducks	Geese	Pigeons	Turkey	Total
Serum	73	67	52	6	9	207
Tracheal swabs	66	64	47	12	11	200
Cloacal swabs	66	64	47	12	11	200
Total	205	195	146	30	31	607

Table (2) Results of examined samples per species in the surveillance for avian influenza type A by RRT-PCR

No.	Name of the village	No. of swabs	No. of houses	Results of RRT- PCR
1	De'bes	26	2	Negative
2	Abu Agwa	40	6	Negative
3	El-Tawaheen	48	2	Negative
4	Kafr Meshref	33	2	Negative
5	Abu Ezbaway	30	6	Negative
6	El-Maseed	34	7	Negative
7	El-Eraki	42	4	Negative
8	El-Abassa	40	5	Negative
9	Kafr Abu Negm	42	5	Negative
10	Mansheit El-Abassa	35	3	Negative
11	El-Abassa El-Kobra	30	3	Negative
Total		400	45	0/11

Table (3) Results of examined serum samples (AI-ELISA, H7 and H5 HI test)

	Village	No. of samples	Antibody detection		
			AI-ELISA*	HI- H7	HI- H5
1	El-Abassa	20	Negative	Negative	Negative
2	Kafr Abu Negm	21	1/5 (chicken)	Negative	1/5 (chicken)
3	Mansheit El- Abassa	36	Negative	Negative	Negative
4	El-Abassa El- Kobra	15	1/5 (chicken)	Negative	1/5 (goose)
5	De'bes	13	Negative	Negative	Negative
6	Abu Agwa	20	Negative	Negative	Negative
7	El-Tawaheen	24	2/7 (chicken)	Negative	6/16**
8	Kafr Meshref	16	Negative	Negative	Negative
9	Abu Ezbaway	15	Negative	Negative	Negative
10	El-Maseed	13	Negative	Negative	Negative
11	El-Eraki	14	Negative	Negative	Negative
	Total	207	4/73	0/207	9/207

* AI-ELISA was available only for chicken sera

** Chickens (1/7), ducks (3/4) and geese (2/5)

DISCUSSION

Surveillance programs on Avian Influenza were conducted either before emerging of the disease (Kilany, 2006 and Safwat, 2006) or after infection both in poultry farms (Selim, 2007) or in backyard birds (Aly *et al.*, 2007 b).

Special attention to wild birds-backyard pathway for introduction of avian influenza viruses either H5 or H7 subtypes were extensively supported worldwide (Račnik *et al.*, 2006, Buscaglia *et al.*, 2007, Terregino *et al.*, 2007). Backyard birds in Egypt play a pivotal role in perpetuation and

circulation of H5N1 virus in the environment (Aly *et al.*, 2007 b). After reporting of low pathogenic AI-H7 in wild migratory ducks by the Egyptian Government in El-Abassa Lake at El-Sharkia governorate, we implement targeted serological and molecular surveillance to the villages in the periphery circumference of the Lake. Samples were taken from the eleven bordering villages. Serological surveillance is crucial to detect the exposure of backyard birds to infection. The samples were taken mostly from adult chickens, ducks, geese while pigeons and turkey were rarely had been found. Negative results of H7 both serology and molecular surveillances provide an evidence of the absence of H7 Avian Influenza from villages in close contact to the Lake. Currently used ELISA isn't applicable in detection of AI antibodies in water fowl as well as for detection of AI subtype, therefore HI test is more specific, applicable regardless the species and time saving (Beck and Swayne, 2003 and Aly *et al.*, 2007a). Results of HI test against H5 might be explained by vaccination or uncontrolled relatives movement of birds rather

than infection based on negative RRT-PCR results.

The incessant threat of wild birds to Egypt should be incite us to keep going on surveillance. Hence, we planned a large scale surveillance program on all Egyptian wetlands will be implemented this winter sooner or later.

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