

# Risk Factors Associated with Highly Pathogenic Avian Influenza in the Household Sector in Egypt

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## Abstract

Influenza epidemics are a major health concern worldwide. Highly pathogenic avian influenza (HPAI) H5N1 viruses in Egypt have been subjected to rapid genetic and antigenic changes since the first outbreak in February 2006 and have been endemic in poultry in Egypt since 2008. The aim of this study was to assess the most prominent risk factors affecting highly pathogenic avian influenza in the household sector in Egypt. For this purpose 80 villages were selected randomly from different Egyptian districts according to the presence of rumors, high density and high morbidity and mortality rate in different poultry species to collect samples for viral detection for this study. In addition, a questionnaire about the hypothesized risk factors was constructed.

The final multivariate logistic regression model showed, a significant association between source of chicken ( $p<0.05$ ), season ( $p<0.05$ ), presence of nursery farms ( $p<0.05$ ), husbandry system ( $p<0.05$ ), carrying out individual interview for data collection ( $p<0.05$ ) and respond ability of governmental organizations in data collection ( $p<0.05$ ).

According to our knowledge, this is the first paper to discuss the risk factors associated with highly pathogenic avian influenza in household sector in Egypt.

**Keywords:** Egypt; Avian influenza; Risk factors; Household

## Introduction

In Egypt, household poultry keeping has been a livelihood strategy since ancient times. Aviculture has been critical for the poor, and represents a cash-income for maintaining the household economy and an important source for animal protein for household keepers. Before the occurrence of HPAI outbreak in Egypt in 2006, poultry diseases weren't a governmental priority so reporting unusual events among birds were scaring [1].

Avian flu, caused by the influenza virus Type 'A', can affect several species of birds (chickens, turkeys, ducks, quails, guinea fowl, etc.), as well as pet birds and wild birds with some strains resulting in high mortality rates. The virus has also been isolated from mammalian species including humans, rats and mice, weasels and ferrets, pigs, cats, tigers and dogs [2]. The Asian origin highly pathogenic H5N1 strain of the avian influenza virus has attracted much attention over the last few years because of significant outbreaks globally in domestic and wild birds. Concern is raised because of the degree of virulence not only in domestic poultry but also in wild birds as well as the ability to infect mammalian species. While AI viruses are species specific, the highly pathogenic H5N1 AI virus has also infected humans [3].

The H5N1 HPAI global disease situation is now relatively stable, but still alarming in some countries where the disease is considered entrenched. In these locations, pockets of infections are closely associated to well-known risk factors, such as high human and chicken densities, large free-grazing duck populations, poor biosecurity in smallholder units and culturally-determined food market habits linked to poor poultry hygiene.

In 2007, the usage of spatial cluster analysis revealed the presence of more specific risk factors supporting the spread of infections either in birds or to human in selected geographical clusters, such as the higher percentage of surface water which would support higher densities of domestic and wild water birds compared with other adjacent regions [3].

## Materials and Methods

### Sampling procedure

The sampling of the live birds was carried out from August 2010 to November 2012. About 80 Villages were selected to carry out the study according to presence of rumors, high density, risk factors, high mortality in different poultry species indicating the presence of HPAI (H5N1) positive cases. Different types of samples were collected for different purposes; blood samples from chickens and ducks were collected for serological tests; tracheal and cloacal swabs were collected for detection of AI virus using real time PCR; and the same samples were used for virus isolation.

By approaching the village early in the morning, the teams observed the village for any diseased birds showing any clinical signs, such as respiratory and nervous symptoms, cyanosis in comb and wattles, and diarrhea. The presence of any of these signs was used to select the bird for sampling. In case there were no diseased birds, healthy birds representing the different categories of age, species and sources were selected randomly.

Sampling preparation took place at Gamasah Regional Laboratory and then samples were transported to the National Laboratory in Cairo for further analysis.

### Laboratory procedures for different purposes

The procedure for pooling of individual samples for AI detection

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using QRT-PCR was as follows: Cloacal and tracheal swabs were pooled separately and the examined pools were a mix of both. his was done centrally in NLQP (National Laboratory for Quality Control on Poultry Production, Cairo) with samples from five birds together in one pool, according to the following criteria:

Species (chicken pools were separated from the duck pools)

Health status, (diseased birds were pooled separately)

### Procedure for real time PCR for detection of AI

The method employed was according to Veterinary Laboratories Agency (VLA), using a one-step real time PCR kit; primers and probes were designed according to VLA sequences and the test was conducted with a Stratagene MX3005P real time PCR machine.

### Procedure for antibody detection of avian influenza by using ELISA test

Detection of AI antibody by using commercial kits (Biocheck, Netherlands) for chicken sera and (ID VET, France) for duck sera.

### Haemagglutination inhibition (HI) test

Detection of avian influenza antibodies was done according to the 2005 Protocol of the World Organization for Animal Health (OIE).

### Composition of the field teams

Field teams surveying each village were composed of two members from General Organization of Veterinary Services (GOVS). They were responsible for interviewing peoples, collecting data and sampling of (tracheal, cloacal, blood samples and internal organs) in rapid field test positive cases according to the method described by OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 2.1.14.

### Gathering and analysis of the questionnaires from the villages

The questionnaires used contained qualitative and quantitative information on the villages, i.e. location, size of the village, species kept, husbandry system, common diseases, time line and seasonal calendar for occurrence of HPAI out breaks. After collecting data, it will be entered into excel sheet and analyzed by using SPSS (statistical software program, version 15, USA).

### Results

Table 1 shows the Classification and levels of risk factors suggested to affect prevalence of highly pathogenic avian influenza in Egypt, where as Tables 2 and 3 show the distribution range and final multivariate logistic regression model for risk factors associated with highly pathogenic avian influenza respectively.

### Discussion

Influenza A (H5N1) is a highly pathogenic avian disease which could cause death to birds where Egypt was affected in 2006 [4].

Respond ability of Governmental organizations (GO) in data collection has a serious role in combating infectious diseases through: (1) Sharing information in an open, timely and transparent manner, (2) Adopt an integrated and comprehensive approach that incorporates animal and public health aspects in managing avian influenza outbreaks and influenza pandemics, (3) Ensure coordination on all aspects of emergency management for an avian influenza outbreak or a human influenza pandemic, by building on existing mechanisms of cooperation and strengthening these mechanisms as required [5].

Variable	Levels
Number of visits to each village to collect data	One visit=1; two visits=2; three visits=3
Rapid field test	Present=1; not present=0
Respond ability of non governmental organizations in data collection	Good response =1; no response = 0
Respond ability of governmental organizations in data collection	Good response =1; no response = 0
Number of interviews in each visit	One interview=1; two interviews=2; three interviews=3
Presence of key informants	Not present =0 ; present = 1
Carrying out individual interviews for data collection	yes = 1; no=0
Carrying out group interviews for data collection	yes =0; no =1
Presence of rumors	Present =1; not present=0
Presence of farms in the vicinity of villages	Present =1; not present =0
Presence of intensive duck breeding	Not present =0; present =1
Presence of intensive geese breeding	Not present =0; present =1
Husbandry system	Scavenging=1; backyard=2; closed room=3; roof top=4
Presence of nursery farms	Present =1; not present=0
Season	Winter=1; summer=2; autumn=3; spring=4
Restocking season for chickens	Winter=1; summer=2; autumn=3; spring=4
Source of chicken	Hatchery=1; peddlers=2; live bird market=3; farms=4; street vendors=5; home brooding=6
Education level of householders	Illiterate=1; primary=2; metric=3; graduate=4; post graduate=5
Presence of disposal areas	Not present=0; present=1
Presence of live bird markets	Not present=0; present=1
Presence of Migratory birds	Not present=0; present=1
Presence of water and drainage canals	Not present=0; present=1
Co-changing of ducks for mating	Not present=0; present=1
usage of live birds as gifts	Not present=0; present=1
Vaccination	Not present=0; present=1
Mixing between different species	Not present=0; present=1
Authority notification of expected cases	Not present=0; present=1
Hygienic disposal of infected birds	Not present=0; present=1
Biosecurity	Not present=0; poor=1; good=2

**Table 1:** Classification and levels of risk factors suggested to affect prevalence of highly pathogenic avian influenza in Egypt.

Individual interview is a pioneer method for data collection as it is so valuable in providing detailed information, appropriate when there is an expected verities of different stories and where the topic to be discussed is sensitive where a respondent may be unwilling to speak about some aspect of their experience in front of others, or where there is a possibility that the story told could contaminate other participants' stories [6].

Husbandry system plays an important role in the occurrence of HPAI household outbreaks in Egypt. Very small poultry flocks are found everywhere from remote rural villages to city rooftops and are owned by enormous numbers of families, many of whom are very poor. Often they are owned and managed by women. Children also take care of birds and may own them and give them names; care of poultry is one of the ways to learn responsibility and independence. In villages and on city streets, they are found scavenging, in contact with other poultry, wild birds and people. Many city flocks, however, are kept enclosed on rooftops or in courtyards by owners who pay close attention to them.

Variable	Positive cases		Normal	
	Number (20)	%	Number(60)	%
Number of visits to each village to collect data				
1	0	0	39	65
2	0	0	16	26.66
3	20	100	5	8.334
Rapid field test				
0	0	0	26	43.333
1	20	100	34	56.667
Respond ability of non governmental organizations in data collection				
0	0	0	0	0
1	20	100	60	100
Respond ability of governmental organizations in data collection				
0	5	25	33	55
1	15	75	27	45
Number of interviews in each visit				
1	0	0	34	56.667
2	0	0	26	43.333
3	20	100	0	0
Presence of key informants				
0	0	0	21	35
1	20	100	39	65
Carrying out individual interviews for data collection				
0	6	30	7	11.67
1	14	70	53	88.33
Carrying out group interviews for data collection				
0	4	20	0	0
1	16	80	60	100
Presence of rumors				
0	0	0	0	0
1	20	100	60	100
Presence of farms in the vicinity of villages				
0	0	0	0	0
1	20	100	60	100
Presence of intensive duck breeding				
0	4	20	10	17.67
1	16	80	50	83.33
Presence of intensive geese breeding				
0	17	85	51	85
1	3	15	9	15
Husbandry system				
1	1	5	13	21.667
2	1	5	26	43.333
3	9	45	18	30
4	9	45	3	5
Presence of nursery farms				
0	12	60	7	11.667
1	8	40	53	88.333
Season				
1	17	85	12	20
2	1	5	17	28.333
3	1	5	24	40
4	1	5	7	11.666
Restocking season for chickens				
1	0	0	27	45
2	0	0	6	10
3	14	70	27	45
4	6	30	0	0

Source of chicken				
1	0	0	21	35
2	20	100	27	45
3	0	0	3	5
4	0	0	3	5
5	0	0	4	6.667
6	0	0	2	3.334
Source of ducks				
1	0	0	0	0
2	0	0	20	33.333
3	20	100	24	40
4	0	0	0	0
5	0	0	0	0
6	0	0	16	26.667
Education level of householders				
1	7	35	11	18.333
2	9	45	30	50
3	4	20	16	26.557
4	0	0	3	5
5	0	0	0	0
Presence of disposal areas				
0	0	0	0	0
1	20	100	60	100
Presence of live bird markets				
0	0	0	25	41.666
1	20	100	35	58.334
Presence of Migratory birds				
0	0	0	0	0
1	20	100	60	100
Presence of water and drainage canals				
0	0	0	0	0
1	20	10	60	100
Co-changing of ducks for mating				
0	0	0	21	35
1	20	100	39	65
usage of live birds as gifts				
0	0	0	0	0
1	20	100	60	100
Vaccination				
0	20	100	30	50
1	0	0	30	50
Mixing between different species				
0	0	0	17	28.333
1	20	100	43	71.67
Authority notification of expected cases				
0	20	100	57	95
1	0	0	3	5
Hygienic disposal of infected birds				
0	16	80	45	75
1	4	20	15	25
Biosecurity				
0	13	65	32	53.333
1	6	30	18	30
2	1	5	10	46.667

**Table 2:** Distribution of risk factors associated with highly pathogenic avian influenza and normal cases

Unregistered, unlicensed nursery farms in Egypt are considered as a reservoir for HPAI infection due to poor biosecurity. So, strict biosecurity is required to prevent dissemination of the infection from certain nursery to another [7].

After 25<sup>th</sup> January Egyptian evolution, there were a significant

shortage of energy sources required by poultry farms to; (1) achieve the optimal temperature required by the chick in winter (2) to enhance the work of ventilators, This shortage let the chick in the worst environmental conditions that enhanced the appearance of many disease conditions such as infectious bronchitis (IB), mycoplasmosis

Variable	<sup>1</sup> $\beta$	<sup>2</sup> SE	Odds	P	<sup>3</sup> CI
Respond ability of governmental organizations in data collection	1.299	.578	5.054	.025	3.667
Carrying out individual interviews for data collection	-2.097-	.767	7.467	.006	.123
Husbandry system	1.686	.432	15.208	.000	5.399
Presence of nursery farms	-2.430-	.608	15.955	.000	.088
Season	-1.573-	.427	13.572	.000	.207
Source of chicken	-1.009-	.470	4.605	.032	.365

<sup>1</sup> $\beta$ : Regression coefficient

<sup>2</sup>SE: Standard error

<sup>3</sup>CI: Confidence interval at 95%

**Table 3:** Final multivariate logistic regression model for risk factors associated with highly pathogenic avian influenza.

and HPAI moreover, many people prefer to purchase baby chicks in winter season due to their adequate growth rate [8].

Hatcheries distribute many live birds either directly or through peddlers. When they hatch, these chicks are not infected with HPAI but may become infected between hatching and distribution if the virus is being brought to the hatchery through contaminated products or maintained if other birds are present. Disposable cardboard trays should only be used once but this is not always the case. Plastic egg trays are designed to protect eggs but their structure makes cleaning and subsequent disinfection very difficult. This is equally true of the trolleys on which the flats are usually stacked [7].

## Conclusion

From the previous mentioned results we conclude that there were main six factors initiating the occurrence of HPAI (H5N1) in the household sector in Egypt which include source of chicken, season, presence of nursery farms, husbandry system, carrying out individual interview for data collection and respond ability of governmental organizations in data collection. Further investigations should be

carried out to study other risk factors affecting the occurrence of HPAI (H5N1) in the household sector in Egypt.

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