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SERO-PREVALENCE OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) VIRUS (H5N1) IN LAYER FARMS

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ABSTRACT

***Background:** Avian influenza (AI) virus is one of the highly devastating viral diseases affecting poultry with a major significance in layers and egg production subsequently causing abnormalities in eggs represented by soft-shelled eggs and stopping laying activities rapidly with disease progression. This study aims to assess the prevalence and occurrence of highly pathogenic avian influenza (HPAI) virus (H5N1) in layer farms and identify the virus persistence in environment.*

***Methods:** Across sectional study was carried out in five layer farms; differ in production system (four battery/cage and one litter) in Dakahlia governorate, Egypt. Different samples were collected; Cloacal and tracheal swabs from live birds, lung and liver specimens from dead birds and fresh droppings, water and feed samples from bird's environment. The collected samples were subjected to sero-identification by Haemagglutination (HA) and Haemagglutination inhibition (HI) serological tests.*

***Results:** Out of five examined layer farms only two farms show positivity of H5N1 (40%). On other hand, this study revealed that bird samples showing high seroprevalence of HPAI virus (H5N1) than environmental samples. The most frequent percent of H5N1 detected in organs from dead birds followed by tracheal then cloacal swabs. Among all environmental samples only fresh dropping samples were positive for H5N1.*

***Conclusion:** Surrounding environment play an important role in transmission of HPAI virus (H5N1) and layer act as a silent risk for AI spread due to lack of any pathognomonic lesion or signs of the disease so, more attentions and early diagnosis and detection of AI in layer farms should be strictly adopted.*

INTRODUCTION

Influenza is one of the most common respiratory infections, affecting millions of birds every year caused by avian influenza viruses which are a highly contagious, extremely variable viruses that are widespread in birds. Wild birds in aquatic habitats are thought to be their natural reservoir hosts, but domesticated poultry and other birds can also be infected **(Brown, 2010)**. Over the past several years, heightened concerns about emergence of a pandemic influenza strain(s) have withdrawn the attention of scientists, policymakers and the general public. Influenza A viruses circulate in a wide variety of animals, including birds, humans, pigs and other mammals (**Webster *et al.*, 1992**). Avian influenza (AI) virus is one the highly devastating viral diseases affecting poultry with a major significance in layers and egg production subsequently causing abnormalities in eggs represented by soft-shelled eggs and stopping laying activities rapidly with disease progression (**Elbers, 2005)**. In the first few years after its introduction to Egypt in 2006,

the highly pathogenic avian influenza (HPAI) H5N1 outbreaks had a seasonal incidence and were usually accompanied with the season of bird migration **(Aly *et al.*, 2008)**. Later on, the diseases became endemic in Egypt, as well as in other five countries worldwide **(FAO-OIE-WHO, 2012)**. The persistence of the virus in Egypt may be contributed to many factors including the early application of vaccination directly after the introduction of the virus to Egypt. The used vaccines were imported from different countries and provide low protection level. Also, this massive vaccination policy encourages the evolution of genetic drift evolution **(Abdel-Moneim *et al.*, 2012)**. Other additional factors enhanced the persistence of the virus include, poultry production in backyards and their marketing as well as fast and randomized movement of poultry, by-products, manure, insufficient human awareness, the unhygienic disposal of dead birds and the use of untreated wastes of poultry farms as a feed to farmed fish. The contaminated materials will be fed by wild and aquatic birds and this allow the virus to persist in the environment **(Aly *et al.*, 2008).** The poor hygienic control program applied in Egypt lead to emergence of a new strain H5N8 in Egypt in 2016 **(WHO, 2017)**. This study aims to assess the prevalence and occurrence of highly pathogenic avian influenza (HPAI) virus H5N1 in layer farms and identify the virus persistence in environment which play an important role in transmission of HPAI virus (H5N1).

MATERIALS AND METHODS

1. Farm description:

Farm / Parameter	I	II	III	IV	V
Housing System	Battery / cage	Battery / cage	Battery / cage	Battery / cage	Deep litter
No. of Pens	4	6	4	8	8
No. of birds/pen	5.000	30000	27000	32000	37000
Distance from the nearest farm	1.5km	1km	2km	1Km	1km
Water source	Surface water	Underground water	Underground water	Underground water	Underground water
Water device	Nipples	Nipples	Nipples	Nipples	Automatic troughs
Water system sanitation	Not present	Not present	Not present	Not present	Not present
Bird Proofing	Wire net on windows	Wire net on windows	Wire net on windows	Wire net on windows	Wire net on windows
Rodent proofing	Bait& traps	Bait& traps	Bait& traps	Bait& traps	absent
All in/ All out	Yes	Yes	Yes	Yes	Yes
disposal of dead birds	Hygienic disposal by incineration	Hygienic disposal by incineration	Hygienic disposal by incineration	Hygienic disposal by incineration	Unhygienic disposal
Disinfection	chlorine, Iodine and Virkon S	formalin and Virkon S	Virkon S and TH4.	Phenol& Virkon S	Phenol& Virkon S
AIV Vaccination program	Adopted	Adopted	Adopted	Adopted	Adopted
Traffic control	High	Fair	High	Moderate	Fair

2. Sampling:

A random sampling technique was adopted. A total of six hundred and seventy two samples (n=672) were collected from live birds; cloacal swabs and tracheal swabs (n= 168/each), dead birds; lung and liver specimens (n=168/each) and surrounding environment; fecal, water and feed samples (n=168/each). The precautions for sampling were done as described by **(OIE, 2014)**. The collected samples were sent to National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), Animal Health Research Institute, P.O. Box 246, Dokki, Giza-12618, Egypt for further examination.

2.1 Live bird samples:

2.1.1 Tracheal swabs

Swabs were obtained by inserting sterile moist swab gently into trachea as described by **Jia (2007)**.

2.1.2 Cloacal swabs

Swabs were obtained by inserting sterile moist swab gently into cloaca as described by **Jia (2007).**

2.2 Dead birds samples (lung and liver):

Tissues samples were aseptically collected under strict condition in NLQP according to method described by **(Swayne and Halvorson, 2003).**

2.3 Environmental samples:

2.3.1 Fecal samples:

A total number of fresh dropping samples were collected from ten different locations from ground in litter system farm (n=48) and from manure belt in battery/cage system farms (n=120) according to method performed by **Reis *et al.* (2012).**

2.3.2 Drinking water samples:

Water samples were collected under aseptic conditions from both automatic water troughs and nipples from litter and battery/cage system, respectively according to method described by **Savita *et al.* (2008)**.

2.3.3 Feed samples:

Feed samples (n=168) were collected from feeder in front of birds under aseptic conditions in a sterile plastic bags and processed as performed by **Savita *et al.* (2008).**

3. Serotyping identification of AIV:

Serological identification of avian influenza virus was carried out according to **(OIE, 2014)**. These tests include; Haemagglutination (HA) and Haemagglutination inhibition (HI) tests.

Statistical Analysis

The statistical analysis was carried out by performing analysis of variance using Chi-square test **(Russel, 1996)** in order to know the significance of occurrence of HPAI virus - H5N1in both birds and their surrounding environment.

Table (1): Percentage of avian influenza virus (H5) identified by HI test in bird and environmental samples in examined layer farms.

Farms / Samples/ Swabs	I	II	III	IV	V	Total
Tracheal	0	33.3	0	0	58.3	23.8
Cloacal	0	13.9	0	0	30.6	11.9
Lung	0	11.11	0	0	31.25	11.3
Liver	0	12.5	0	0	25	8.3
Total	0	16	0	0	36.5	13.8
Water	0	0	0	0	8.3	2.4
Fecal	0	5.5	0	0	20.8	7.1
Feed	0	0	0	0	0	0
Total	0	1.8	0	0	9.7	3.2

RESULTS & DISCUSSION

There is a high possibility for spreading of AI viruses is spreading among humans, wild birds and poultry and may cause new outbreaks after its mutation during interspecies transfer and replication. Poorly controlled movement of birds and lack of biosecurity caused AI to become endemic in poultry populations **(Swayne and Halvorson, 2003)**. Presence of different subtypes of AI viruses in the field indicates that there is always a likelihood of generating new virus by gene reassortment between subtypes pathogenic in birds and mammals**.** Vectors including humans (farm personnel, veterinarians), vehicles used to transport poultry, equipment used on poultry farms, rodents and flies could result in secondary spread of AIV (**Sawabe *et al.*, 2006)**. All of the previuos risk factors could resulting in AI virus transmission. On other hand, the probability of AI transmission was increased on the basis of inter-farm distance, also the density of farms in an area was a good indicator for major outbreak occurrence (**Boender *et al.*, 2007)**.

Non significance of HPAI virus (H5N1) prevalence between bird and environmental samples in different examined layer farms **(▪ 2= 10.6, D.F=24, P.V=0.991)** contributed to low prevalence of virus (two farms were positive among five examined farms. The above table shows the sero-prevalence of H5N1 virus in birds and their surrounding environment in five examined layer farms. There are 2 farms (40%) out of 5 examined layers farms show positivity of AI. In brief, sero-prevalence of AI virus in positive farms no. (II and V) was 9.9% and 25%, respectively. This result is higher than the results reported by **(Mohammed, 2011)** who detect AI in one farms out of 43 farms (2.3%). Also, This result lower than reported by **(Wesam, 2008)** who detect AI in 12 farms out of 15 examined farms (80%). This variation in recorded results may be contributed to seasonal and geographical variations. In birds, either live or dead were seropositive but in environment, only fecal samples was seropositive for H5N1. Organs of dead birds showing high percent of AI virus followed by tracheal swabs then cloacal swabs. Briefly, two seropositive farms (II and V) show percent of tracheal swabs 33.3 % and 58.3%, respectively. While, cloacal swabs observed in farm (II and V) 13.9% and 30.6%, respectively. All dead birds' organs (lung and liver) showed 11.1% and 31.25%, respectively for each organ samples in farm (II). On other hand, farm (V) shows positive lung and liver samples (12.5%, 25%, respectively). Influenza A viruses were detected by high percent in tracheal then cloacal swabs and internal organs. This current findings as reported in previous study

conducted by **(Kayali, 2014)** 42.4% of dead birds (organs), 20.8% of sick birds (cloacal and tracheal swabs).

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الملخص العربي

مدى الانتشار الفيروسى لفيروس انفلونزا الطيور العالى الضراوه H5N1 فى مزارع الدجاج البياض

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يعتبر مرض انفلونزا الطيور من الامراض الفيروسيه الخطيرة التى تؤثر على صحه الطيور وخاصه دجاج البياض منها وعلى شكل ومعدل انتاج البيض. تهدف هذه الدراسه الى تقييم مدى انتشار السيرولوجى وحدوث فيروس الانفلونزا عالى الضراوة فى مزارع البياض ومعرفه مدى استمرار تواجد هذا الفيروس فى البيئه.لذلك اجريت هذه الدراسه المقطعيه على خمسه مزارع بياض فى محافظه الدقهليه . هذه المزارع مختلفه فى نظام التربيه بيطاريات وفرشه عميقه. مسحات من الشرج والقصبه الهوائيه والكبد والرئه تم اخذها من الطيور الحيه والنافقه على التوالى. كما تم اخذ عينات من البيئه مثل مياه الشرب والعلف و الزرق وتم اخضاع العينات الى الاختبارات السيرولوجيه مثل اختبار تخلط الدم والتجلط المثبط. ووجدنا حوالى مزرعتين من اصل خمسه مزارع مصابه بالفيروس ووجدنا ايضا ان نسبه تواجد الفيروس فى البيئه اقل من تواجده فى الطيور. وقد كشفت الدراسه تواجد الفيروس فى جميع العينات المأخوذه من الطيور لكن فى البيئه تواجد الفيروس فى عينات الزرق فقط مما يشير الى دور البيئه الفعال فى انتشار الفيروس.