

**Short Communication****Seroprevalence of Avian influenza H<sub>9</sub> among the free-range ducks in Moshtohor**

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**ARTICLE INFO****ABSTRACT****Keywords**

Avian Influenza H9N2,  
Seroprevalence,  
Free-range Ducks,  
Hemagglutination Inhibition  
Assay,  
Pandemic Potential  
Egypt

Received 29/03/2023

Accepted 25/04/2023

Available On-Line

01/07/2023

H9N2 Avian Influenza viruses have become globally prevalent in poultry, posing a genuine threat to the poultry industry and humans due to zoonotic infection and a potential pandemic. Because the H9N2 avian influenza virus subtype is known to cause respiratory diseases in a variety of avian species, the emergence of novel, highly pathogenic influenza viruses is possible. The purpose of the study was to look into the prevalence of avian influenza H9N2 in free-range ducks in Moshtohor village, Qalubia, Egypt. Blood samples were gathered from 50 free-range ducks from 13 households, and serum samples were tested for H9N2 antibodies using the hemagglutination inhibition assay (HI). The study discovered 76.9% seroprevalence of AI H9N2 in the tested ducks, indicating H9N2 infection. The findings emphasize the significance of surveillance and control measures in preventing the spread of avian influenza viruses, which can cause pandemics in humans as well as severe economic losses in poultry production.

**1. INTRODUCTION**

Avian influenza viruses (AIVs) are a major public health concern worldwide due to their potential to cause pandemics in humans and severe economic losses in poultry production (Blagodatski et al., 2021). H9N2 is an AIV subtype that has been identified in poultry across several countries worldwide, including Egypt. The virus is endemic to Egyptian poultry and is associated with low pathogenicity in chickens and ducks, although it can cause severe respiratory disease in other avian species (Kim, 2018).

Egypt has been experiencing H9N2 AIV outbreaks since 2010, with several reports of the virus in poultry and humans (Nagy et al., 2017). In poultry, H9N2 AIV infection results in a reduction in egg production and weight gain, leading to substantial economic losses (Nguyen et al., 2019). Furthermore, H9N2 AIV can serve as a reservoir for genetic reassortment with other AIVs, potentially leading to the emergence of novel, highly pathogenic influenza viruses, particularly in Egypt (Kim, 2018).

Ducks have been identified as key hosts for AIVs, with high viral shedding and transmission rates reported in domestic and wild ducks (Hulse-Post et al., 2005). Eladl et al. (2019) investigated the seroprevalence of AI H9 between 2012 and 2015 in Dakahlia Governorate, Egypt which recorded 57.7% in backyard chickens and 12.2% in backyard ducks. However, limited data are available on the seroprevalence of H9N2 AIV in free-range ducks in Egypt, particularly in rural areas where backyard poultry farming is common.

Moshtohor village, located in Qalyubia governorate, is one such area where backyard poultry farming is widespread. Therefore, we conducted a study to determine the seroprevalence of H9N2 in free-range ducks of different breeds and of age ranged from 1 month to 1 year in Moshtohor village. We collected blood samples from randomly selected ducks and tested them for H9N2 antibodies using the hemagglutination inhibition assay (HI).

**2. MATERIAL AND METHODS***Ethical statement*

The study was conducted following the guidelines of the animal welfare committee, and the protocols were approved by the Research Ethics committee, Faculty of Veterinary Medicine at Benha University, (Approval number BUFVTM 09-03-23)

*2.1. Study Design*

A cross-sectional study was conducted in March 2020 to investigate the seroprevalence of avian influenza H9N2 among free-range ducks in Moshtohor village, Qalyubia, Egypt. Thirteen households that raised ducks were randomly selected for sample collection, and data on breed, age, AI vaccination status, and exposure to other bird species (domestic and wild) were collected. A total of 50 blood samples were collected from the medial metatarsal vein of the ducks (Table 1).

*2.2. Laboratory Methods*

The collected blood samples were transported on ice to the laboratory on the same day of collection. The serum was separated through centrifugation at 1500 × g/15 min and

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stored at -20°C until use. A hemagglutination inhibition (HI) assay was performed to determine the seroprevalence of antibodies against the AI H9 virus, following the guidelines of the World Organization for Animal Health (OIE) in 2015. The reference AI strain (H9N2) used in the test was obtained from MEVAC, Cairo, Egypt.

### 2.3. Data Analysis

The collected data on breed, age, AI vaccination status, exposure to other bird species, and HI titer results were entered into Microsoft Excel. Descriptive statistics, including means, were calculated.

## 3. RESULTS

The data collected showed that the ducks were of different species and freely roamed on the house roof (Table 1). The duck age in this study ranged from 1 month up to 1 year. The number of ducks in the tested flocks ranged from 3 to 13 birds per flock. All ducks from which samples were taken had previous contact with other birds, whether wild or domesticated. Also, they have never been vaccinated against AI. Our findings indicate a high seroprevalence of AI H9N2 by 76.9% in the tested ducks, with an HI titer of AI H9N2 above log 5. As showed in Figure 1, The HI titers of AI H9 in backyard ducks in our study ranged from 5 to 9.7 log<sub>2</sub>.

Table 1 Descriptive data for the backyard ducks examined for AI H9 seroprevalence.

House No.	Duck breed#	Duck No.	Duck age	Mean HI titer (Log <sub>2</sub> )
1	Mallard	10	1 month	4
2	Balady	13	5 months	7.3
3	Balady	>10	5 months	6.7
4	Balady	4	1 month	7.3
5	Balady	>10	5 months	7.3
6	Hybrid	>10	2 months	7
7	Mallard	10	1 month	5
8	Balady	3	1 year	6.7
9	Balady	8	2 months	4
10	Mallard	6	3 months	7.3
11	Balady	6	5 months	9.7
12	Balady	7	4 months	6.3
13	Balady	10	1 month	4

All ducks from which samples were taken had previous contact with other birds, whether wild or domesticated. Also, they have never been vaccinated against AI

Fig. 1: Mean HI titer

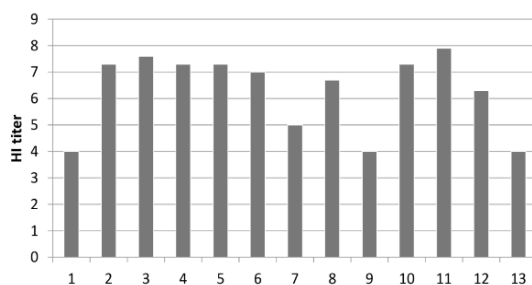


Fig (1) Mean HI antibody titer in different backyard duck flocks.

## 4. DISCUSSION

The present study investigated the seroprevalence of avian influenza H9N2 among free-range ducks in Moshtohor village, Qalyubia, Egypt. The backyard reared ducks are exposed to other birds such as pigeons, sparrows, and

crows. The absence of vaccination against any diseases increased the risk of infection transmission between different bird species. The presence of antibodies against AI H9N2 in the serum of tested birds confirms that they were exposed to AI H9N2 infection. Our results are in agreement with Eladl et al. (2019), who recorded a seroprevalence of AI H9N2 with a history of no AI vaccination as 57.7% in backyard chickens and 12.2% in backyard ducks. The high seroprevalence of AI H9N2 in ducks may be attributed to a recent infection, which could be due to the seasonal changes in March, the age of ducks, and the introduction of new ducks into the infected place. Our findings disagree with Eladl et al. (2019), who recorded that the low HI titer of duck ranged from 0.66 to 4.33. This discrepancy may be due to the difference in the time and locality of the sera collection.

## 5. CONCLUSION

Based on our findings, we concluded that exposure of ducks to AI H9N2 is a significant risk factor that threatens human health and can aid in the spread of AI. Therefore, it is necessary to apply vaccination programs to protect backyard ducks from AI H9N2 infection and limit its transmission to other birds and humans.

## ACKNOWLEDGEMENT

The acknowledgement expresses sincere appreciation to Prof. Dr. Mohamed Faisal (Department of Fisheries and Wildlife, College of Agriculture & Natural Resources, Michigan State University), for his valuable financial and technical support during the research. The authors acknowledge the expertise and guidance of Prof. Dr. Mohamed Faisal, which was instrumental to the accomplishment of the research objectives. They express their gratitude for the opportunity to work under his supervision and hope to continue benefiting from his guidance and mentorship in the future.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the research presented in this paper. The authors have no financial or personal relationships with any individuals or organizations that could potentially bias their work. Any sources of funding or support are acknowledged in the paper.

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