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Surveillance Of Highly Pathogenic Influenza A And Newcastle Disease Viruses In Some Wild Birds In Egypt

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ABSTRACT

Screening wild healthy birds for antibodies against some viral pathogens of poultry has become of epidemiological importance. Serum samples from wild White Ibis and Crows in Egypt were screened by hemagglutination inhibition (HI) test for (HPAIV) subtypes and (NDV). HPAI (H5N1, H7N1) antibodies were detected in Ibis sera with 13.9% & 12.9% and with 4.3% & 0.0% respectively in Crows. Antibodies to H9N1 neither detected in Ibis nor in Crows. NDV antibodies were detected in Ibis with 6.3% while in Crow were with 15.4%. These results show the possible role of wild birds in the transmission of HPAI and ND viruses to poultry and humans populations.

INTRODUCTION

Wild birds have played a significant role in the global spread of highly pathogenic avian influenza H5N1, which has killed more than 240 people, many millions of poultry, and an unknown number of wild birds and mammals, including endangered species, since 2003 (*1*). **Capua and other's** *(2)* isolated HPAI from different species of wild birds that live near or around Italy in 1999-2000. Wild Crows and White Ibis have increased significantly around the urban environment in Egypt. They may carry causal agents of highly pathogens with zoonotic infectivity through shedding which may contaminate the environment threatening public health and poultry industry *(3)*. Avian influenza (AI) and Newcastle disease (ND) are two serious infectious viral diseases which disserve poultry and humans. At present, all subtypes of avian influenza virus (AIV) and some serological subtypes of avian paramyxovirus 1 (APMV) have been isolated from wild birds, so it has been believed that they are natural reservoirs of AIV and APMV virus, and as transmismitting agents, they play an important role in spreading of these diseases among domestic poultry *(4)*. Once the pathogenic strain is introduced into domestic poultry, it leads to a great loss to the economy *(5)*.

Avian influenza has had a tremendous impact around the world as the current highly pathogenic strain, H5N1, continues to spread across the world. This problem requires a global response. Since 2003, this virus has resulted in stamping of millions of domestic fowl and infected more than 130 persons *(6)*.

Avian influenza viruses originate in wild birds, particularly water birds, and are usually not dangerous to their hosts. However, after transfer to a new type of host, either avian or mammalian, influenza viruses sometimes undergo rapid evolution and may develop into new, highly pathogenic strains that pose serious threats to humans, poultry and wild bird population. The infamous Spanish flu pandemic, which killed between 40 and 100 million people in 1918, was a highly pathogenic H1N1 strain that originated in birds *(7)*.

The first reported isolation of an influenza virus from wild birds was in 1961 from common terns (*Sterna hirundo*) in South Africa, where HPAI virus (H5N3 subtype) existed. Systematic surveillance studies revealed the widespread distribution of influenza viruses in wild avian population *(8)*. The outbreak of highly pathogenic avian influenza of the H5N1 subtype in Asia has subsequently spread to Russia, the Middle

East, Europe, and Africa. It has put an increased focus on the role of wild birds in the persistence of influenza viruses. The ecology, epidemiology, genetics, and evolution of pathogens cannot be fully understood without taking into account the ecology of their hosts *(9)*. So far, the HPAI (H5N1) strain that originated in poultry in Southeast Asia has caused mortality in >60 wild bird species *(10&11)*. In addition, during the devastating outbreaks in poultry, the H5N1 virus was transmitted to 175 humans, leading to 95 deaths (as of 6 March 2006). It has also been isolated from pigs, cats, tigers, and leopards *(9)*. Human and avian influenza viruses are closely related and it is likely that some genetic material of all human influenza viruses originated in birds *(12)*. However, only a small number of avian influenza virus is known to infect humans directly, including H5N1, H7N2, H7N3, H7N7 and H9N2. HPAI, H5N1, primarily in particular, has caused international public health concern. Nowadays, H5N1 primarily causes disease in poultry and unusually has caused mortality in wild birds *(13)*.

The current HPAI strain, subtype H5N1, is believed to have emerged in 2002. At present, over 200 million domesticated birds have been killed by the virus or culled to stem its spread. This strain has acquired the capability to infect humans: the World Health Organization (WHO) reported that more than 190 people have been infected, over 100 of whom have died, predominantly in South-East Asia. Currently, the virus does not spread from human to human, but it is feared that only a minor adaptation is needed for it to develop the necessary characteristics. Such an adaptation would allow the virus to become pandemic *(7)*.

Wild birds were also thought to play a role in the transmission of avian viruses like Newcastle disease. NDV has been reported from many species of free-living birds and wild waterfowl which are considered as a potential natural reservoir *(14,15)*. Information on HPAI remains scarce and also details including information on its adaptability, contagiousness, and dissemination through wild birds.

The objective of this study was to confirm the role of wild Ibis and Crows as a possible source in spreading of HPAIV or NDV and to provide some basic information.

MATERIAL AND METHODS

Birds

A total of 55 White Ibis and 25 Crow were hunted from different localities in Sharkia and Ismailia provinces which were thickly populated with wild birds and so close to poultry farms and humans. These birds were subjected to clinical and postmortem examination.

Serum samples

Eighty serum samples were collected from hunted birds by slaughtering. Sera were subsequently stored at -20 °C until used.

Heamagglutination inhibition (HI) tests

In order to monitor the antibody titers against HPAIV and NDV in wild birds, HI test was performed according to the protocols described earlier *(16)* against HPAI (H5N1/H7N1/H9N2) subtypes and NDV using 4 heamagglutination units (4HA). The HI test was carried out at **National Laboratory for Veterinary Quality Control on Poultry Production, Dokki, Giza.**

Histopathological examination

Specimens from spleen and brain with characteristic lesions were taken and fixed for histopathological examination *(17)*.

RESULTS AND DISCUSSION

Hunted birds appeared apparently healthy. This may be related to the natural resistance of wild birds. In most reports, wild birds with HPAIV consistently showed no disease signs or had mild form of the disease without showing noticeable clinical symptom after infection by AIV, but they can spread virus persistently through alimentary canal polluting ambient water resource and habitats. So they are virus resource of poultry. Because wild birds do not fall ill after infection, their outward appearance is healthy *(18).*

Out of 80 serum samples collected from wild Ibis and Crows, total positive 10 samples (12.5%) for both wild birds showed HI antibody titers against HPAIV (H5N1 & H7N1) serotypes and 3 positive samples (10.3%) HI antibody was detected against NDV **(Table 1& 2).**

Table 1. Seroprevalence of (HPAIV) serotypes antibodies in Ibis and Crow as determined by HI assay.

Bird species	Serum samples (N)	Number of HI positive samples												Total positive %
		Tested No	H5N1				Tested No	H7N1				Tested No	H9N2	
			positive	Titer range	GMT	%		positive	Titer range	GMT	%		positive	
White Ibis	55	36	5	3-7 Log^2	4.7	13.9	31	4	2-4 Log^2	3	12.9	31	0	16.3
Crow	25	23	1	4 Log^2	-	4.3	18	0	0	-	-	18	0	4

Table 1 and 2 showed the seroprevalence of AI antibodies. They were higher in White Ibis compared to Crows (16.3% vs. 4%, respectively). Ibis showed HI antibodies against most HPAI serotypes, except for the subtype H9N2, meanwhile, Crows were seropositive only to the H5N1 subtype (4.3%). Ibis showed the highest HI titers (7, 6, 4, 3, 3) **Log^2** against H5N1 subtype with (13.9%) as well as HI titers against H7N1 subtype which were 4, 4, 2, 2 **Log^2** with 12.9% **(Fig.1)**. According to the percentage of serum HA antibody for H5N1 subtype, it could be, concluded that H5N1 may be a dominant AIV subtype in Egypt than for any other subtype, also may indicate that wild Ibis and Crows were a potential source and reservoir of HPAIV (H5N1, H7N1) in Egypt to poultry and humans.

Table 2. Seroprevalence of NDV antibodies in Ibis and Crows by HI assay.

Bird species	Serum samples	NDV HI positive samples			
		Tested No	positive	Titer range	%
White Ibis	55	16	1	2	6.3
Crow	25	13	2	3	15.4

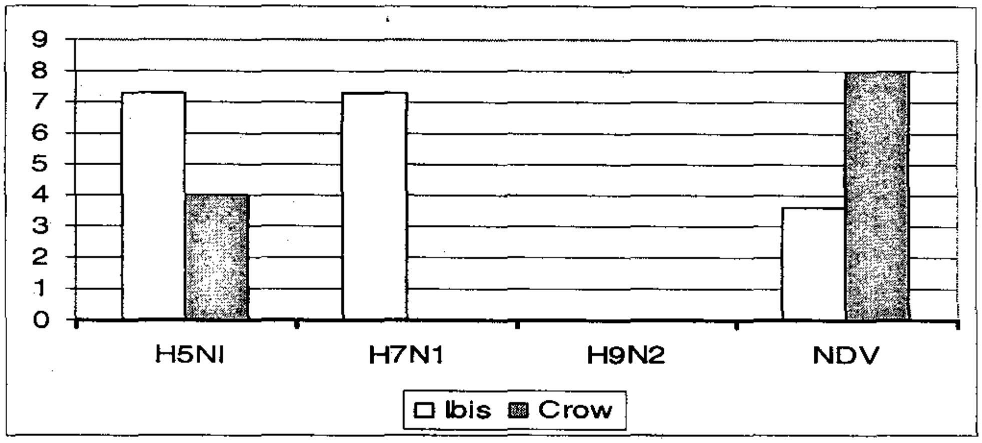

Fig. 1. Prevalence % of HPAI and ND viruses in wild birds.

These results are comparable to some extent with that reported previously which showed that 41% and 31% of Ibis had antibodies to Influenza A in 1997 and 2000 respectively *(19)*. In Egypt, **Mosa,** *(20)* documented the recovery of HPAIV subtype H5N1 (5.1%) from wild migrating quail in Egypt during migration season September and October 2005. He asserted that these isolates were highly pathogenic to our domestic population.

Our results revealed that the presence of H7N1 antibodies in sera of white Ibis. It is believed that, the absence of HA antibody against H7N1 subtype in our Crows sera may be due to the limited number of available samples as a result of Crows hunting is very difficult as this bird is very smart and intelligent. Evidence of anti-H7 antibodies was observed in 3.8% of serum samples collected from poultry workers during the period in 2003 where low pathogenic avian influenza (LPAI) H7N3 virus was circulating *(21).* These findings highlight the need for surveillance in people occupationally exposed to avian influenza viruses, so that they can be monitored for the risk of avian-to-human transmission during outbreaks of avian influenza caused by both LPAI and HPAI viruses.

Sera tested for antibodies against H9N2 were found negative for both Ibis and Crows. These results differed from that previouly recorded which found 17% of Crows had HA antibody against AIV H9N2 in Pakistan *(22)*.

Postmortem of birds showed congestion of the internal organs (brains, lungs, livers, hearts, spleen, kidneys and intestine), in addition to necrotic foci distributed on the liver, pancrease and spleen which enlarged and mottled. Brain showed foci of hemorrhage and congestion with neural degeneration (**Fig. 2&3**).

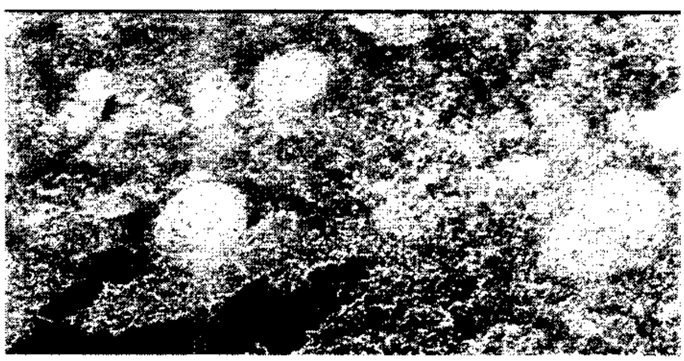

Fig. 2. Photomicrograph of the spleen showed congestion, hemorrhage, lymphoid depletion and necrosis of lymphocytes. H&E. X 400.

Fig.3. Photomicrograph of the brain, showed neuronal degeneration accompanied by satellitosis and neuronophagia. H&E. X 400.

Tanimura and others *(23)* reported that HPAIV was associated with clinical disease, severe pathologic changes and death in Crows. They demonstrated that H5N1 avian influenza antigen from died crows could be detected using standard histological methods and immunohistochemistry. The most prominent lesions were gross patchy areas of reddish discoloration and necrotic foci in the pancreas. The consistent histologic lesions included severe multifocal necrotizing pancreatitis and the virus was isolated from the brain, lung, heart, liver, spleen, and kidney. HPAI (H5N1) was isolated and identified a highly pathogenic H5N1 from dead magpies (*Pica pica sericea*) in which the prominent lesions were multifocal or coalescing necrosis of the pancreas with enlargement of the livers and spleens *(24)*. Microscopically, there were severely necrotizing pancreatitis. The obtained gross lesions and serological results are consistent with previous studies *(23,24)*. We could conclude that, our finding could be attributed to HPAI V.

HI antibodies against NDV were detected one time (6.3%) from Ibis with titer of 2 (Log^2). Meanwhile, Crow showed antibodies to NDV with percent of 15.4% with titers 3 (Log^2). These finding reflect, to some extent, the situation of NDV infection in wild birds in this area and indicated that ND, infection existed in both Ibis and Crows and it was possible for them to spread virus. Several reports supported our results. HI antibody against ND was detected in 11 serum samples of wild mallard ducks *(25)*. It has been recorded that NDV antibody prevalence in wild birds was 10% *(26)*. HI antibodies were detected in Inland great cormorants (*Phalacrocorax carbo*) for NDV*(27)*. In a study carried out with the birds of RioZOO Foundation, 194 birds from different families of wild birds were tested for antibody against NDV using HI. Nine serum samples belonging to the families Falconidae, Phasianidae, and Strigidae were found positive *(28)*.

The potential for an influenza pandemic is very real and necessitates international cooperation to prevent its occurrence. So effective influenza surveillance requires new methods capable of rapid and inexpensive genomic analysis of evolving viral species for pandemic preparedness, to understand the evolution of circulating viral species, and for vaccine strain selection *(29)*.

The transmission of HPAI H5N1 from infected poultry to humans is inefficient, and serious infections in humans have typically only been associated with direct contact with sick or dying poultry. Although transmission of HPAI to humans via wild birds is possible *(30-32)* and should not be dismissed *(33)*. The major risk factor for humans is contact with poultry during an epizootic. Therefore, in Australia the most effective preventative measure from a human health perspective is to prevent and control epizootics in poultry, and in the event of an epizootic, to impose additional health precautions. Data indicated that, the outbreak of AIV in poultry have spatial and transient relationship with wild birds *(34)*, and the veneniferous migratory birds can spread AIV all over the world. Thus investigating the situation of AIV and NDV infection and carrying of wild birds has very important values in theory and economy.

These data provide evidence regarding the wild Ibis and Crow as one of the carrier of HPAIV infection to poultry and humans population in Egypt. We believe that the results obtained in this study could to a certain extent reflect the status of AIV and NDV in wild birds in some Egypt Provinces. To gain overall and profound recognition and comprehension we should rely on long-term and extensive monitoring work.

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الملخص العربي

متابعة لفيروس أنفلونزا الطيورالضارى وكذلك مرض النيوكاسل في بعض الطيور البرية بجمهورية مصر العربية

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المسح السيرولوجى للأجسام المناعية ضد بعض الأمراض الفيروسية الهامة المنتشرة في الطيور أصبح له أهمية وبائية. تم أخذ عينات مصل الدم لبعض طيور ابوقردان وكذلك الغربان المنتشرة في بعض محافظات جمهورية مصر العربية لإجراء اختبار منع التلازم الدموي لفيروس أنفلونزا الطيور الضاري وكذلك فيروس النيوكاسل .

وقد أسفرت النتائج عن وجود أجسام مناعية لفيروس أنفلونزا الطيور للعترة الضارية H7N1; (H5N1) بنسبة مئوية (١٣،٩% و ١٢،٩%) في طيور أبو قردان وكذلك (٤،٣%; 0.00 %) في الغربان على التوالي ولم يتم إيجاد اى أجسام مناعية للعترة الضارية (H9N2). أما الأجسام الناعية ضد فيروس النيوكاسل فقد وجد في طيور أبو قردان بنسبة (٦،٣%) و بنسبة (١٥،٤%) في الغربان.

أثبتت هذه الدراسة عن وجود العترات (H7N1; H5N1) لفيروس أنفلونزا الطيور الضاري في طيور أبو قردان أما فى الغربان فقد ثبت وجود H5N1 فقط مما يترتب علية احتمالية انتشار هذا الفيروس عن طريق هذه الطيور البرية للقطعان المختلفة من الطيور وكذلك الأنسان نظرا لوبائية هذا المرض العالية .