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Detection of Antibodies Against Avian Influenza Virus in Wild Pigeons and Starlings

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Abstract: Sixty wild birds for each of pigeons and starlings were captured in Mosul and examined clinically and serologically for avian influenza virus (AIV) infection. ELISA and haemagglutination inhibition (HI) tests were used for the detection of (AIV) antibodies in both types of examined birds. Clinically the pigeons appeared dull with loss of appetite. The percentage of positive serum antibodies titers against (AIV) was 81.8 and 50% with ELISA and HI tests, respectivly. The starlings did not show any abnormal clinical signs and all serum samples showed negative results by ELISA and HI tests. In conclusion, pigeons only showed ability to be infected with AIV subtype H9N2 and they may play an important role in spreading (AIV) as natural carriers.

Key words: Avian influenza, virus, starling bird, pigeons, ELISA test

INTRODUCTION

Avian influenza (AI) which is also known fowl plaque, is a zoonotic viral disease characterized by respiratory, gastrointestinal and nervous system findings with high morbidity and mortality in *Avian* sp. (Jordan, 1996).

Many species of domesticated or wild birds can be infected with AI virus.It is observed in the various wild winged animals and water birds such as hens, turkeys, ducks, geese and pheasants (Astorga *et al*., 1994; Swayne, 1997).

The disease is caused by avian influenza virus (AIV) type A which is classified under the family Orthomyxoviridae. Influenza viruses vary widely in their pathogenicity and their ability to spread among the birds. which usually act as carriers. Some strains of influenza virus cause severe Illness or death in chickens, turkeys and guinea fowl (Alexander, 2003). Among the 16 haemagglutinin and 9 neuraminidase Ag of Type A influenza virus (Akey, 2003), H5N1 has been proven to be the most virulent and causes 100% morbidity and mortality to the poultry population (Cherbonnel *et al*., 2003). In addition to their effect on avian spp, the virus is found to be associated with death in human, creating a serious public health concern (Sins *et al*., 2003).

The present study was under taken to detect the presence of subclinical infection or carrier features in the pigeons and starlings as they are migrating birds, which can be considered reservoir or carriers for most subtypes of (AIV) type A in the world (Webster *et al*., 1992).

MATERIALS AND METHODS

Specimen collection: sixty pigeons (*Columba livia*) and sixty starlings (*Sturnus vulgars*) were captured from Ninevah province around Mosul city in 2007.

Clinical signs: The clinical signs in both types of birds were observed and recorded during the period from 2-3 weeks after capturing the birds.

Blood samples: Blood samples were taken from each bird, 1-2 mL of blood was drawn and allowed to clot. The serum was removed after centrifugation at 800Xg for 10 min. Sera were subsequently stored at -20°C until used (Khawaja *et al*., 2005).

Serological tests

ELISA: Detection of AI antibodies was done by the ELISA test using AI Indirect ELISA Kit (Jovac-Jordan). This test has been done according to the procedure of the company and readings of the results were done using ELISA reader in the Virology Lab-College of Veterinary Medicine-University of Mosul.

Haemagglutination inhibition test: It was done using a reference (AIV) subtype (H9N2) with 4 agglutinating virus unit using of 0.5% chicken RBCs (AL-Attar, 2007).

RESULTS

Clinical signs: The pigeons showed only dullness and loss of appetite as the only clinical signs, where as in starlings there were no abnormal clinical signs.

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Table 1: Results of ELISA test

Bird sp.	Mean of Sp value	Percentage of positive samples	Percentage of negative samples
Pigeon (*Columba livia*)	780	81.82	18.18
Starling (*Sturnus vulgars*)	554	0.00	100.00

Table 2: Results of Haemagglutination inhibition test (HI)

Bird sp.	Mean of HI test (Log 2)	Percentage of positive samples	Percentage of negative samples
Pigeons (*Columba livia*)	8Log2	50	50
Starlings (*Sturnus vulgars*)	0	0	100

Serological test

ELISA test: The result of Abs Titer in serum samples of pigeons showed that 81.82% of samples were positive for AI by the ELISA test, where as 18.18% of samples were negative. All Starlings serum samples showed negative results (Table 1).

Haemagglutination inhibition test: Pigeons serum samples showed positive results at 50% and the mean titer 8 Log2 against (AIV) subtype H9N2, but Starling serum samples showed no antibodies titer (Table 2).

DISCUSSION

This study was carried out to find the role of wild birds in the spread of avian influenza viruses as carrier birds. For this purpose, blood samples were collected from pigeons (*Columba livia*) and starlings (*Sturnus vulgars*). The antibody titers against AIV Type A were tested by ELISA test which is a highly sensitive test in this respect (Snyder *et al*., 1984). Pigeons showed high antibody titers against AIV, which is in agreement with that founded in chickens (78%) at the same area of study (AL-Attar, 2007). This indicates that the pigeons may play an important role in spreading of AIV as natural carriers like other wild birds (ducks, geese, crows, eagle and Indian fowl).Therefore it is very dangerous to let pigeons in freely management between poultry farms (Khawaja *et al*., 2005).

Samples taken from *Sturnus vulgars* show no titer of antibodies against AIV. This indicates that this type of birds may have natural resistance against AIV or due to its short period contact with other wild birds. These birds appears for a short time in the area of the present study through its migration period, so this interpretation need further investigations in the future.

AIV-infection depends on multiple factors including pathogenicity of the virus, host species, age of the host, routes of infection. All these factors affect the AIV infection in different wild birds which need more study too (Synder *et al*., 1984; AL-Nasraway *et al*., 2005).

HI test was used to determine the subtypes of virus infection which was H9N2 in this study and this subtypes also recorded in Iraq by in chickens (AL-Attar, 2007; AL-Nasraway *et al*., 2005).

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