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RESEARCH ARTICLE

Surveillance of Influenza A/ H5, H7, H9 Viral Subtypes in Domestic and Wild Birds at Many Geographical Regions of Iraq

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Abstract

Background: Influenza A viruses are zoonotic agents, capable of crossing the species barriers. Nowadays, they still constitute a great challenge worldwide. The natural reservoir of all influenza A viruses are wild bird particularly water fowl, despite the fact they have been isolated from a number of avian and mammalian species, including humans.

Methods: Nasal swab or fresh tracheal swab in case of dead bird was taken from 141 broiler chickens suffering from respiratory signs, 139 of local chickens and 109 of wild birds from many regions in Iraq, advance molecular technique, Reverse Transcription Real Time PCR (RT- qPCR) was done to investigate the presence of the most important viral subtypes (H5, H7, H9).

Results: Influenza A virus was found in 63.1% of the commercial broiler chickens with respiratory signs, in 4.31% of local chickens and in 6.42% of the wild birds; subtype H9 was present in all positive samples (100%) that collected from both commercial broiler chickens and the local chickens besides to 57.14% of the positive samples in wild birds, subtypes H5 was recorded in 42.85 % of the positive samples in wild birds, where as H7 subtype not found in this study.

Conclusions: The study has demonstrated the occurrence of influenza A viruses in the wild birds as well as the domestic chickens in Iraq in addition to public health importance of AIV; according to our knowledge, this research is the first that recorded presence of H5 subtype in poultry at our country.

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Introduction:

Avian influenza is a zoonotic highly contagious and worldwide distributed disease caused by Avian influenza A viruses (AIV) which belong to the family *Orthomyxoviridae*, can cause significant economic losses in domestic poultry (1,2).

The virus was classified into subtypes on the basis of their two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA), all of sixteen HA (H1-H16) and nine NA (N1-N9) subtypes have been isolated from wild birds particularly aquatic. which are the major natural reservoir (3, 4). But the subtypes H5, H7 and H9 considered as the most common avian subtypes. However, viruses of avian origin have been isolated from a number of mammalian species, including humans (5 , 6).

In addition to the sixteen previously known haemagglutinin and nine neuraminidases subtypes identified from avian species, H17N10 and H18N11 subtypes have recently been detected in bats, representing the entire pool of influenza A viruses known today (7).

Avian Influenza viruses are also typed by their degree of pathogenicity or virulence. Highly pathogenic avian influenza (HPAI) has a high mortality rate in poultry (90 and 100%) whilst Low pathogenic avian influenza (LPAI) was either asymptomatic or causes a less severe symptom (8).

In essence, AIVs are nonpathogenic in wild birds, in spite of they sometimes cause significant morbidity and mortality when transmitted to domestic poultry (9).

The worldwide spread of AIV results from the migration of waterfowl, when, in occasional cases of domestic birds and mammals infection, self-limiting or sustained epidemics or even pandemics arise (2).

Wild bird might transmit infectious material either by active shedding, or by mechanical transfer of water droplets or water bird feces containing AIV. Wild bird species that enter poultry barns could carry AIV particles directly into the poultry flock or may spending time near poultry barns to contaminate surfaces near barns from which AIV could be carried into poultry barns by farm workers, equipment, pets, or rodent or insects (10). Depending on environmental conditions, AIV in water droplets, fecal material, or organic debris could remain infective for days to weeks (11).

Previous studies was involve AIV in domestic chickens at in Iraq (12,13), but neither of them include wild birds.

As wild birds are the ultimate source of influenza A viruses for domestic birds and mammals(2), so the knowledge of the epidemiology of these avian influenza viruses (AIVs) among wild and domestic birds is necessary to improve surveillance at least in our regions and better clarify underlying factors in host-switching of AIV. Therefore, the aim of this study is searching of the most common influenza A subtypes (H5, H7 and H9) in both domestic flocks and wild birds at many geographical areas in our country.

Materials and Methods:

The study was performed from the beginning of September 2013 to the end of June 2014, both domestic chickens and wild birds were included in our study from different geographical regions of five Iraqi governorates: Najaf, karballa, Qadisyia, Muthana, and Thiqaar as following: 141 broiler chickens that were sent to laboratory of Veterinary Hospitals from different flocks suffering from respiratory signs; random samples taken from the life birds markets (LBMs) at the study areas from 139 and 109 of local chickens and wild birds respectively; wild birds types that involved in the present study were mallard, coot, wild geese, teal duck and flamingo.

The commercial broiler chicken were not vaccinated to the avian flu H9 but vaccinated only attenuated live ND by drinking water in the 7, 18, 30 days of age and vaccinated IBD (intermediate D78) in 7day and intermediate in 14 day of their age.

Nasal swab was taken from each bird or fresh tracheal swab in case of dead birds; Dacron tipped swabs were used for sample collection, each samples was collected in specific sterile viral transport medium, M199 solution [0.5% (w/v) bovine serum albumin (BSA), 26106 U/L penicillin,200 mg/L streptomycin, 26106 U/L polymyxin B, 250 mg/L gentamycin, 60 mg/L levofloxacin hydrochloride, and 56105 U/ L nystatin](15).

Samples were kept on ice during collection and immediately preserved in a nitrogen dry shipper for shipment to the laboratory.

In the laboratory, samples were preserved at -70°C until use. 100 µL of each sample were prepared for RNA extraction which done by using the AniGen viral RNA purification kit, Bionote, Korea.

Influenza A virus was screened using RT-qPCR assay that targeted the influenza Matrix gene. Samples were amplified using One-step(Reverse Transcription and Amplification) Real Time RT-PCR Kit (Qiagen, Germany) for detection of type A avian influenza viruses using. Exicycler thermal block Real-Time PCR device (Bioneer, Korea), targeting the matrix gene through primers and probe mentioned by Spackman *et al*. (16); forward primer: 5′-AGA TGA GTC TTC TAA CCG AGG TCG-3′, reverse primer: 5′-TGC AAA AAC ATC TTC AAG TCT CTG-3′and probe: 5′ FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA-3.

Positive samples for AIV were used for specific searching of most common HA subtypes: H5, H7, H9 by using one-step specific kit for the viral subtypes QuantiTect Probe RT–PCR kit (Qiagen. Germany) at following conditions: 50˚C for 30 min, 95˚C for 15 min, and 45 cycles of 94˚C for 1 sec and 60˚C for 30 sec.

Results:

Our study reveal that the percentage of infection with Influenza A virus was 63.1% (89/141) in the commercial broiler chickens that suffering from respiratory signs at the study regions; the highest infection rate was reported in

chickens from flocks of Karbala province which was 71.42% (20/28), where as the lowest infection rate occurred at Qadisyia province, 47.61 (10/21). (Table 1).

The examination of random samples from local chickens that growing in the backyards demonstrate that the percentage of infection in these chickens was 4.31% (6/139); the highest infection rate recorded at Muthana province, 8% (2/25), the lowest infection rate was in Thiqaar province, 3.33% (1/30), whilst there was no infection had been found in the local chickens at Karbala province. (Table 1).

Table (1): Percentages of Infection in Commercial Broiler Chickens that Suffering from Respiratory Signs and in Local Chickens at the Study Areas.

Governorate	Commercial Broiler Chickens			Local Chickens		
	NO. of Samples	NO. of positive Samples	Percentage of Infection	NO. of Samples	NO. of positive Samples	Percentage of Infection
Najaf	43	29	67.44%	35	2	5.71%
Karbala	28	20	71.42%	26	0	0.0%
Qadisyia	21	10	47.61%	23	1	4.34%
Muthana	18	13	72.22%	25	2	8.0%
Theqaar	31	17	54.83%	30	1	3.33%
Total	141	89	63.1%	139	6	4.31%

The surveillance of the virus by collection of random samples from wild birds at the study regions demonstrate that the percentage of infection was 6.42% (7/109); the highest infection rate was in the coot, 11.11% (4/36), the lowest infection rate was found in the flamingo, 4.76% (1/21), while infection not reported in both teal duck and mallard. (Table 2).

Table (2): Percentages of Infection in the Wild Birds at the Study Areas.

Type of Wild Birds	NO. of Taken Samples	NO. of Positive Samples	Percentage of Infection
Wild Geese	23	2	8.69%
Coot	36	4	11.11%
Teal Duck	16	0	0.0%
Mallard	13	0	0.0%
Flamingo	21	1	4.76%
Total	109	7	6.42%

Further examination of all positive sample by use of specific RT-qPCR kit searching of the major AIV subtypes : H5, H7, H9, revealed that subtype H9 is the prevalent type, which had been recorded in all positive samples (100%) that collected from both commercial broiler chickens and the local chickens and also in 4 out of 7 samples (positive samples) of wild birds (57.14%) .

Three of the seven (42.85 %) of AIV positive samples in wild birds were belonged to the subtype H5; two of them were in wild geese and one in flamingo. Whilst the subtype H7 not found in all samples (Figure 1).

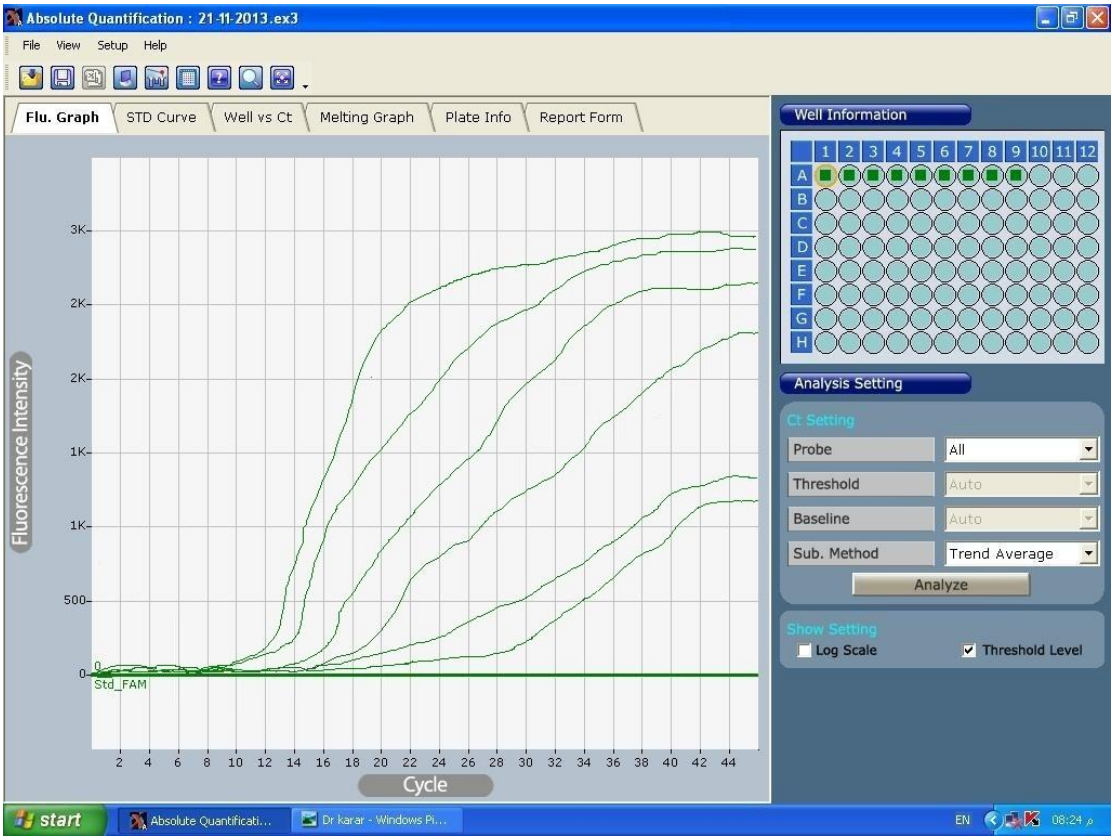

Figure (1): The Graphic Results of Positive Cases with Influenza A/ H9 and H5 Obtained by The Real Time PCR Thermocycler: Exicycler™ Quantitative Thermal Block, Version 3.0.
Xaxis: cycle number, Y axis: Log. Fluorescence.

Discussion:

Infection with AIV in Iraq caused marked economic losses to the industry of poultry especially at the broiler chicken farms through the past years and is continuing, moreover it considered as a serious risk for both man and animals(16). We use RT-qPCR assay for all samples in our study to obtain accurate result as this assay is highly sensitive and highly specific (17).

At the study regions, the percentage of infection with Influenza A virus was 63.1% (89/141) in the commercial broiler chickens that suffering from respiratory signs; this infection rate was lower than that found by Al-Mohana *et al.* , 2013 (12), they registered that AIV present in 100% (53/53) of broiler flocks in Najaf province with history of respiratory signs; lower finding was recorded by Al-Dabhawe *et al.* , 2013 (13), they found that the virus found in 47.3% (18/38) of broiler chickens suffering from respiratory signs in many regions of Iraq.

This variation may be due to difference in the number of samples, tests used in the diagnosis and managements of the flocks. However, the infection rate in each situation is high and the lacking of compensation may hampers the reporting of outbreaks and might result in unactual or blurred picture of the actual field picture at our flocks.

But both of the aforementioned orphan studies in Iraq do not deal with neither wild birds nor local chickens that grow at backyards. The present study is the first study that involve wild and local birds in Iraq which is highly important as such researches are crucially beneficial to us in order to obtain an active surveillance on avian influenza virus and to predict the future outbreaks of new viral subtypes in domestic chicken farms and to introduce of a specific vaccine for these subtypes as soon as possible to prevent or minimize of any future disaster in the industry of poultry at our regions. In addition to obtaining of a sanitary information for human health, as the wild birds are responsible for the distribution of different AIV subtypes for man animals all over the world (18,19).

Results of RT-qPCR assay to a random samples collected from local chickens that growing in the backyards explained that the infection was detected in 6 out of 139 (4.31%).

The above finding came higher than that was reported by Kayali *et al.* , 2011(20) and lower than that of El-Zoghby *et al.* , 2013(21) in Egypt which was 0.9% (12/1318) and 11.5% (151/1435), respectively, in local bird grew in backyards. Such difference may belong to number and season of the samples collection in addition to the tests or assays that used and the growth conditions such as crowding.

The illegal trading of unexamined commercial poultry and backyard birds into LBMs is common besides to continuous exposure of these chickens to the wild birds during their life might explain the elevated incidence of the virus in local chickens that grew at backyards.

The current study revealed that the percentage of infection in the wild birds was 6,42% (7/109); which is in contrast to the result of El-Zoghby *et al.* , 2013(21) in Egypt, they found higher incidence, 11.4% (108/944), but our finding clearly higher than that registered by Kirunda *et al.* , 2014 (22) in Uganda which was 1.3% (12/929).

Variation in prevalence of influenza A viruses in wild birds at different regions not uncommon and could probably be associated with persistent migration and diversity of these birds, herein finding came in comparable to observation of Smietanka and Minta (2014) (23).

The present study manifest that H9 is predominant subtype, it was found in all positive samples that collected from both commercial broiler chickens and local chickens in the study areas, besides to 57.14% of positive wild bird samples.

The domination of H9 subtype in this study came in alignment with majority of the global studies, for instance, the studies of Al-Mohana *et al.* , 2013 (12) in Iraq, Hadipour , 2011 in Iran and Wang *et al.* , 2014 in China. Such scenario might be due to that this subtype is the original type in birds (26).

The present study was recorded that 3(42.85 %) of AIV positive samples in wild birds were belonged to the subtype H5; two of them were in wild geese and one in flamingo. Whilst the subtype H7 not found in all samples.

According to our knowledge, The current study is the first, at least in the study areas, that record presence of H5 in birds Iraq.

It is highly important to investigate the presence of the new subtypes of the virus through a consecutive and comprehensive surveillances in a periodic way at our country. The updating of the information about viral subtypes are crucially needed in everywhere at every time in order to predict and prohibit the future pandemic (27).

Indeed, commercial poultry-LBMs-wild birds-backyards cycle in Iraq is closely integrated and any breach will eminently affect poultry and endanger public health. Therefore we crucially suggest the enforcement of bio-security measures which should be the first line of defense whilst the vaccination acts only as an ancillary tool for control of AIV.

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