Strong Evidence of Highly Pathogenic and Low Pathogenic Avian Influenza Viruses in Domestic Poultry in Iraq

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Abstract

Background: Avian influenza is very important contagious disease caused by Avian Influenza A viruses which continue to pose real threats for both human and animal. The virus subdivided into many HA and NA subtypes, some of them are highly pathogenic while others are low pathogenic for poultry.

Method: Nasal swabs or fresh tracheal swabs in case of dead birds were taken from 417of chicken farms and flocks besides to 689 of local poultry that grew in the backyards all over Iraq to detect and identify viral subtypes.

Results: Avian influenza viruses were observed in 7.04% of commercial chicken farms and in 4.93% of local poultry that growing in the backyards at Iraq; following subtypes were recorded: H9N2, H5N1, H7N9, H5N8 and H5N2. Subtype H9N2 is the prevalent type, all infections with H5N1, H5N8 and 28.57% of H7N9 subtypes were highly pathogenic; 100% of infections with H9N2, H5N2 and 71.42% of H7N9 appeared as low pathogenic.

Conclusions: According to the author's knowledge, the present study is first study recordedavian Influenza virus subtypes: H5N1, H7N9, H5N8 and H5N2 in domestic birds at Iraq.

Keywords: *Avian Influenza Virus; Low pathogenic AIVs; Highly pathogenic AIVs.*

Introduction

Avian influenza viruses (AIVs) are type A influenza viruses which considered as omnipresent pathogen that continue to pose a cosmopolitan and real threat for animal and human health, they belong to Orthomyxoviridae family; characterized by enveloped virion with single-stranded, eight segmented negative polarity RNA genome. According to their surface glycoproteins, it classified into 18 HA and 11 NA subtypes, of which about 16 HA (H1–H16) and also 9 NA (N1–N9) subtypes that circulate in birds[1,2].

Avian influenza H5 and H7 subtypes were further classified into low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) viruses depending on certain molecular markers besides to the morbidity and mortality rates which may reach at least 70% at intravenously *in vivo* inoculated specific pathogen free (SPF) chickens[3].

Markedly, HPAI viruses cause severe respiratory signs concomitant with high morbidity and mortality rate in poultry farms or that grow in backyards, in a contrast manner, LPAI viruses are just induce subclinical infections or sometimes case mild respiratory disease and slight reduction in egg production ensuing low mortality rate or mild spectrum of diseases in the wild birds which are considered as the main natural reservoir of influenza A viral subtypes[4].

Infection with HPAI viruses not only cause marked effect on poultry production leading to significant economic consequences, but it also had been reported to infect human, due to the affinity of Sialic acid receptors, representing real public health problem[5].

Unfortunately, LPAI viruses of HA subtypes H5 and also H7, may mutate under vague circumstances and adversely change into HPAI variants causing severe progressive disease that conducive to high mortality rates in both domestic and wild birds[6].

Globally, HPAI viruses of the H5N1 subtype were recorded in Asia, Middle East, Europe and Africa, resulting to heavy economic losses as a result of numerous harmful epidemics affecting both domestic poultry and wild birds; More recently, three another certain HPAI H5 subtypes emerged: H5N8 and H5N6 at the years, 2014 and 2017, respectively. In addition, to new LPAI subtypes, H5N2 and H9N2 were also recorded; many studies worldwide referred to that the last subtype is the more prevalent low pathogenic avian influenza subtype in poultry [7].

Generally, few Previous studies were involved AIV in domestic chickens in Iraq but neither of them involve most common viral subtypes, while, only one orphan study of them had included local poultry in backyards [8].

Studying of HPAI and LPAI infections are crucially important to obtain an active surveillance at our country to establish a reliable ground-work, in order to pinpoint and minimize the insidious hazards of these subtypes. Therefore, this study aims to determine the incidence, temporal spreads and spatial distribution of HPIVs and LPIVs in the domestic poultry throughout Iraq.

Materials and Method

Well sophisticated team of veterinarians were participated in sample collection randomly from 417 of chicken farms and flocks all over Iraq, ten random samples were taken from live birds at different sites of each farm and flocks besides to 5-10 of dead birds. In addition to 689 of local poultry (287 of local chickens,165 of local ducks, 132 of local turkeys and 105 of local geese) that grew in the backyards from all Iraqi provinces.

Many but not all commercial chickens were vaccinated against avian flu subtype H9N2, Newcastle disease (ND) and infectious bronchitis disease (IBD).

The study was conducted from the beginning of August 2018 to the end of September 2019, farms that designated as infected with respiratory signs were defined as flocks characterized by core clinical respiratory signs such as, sneezing, coughing, rattling, dyspnea, nasal discharges snickering, depression and/ or diarrhea or central signs nervous which concomitant with clear rising of mortality rates within at least 3 days; respiratory signs were determined after excluding all positive samples that co-infected with ND and IBD.

Special Dacron tipped swabs were aseptically used for sample collection, Nasal swab had been taken from the each bird or fresh tracheal swab in case of newly dead birds, each sample was put in sealed insulated tubes, contain viral transport medium, termed as M199 solution [0.5% (w/v) certain sterile bovine serum albumin (BSA), 26106 U/L of penicillin, 200 mg/L of streptomycin, 26106 U/L of Polymyxin B, 250 mg/L of Gentamycin and 60 mg/L of Levofloxacin hydrochloride besides to 56105 U/L from Nystatin], all these tubes were kept on ice during collection and immediately preserved in a liquids nitrogen dry shipper for shipment to our laboratory and preserved inside specific deep freezer at -70°C until use [9,10].

Samples were prepared for RNA extraction through a prepared suspensions using a High Pure Viral Nucleic Acid extraction and purification Kit (Roche, Germany), based on the manufacturer's protocol. The RNA suspension was divided into three equivalent parts of 100 µL and each part was used for AIVs, IB and ND viruses diagnostic tests; For diagnosis of avian influenza, infectious bronchitis besides to Newcastle disease viruses, we used One-step reverse transcription real time PCR (rRT-PCR) assay, through involving following primers and probes for AIV according to [11]:

Forward primer 5′-AGATGAGTCTTCTA-ACCGAGGTCG-3. 100 Matrix protein and Reverse 5′-TGCAAAAACATCTTCAAGTCTCTG-3. Probe 5′-TCAGGCCCCCTCAAAGCCGA-3.

Certain amplification condition consisted of a starting first reverse-transcription initial step at 45°C for 10 minutes, followed by Thermo-Cycling of 10 minutes at 95°C hot start and then by 45 PCR cycles of denaturation at 95°C for 10 seconds, annealing and extension at 60°C for 45 seconds and 65°C for 45 seconds, respectively, as originally mentioned by[11].

All AIV positive samples were subjected again for further examination searching for most common avianinfluenza HA and NA types which performed through using one-step rRT-PCR kit (Enzynomics, Korea), based on recommended method through utilizing multiple sets of numerous array of forward and reverse primers and probes, which done under implementation of manufacturer's instructions[12].

The infections were classified as HPAI or LPAI in the present study depending on the results of intravenous inoculation of a ten susceptible 4 to 8 week old SPF

chickens with viral subtypes; the strains were considered as a highly pathogenic if they cause more than 75% mortality within 10 days, if not, they are low pathogenic, based on original method described by[13].

Statistical analysis: We use SPSS software version 26 (IBM,NY,USA) through application of Chi-square (x2), to determine parameters difference among categorical data, p-value ≤ 0.01 were considered as statistically significant.

Results

The study reveal that the percentage of infection with AIVs was 7.04% (45/639) in the commercial chicken farms that suffering from respiratory signs at the study regions; Whereas, examination of random samples from local poultry growing in the backyards demonstrate that the percentage of infection in these birds was 4.93% (34/689)(Table 1).

Table (1): Percentages of Infection in the domestic poultry throughout all Iraqi Provinces.

Provinces	Commercial Chicken Farms			Local Domestic poultry		
	No. of Samples	No. of positive Samples	Percentage of Infection	No. of Samples	No. of positive Samples	Percentage of Infection
Baghdad	49	4	8.16%	43	3	6.97%
Al-Basrah	47	3	6.38%	32	2	6.25%
Babil	46	7	15.21%	51	2	3.92%
Arbil	44	2	4.54%	33	0	0.00%
Ninawa	41	3	7.31%	64	8	12.50%
Al-Najaf	41	4	9.75%	32	1	3.12%
Al-Karbela	39	3	7.69%	45	0	0.00%
Al-Anbar	38	2	5.26%	31	1	3.22%
Wasit	36	1	2.77%	37	2	5.40%
Al-Qadisiyyah	35	2	5.71%	29	3	10.34%
Dahuk	33	3	9.09%	36	0	0.00%
Dhi-Qar	32	4	12.50%	39	1	2.56%
Diyela	30	0	0.00%	28	2	7.14%
As-suleymaniyyah	28	1	3.57%	46	1	2.17%
Salahad-Din	27	3	11.11%	35	3	8.57%
Al-Muthanna	25	0	0.00%	29	0	0.00%
Maysan	24	2	8.33%	41	2	4.87%
Kirkuk	24	1	4.16%	38	3	7.89%
Total	**639**	**45**	**7.04%**	**689**	**34**	**4.93%**

Further examination of all positive sample by use of specific rRT-PCR assay searching of the common AIV subtypes was recorded presence of following subtypes: H9N2, H5N1, H7N9, H5N8 and H5N2 at domestic poultry in Iraq, other viral subtypes were not observed.

The current study was reported that subtype H9N2 is the dominant type at both commercial chicken farms and local poultry that grew at backyards which came in percentages of 60.00% and 61.76%, respectively; with significant difference at level of p≤0.01 among all subtypes; Subtype H5N1 rank below in percentage of 17.77% at commercial chicken farms and 11.76% at domestic poultry that grew in the backyards; while the lowest incidence occur in H5N2 subtype at commercial chicken farms, 2.22%, in the other hand, the lowest subtype rate at local poultry was recorded with H5N8 subtype, 5.88% (Table 2).

The study revealed that all infections withH5N1, H5N8 and 28.57% (2/7) of H7N9 subtypes were highly pathogenic, whilst, 100% of infections with H9N2, H5N2 and 71.42% (5/7) of H7N9 appeared as low pathogenic pattern results shown in Fig. 1.

Table (2): Percentages of Infection in domestic poultry with Different Viral Subtypes.

Viral Subtypes	Commercial Chicken Farms		Local Domestic poultry	
	No. of Positive Samples	Percentage of Infection	No. of Positive Samples	Percentage of Infection
H9N2	27	60.00%*	21	61.76%*
H5N1	8	17.77%	4	11.76%
H7N9	5	11.11%	4	11.76%
H5N8	4	8.88%	2	5.88%
H5N2	1	2.22%	3	8.82%
Total	**45**	**100%**	**34**	**100%**

*p≤0.01

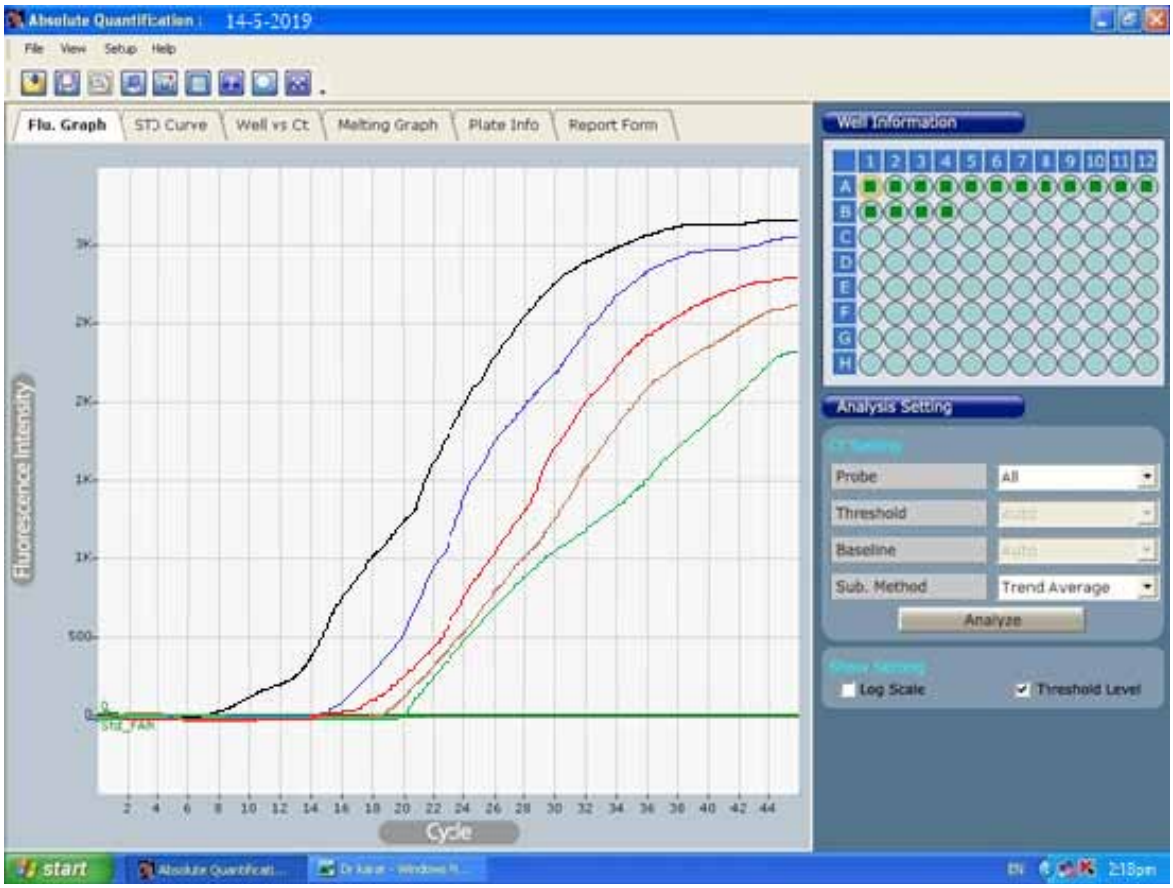

Figure (1): Graphic Results for AIVs Positive Samples that Obtained by rRT-PCR, Thermo-cycler: Exicycler™ technique, Quantitative Thermal Block, Korea. Five Amplification curves belong to the Five recorded subtypes.

Discussion

Avian influenza viruses are ubiquitous pathogens imposing great human and animal health problems, conducive to heavy economic loses in poultry production besides to their risks for all mammals including man; Since about three of four consensus pandemic human influenza outbreaks during past century were ensued from a genetic re-assortment of avian influenza viruses[14].

Our study reveal that the percentage of infection with AIVs was 7.04% (45/639) in the commercial broiler chicken farms and flocks at the study regions; this infection rate was drastically lower than that found by Abdul-Sada, 2015 [8] in Iraq, who registered that AIVs were present in 63.1% (89/141) of the broiler flocks.

This remarkable variation might be attributed to involving flocks with respiratory signs only, in the last two studies, while in the current study we collected the samples randomly from commercial farms and flocks, in addition to the difference in the number of samples and numerous managements conditions of the flocks.

The examination of random samples from local poultry that growing in the backyards was pointed out that the percentage of infection in these birds was 4.93% (34/689).

The illegal stochastic trading of un-examined commercial poultry and also backyard birds into live bird markets (LBMs) is common besides to continuous exposure of these chickens to the wild birds (natural reservoir of AIVs) during their life might emphasize the elevated incidence of the virus in local chickens that grew at backyards.

Further examination of all positive sample by use of specific rRT-PCR assay searching of the common AIV subtypes was recorded presence of following subtypes: H9N2, H5N1, H7N9, H5N8 and H5N2 at domestic poultry in Iraq, other viral subtypes were not observed.

According to our knowledge, this is the first study that recorded H5N1, H7N9, H5N8 and H5N2 subtypes at the domestic poultry in Iraq.

It is clearly important to investigate the spreading and occurrence of AIVs subtypes through a consecutive and comprehensive surveillances in a periodic way at our country. The updating of the information about viral subtypes are substantially needed in everywhere at every time in order to predict or prohibit of future pandemics.

The current study explained that subtype H9N2 is the predominant type at both commercial chicken farms and local poultry that grew at backyards which came in percentages of 60.00% and 61.76%, respectively with significant difference at level of $p \leq 0.01$ among all subtypes; also it is geographically distributed more than other subtypes; Subtype H5N1 rank below in percentage of 17.77% at commercial chicken farms and 11.76% at domestic poultry that grew in the backyards. .

The domination of H9N2 subtype in this study came in alignment with majority of the studies, worldwide, like the study of Hassan *et al*., 2016 [15]in Egypt (nearby country) who found that this subtype is predominant and represent 41.7% from AIV subtypes followed by H5N1 in 26.7% and study of Kandeil*et al*., 2019 [16] which reported that H9N2 was formed 48% of all AIV in Egypt followed H5N8, 37.1%. Such scenario might be due to that this subtype is the original type in birds[6].

Contrast finding was observed by Lee *et al*., 2017[17] in South Korea. They Observed that H5 was the most prevalent among all AIVs subtypes and came in 23.9% of these subtypes; that might be attributed to geographical distribution, kind of assay, sample size and ornithogenic causes.

We observed that all infections with: H5N1, H5N8 and 28.57% (2/7) of H7N9 subtypes were highly pathogenic, whilst, 100% of infections with H9N2, H5N2 and 71.42% (5/7) of H7N9 appeared as low pathogenic pattern.

Interestingly, the cosmopolitan distribution of most AIVs subtypes and the pathogenic patterns of some subtypes like H5Nx and H7Nx remain unclear and vague under certain circumstances particularly regarding the shifting from low to high pathogenic forms, further global studies are required to illuminate their epidemiology and pathogenicity [4,18].

Indeed, the commercial poultry - backyards cycle at LBMs in Iraq is closely integrated that can create a good niche for subtypes spread or even re-assortment events and any breach may eminently affect poultry production and endanger public health, since its seemingly can easily pass host species barriers, so, providing of a precise and sufficient informative data concerning AIVs subtypes at our country is of crucial necessity.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

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