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The Economic Importance of the Avian Influenza in Poultry Industry

A Research

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

صَدَقَ اللهُ العَظِيمُ

من سورة طه

Certificate of Supervisor

I certify that the research entitled " **The Economic Importance of the Avian Influenza in poultry Industry** " was prepared under my supervision at the College of Veterinary Medicine/ University of Al-Qadissiya .

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Dedication

I dedicate this project To my father soul, my mother for their endless Love, Support & Encouragement

To my sisters & brothers.

List of Contents

Summery

Avian Influenza (AI) is considered to be the most contagious poultry diseases and may cause severe economic losses in poultry industry . Avian Influenza virus belongs to the family Orthomyxoviridae and the viral genome ,segmented-RNA. This review aimed to focus spot light on the role of AI in poultry industry. The review comprise the etiological agent , morphology and genomic feature .The study included the epidemiological characteristics and routs of transmission of the virus , occurrence , morbidity and mortality rates were mentioned , risk factors of the virus and host Susceptibility were also studied . The review was also studied the important characteristics and pathognomonic clinical signs of the disease .There was a part of study to investigate the gross lesions and postmortem findings . We also study the diagnostic methods used for detection of the disease as field and laboratory diagnosis, immunization and control measures was included . In conclusion , AI is one of the most important viral disease in poultry and caused heavy economic losses and impact poultry industry .

Chapter One

Introduction

Introduction.

Chapter One :

1. 1 Introduction:

Poultry industry has expanded rapidly over the last fourth decades and is playing a vital role in the economy of the country. However, the industry is confronted with a variety of problems, particularly the diseases of viral origin, poultry production is one of the best available sources for the production of high biological value animal protein in terms of meat and eggs, commercial hybrids, both broilers and layers, are being propagated for meat and eggs production throughout the world (Thomazelli et al., 2012).

Avian influenza (AI) is a viral, highly contagious disease of domestic and wild birds characterized by respiratory, digestive, nervous signs, depression, reduced feed, water intake, decline in egg production and egg quality. Avian influenza results from infection by viruses belonging to the species influenza A virus, genus influenzavirus A and family Orthomyxoviridae. These viruses are also called type A influenza viruses. Influenza A viruses are classified into subtypes based on two surface proteins, the hemagglutinin (HA) and neuraminidase (NA). (CDC; 2015)

AI viruses can be divided into two distinct pathotype groups based on their ability to cause disease, Highly pathogenic avian influenza virus (HPAIV) and low pathogenic avian influenza virus (LPAIV) (Woo and Park, 2008) . Influenza viruses belong to the family Orthomyxoviridae which is divided into five genera : Influenza A , Influenza B , Influenza C , Isavirus and Thogotovirus on the basis of antigenic differences in internal nucleoprotein and matrix protein . And another six has recently be described (Presti et al., 2009)

1-2 The aims of the study :

The goal of present study was to focus spot light on the infections of

Avian Influenza virus and to evaluate the economic importance of

Avian Influenza in poultry industry.

Chapter Two

Literatures Review

2 . Review of literature

2.1. Definition:

Avian influenza (AI) is an acute highly contagious and infectious viral disease of all species of birds in general, and poultry in particular and is worldwide in distribution (**Racnik *et al*., 2008**). Influenza A viruses of subtype H9N2 are now considered to be wide spread in poultry and have demonstrated the ability to infect human (**Swayne and Halvorson, 2003**) . The disease characterized by rapidly spread to other flocks with cardinal signs of respiratory distress, poor growth ,decrease production and at last mortality (**Hadipour *et al*., 2011**).

2.2. History and epidemiology of Avian Influenza:

The earliest evidence of avian influenza in poultry that's back to 1878 when highly pathogenic avian influenza was initially recognized as an infectious disease of chickens in Italy and called "fowl plaque" by Perroncito (Kaleta and Rulke, 2008) . Avian influenza has a worldwide distribution which includes Asia and Middle East, Europe, Africa, North and South America (NADIS INFO., 2006) . Further detections followed in Balkan countries (Bosnia-Herzegovina, Bulgaria, Croatia, Greece, Romania, Serbia, Montenegro and Slovenia), more broadly in the Middle East (Egypt, Iraq, Iran, Jordan, Kuwait, Palestinian Territories) and the Caucasus (Azerbaijan and Georgia) (Williams and Peterson, 2009) .

H9N2 infections have been reported in the middle east and Asia causing widespread outbreaks in commercial chickens in Iran ,Saudi Arabia ,Pakistan, China ,Korea ,United Arabian Emirate , Jordan ,Kuwait, Lebanon ,Libya (Mosleh *et al.* 2009).

Office International des Epizooties (OIE) (2006) has

announced outbreaks of Avian influenza type H5N1 in domestic poultry in Iraq. While H9N2 type was diagnosed as a causative agent of mortality in poultry (**AL-Nassrawe, 2002; Hassan, 2007; Khamas, 2008; Jasim, 2009; AL-Nakshabandi,2009; Zahid, 2010; Tuma, 2012**)

In some of these countries, vaccines have been deployed to bring the disease under control ,but nevertheless it appears that H9N2 infections have endemic in commercial poultry in a significant number of countries (**Naeem and Saddique, 2006**) . Avian influenza viruses mainly infect birds ,but can also cause disease in horses, swine, mink ,cats ,dog, white tigers and clouded leopard ,marine mammals and humans (**Yoon *et al.*, 2005b**) . Type A influenza viruses can infect a wide range of hosts and can be pathogenic to both humans and Birds (**Yee *et al.*, 2009**) . Outbreaks of avian influenza virus (AIV) caused great economic losses in the poultry industry and are threats to human health (**Sun *et al.*, 2013**) .

2. 3 Etiology :

2. 3.1 Classification :

Influenza viruses belong to the family *Orthomyxoviridae* which is divided into five genera : Influenza A , Influenza B , Influenza C , Isavirus and Thogotovirus on the basis of antigenic differences in internal nucleoprotein and matrix protein . And another six has recently be described (**Presti *et al.*, 2009**) . Influenza A viruses are classified into different subtypes by the antigenicity of surface protein, hemagglutinin (HA) and neuraminidase (NA), and sixteen subtypes of HA (H1 to H16) and nine N subtypes of NA (N1 to N9) have been described in birds. **(Cheema *et al.*, 2011**) .

Recently , another study by **Tong *et al.* (2012)** described seventeen subtypes of HA (H1 to H17) and ten subtypes of NA (N1 to N10) found

only in bats . According to their ability to cause disease in poultry, especially in chickens, avian influenza viruses (AIV) are subclassified into two pathotype groups of highly pathogenic avian influenza (HPAI) viruses and low pathogenic avian influenza (LPAI) viruses (**Martins**,**2012**) .

2. 3.2 Morphology and Genomic structure :

Influenza virus particles are considered pleomorphic and can appear spherical with particles 50 to 120 nm in diameter, or filamentous virions 20 nm in diameter or longitudinally shaped . (**Wise *et al*., 2009**). Influenza viruses are RNA viruses with segmented negative single-stranded RNA . The genome of influenza A viruses is consists of eight segments coding for 11 proteins :

-Segment 1: Polymerase – basic protein-1(PB1) and some time (PB1-F2)

-Segment 2 : Polymerase –basic protein-2 (PB2)

-Segment 3 : Polymerase – acidic protein (PA) .

-Segment 4 : Haemagglutinin (HA or H) .

-Segment 5 :. Nucleoprotein (NP)

-Segment 6 : Neuraminidase (NA or N)

-Segment 7 : Matrix protein (M1 and M2) .

-Segment 8 :Non-structural protein (NS1 and nuclear export protein (NEP) /NS2) . Figure (2-1) **(Samji, 2009**) .

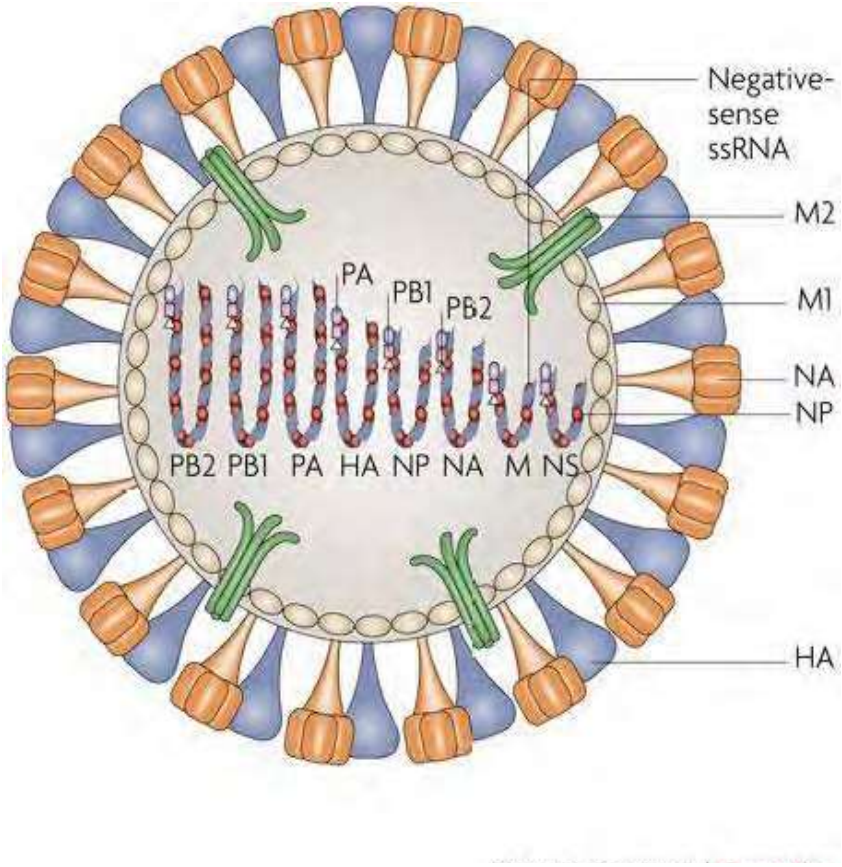

Figure (2-1) : A schematic representation of the structure of influenza A virus. (Nelson and Holmes , 2007) .

The ability of virus to change through antigenic drift and shift increase the potential of emerging virulent strain of AIV(**Marjuki *et al.*, 2009**). Antigenic drift : is a gradual change in gene due to accumulation of point mutations and gets in the (HA) and (NA) . This small change leads to a change in the amino acid sequence. And this change does not allow the antibody to link with the virus and will allow the virus to avoid the immune system highly motivated as a result of exposure to antigenic previous installation , and antigenic drift more deviation severity in humans than in birds (**Cattoli *et al.*, 2011**) .

Antigenic shift : major antigenic changes in HA or NA result in the appearance of a new subtype . These newly introduced proteins are

immunologically different from the previously circulating strains and will result in high infection rates in immunologically naïve populations and will cause pandemic .(**Wright *et al*., 2007**) . Antigenic shift can result from different mechanisms . First mechanism is genetic reassortment between subtypes (between human and animal strains inside of human or pig host cell, giving rise to novel HA ,NA strain (**Peiris *et al*., 2007**) . A second mechanism for antigenic shift is through direct transfer of whole virus from one host species into another (without undergoing genetic reassortment (**Smith *et al*., 2009**) .

2.4 Transmission :

Transmission occurs directly or indirectly through aerosols, water, feed and other materials that have been contaminated by feces. Wild aquatic birds, such as geese, shorebirds and wild ducks are the natural reservoirs of all influenza A viruses (**Kalthoff *et al*., 2010**) . AI surveillance in wild birds may be useful for risk assessments in poultry, humans, pigs, and other animals (**Van Dalen *et al*., 2010**).

2. 5. Pathogenicity of AIV :

Influenza A viruses that infect poultry can be divided into two groups on the basis of their ability to cause disease in chickens which are Highly Pathogenic Avian Influenza and Low Pathogenic Avian Influenza (**Pantin-Jackwood and Swayne, 2009**). Pathogenicity of influenza viruses also depends on the role of surface HA antigen . HA0, precursor of HA, consists of two HA1 and HA2 subunits and between HA1 and HA2 there are multiple basic cleavage site which contributes to the influenza virus infectivity , and compulsory for efficient viral entry and replication into host cells (**Peiris *et al*., 2007)**.

It has been demonstrated that the HA0 precursor proteins of AIV of low Virulence for poultry are limited to cleavage by host proteases such as trypsin and trypsin- like enzymes , thus AIV remains restricted to replication at sites in the host where such enzymes are found, i.e. the respiratory and intestinal tracts . In contrast the HA0 of HPAI is cleavage by cellular proteases such as ubiquitous furin and PC 6 (proprotein convertases) (**Swayne and Suarez, 2000**) .

Pathogenicity of HPAI viruses are associated with multiple basic amino acids (arginine and lysine) at their HA cleavage site motif which enable them to replicate and damage all the vital organs and tissues ultimately resulting in death of the infected birds**.** In contrast, LPAI viruses contain single basic amino acid (arginine) at cleavage site and are capable to replicate only in limited tissues of respiratory and digestive systems (**Perdue *et al.*, 1997**) . The HPAI is extremely contagious and rapidly fatal, with a mortality approaching 90-100% in domestic fowls (**Capua & Alexander, 2009**). While LPAIV produce asymptomatic infection . Most HPAI viruses belong to H5 and H7 subtypes, even though not all H5 or H7 viruses are truly pathogenic but most of them are non pathogenic also (**OIE**, **2005**) .

2.6 Clinical Signs :

The incubation period of avian influenza viruses range from as short as a few hours in intravenously inoculated birds to 3 days in naturally-infected individual birds and up to 14 days in a flock depending on the subtype, the viral dose, the species and age of the birds and route of infection **(Abdu *et al*., 2005)** .

The clinical signs of AI are variable and influenced by the virulence of the virus involved, the species affected, age, concurrent viral or bacterial infection and the environment (**Elbers *et al*., 2005) .**

Clinical signs in birds generally range from asymptomatic infection to drops in egg production and mild respiratory disease (**Capua & Alexander, 2004**) .The symptoms following infection with low pathogenic AIV may be as discrete as huddling ,ruffled feathers, depression, decrease activity, decreased feed and water consumption coughing, sneezing, rales, lacrimation and sinusitis also there is mild to moderate inflammation of the trachea, air sacs and conjunctiva (**Ladman *et al* ., 2008**) . Some low pathogenic strains such as certain Asian H9N2 lineages, adapted to efficient replication in poultry, may cause more prominent signs and significant mortality. LPAI viruses can sometimes lead to high mortality, especially if infection is complicated by secondary pathogens (**Swayne and Pantin-Jackwood, 2008)** .

In highly pathogenic form, the illness in chickens and turkeys is characterized by a sudden onset of severe symptoms and a mortality that can approach 100 % within 48 hours (**Capua & Alexander, 2009**) . Many birds die without premonitory signs .Generally, there were edema of the head and eyelids, cyanosis of the combs, wattles and legs, greenish diarrhea and labored breathing may be inconsistently present . In layers, soft-shelled eggs are seen initially, but any laying activities cease rapidly with progression of the disease , Nervous symptoms including tremor, unusual postures (torticollis), and ataxia (**Wenhua *et al*., 2006**) .

2.7 Gross Lesions :

The appearance of gross lesion is variable depending on the virus strain, pathogenicity of the virus , the age and species of poultry affected and the presence of secondary infections (**Swayne *et al*., 1997**) .

Lesions may include swelling of the face and area below the beak. Removing skin from the carcass will show a clear straw- colored fluid in the subcutaneous tissues. When infection with HPAI viruses , in most cases , per acute infections with death (day one to two of infection) poultry have lacked visible gross pathological lesions (**Lamb and Krug, 2001**) .

During the acute stages of infection in chicken there are severe lung congestion, hemorrhage and edema in dead chickens , such that excised tissue exuded serous fluid and blood (**Joannis *et al*., 2006**) . More varied visible lesions are seen in chickens surviving 3 to 5 days post infection , including congestion and /or cyanosis of the comb and wattles and swollen head, especially prominent from periorbital and intramandibular subcutaneous edema , the change in the comb and wattles progress to depressed areas of dark red to blue areas of ischemic necrosis as the result of vascular infarction (**Ellis *et al*., 2004**) .

Lesions of LPAI in the respiratory tract are most frequently seen . It is characterized as catarrhal, fibrinous, serofibrinous , mucopurulent , or fibropurulent inflammation . The tracheal mucosa can be edematous with congestion and occasionally hemorrhage, tracheal exudates may vary from serous to caseous, with occasional occlusion of airways and resulting asphyxiation, the air sacs may be thickened and have fibrinous to caseous exudates (**Swayne and Halvorson, 2003**). Petechial to ecchymotic hemorrhages may be present in multiple visceral organs or on

the serosal surface such as the epicardium of the heart, serosa of small intestine , abdominal fat, serosa of sternum, caecal tonsils, Meckel´s diverticulum, Payer's lymphoid patches of the small intestine, (**Zahid, 2010**) . Hyperemia and hemorrhage in mucous membrane, edema and congestion in the lung, swelling and congestion in the liver , hemorrhageson the mucosal surface of the proventriculus, hemorrhages and erosions of the gizzard lining , hemorrhagic foci on the lymphoid tissues in the intestinal mucosa, kidney may be severe congestion and swelling due to uric acid deposition in the renal tubules and ureters , edema and hemorrhage in the mucous membrane of the bursa of fabriciuos and wide spread hemorrhage in the gastrointestinal tract (**Jassim, 2009)** . Occasionally , spleen have white foci of necrosis and the pancreas may have red to light orange to brown mottling, hemorrhages and degeneration of the ovary , ruptured ova with yolk peritonitis have been reported in layers and broilers turkey breeders (**Li *et al*., 2005**) .

2.8 Diagnosis :

Effective strategy to control the spread of influenza viruses depends on the availability of accurate and sensitive diagnostic methods. There are two types of AI diagnosis : presumptive or definitive .

Presumptive diagnosis of avian influenza depends on the clinical signs , whether the infection is HPAI or LPAI. For HPAI, clinical signs help in diagnosis whereas LPAI infection in birds is asymptomatic (**Swayne and Halvorson, 2003**) . Definitive diagnosis of AIV depends on various laboratory methods including indirect evidence of infection by detecting anti-influenza antibodies by serological methods and direct detection methods for either live virus , viral antigen, or viral nucleic acid (**Suarez *et al*., 2007**) .

2. 8.1 Virus Isolation :

Virus isolation in embryonated eggs inoculation or tissue culture is a technique where a sample is inoculated into a live culture system and the presence of live virus infection is then detected in this culture system . AI virus is isolated by inoculation of swab fluid or tissue homogenates into 9 to 11day-old embryonated chicken eggs, usually by the chorioallantoic sac route (**Swayne *et al*., 2008**) .

2. 8.2 Serological Methods :

Used for the detection of influenza virus – specific antibodies in serum. Serology can either detect total antibodies or be class-specific (IgG, IgA, or IgM) . Different serological techniques are available for influenza diagnosis such as Enzyme linked immunosorbant assays (ELISA), haemagglutination inhibition (HI),neuraminidase inhibition (NI), Virus neutralization (VN), complement fixation (CF) , Agar gel immunodiffusion (AGID) and indirect immunofluorescence are common serological tests for AIV employed in the diagnostic laboratory (**Petric *et al*.,2006**).These tests are based on the presence of influenza specific antibodies that first appear approximately two weeks after initial infection and peak levels occur after 4-7 weeks after initial infections (**Abdulla, 2010**) .

2. 8.3 Molecular Diagnosis :

Molecular technique in avian influenza diagnosis can be used for multi purposes : screening for the influenza A matrix gene , determination of HA and NA subtypes and determination of the pathogenicity of the virus through molecular sequencing of the virus cleavage site . Molecular methods are rapid , and of acceptable sensitivity as compared to viral isolation, and provide results within short time **(Kariminejhad *et al*.,2012**) .Available several different types of molecular diagnostic tests

,the most commonly used are conventional or gel-based RT-PCR, where the PCR product is analyzed by ethidium bromide staining after electrophoresis on agarose gel (**Cattoli and Capua, 2006**) .

Real time RT-PCR test is an alternative detection method , it typically uses a fluorescently-labeled probe to detect the increase in PCR product while the test is being performed: i.e. the results are reported in real-time . The other test used for avian influenza is the Nucleic Acid Sequence Based Amplification (NASBA) test, which directly amplifies and detect RNA (**Collins *et al*., 2003**) . Real –time PCR eliminates post-PCR processing of PCR products . This help to increase throughput and reduce the chances of carryover contamination . The real-time PCR system is based on the detection and quantification of the PCR products in real time while the amplification is in progress (**Siddique *et al*., 2008**) . Sensitivity of PCR may be affected by the amount of viral material present , the timing of sample collection post exposure and the quality of the sample (**Cattoli *et al*., 2004**) .

2.9 Immunity :

Influenza causes an acute infection of the host and initiates a cascade of immune reactions, almost all parts of the immune defense system ,most of the initial innate response , including cytokine release (IFmα/B) , influx of heterophil granulocytes or natural killer cells (**Azad e*t al*.,2004) .** Immune cell activation ,is responsible for the acute onset of the clinical symptoms, innate immunity is an essential prerequisite for the adaptive immune response ,firstly ,to limit the initial viral replication and antigen load ,and secondly ,because the antigen specific lymphocytes of the adaptive immune response are activated by co-stimulating molecules that are induced on cells of the innate system during their interaction with viruses (**Jacob *et al*.,2003**) . Influenza induce both systemic and local

antibody production (humoral immunity) , as well as Cytotoxic T cell responses (cellular immunity) ,each of which is important in recovery from acute infection and resistance to re-infection (**Jawetz *et al.*,2001**) .

Humeral immunity is responsible for stimulating the production of systemic immunoglobulines (IgM, IgG) and mucosal antibodies (IgA) . Following infection circulating systemic IgM immunoglobulins develop within 5 days followed by IgG (**Suarez, 2005**) . The mucosal immune response against influenza , as measured in nasal secretion , is characterized by the presence of IgA and IgG against HA, the mucosal anti-HA IgG levels correlate well with the respective serum levels , indicating passive diffusion from the systemic compartment , whereas IgA is produced locally (**Treanor *et al*., 2000**) .

2. 10 Vaccination :

Vaccination has proven to be a powerful tool for control of Avian influenza outbreaks vaccination increases the bird resistance to field virus transmission , therefore the vaccine is given in the fall before the outbreak occurrence of AI. (**Capua and Marangon, 2007a) .**Protection against HPAI in poultry largely depends on HA- specific antibodies. Therefore, the vaccine virus should belong to the same H subtype as the field virus. An ideal match of vaccine and field virus (**Lee and Suarez, 2005) .**

Vaccination with Inactivated vaccine against H9N2 LPAI virus has been used extensively in Asia and the Middle East (**Swayne and Kapczynski, 2008a**). In addition, adjuvant killed vaccines can provide a strong humoral immune response and they provide an effective protection against High Pathogenic Avian Influenza and Low Pathogenic Avian

Influenza challenges (**Pan *et al*., 2009**) . Recombinant vaccines for AI viruses have been produced by inserting the gene coding for the influenza virus haemagglutinin into a live virus vector and using this recombinant virus to immunize poultry against AI (**Swayne, 2004**) . The poxvirus was further modified with the gene deletion in the genome to ensure safety and genetic stability and inserted into a non-essential location in the poxvirus genome resulting in a virus that protect poultry from two infectious diseases, fowl pox and AI, it stimulated an antibody titer faster than inactivated oil-emulsion vaccines and it prevented mortality after exposure to lethal AI viruses (**Swayne, 2007**) .

In China, **Veits, (2006)** referred that the inactivated avian influenza vaccine H5N1 as a vector of fowl pox virus can induce more than (10) months protective immune response in chickens after one dose inoculation, and most importantly, this vaccine is immunogenic for geese and ducks in compassion with the H5N1 fowl pox vectored live vaccine which induce an over than 40 weeks protective immune response against H5N1 virus challenge . **Swayne, (2009)** referred that SPF chickens vaccinated at one day of age with a live fowl pox vaccine and vaccinated twice with recombinant fowl pox–Avian Influenza vaccine protected 85% of the birds against morbidity and mortality after a challenge with a highly pathogenic AI virus .

3. Conclusions and Recommendations

3.1 Conclusions :

1- The disease is widely spread and outbreaks occur even in farms receiving of several vaccination programs and the virus remains an ongoing threat to commercial flocks.

2- Avian Influenza virus has many Sero groups and the viral glycoproteins, Hemagglutinin (HA) and Neuraminidase (NA) play important role in the virulence and epidemiology of the disease .

3- Influenza A viruses that infect poultry can be divided into two groups are Highly Pathogenic Avian Influenza and Low Pathogenic Avian Influenza .

4- Spread of the virus very fast as it was Segmented -RNA virus and the replication is most rapid due to mutation.

5-According to the review studies which showed the Avian Influenza was endemic in different countries including Iraq .

3.2 Recommendations :

1- Further field studies for detection of Avian Influenza that included all endemic areas .

2-Application of many surveys from time to time to identify virulent local strain of AIV .

3- Detection of efficient programs that apply for controlling the spreading of AI and evaluation the programs the vaccination and immunization to eradicate the spreading AI .

References

Abdu, P.A.; Wakawa, A.M.; Sa‟idu, L. and Umoh, J.U. (2005). Avian influenza: A review. *Nigerian Veterinary Journal*, 26(1): 34-43.

Abdulla, A.K. (2010) . H9N2 AI vaccine in broiler, influenced on maternal immunity and it´s effect in immune response of Newcastle and Gumboro and body weight . M.Sc. thesis - Coll. Vet. Med. - University of Mosul .

Al-Nakshabandi, A. A. R. (2009) . Investigation of the high- mortality outbreaks of broiler chickens in Erbil area with special reference of Newcastle disease and Avian influenza H9N2 . *PHD. Thesis. University of Duhok-Iraq* .

Al-Nassrawei, H. A. (2002) . Study of Influenza infection in human and birds. *M.Sc. Thesis. Vet. Coll. University of Baghdad* .

Azad , T.M .; Mohammadi , H . ; Masoovi , Z. ; Saadatmand and Nateah , R. (2004) . Influenza Surveillance in Islamic Republic of Iran from 1991-2001. Eastern Mediterranean Health . *Journal*, 10(3) : 315-321.

Capua, I. and Alexander, D.J. (2004) . Avian influenza: Recent development. *Avian Pathology*, 33:393-404 .

Capua, I. and Alexander, D. J. (2009) . Avian influenza infection in birds: A challenge and opportunity for the poultry veterinarian . *Poultry Science*, 88(4): 842-846 .

Capua, I. and Marangon, S. (2007a) . The challenge of controlling notifiable Avian Influenza by means of vaccination . *Avian Diseases*, 51:317-322 .

References

Cattoli , G . ; Drago , A .; Maniero , S .; Toffan , A .; Bertoli , E . ; Fassina , S . ; Terregino , C . ; Robbi , C . ; Vicenzoni , G .and Capua , I . (2004) . Comparison of three rapid detection systems for type A influenza virus on tracheal swabs of experimentally and naturally infected birds . *Avian pathology* , 33 : 432-437.

Cattoli, G. and Capua, I. (2006) . Molecular diagnosis of avian influenza during an outbreak . *Developments in Biologicals*, 124:99-105 .

Cattoli, G.; Milani, A.; Temperton, N.; Zecchin, B.; Buratin, A.; Molesti, E.; Aly, M. M.; Arafa, A. and Capua, I. (2011) . Antigenic drift in H5N1 avian influenza virus in poultry is driven by mutations in major antigenic sites of the hemagglutinin molecule analogous to those for human influenza virus. *J. Virol*.,85(17) : 8718–8724 .

Caupa, I. and Alexander, D.J. (2009). Avian Influenza and Newcastle Disease A Field and Laboratory Manual. Milan: Springer-Verlag. ISBN 978-88-470-0825-0.

Centers for Disease Control and Prevention [CDC]. (2015). Avian flu [Website online].. Available at: http://www.cdc.gov/flu/avianflu/. Accessed 2 Nov 2015.

Sun X, Xu X, Liu Q, Liang D, Li C, He Q, Jiang J, Cui Y, Li J, Zheng L, Guo J, Xiong Y, Yan J. (2013) Evidence of avian-like H9N2 influenza A virus among dogs in Guangxi, China. Infect Genet Evol.;20:471-5.

Cheema, B.F.; Siddique, M.; Sharif, A.; Mansour, M.K. and Iqbal, Z. (2011) . Sero-prevalence of avian influenza in broiler flocks in district Gujranwala (Pakistan) .*Int. J. Agric. Biol*.,13(6):850–856.

Collins, R. A., Ko, L. S., So, K. L., Ellis, T., Lau, L. T. and Yu, A. C. (2003) . A NASBA method to detect high- and low- pathogenicity H5 avian influenza viruses . *Avian Dis*eases , 47:1069-1074 .

Ellis, T.M.; Bousfield, R.B.; Bissett, L.A.; Dyrting, K.C.; Luk, G.S.; Tsim, S.T., et al.(2004) . Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathol*. 33(5):492-505.

Hadipour, M. M.; Habibi, G.H.; Golchin, P.; Hadipourfard, M.R., and Shayanpour, N. (2011b) . The role of avian influenza, Newcastle disease and infectious bronchitis viruses during the respiratory disease outbreak in commercial broiler farms of Iran. *Int. J. Ani. Vet. Adva*. , 3: 69-72.

Hassan, A. A. S. (2007) . Epidemiological and diagnostic study on avian influenza disease in Iraq . M.Sc. *Thesis Vet. Med. Coll., Baghdad Uni.*

Jacob , J . P . ; Butcher , G .D . ; Mather , F .B . and Fener. R.D . (2003) . Avian influenza in poultry . *Institute of Food and Agriculture Science , University of Florida , Gainesville,* 32611.

Jasim, N. S. (2009) . The isolation and molecular identification of avian influenza virus and its cytopathic effect on tumor cell lines *in vitro*. *Ph. D. Thesis Vet., Med. Coll., Baghdad Uni.-Iraq* .

Jawetz , E. M.D . ; Melnick , J. L . and Adelberg's , E . A . (2001). Orthymxoviuses. (influenza viruses) .In : *Medical microbiology* . 20th ed. Lange Medical book , McGraw, Hill , PP: 459-469.

Joannis, T.; Lombin, L.H.; De Benedictis, P.; Cattoli, G. and Capua, I. (2006) . Confirmation of H5N1 avian influenza in Africa. *Vet Rec.*,158(9):309-10.

Kaleta, E. F. and Rülke, C. P.A. (2008) . *Avian Influenza*. Blackwell Publishing Ltd;. The Beginning and Spread of Fowl Plague (H7 High Pathogenicity Avian Influenza) across Europe and Asia (1878-1955) pp. 145–189.

Kalthoff, D.; Globig, A. and Beer, M. (2010) . Highly Pathogenic Avian Influenza as a zoonotic agent. *Vet. Microbiol.* 140: 237-245 .

Kariminejhad, E. and Mehrabanpour, M. J. (2012) . Serological and Molecular Assay for Detection of Avian influenza virus in broiler chicken flocks of Fars Province -Iran . *Int. J. Anim. Veter. Adv*. 4:125-129 .

Khamas, I. J. (2008) . Avian influenza virus H9N2 in Iraq. *Iraqi Journal of Veterinary Medicine* . 32:223-230 .

Ladman, B. S., Rosenberger, S. C., Rosenberger, J. K., Pope, C. R. and Gelb, J. Jr. (2008) . Virulence of Low Pathogenicity H7N2 Avian Influenza Virus from the Delmarva Peninsula for Broiler and Leghorn Chickens and Turkeys. *Avian Biosciences Center*, University of Delaware, Newark, Delaware 19716- 2150 .

Lamb, R. A. and Krug, R. M. (2001) . *Orthomyxoviridae* : the virus and their replication . *In field virology* (4th Ed.) (Knipe, D. M. and Howley, P.

M., Ed.), pp. 1487-1531 . Philadelphia . Lippincott Williams and Wilkins .

Lee, C. W. and Suarez, D. L. (2005) . Avian influenza virus : Prospects for prevention and control by vaccination . *Animal. Health Res. Rev*., 6(1) : 1-15 .

Li, C.; Yu, K.; Tian, G.; Yu, D.; Liu, L.; Jing, B.; Ping, J. and Chen, H. (2005) . Evolution of H9N2 influenza viruses from domestic poultry in Mainland China .*Virology*, 340: 70–83 .

Marjuki, H.; Wernery, U.;Yen, H. L.; Franks, J.; Seiler, P.; Walker, D.; Krauss, S. and Webster, R. G. (2009) . Isolation of highly pathogenic avian influenza H5N1 virus from Saker Falcons (*Falco cherrug*) in the Middle East . *Adv. Virol*. Doi: 10.1155/2009/294520

Martins, N.R. (2012). An overview on avian influenza . *Rev Bras Cienc Avic*., 14(2): 71-87.

Mosleh, N.; Dadras, H. and Mohammadi, A. (2009) . Molecular quantitation of H9N2 avian influenza in various organs of broiler chickens using Taqman real time PCR . *Journal of Molecular Genetic Medicine*, 3 :152-157 .

NADIS INFO. (2006a). *Special avian flu edition. National Animal Disease Information and Surveillance (NADIS INFO) Bulletin*. Pan African Programmed for the Control of Epizootics (PACE). *Federal Department of Livestock and Pest Control Services*. No.9, pp.1-3.

Naeem , K . and Siddique , N , (2006) . Use of strategic vaccination for the control of avian influenza in Pakistan . *Development in Biology* (Basel) , 124:145-150 .

Office International des Epizooties [OIE] . (2005) (*world organization for animal health*). Highly pathogenic avian influenza in Mongolia : migratory birds.

OIE (World Organization for Animal Health) , (2006) . Avian Influenza in Iraq : follow-up report No.1 .

Pan, Z.; Zhang, X.; Geng, S.; Cheng, N.; Sun, L.; Liu, B.; Huang, J. and Jiao, X. (2009) . Priming with a DNA vaccine delivered by attenuated *Salmonella typhimurium* and boosting with a killed vaccine confers protection of chickens against infection with the H9 subtype of avian influenza virus . *Vaccine*. 27: 1018-1023 .

Pantin-Jackwood, M.J. and Swayne, D. E. (2009) . Pathogenesis and pathobiology of avian influenza virus infection in birds . *Rev. Sci. Tech.* 28: 113-136 .

Perdue, M. L.; Garcia M. and Senne, D. (1997). Virulence-associated sequence duplication at the hemagglutinin cleavage site of avian influenza viruses. *Virus Res*., 49:173–186.

Petric, M.; Comanor, L. and Petti, C. A. (2006) . Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics . *The Journal of Infectious Diseases*, 194: S98-S110 .

Presti, R. M., Zhao, G., Beatty, W. L., et al. (2009). Quaranfil, Johnston Atoll, and LakeChad viruses are novel members of the family *Orthomyxoviridae*. *J. Virol, 83*(22), 11599-11606.

Račnik, J. ; Slavec, B. ; Trilar, T.; Zadravec, M.; Dovč, A.; Krapež, U., *et al*. (2008) . Evidence of avian influenza virus and paramyxovirus subtype 2 in wild-living passerine birds in Slovenia. *European Journal of Wildlife Research*. 54:529–32.

Samji, T. (2009). Influenza A: understanding the viral life cycle. *Yale J Biol Med*, *82*(4): 153-159.

Siddique, N.; Naeem, K. H.; Ahmed, Z. and Malik, S. A. (2008) . Evaluation of RT-PCR for the detection of influenza virus serotype H9N2 among broiler chickens in Pakistan. *Int. J. Poul. Sci.* 7(11):1122-1127 .

Smith, G.J.D.; Vijaykrishna, D.; Bahl, J.; Lycett, S.J.; Worobey, M.; Pybus, O.G.; Ma, S.K.; Cheung, C.L.; Raghwani, J.; Bhatt, S.; Peiris, J.S.M.; Guan, Y. and Rambaut, A.(2009) . Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature*, 459 (7250): 1122–1125.

Suarez, D. (2005). Overview of avian influenza DIVA test strategies. *Biological*, 33: 221-226.

Suarez, D.L.; Das, A. and Ellis, E.(2007) . Review of rapid molecular diagnostic tools for avian influenza virus. *Avian Diseases*, 51:201–208.

Swayne , D . E . and Halvorson , D . A . (2003). Influenza In : Saif. Y . M . ; Barns , H .J . , Glisson, J .R . , Fadly , A .M . , Mc Dougald , L . R . and Swayne , D . E (Ed.) . *Disease of Poultry* . Iowa State University Press . Ames. 135 -160 .

Swayne , D .E . and Suarez , D . L . (2000) . Highly Pathogenic avian influenza Rev . *Sci* . *Tech* . *off. In. Epizoot* . 19 : 463-482.

Swayne, D.E. (2004). Application of new vaccine technologies for the control of transboundary diseases. *Develop. Biol*ogy , (Basel), 119, 219–228.

Swayne, D. E., and Pantin-Jackwood, M. (2008) . Pathobiology of avian influenza virus infections in birds and mammals. In: *Avian influenza*. Swayne, D. E., (Ed.), Blackwell Publishing, Ames, Iowa. pp. 87–122.

Swayne, D. E.; Perdue, M. L; Garcia, M; Rivera-Cruz, E. and Brugh, M. (1997) . Pathogenicity and diagnosis of H5N2 Mexican avian influenza viruses in chickens. *Avian Dis*., 41:335–346.

Swayne, D.E. (2007) . Understanding the complex pathobiology of high pathogenicity avian influenza viruses in birds. *Avian Disease* 51:242–249.

Swayne, D.E. (2009) . Avian influenza vaccines and therapies for poultry. *Comp. Immunol. Microbiol. Infect. Dis*., 32:351-363 .

Swayne, D.E. and Kapczynski, D. (2008a). *Vaccines*, vaccination, and immunology for avian influenza viruses in poultry. *In:* Avian Influenza. Swayne D.E. (Ed.), Wiley-Blackwell, Ames, Iowa, USA, 407–451.

Swayne, D.E.; Senne, D.A. and Suarez, D.L. (2008) . Avian influenza . *In*: A laboratory manual for the isolation , identification and characterization of avian pathogens, 5th Ed. , Dufour –Zavala, L., (eds.), American Association of Avian Pathologists, (pp.128-134), Athens , Georgia, U.S.A.

Thomazelli, L. M.; Araujo, J.D.; Ferreira, C.S.; Hurtado, R.; Oliveira; D.B.; Ometto, T.; Golono, M.; Sanfilippo, L. Demetrio, C.; Figueiredo,

M.L. and Durigon; E.L. (2012). Molecular Surveillance of the Newcastle Disease Virus in Domestic and Wild Birds on the North Eastern Coast and Amazon Biome of Brazil. *Brazilian J. Poult. Sci*. 14(1):01-07.

Tong, S.; Li, Y.; Rivailler, P.; Conrardy, C.; Castillo, D. A.; Chen, L. M., et al. (2012) . A distinct lineage of influenza A virus from bats. *Proc. Natl. Acad. Sci*. U S A. ,109(11):4269-4274.

Treanor , J .J ; Hayden , F. G. and Oidvrooman , P .S . (2000). Efficacy and safety of oral neuraminidase inhibitor Oseltamovor in treating acute influenza a randomized controlled trial . US. Oral neuraminidase study group . *JAMA* ., 283 : 1016-24.

Tuma, M. A. (2012) . Pathogenicity of Avian Influenza H9N2 Virus isolate in Broilers . M.Sc. Thesis – Collage of Veterinary Medicine – Baghdad University – Iraq .

Van Dalen, K.K. ; Franklin, A.B.; Mooers, N.L.; Sullivan, H.J. and Shriner, S.A. (2010) : Shedding light on avian influenza H4N6 infection in mallards: modes of transmission and implications for surveillance. *PLoS ONE*, 20 (5): e12851.

Veits, J.(2006). Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. *Proceedings of the National Academy of Sciences USA*; 103: 8197–8202.

Wenhua, K.; Wanyong , P.; Junfeng , H. and Deming, Z. (2006). Isolation of avian influenza virus (H9N2) from emu in China . *Irish Veterinary Journal*, 59(3): 148-152 .

Williams, R.A.J., Peterson, A.T.(2009) . Ecology and geography of avian influenza (HPAI H5N1) transmission in the Middle East and northeastern Africa. *International Journal Health Geographic*. 8: 47.

Wise, H.M.; Foeglein, A.; Sun, J.; Dalton, R.M.; Patel, S.; Howard, W.; Anderson, E.C.; Barclay, W.S.; Digard, P.(2009) . A complicated message: identification of a novel PB1-related protein translated from influenza A virus segment 2 mRNA. *Journal of Virology*, 83: 8021–8031.

Woo, J.T. and Park, B.K. (2008) . Seroprevalence of low pathogenic avian influenza (H9N2) and associated risk factors in the Gyeonggi-do of Korea during 2005–2006. *J. Vet. Sci*. 9: 161–168.

Wright, P.F.; Neumann, G. and Kawaoka, Y. (2007) . *Orthomyxoviruses*. *In* Knipe, D. and Howly, P., (Eds.), *Field Virology*, 5th ed., pp. 1691-1740 , Lippincott Wilkins, Philadelphia, Pennsylvania, USA .

.

Yoon, H.; Park, C. K.; Nam, H. M. and Wee S. H. (2005 b) . Virus spread pattern within infected chicken farms using regression model: the 2003–2004 HPAI epidemic in the Republic of Korea. *J. Vet. Med*. 52, 428–431.

Zahid, A. A. H. (2010) . Histopathological study of important Lymphoid organs in broiler infected with Avian Influenza type (H9N2) in Iraq . *The tenth scientific conference* . Vet. Coll. University of Baghdad – Iraq .