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Evaluate The Efficacy of Commercial Vaccine H9N2 in Broiler Chickens Against Experimental Infection

A Thesis
Submitted to the College of Veterinary Medicine-University of Diyala in as Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Microbiology

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Abstract

The present study aims to determine the efficacy of H9N2 vaccine in poultry farms in Governorate of Diyala , and to point out the susceptibility of unvaccinated flocks to H9N2 AIV by means of clinical signs and pathogenicity in experimentally infected broilers. This local isolate of H9N2 was successfully propagated in allantoic cavity of (9 days) old embryonated hen's eggs. The propagated virus gave a titer of (2048 HAU/0.1) by HA test. After a number of optimization the EID_{50} of the propagated stock virus was determined and revealed as ($10^{10.5}$EID50/0.1 ml) of stock virus. Hyperimmune serum was prepared for the same local isolate in rabbits. The collected serum from such rabbits gave a titer of (2^{11}HIU/0.1 ml of stock serum) by HI test. To achieve the aims of the study, 180 broilers of one day old were used and subdivided into (3) groups (60) birds each as group A, B, and C. They were separated completely from each other to avoid the possibility of virus transmission between groups. Maternal antibody levels were determined for all groups using ELISA kit. Groups A and B were vaccinated with La-Sota NDV vaccine. Group A was further vaccinated with NDV vaccine at (14) days old, and group B was further vaccinated with bivalent inactivated H9N2 commercial vaccine, whereas group C was used as control. Groups A and B were infected by intranasal dropping with 0.3 ml of

stock virus of a titer ($10^{10.5}$EID50/0.1 ml) at day (28).After challenge the clinical signs did not appear in group (A) until day (4)post infection and appeared till the end of observation period in this group.

The clinical signs characterized by moderate to severe nasal discharge, prominent depression, mild edema of the head and ruffling feather. The mortality rate among such broiler group was recorded as 2 birds with a ratio of 3.3%. Group (B) after 12 days post inoculation showed clinical signs characterized by mild nasal discharge; mild depression with mild diarrhoea and the mortality rate among B broiler group was recorded as zero bird that reached 0%. In control group (C) no remarkable signs or gross lesions were observed, also the mortality rate among such broiler group was recorded as zero. Maternal immunity appeared with the IgG mean titer of 6541.66 for the birds from all groups at the age of 3 days. Also the mean titer of anti-AIV IgG was significantly decreased among the vaccinated (A, B) and control group (C) at 14 and 35 days. The anti AIV at the age of 14 days appeared at mean of 1173.16 for group A, whereas the mean titer of the same antibody appeared as 2503.77 for group B, and 373.94 for group C. The mean titer of IgG against AIV at the age of 35 appeared as 337.77, 2303.22, and 174.27 for group A, B, and C respectively. Real time PCR was used for the detection of H9N2 in tissue samples from challenged birds showed that group A samples (trachea,

lung and liver) were positive for H9N2 with matrix gene(gene M 42), whereas, same types of tissue samples collected from birds of group B were negative for H9N2.

Histopathological microscopic observation showed clear degeneration in tracheal epithelium and mucus gland with sever congestion and haemorrhage extended to sub mucosa and also there was observed vacuoles filled with blood. Lung's tissue showed the local infiltration of mononuclear inflammatory cells, with thickened in para bronchi wall. Kidney's tissue showed the pathological changes in cortical region appeared as sever infiltration with the inflammatory cells, sever congestion and multifocal necrosis and sever degeneration on the subcapsular area extended towards the medullary cones. Pathological changes in liver appeared as multifocal necrosis accompanied with infiltration of inflammatory cells and sever congestion. Splenic parenchyma accompanied with sever infiltration of inflammatory cells and sever congestion. Infiltration of inflammatory cells in peri-arteriolar sheaths was observed with the prominent of fibrinoid necrosis. Mild pathological changes were observed in intestinal tissues.

As concluded from above, available commercial inactivated H9N2 vaccine, could not completely protect broilers of local farms from the infection with same virus H9N2 but local isolate. The virus induced mild

clinical signs in both vaccinated and non-vaccinated birds, but caused mortality rate of 3.3% among unvaccinated bird.

Dedication

To my parents ..... Without them I would not have existed in this life, and from them I learned to stand up, no matter what the difficulties.

To brothers & sister..... for their continoues support for me during the period of study.

To my husband ..... who accompanied me all the time working in this thesis.

Duaa

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Duaa

Supervisor Certification

We certify that this thesis entitled **(Evaluate the Efficacy of commercial vaccine H9N2 in broiler Chickens Against Experimental Infection)** was prepared by **(Duaa Adnan Mousa)** under our supervision at, Department of Microbiology, College of Veterinary Medicine-University of Diyala in as partial fulfillment of the requirements for the Degree of Master of Science in Veterinary/ Microbiology**.**

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We, the Examination Committee, certify that we have read the thesis prepared by the student (**Duaa Adnan Mousa**) and which was entitled (**Evaluate The Efficacy of Commercial Vaccine H9N2 in Broiler Chickens Against Experimental Infection**) and we examined the student in it, we found that the thesis is adequate for awarding the Degree of Master in Veterinary Microbiology

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I hereby declare that this thesis entitiled tittled “Evaluate The Efficacy of Commercial Vaccine H9N2 in Broiler Chickens Against Experimental Infection ” presented at the College of Veterinary Medicine-University of Diyala in 2018, is my original work**,** except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at University of Diyala or other Universities.

Duaa Adnan Mousa

Date: / / 2020

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List of abbreviations

Abbreviation	Explanation
AI	Avian Influenza
APCs	Antigen Presenting Cells
CD	Cluster of Differentiation
cRNA	Complementary RNA
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
EID50	50% Egg Infactive Dose
ELISA	Enzyme Linked Immune Sorbent Assay
ER	Endoplasmic Reticulum
HA	Heamagglutination
HI	Hemagglutination Inhibition
HPAI	High Pathogenic Avian Influenza
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgY	Immunoglobulin Y
INF	interferon
LPAI	Low Pathogenic Avian Influenza
M	Matrix
MHC	Major Histocompatibility Complexes
mRNA	Messenger Ribonucleic Acid
NA	Neuraminidase
NCBI	National Central for Biotechnology Information
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NEP	Nuclear Export Protein
NK	Natural Killer Cells
NP	Nucleocapsid Protein
NS	Nonstructural Proteins

OIE	Office International Des Epizooties
PA	Polymerase Acidic Protein
PAMPs	Pathogen Associated Molecular patterns
PB	Polymerase Basic Proteins
PBS	Phosphate Buffer Saline
PKR	Protein Kinase R
PPLO	Pleuropneumonia Like Organisms
PRRs	Pattern Recognition Receptors
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
RBCs	Red Blood Cells
RIG-I	Retinoic Acid-Inducible Gene I
RNA	Ribonucleic Acid
RNase	Ribonuclease
RNP	Ribonucleoprotein
rpm	Rotation per Minute
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SPF	Specific pathogen Free
TLRs	Toll Like Receptors
TNF	Tumor Necrotic Factor
UTR	Un-translated
WHO	World Health Organization

Chapter one

Introduction

Chapter One

1.1.Introduction

Poultry industry is greatly affected by outbreaks due to avian influenza viruses that spread worldwide. These outbreaks are associated with high morbidity and low mortality (Alexander and Capua 2009 ; Goudrazi *et al.*, 2013). Avian influenza viruses are RNA genome viruses, which is single stranded and negative sense RNA. The genome is segmented into 8 segments and their total length of 13.5 kilo base. The virions are enveloped and classified within the family Orthomyxoviridae (Lamb, 2001). All avian influenza viruses are grouped within the type Influenza A of the family Orthopmyxoviridae, and this family have another four genera which are, Influenza B, Influenza C, Thogotovirus, and Isavirus (MacLachlan and Dubovi 2011). This classification is attributed to the differences in genetic structures of both matrix proteins (MP) and nucleoproteins (NP) of these viruses (Alexander, 1995; MacLachaln and Dubovi, 2011). Many workers described the ability of human, other mammals to be infected by avian influenza viruses. Whereas, type A influenza virus was reported to infect birds. This type might cause pandemic, epidemic, and endemic diseases among different birds including domesticated, aquatic, and wild species in addition to mammals (lee *et al.*, 2001, Capua and Alexander,2009,).The type

Influenza A included many viruses, these are although related to each other serologically and from genetic point of view, but differences are also detected between each other depending on the structure of their glycoprotein spikes. Accordingly viruses of Influenza A are divided into subtypes (Alexander, 1995; Tzarum *et al.,* 2017).These glycoprotein surface spike of the virus are hemagglutinin (HA) and neuraminidase (NA). Attachment of viruses to specific receptors of host is mediated by HA, whereas NA is mediated the release of the virus from infected host cell after viral replication (Pales *et al.,* 2007).

Such genetic differences between HA and NA distinguished 18 H subtypes (H1 to H18) and 11 NA subtypes (N1 to N11) including those isolated from bats (H17 and H18, N10 and N11). The name of each subtype is a combination of HA subtype and NA subtype (Tong *et al.,* 2013; Ellakany *et al.,* 2018; Wang *et al*., 2018).

Aquatic birds and migratory birds like gulls, waterfowl and shorebirds are the main reservoirs of Influenza (A) viruses. New Influenza (A) viruses those are detected in bats and classified as H17N10 and H18N11 led to a suggestion that wild and aquatic birds are not the only reservoir for Influenza (A) viruses (Ton; 2013). Avian influenza viruses are not only infected birds, as many reports mentioned the transmission of such viruses to humans and some other mammalian (Webster, 1997; MacLachaln and Dubovi, 2011).

Influenza (A) viruses are subjected to another classification when some of the high virulent and caused high morbidity and mortality among domesticated poultry and showed high levels in pathogenicity indices and according grouped as highly pathogenic avian influenza viruses (HPAIVs), the mortality caused by such viruses in infected birds reached sometimes 100%. The highly virulent subtypes that affecting domesticated poultry are those of H5 and H7 with high pathogenicity (Webster *et al.*, 1997).

The other Influenza (A) viruses that are characterized by low pathogenicity indices, mild or no clinical signs, and low mortality are grouped or known as low pathogenic avian influenza viruses (LPAIVs) (Alexander, 2007; Swayne *et al.*, 2013). It was noticed that LPAIVs are mostly associated with mild respiratory signs and localized intestinal infections in birds, whereas viruses of HPAI induced systemic infections in susceptible birds and animals with high morbidity and mortality rates (Taubenberger and Morens, 2006). Furthermore, these HPAIVs caused high economic losses in poultry industry and they may have bad an effect on public health, due to the possibility of continuous mutation among these viruses that may lead to the emerging of new virulent virus and make the used vaccines ineffective (Alexander, 2007).

Emerging of new influenza virus strain may be associated to gene segment moved from human pandemic strain originated from old outbreaks since 1918 to newly circulated avian viruses (Byrd-Leotis *et al.*, 2017).Genetic

reassortment (genetic shift) between two avian and human influenza viruses was mentioned as the cause of many outbreaks of influenza in Europe, Asia, and Africa. These reassortments might result in emerging of highly contagious and virulent strains that caused human pandemic infections among the years, 1918, 1957, 1968, 1977 and 2009 (Taubenberger and Morens, 2006; de Wit and Fouchier, 2008; Smith *et al.,* 2009; Liu *et al*., 2013).

Longtime ago the accepted idea was that avian influenza viruses could not transmitted naturally to human, but the first evidence of 1997 when H9N2 and H5N1 avian influenza were transmitted to humans from poultry had changed the idea (Shortridge *et al.,* 1998). Many avian influenza virus outbreaks had hit poultry industry of Asian and Middle East poultry farms during late 1990s and early 2000s. These outbreaks are reported to be due to LPAI subtype H9N2 (AL-Natour *et al.,* 2005).

In Iraq, avian influenza virus was firstly isolated from flocks of layers by (Al-Nasrawi 2002) , and this was followed by the isolation of subtype H9N2 (AMR.ANT/Iraq?2005) from broilers (Sabbar 2007). The last report from office international des epizooties OIE(Follow-up report No.8 Report reference: ,

Reference OIE : 26150, Report Date : 11/03/2018, Country : Iraq) confirmed presence of two HPAI (H5N8) in Governorate of Diyala that

caused high mortality in layer chickens reached to 80-90%. This was followed by massive use of H9N2 killed vaccine in vaccination programs in Diyala Governorate.

1.2.Aims of the study:

1- The present study was an attempt to better understand the situation of H9N2 virus in Diyala Governorate as well as to assess the pathogenicity of H9N2 avian influenza virus in experimentally infected commercial broiler chickens.

2- To study the Efficacy of H9N2 vaccine in poultry of Diyala Governorate .

3- To evaluate Susceptiblity of unvaccinated flocks to H9N2 AIV.

4- To study the Pathogenecity of H9N2 AIV in flocks of Diyala Governorate .