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Seroprevalence and Molecular Detection of Infectious Laryngotracheitis in Layers of Diyala Province

A thesis
Submitted to the Council of the College of Veterinary Medicine/University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Medicin/Veterinary Microbiology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Dedication

To my father..... *The most precious person in my life, to the source of my strength and success, to the warm embrace, to which by their silent prayers brouth me to success. My Allah protect you for me all the life.*

To my mother.....*The source of light in my life who surrounds me with her love and kindness and never forgets me in her sincere prayer. My Allah protect you for me all the life.*

To my brothers and my sisters*.....The most wonderful persons in the world, with my respect and love.*

To my husband*.....The warm heart which is the my second half,which helps me and shares me every step to glory.*

To my children*.....(Abdulla,Tuqa) symbol of love and pride.*

I dedicate this humble work

Anmar

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Anmar

Declaration from

I here by declare that this thesis entitled **(Seroprevalence and Molecular Detection of Infectious Laryngotracheitis in Layers of Diyala Province) presented at the College of Veterinary Medicine-University of Diyala in 2022,** is my original work, except for quotations and citations which have been duly acknowledged.I also declare that it has not been submitted previously or concurrently, for any other degree at the University of Diyala or other Universities.

Anmar Ayoub Kadhim

Date: / / 2020

SUMMARY

Avian infectious laryngotracheitis (ILT) is a viral respiratory disease which is included within List B of the Office International des Epizooties (OIE). Avian infectious laryngotracheitis caused by: Gallid Herpesvirus I. Infectious laryngotracheitis virus is a member of the family Herpesviridae, subfamily Alphaherpesvirinae, genus Iltovirus. The species is named Gallid Herpesvirus-1(GHV-1) double stranded linear DNA virus . Gallid Herpes virus causes respiratory disease in chickens and pheasants. Many Iraqi workers studied the widespread disease such as Alaraji and Al-Saadi. In Diyala Province, no research was done to identify the disease in chicken farms, especially the layer farms.

Five (5) tissue sample(tracheas and larynxes) and(270) serum samples were collected from chickens with respiratory tract infection after post mortem examination; these samples were collected from December 2021 to May 2022 from different areas in Diyala province include five region Baqubah, Khan Bani Saad, AL-ghalibia, Kanaan, and AL Khales.The flocks were 70 to 180 days old. Due to trachea occlusion by hemorrhage or exudate, mortality was 10-20% and morbidity was 50%. Not all layer farms received the ILTV vaccine, thus serum samples were ELISA-tested. RT-PCR assays were performed on collected samples using field and vaccine strain primers to rule out vaccine strain dissemination due to latent infection. Infectious laryngotracheitis (ILT) virus was isolated on Chorioallantoic memberan of embryonated chicken egg. In this study's sequencing analysis, just one sample represented this location and also

30 layers of 72-day-old were infected with ILTV from PCR positive homogenized infected CAM and 10 were controls.

Serological ELISA test that performed on collected serum samples from infected live birds showed positivity to ILTV IgG.Statistically the result were showed significant at ($P<0.05$) in Baqubah and Khan Bani Saad in comparison of Al-ghalibia ,Kanaan and AL-Khales according to region.According to age were showed significant at ($P<0.05$) at 120 and 180 days. All flock tissue samples tested positive for the ILTV field strain and negative for the viral vaccine strain using RT-PCR.On embryonated chickens' egg CAM, the virus generated pock sores. The 247 bp sample showed no nucleic acid variations, unlike most reference sequences (GenBank acc. no. ON572193).

In the current study, a phylogenetic tree was generated based on nucleic acid differences found in the amplification 247 bp of the UL22 genetic amplicon. Sample S1 was a type 1 (Gallid alphaherpesvirus 1) main clade virus The S1 sample under study did not differ from its Gallid alphaherpesvirus type 1 neighbors. This sample matched many Gallid alphaherpesvirus type 1 sequences, including the GenBank accession number (JX646899.1, MK895003.1, MN335811.1, MF405080.1, and MG775218.1). These Gallid alphaherpesvirus 1 strains are from Australia, Russia, and Peru.

In expremintal infection symptoms included despondency, rales, wet eyes (mild conjunctivitis), coughing, and minor gasping. These symptoms appeared 3 days after ILTV droppling in the eyes,inoculation intranasal and intraltracheal from a nearby field isolate. After 15 days of observation, no death was detected.

Postmortem inspection of these layers indicated minimal mucus in the trachea after 24 hours and tracheitis 4-6 days after infection. The IgG antibody titer in experimentally infected chickens was determined by ELISA at 7, 14, and 21 days post-infection and show significant titer at 21 days P.I.

According to histological examinations of infected larynx and trachea after 24,48,72 hours, ILTV virus induced fusion of localized and/or multifocal mucosal epithelial cells. These caused intraepithelial syncytial (multinucleated) cells with cilia and intranuclear inclusion bodies. Basophilic and eosinophilic inclusion bodies might fill the nucleus or be surrounded by a halo.

Histological investigation of the chorioallantoic membrane infected with ILTV after (4-5) days post inoculation, showed pathological changes included congestion, hemorrhage and epithelial hyperplasia with appearance of basophilic/eosinophilic intranuclear inclusion bodies.

Conclusion in this study was the ILTV is field strain and not originated from vaccine strain,no variation in nucleic acid sequences of ILTV genom,the detected virus is mild strain and the severity of disease may be co-infection ,crowding, poor ventilation.

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Results

	inflammatory cells (in), congestion (co), and hemorrhage (h) (A, B at 40X) (H&E).	

List of abbreviations

Abbreviation	Explanation / Meaning
AI	Avain influenza
Bp	base pairs
CAM	Chorioallantoic memberane
CEF	Chicken embyo fibroblast
CEL	Chicken embyo lung
CEO	Chicken embyo origen
Ct	Cycle threshold
DNA	Deoxyribonucleic Acid
DsDNA	Double strand Deoxyribonucleic Acid
ECE	Embryonated chicken egg
ELISA	Enzyme linked immune sorbent assay
GaHV-1	Gailld herpesvirus-1
H-E	Hematoxylin-Eosin stain
IBV	Infectious bronchitis virus
ICP4	Infected cell protein 4
IgG	Immunoglobulin G
IHC I	Immunohistochemistry
ILT	Infectious laryngotracheitis
ILTD	Infectious laryngotracheitis disease
ILTV	Infectious laryngotracheitis virus
IR	Inversion repeat
Kbp	Kilobase pairs
NCBI	National central for Biotechnology Information
ND	Newcastle disease
ORF	Open reading frame
P.I	Post infection
PBS	Phosphat buffer saline
PCR	Polymerase-chain Reaction
Pmol	Picomole
qRT-PCR	Quantitative Reverse Transcriptase Polymerase-chain Reaction
RFLP	Restriction fragment length polymorphism
Rpm	Rotation per Minute
RT-PCR	Real time polymerase chain reaction
TCO	Tissue culture origin
TR	Termial repeats

TRG	Trigeminal ganglion
UL	Unique long
US	Unique short

Chapter One

Introduction

Introduction

1.1.Introduction

The poultry industry is expanding quickly and has an impact on world food security.Various illnesses have been spread through globalization, climate change, and an increase in the number of chickens. Among developing infections, infectious laryngotracheitis (ILT) posses a serious threat to poultry economical industry (Bagust *et al*., 2000). Peafowl and pheasants have displayed spontaneous illness, despite the fact that poultry are the main target (Garca and Spatz, 2020; Gowthaman *et al*., 2020; Oliga *et al.*, 2021).

Many bird species are immunized to ILTV infection such as, sparrows, pigeons, ducks and crows, but Galliformes are not (Guy and Garcia, 2008). Losses in productivity come from morbidity and mortality, lower weight gain, lower egg yield, immunization, biosecurity precautions, and cost of medication to avoid subsequent illnesses. These losses are reported by many authors (Jones 2010; Garcia *et al*., 2014). There are two types of this viral disease of tracheitis in chickens that have been reported field levels: a difficulty in breathing, sneezing, expectoration of mucus mixed with blood, conjunctivitis and severe tracheitis of haemorrhagic type and, bird death ranged from five to seventy percent describe as the severe acute or epizootic form. A milder variant is characterized by moderate to mild to catarrhal tracheitis, and inflammation of sinuses (Kirpatrick *et al*., 2006; Oldoni *et al*., 2008; Ou and Giambrone, 2012).

Virus-free birds can carry GaHV-1 for years. This viral infection was reported to cause latency in central nervous system (CNS) (especially trigeminal ganglions) after seven days of severe infection

(Gowthaman *et al.,* 2020). The disease was first described in the US by May and Thittsler in 1925, then followed by in Australian country, United Kigdom, and then in European countries (Cover, 1996). ILT was approved in 1931 by USA Veterinary Association (Guy and Garcia, 2008). The use of a cloacal immunization against ILT was a first for a chicken virus (Gibbs, 1934).

The ILT disease is caused by Gallid herpesvirus-1 which grouped in subfamily *Alphaherpesvirinae* that classified within family of herpesvirus (*Herpesviridae*). It is contained 150 kilobase pairs of dsDNA encode eighty proteins of the virus as reported by Menendez *et al*. (2014). ILT virus (ILTV) infection produces a severe respiratory disease with varied fatality. Unwell birds, dust that is tainted with the virus, garbage, water, wind and utensils can transmit viruses. Natural infections incubate for 6-14 days and the strain of the virus and presence of co-infection in respiratory tract of infected determine the infection severity (Garcia and Spatz, 2020; Gowthaman *et al*., 2020).

Cell mediated immunity and antibody responses in infected birds are stimulated by the enclosing glycoproteins of ILT (York and Fahey, 1990). It is believed that ILTV glycoproteins are essential for both entrance of the virus and its replication as reported by Goraya *et al*, (2017). The disease is very hazardous and has been reported in almost every country between 2000 and 2013, the disease was documented in a number of countries throughout the world (Menendez *et al*., 2014). In Al-Diwaniyah, Iraq , ILTV was recently molecular proven (Alaraji *et al*., 2019; Al-Saadi, 2022).

There were many outbreaks of ILT in Windhoek, Namibia, in 2018 resulted in devastating layer and broiler mortality (Molini *et al*.,

2019). Inadequate vaccination, biosecurity breaches, dealing with condensed layer flocks, using short cycles of production, and using of layer flocks with multiple ages and multifunctional poultry in the same location, all contribute to an increase in ILT outbreaks (Garcia *et al*.,2019; Blakey *et al*., 2019).

Since middle of the 1920s , ILT has been a menace to the world poultry industry. All birds, regardless of age, from 8 days to 4 years, can contract the ILTV virus (Linares *et al.,* 1994). Birds older than three weeks are more sensitive (Dufour-Zavala, 2008). ILT pathogenicity, viral dose, and co-infections with other respiratory diseases have an influence on morbidity and death. ILT outbreaks are frequently brought on by intensive chicken husbandry, mixing several bird species in one region, and inadequate biosecurity (Guy and Garcia, 2008). Iraqi Taha *et al*. (2016) isolated the virus on chorioallantoic membrane (CAM) and discovered ILT antibodies in non-vaccinated layers in Baghdad (Taha *et al.,* 2017). Using PCR, Mohammed *et al.* (2019) reported ILTV in Baghdad layers.

Numerous ILT outbreaks were unofficially recorded in Diyala Province, but no research was done to identify the disease and its cause. Furthermore, since the virus may induce latent infection, no layer farm utilized the ILT immunization program, making it impossible to claim that the disease was caused by vaccination. As a result, this research was designed:

1.2.Aims of Study

1.Detection of ILTV virus in Diyala farms through:

A.Serological identification of (ILTV) of some flocks of layer in Diyala Governorate.

B.Detection of ILTV by molecular test(RT-PCR) performing on some flocks of layer in Diyala Governorate by the use of specific primers that can find out its origin as a vaccine strain or not.

C.Sequencing and Phylogenetic analysis of the detected ILTV to find out the relatedness of local Iraqi strains and references strains from Genebank (NCBI).

D.Experimental infection in layers by isolated ILTV in CAM after confirmed by RT-PCR.