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Serological and Molecular Detection of Chicken Anemia Virus in Chickens and Japanese Quails in Diyala Province

A Thesis

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وَلَوْ أَنَّمَا فِي الْأَرْضِ مِن شَجَرَةٍ أَقْلَامٌ وَالْبَحْرُ يَمُدُّهُ مِن بَعْدِهِ سَبْعَةُ أَبْحُرٍ مَّا نَفِدَتْ

كَلِمَاتُ اللَّهِ ۗ إِنَّ اللَّهَ عَزِيزٌ حَكِيمٌ (27)

سُورة لقمان: الآية [27]

Dedication

To

My mother

A strong and gentel soul who taught me to trust in Allah, believe in hard work and that a lot could be made from little.

My father

My guardian angel and the light of my life who always supported me whatever path I took.

My sister Raghad

My soul mate who was with me in all steps of my work.

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(Declaration form)

I hereby declare that this thesis entitiled tittled **"Serological and Molecular Detection of Chicken anemia virus in Chickens and Japanese Quails in Diyala Province" presented at the College of Veterinary Medicine-University of Diyala in 2018,** 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.

Haneen Ghazwan Yahya

Date: / / 2018

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Summary

Chicken infectious anemia or chicken anemia virus is a viral disease found worldwide, and caused by a single DNA virus from *Circoviridae*. The virus targeted the bone marrow of susceptible chicken and led to hemocytoblast destruction that was conjugated to erythroid and myeloid cell depletion. This led to severe anemia. Severe atrophy of thymus associated with destruction of progenitor cells of T lymphocytes was attributed to the infection with this virus and led to depletion of T cytotoxic and T helper cells.

This study was conducted from October 21st, 2017 to June 5th, 2018 and aimed to investigate, for the first time in Iraq, the seropositivity rate of anti chicken anemia virus antibodies and chicken anemia virus viral protein1 gene in poultry group of Diyala province. Accordingly 17, chickens and quails (broiler, layer, backyard and Japanese quail) of different ages and from 13 different sites.

Serum samples from 400 birds 100 samples each of a bird's type (broilers, layers, Japanese quails, and backyard indigenous fowls), were collected and subjected to chicken anemia virus Enzyme Linked Immuno Sorbent Assay test. Furthermore, 50 egg embryo tissue samples from five hatcheries, 30 Enzyme Linked Immuno Sorbent Assay test chicken anemia virus positive serum samples, and 4 commercial poultry virus vaccines were subjected to DNA extraction by the use of specific kit and subjected to Polymerase chain reaction. All the tests were performed in virology and molecular biology laboratory of the College of Veterinary Medicine, University of Diyala, Iraq.

The results showed that was 54.75% as 219 serum samples were positive out of 400 tested serum samples.

Summary

In broilers the overall seropositivity rate was 43%. The high seropositivity rate (30.23%) was observed in broiler group of one week of age. This was followed by seropositive rate of 23.25%, 20.93%, 16.28%, and 9.3% for the age groups of 4 weeks, 3 weeks, 5 weeks and 2 weeks respectively.

The overall seropositivity rate in layers was 60% . The age group of 30 weeks of age showed seropositivity rate of 33.3% followed by seropositivity rate of 31.7%, 26.7% and 8.3% for the age groups 28 weeks, 8 weeks and 10 weeks respectively.

Japanese quails group showed an overall seropositivity rate of 29.0% among which the age group of 2 weeks showed high seropositivity rate of 44.8%. Other 3 groups reported seropositivity rate of 31.0%, 17.2% and 6.9% for the age groups of 3 weeks, 4 weeks and 5 weeks respectively.

High seropositivity rates were noticed with local (indigenous) fowl group when overall seropositivity rate of 87% was reported. The seropositivity rate among age groups ranged from 23% to 27.6%.

The overall seropositivity rates of 4 studied bird groups was 54.75%. The local fowls group reported the highest seropositivity rate of 39.7% and flowed by layers (27.4%), broilers (19.6%) and quails (13.2%).

According to Sample/Negative ratio of detected anti-chicken anemia virus antibodies, 85 birds (21.3%) appeared with high level of anti-chicken anemia virus antibodies and low Sample/Negative ratio ranged from 0.001-0.199, 64 birds (16%) with medium level of anti-chicken anemia virus antibodies and moderate Sample/Negative ratio ranged from 0.200-0.399

and 70 birds (17.5%) with low anti-chicken anemia virus antibodies level and high Sample/Negative ratio ranged from 0.400-0.599.

All the tested 17 group from 13 locations of Diyala province were serologically positive to anti-chicken anemia virus antibodies and the highest positivity was detected in Al-Khalis (16.9%).

Polymerase chain reaction results showed that all tissue samples from 50 egg embryos and the 4 commercial vaccines were negative to chicken anemia virus DNA. Furthermore, 2 serum samples from young broilers were positive to Polymerase chain reaction out of 30 Polymerase chain reaction tested serum samples.

Antibodies to chicken anemia virus was widely distributed in poultry farms of Diyala province that required restricted and effective control measures to avoid the possibility of complicated infections and vaccines failure as the virus was reported to cause immune suppression in infected birds.

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List of Abbreviations

Abbreviation	Description
MDCC-MSB1	Marek's Disease Chicken Cell
LSCC-1104B1	Lymphoma Spleen Chicken Cell
AGID	Agar gel immune-diffusion
B lymphocyte	Bone marrow lymphocyte
CAV	Chicken anemia virus
CD3	Cluster of differentiation 3
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
ConA	Concanavalin A
Cux-1	Cuxhaven
CT1	Cytotoxic T lymphocyte
CMI	Cell Mediated Immunity
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FcR	Fragment crystallizable receptor
Gyv3	Gyrovirus 3
HI	Hemagglutination inhibition
ILT	Infectious Laryngotracheitis
IDE	IDEXX ELISA
IBDV	Infectious Bursal Disease Virus
INF	Interferon

IL	Interleukin
MD	Marek`s disease
mRNA	Messenger ribonucleic acids
MAb	Monoclonal antibody
MBE	Monoclonal blocking ELISA
ND	Newcastle disease
ORF	Open Reading Frams
PCV	Packed cell volume
WK	Week
PCR	Polymerase chain reaction
PI	Post infection
RBCs	Red blood cells
RFLP	Restriction fragment length polymorphism
SPF	Specific pathogen free
TCR	T-cell receptor
T lymphocyte	Thymic lymphocyte
VP	Viral protein
S/N	Sample/Negative

Chapter One

Introduction

1.1.Introduction

Chicken anemia virus (CAV) infection is an important disease of poultry from economic and health points of view for the poultry industry, and it is worldwide in its distribution (Schat, 2009; Oluwayelu, 2010; Bhatt *et al.,* 2011).

The disease was first recognized and isolated from affected chicks in Japan in 1979 (Yuasa *et al*., 1979) but Toro *et al.,* (2006) mentioned that the disease was circulated in USA since 1959 as was detected in stock sera. The disease was contagious and effecting mainly young chicken one to four weeks old, and resulted in severe anemia with lymphoid atrophy (Dhama *et al*., 2008). The infection also caused severe immune suppression that increased the susceptibility of infected bird to secondary infections of bacterial, fungal and/or other viruses due to low immune responses to such agents or to vaccination programs (Hangood *et al*., 2000; De Herdt *et al*., 2001; Hoerr, 2010).

The only known host of CAVwas chicken, but many other studies suggested the infection of other birds when certain levels of antibodies to CAVwere detected in Japanese quails (Gholami-Ahangaran and Zia-Jahromu, 2012), whereas, pigeons, ducks, crows and some other domestic poultry were reported to be negative to carry such antibodies (McNulty *et al.,*1988; Farkas *et al*., 1998). These findings indicated that the virus was well adapted to its host (Miller and Schat, 2004) and was worldwide in its distribution (Schat 2003).

Clinically, the disease infected young chicks that showed depression, with pale mucous membranes, and lethargic, hemorrhages could be observed

under skin of different body parts including wings (Blue wing disease). Mortality rates generally were variable and increased with presence of secondary infections due to immune suppression (Toro *et al*.,2000). Other hemorrhagic diseases like hemorrhagic syndrome, gangrenous dermatitis, anemia dermatitis, hemorrhagic anemia syndrome, and infectious plastic anemia might be attributed to play a key role of CAV(Toro *et al*., 2000; Schat, 2003; Dhama *et al*., 2008).

Blood samples from infected chickens were watery with low PCV, and increased time to clot, whereas blood smears showed anemia with pancytopenia or leukopenia. Postmortem findings revealed hemorrhagic lesions in the muscular tissue, and other organs. Most organs of infected birds appeared pale, with atrophied thymuses and bursae (Taniguchi *et al*., 1983). Histological microscopic testing of lymphoid organs showed depleted tissues. Atrophic and hypoplastic lesions were also observed in bone marrow of infected birds (Dhama *et al*., 2002b; Schat, 2003).

The economic losses in poultry industry due to CAVmight be attributed to poor weight gain of infected birds in comparison to uninfected birds, immune suppression and susceptibility to secondary infection, and losses from mortality due to active infection (Adair, 2000; Dhama *et al*., 2008).

The causative virus was found to be transmitted horizontally by fecal-oral route (Yuasa et al., 1983) or vertically from infected male and female parents (Hoop, 1992; Cardona *et al*., 2000a),regardless of their immune status or antibody titer (Miller *et al*., 2003; Brentano *et al*., 2005). Furthermore, detection of anti-CAVantibodies in specific pathogen free birds at the lay onset gave a suggestion of the possibility that the virus might

maintained itself somehow in the reproductive organs of such chickens in a way similar to latency or persistent infections, later the virus reactivated at sexual maturity stage of such birds (Cardona *et al*., 2000b; Miller and Schat 2004; Miller *et al.,* 2008). Detection of the virus in the shaft of feathers of some infected birds increased the possibility of the presence of other modes of transmission due to dissemination of the virus that might lead to other contaminations (Davidson *et al*., 2008).

Chicken anemia virus is a single stranded circular negative sense or ambisense DNA virus. It is the smallest among DNA viruses and classified within the genus *Gyrovirus.* This genus and the genus *Circovirus* were classified with the family *Circoviridae*. The virus DNA was encoded for viral proteins VP1, VP2 and VP3 (Ducatez *et al.,* 2008).

Many other viruses were found to be associated with CAVinfection like infectious bursal disease virus (IBDV) and gallid herpesvirus 2 (Mahzounieh *et al*., 2005).These viruses might play a role on CAVpotential effects as immune suppressive disease agent. Furthermore, this factor with the contagious nature of the virus, its worldwide distribution, the ability to cause latent infections and its vertical transmission might gain the virus much importance (De Herdt *et al*., 2001; Schat, 2003; Eltahir *et al.,* 2011).

In some other countries like India, CAVwas given special attention and importance when it was regarded as emergent viral disease of poultry that needed urgent screening and high control measures (Natesan *et al*., 2006; Dhama *et al*., 2008; Bhatt *et al*., 2011).

Reports mentioned the detection of CAV-related virus in feces, skin and blood of humans, and in feces of stray cats, may threaten potentially the

healthy human (Sauvage *et al*., 2011; Ye *et al.*, 2015). In case of active infections of birds, it was recommended to depend on clinical findings for the purpose of CAVdiagnosis. Furthermore, this can be confirmed by many serological tests like Immunofluorescent antibody test, enzyme linked immunosorbent assay (ELISA) and virus neutralization using of reference serum. Molecular techniques like polymerase chain reaction (PCR) and genomic analysis by sequencing or restriction enzyme fragment length poly morphism might be required when such virus was isolated or its genomic genes were detected. The same above mentioned techniques can be used for screening the virus for epidemiological purposes (Dhama *et al*., 2002a; Schat, 2009; Manoharan *et al*.,2012).

Many vaccination programs were followed to control the virus. These programs were used recombinant DNA vaccines, live-attenuated and inactivated vaccines, and immune complex vaccines. These vaccines were reported to be effected and protective against the infection with CAVboth in broilers and layers (Dhama *et al*., 2008; Schat, 2009). Some other strategies of protection depend on administration of broad spectrum antibiotics to avoid secondary complications resulted from bacterial or fungal infections due to weak immune responses du to CAVinfection (Dhama *et al*., 2002; Schat, 2003).

In Iraq, no data were available on screening poultry for the presence of CAVinfection in broiler, layers and other birds. Furthermore, virus gens were not detected and/or the virus was not isolated. Accordingly, the present study was designed and aimed to:

1.2. Aims of the study

The study aims at:

1- Detection of anti-CAVantibodies (sero-positivity) in the poultry farms (Broilers and Layers), local backyard (indigenous) fowls, and Japanese quails in Diyala province.

2- Detection of CAVgene, by the use of conventional polymerase chain reaction (PCR) in embryonated hens eggs, available commercial vaccines, and positive sera of screened birds.