Cairo University
Faculty of Veterinary Medicine

Isolation, Molecular Identification and Pathogenesis of Fowl Adenovirus

A thesis submitted by

Mohamed Mounir Abd El-Fattah Radwan
(BVSc, Cairo University, 2006; MVSc, Cairo University, 2012)

For the degree of the Ph.D
(Virology)

Under Supervision of

Mohamed A. Shalaby
Professor of Virology and Immunology
Faculty of Veterinary Medicine
Cairo University

Ahmed A. El-Sanousi
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Ayman H. El-Deeb
Associate Professor of Virology
Faculty of Veterinary Medicine
Cairo University

2018

Approval Sheet

This is to approve that Thesis presented by

Mohamed Mounir Abd El-Fattah Radwan

For the degree of PhD. (Virology) has been approved by the examining committee:

Prof . Dr- Abd El Satar A.Mohamed
Chief Researcher in reference
Laboratory for quality control on poultry production
Animal Health Research institute
Dokki –Giza

Prof. Dr. Ausama Abd El-Raouf Abd El- Moneim Yousif
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

Prof ***. Dr- Ayman Hanea El-Deeb***
Ass. Prof. of Virology
Faculty of Veterinary Medicine
Cairo University(Supervisor)

Prof. Dr. Ahmed Abd El-ghani El-Sanousi
Professor of Virology
Faculty of Veterinary Medicine,
Cairo University (Supervisor)

Prof. Dr. Mohamed Abd El–Hamid Shalaby
Professor of Virology
Faculty of Veterinary Medicine,
Cairo University (Supervisor)

Cairo University
Faculty of Veterinary Medicine
Department of Virology

Supervision Sheet

Prof. Dr. Mohamed Abd El-Hameed Shalaby

Professor of Virology and Immunology, Faculty of Veterinary Medicine, Cairo University, Giza,

Egypt.

Prof. Dr. Ahmed Abd El-Ghani El-Sanousi

Professor of Virology, Faculty of Veterinary Medicine, Cairo University, Giza,

Egypt.

Dr. Ayman Hany El-Deeb

Associate Professor of Virology, Faculty of Veterinary Medicine, Cairo University, Giza,

Egypt.

2018

Name: Mohamed Mounir Abd El-Fattah Radwan

Nationality: Egyptian

Specialty: Virology

Degree: PhD in Veterinary Medical Sciences.

Title: Isolation, Molecular identification and pathogenesis of fowl adenovirus

Supervisors:

- **Prof. Dr. Mohamed Abd El-Hameed Shalaby**
  Professor of virology and immunology, Faculty of Veterinary Medicine, Cairo University.
- **Prof. Dr. Ahmed Abd El-Ghani El-Sanousi**
  Professor of virology, Faculty of Veterinary Medicine, Cairo University.
- **Dr. Ayman Hany El-Deeb**
  Associate professor of virology, Faculty of Veterinary Medicine, Cairo University.

Abstract

This study was performed to detect fowl adenovirus (**FAdV**) circulating in commercial meat-type chicken in Egypt during 2015 and to isolate and identify the pathogenicity of the isolated virus. Cloacal swabs were collected from nine commercial broiler farms from chickens of 3-5 weeks of age in Behira province. FAdV was isolated on chicken embryo liver cells. The virus was identified by conventional polymerase chain reaction targeting a conserved region in the hexon gene. Moreover, phylogenetic analysis of the L1 loop of the hexon gene revealed that the isolated viruses clustered with reference strains belonging to FAdV serotype 8a. This is the first record of FAdV from Egypt with complete genotyping. The isolated virus is closely related to strains directly associated with inclusion body hepatitis (**IBH**) causing considerable economic losses. Pathogenicity study of the virus did not show any mortality, although necropsy and histopathological examination displayed severe hepatitis and degenerative changes in the primary and secondary organs of the immune system after 5 days from infection, proving that the virus can cause IBH with intermittent shedding.

Keywords: Fowl adenovirus, Inclusion body hepatitis, Pathogenicity, Egypt, hexon gene, PCR.

Acknowledgements

"Praise to Allah, who has guided us to this; and we would never have been guided if Allah had not guided us."

I would like to express my gratitude and appreciation to the following:

Prof. Dr. Mohamed Abdel-Hameed Shalaby *(Professors of Virology & Immunology, Faculty of Veterinary Medicine, Cairo University) for his supervision, patience, unlimited trust, believe that I can do it, guidance and support throughout the study.*

Prof. Dr. Ahmed Abdel-Ghani El-Sanousi *(Professors of Virology, Faculty of Veterinary Medicine, Cairo University) for his supervision, patience, unlimited trust, believe that I can do it, guidance and support throughout the study.*

Dr. Ayman Hany El-Deeb *(Associate Professor of Virology, Faculty of Veterinary Medicine, Cairo University) for his supervision, patience, unlimited trust, believe that I can do it, guidance and support throughout the study.*

Dr. Samah Fekry Mohamed Ali *(Senior Researcher, Biotechnology Research Unite, Animal Reproduction Research Institute, Agriculture Research Center) for her continuous advice, efforts, technical assistance that facilitate the completion of the study and assistances that are too numerous to mention.*

Prof. Dr. Mohamed Hussein Hassan Ahmed *(Head of Researcher, Poultry Disease Department, Animal Health Research Institute) for his continuous advice, supports, and guidance throughout the study.*

Mahmoud Samir *(Assistant Researcher, Virology Department, National Laboratory for Quality Control on Poultry Production, Animal Health Research Institute) for his continuous support on virus isolation.*

Mohamed Refaat Mousa *(Assistant Lecturer, Pathology Department, Faculty of Veterinary Medicine, Cairo University) for his continuous support in histopathological examination.*

Mohamed Abd El-Razek Salah El Din *(Lecturer, Histology Department, Faculty of Veterinary Medicine, Cairo University) for his continuous support in histopathological examination.*

Staff members of the Virology Department, Faculty of Veterinary medicine, Cairo University for their support.

Staff members of the Biotechnology Research Unite, Animal Reproduction Research Institute, Agriculture Research Center for their assistances.

Members of the Arab Poultry Breeders Co. "OMMAT" for their assistances and support.

Finally, my Mother and Father for their duáa, patience, sacrifice, moral and financial supports.

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Arabic Summary

Arabic Abstract

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List of Abbreviations

AT	:	Adenine-thymidine
Ad	:	Adenovirus
bp	:	Base pair
CAR	:	Coxsackie-adenovirus receptor
CEL	:	Chicken embryo liver
CELO	:	Chicken embryo lethal orphan
CK	:	Chicken Kidney
CPE	:	Cytopathic effect
CTL	:	Cytotoxic T-Lymphocytes
DBP	:	DNA-Binding Protein
DNA	:	Deoxyribonucleic acid
ECE	:	Embryonated chicken egg
EDS	:	Egg drop syndrome
ELISA	:	Enzyme linked immune-sorbent assay
FAdV	:	Fowl Adenovirus
GE	:	Gizzard erosion
HPS	:	Hydropericardium Syndrome
HVR	:	Hypervariable region
IBH	:	Inclusion Body Hepatitis
ITRS	:	Inverted terminal repeats
ISH	:	*In situ* hybridization
Kbp	:	Kilo base pair
LBM	:	Live bird market
LMH	:	leghorn male hepatocytes
MEGA	:	Modern Evolutionary Genetic Analysis
MEM	:	Minimal Essential Medium
MHC	:	Major Histocompatibility Complex
mRNA	:	Messenger RNA
nt	:	Neucleotide
NTC	:	No template control
OIE	:	Office international des epizooties
ORF	:	Open reading frame

PKR	:	Protein Kinase
P-protein	:	Phosphoprotein
PCR	:	Polymerase chain reaction
RE	:	Restriction Enzyme
REA	:	Restriction endonuclease analysis
RFLP	:	Restriction fragment length polymorphism
RNA	:	Ribonucleic acid
RNAi	:	Interfering RNA
RNP	:	Ribonucleoprotein
SPF	:	Specific Pathogen Free
ssDNA	:	Single stranded DNA
TV	:	Trypsin Versin
VA-RNA	:	Virus-associated RNA

Amino acids abbreviations

A	:	**Ala**	:	Alanine
R	:	**Arg**	:	Arginine
N	:	**Asn**	:	Asparagine
D	:	**Asp**	:	Aspartic acid
C	:	**Cys**	:	Cysteine
E	:	**Glu**	:	Glutamic acid
Q	:	**Gln**	:	Glutamine
G	:	**Gly**	:	Glycine
H	:	**His**	:	Histidine
I	:	**Ile**	:	Isoleucine
L	:	**Leu**	:	Leucine
K	:	**Lys**	:	Lysine
M	:	**Met**	:	Methionine
F	:	**Phe**	:	Phenylalanine
P	:	**Pro**	:	Proline
S	:	**Ser**	:	Serine
T	:	**Thr**	:	Threonine
W	:	**Trp**	:	Tryptophan
Y	:	**Tyr**	:	Tyrosine
V	:	**Val**	:	Valine

Chapter (1)

Introduction

Introduction

Members of the family *Adenoviridae* have been isolated only from vertebrates (from fish to human). Recent bioinformatics analysis of the family genome sequences defined five clades corresponding to previously classified genera: *Mastadenovirus*, *Atadenovirus*, *Siadenovirus*, *Aviadenovirus* and *Ichtadenovirus*. **(Berk, 2007; Fitzgerald 2013)** The genus *Aviadenovirus* includes the conventional group 1 avian adenovirus **(Smyth and McNulty, 2008)**, which are important chicken adenoviruses that are also classified into five different species (A-E), into which all the previous 12 European serotypes [fowl adenovirus (**FAdV**) 1-8a and 8b-11] have been placed **(Hess, 2013)**.

Although FAdV has been isolated from almost every part of the world from apparently healthy flocks, even those with exceptionally high levels of production and fertility, it has been also isolated from sick birds. These were suffering from different clinical signs that varied from respiratory signs to depressed egg production, arthritis/tenosynovitis, uneven growth or even enteritis with variable degrees of mortality **(Adair and McFerran, 2008)**. This variation in disease association makes it difficult to assess the economic importance of the FAdV or even its direct role as primary pathogens **(Hess, 2013)**. However, the very virulent FAdV-4 (species C), which appears to have a more serious role than others in the aetiology of hydropericardium syndrome (**HPS**), leads to high mortality that varies from 20% to 80% **(Asthana *et al.*, 2013)**. Some FAdV-1 strains (species A) cause gizzard erosion associated with growth retardation, while other strains can cause serious liver injury and play an important role in the disease condition known as inclusion body hepatitis (**IBH**). All the previous data strengthen the role of some FAdV strains as primary pathogens **(Hess, 2013)**.

IBH caused by FAdV of almost all serotypes was reported in different geographical regions. Certain serotypes, mainly those belonging to species D or E (serotype 2, 3, 6, 7, 8a, 8b, 9 and 11), are directly associated with IBH and

mainly infect broilers. The infection may start at as young as 7 days of age, causing sudden onset of mortality that ranges from 5% to 10%, peaking after 3-4 days and usually stopping on day 5; however, the course of the disease occasionally continues for 2-3 weeks and is associated with intra-nuclear inclusion bodies in the hepatocytes and pale swollen liver with haemorrhages **(Hess, 2013; Adair and McFerran, 2008)**. The great variation in the mortality rate associated with FAdV is due to the degree of virus pathogenicity, presence of secondary infections and chickens' susceptibility **(Grgic *et al.*, 2011)**.

FAdV has never been isolated or even recorded by any means of molecular characterization before in Egypt neither in commercial nor live bird market. This is spite it has been reported worldwide and clinical cases of IBH and hydropericardium syndrome continue increase in the world, provoking substantial economic losses in various geographical areas (**Zhao *et al.*, 2015**). Furthermore, none of the poultry flocks is vaccinated against FAdV.

So this study aims to:

1. Identify the presence of fowl adenovirus circulating in commercial poultry farms in Egypt.
2. Isolation & molecular characterization of Fowl Adenovirus.
3. Determination of the pathogenicity degree of the isolated virus following oropharyngeal administration.

The information is fundamental to monitor and establish a better control strategy for FAdV in Egypt.

Chapter (2)

Review of Literature