



Cairo University
Faculty of Veterinary Medicine



**Molecular Epidemiological Studies on Avian Influenza (H9N2) and
Infectious Bronchitis (IB) Viruses Affecting Respiratory System
in Chickens in Lebanon**

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Abstract

Avian influenza and infectious bronchitis viruses are pathogens with economic importance in poultry industry. To determine the role of these viruses in respiratory diseases outbreak in Lebanese poultry farms, 347 serum samples from 26 flocks with respiratory disease symptoms were examined by ELISA tests. Some of these flocks had received influenza A and infectious bronchitis viruses' vaccines. The overall antibody titers and of AIV H9 subtype and infectious bronchitis viruses were recorded. The results revealed the presence of both viruses in some flocks at the same time.

Also; tracheal swabs and tissue organs were collected from 60 poultry flocks from different ages, different type of raising which were housed in different Governorates in Lebanon including, broilers, layer hens, breeders, backyard chickens and ducks for molecular characterization by real time- RT-PCR. Sequence and phylogenetic analysis of some of the H9N2 and IBV-positive samples have been conducted. All flocks had shown respiratory signs and mortality. The real time- RT-PCR results have shown 12 flocks to be positive to infection with IBV and eight were positive to infection with H9N2. A new lineage of H9N2 virus from the last analysed sequences and VAR2 IBV has been detected for the first time among Lebanese poultry flocks and had shown 99% similarity with VAR2 genotype present in Egypt. even in vaccinated chickens against H9N2 and IBV respectively.

Conclusion: H9N2 and VAR2-IBV are reported to be circulating and affecting chickens with mortalities and loss of production in Lebanese poultry farming.

Key words: H9N2, IBV, Lebanon, respiratory disease, ELISA, rt-RT-PCR.

Dedication

To my Beloved Mother,

My Sisters Abir & Lina

My Son Bassem

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List of Abbreviations

Ab/s	Anti-body/ies
AC-ELISA	Antigen capture-enzyme linked immunosorbent assay
ACoVs	Avian coronaviruses
Ag-C-ELISA	Antigen-capture ELISA
AGID	Agar gel immunodiffusion
AI	Avian influenza
AIV	Avian influenza virus
Au	Gold
bELISA	Blocking ELISA
BLAST	Basic local alignment search tool
CAM	Chorioallantoic membrane
cDNA	Complementary DNA or copy DNA
CL^{pro}	Chymotrypsin-like proteinase
CoV/s	Corona virus/es
CT	cycle threshold
CTD	C-terminal domain
DAS-ELISA	Double antibody sandwich-ELISA
DIVA	Differentiating infection from vaccination administration
dsDNA	Double stranded DNA
E	Small envelope protein
ECE	Embryonated chicken eggs
ELISA	enzyme linked immunosorbent assay
EM	Electron microscopy
FP	Fusion peptide
H9N2	hemagglutinin 9, neuraminidase 2
HA	Hemagglutinin/ hemagglutination
HI	Hemagglutination inhibition
HPAI	Highly pathogenic avian influenza
HR	Heptad-Repeat region
HRP	Horse radish peroxidase
HVR	Hypervariable region

IA	Influenza virus
IAV	Influenza virus type A
IBD	Infectious bursal disease
IBV	Infectious bronchitis virus
IFA	Immunofluorescent assay
ILTV	Infectious laryngotrachitis virus
IPA	Immunoperoxidase assay
LP	Low pathogenic
LPAI	Low pathogenic avian influenza
LPM	Live poultry market
LT	Laryngotrachitis
M or M1	Membrane/ matrix protein
M2	Membrane ion channel
Mab/s	Monoclonal antibody/ies
Mass	Massachusetts
M/RT-PCR	Multiplex RT-PCR
N	Nucleoprotein
NA	Neuraminidase
NASBA	Nucleic acid sequencing-based amplification
NDV	Newcastle disease virus
NEP	Nuclear export protein
NES	Nuclear export signal
NP	Nucleoprotein
NS	Non-structural
nsps	Nonstructural proteins
NTD	N-terminal domain
ORF/s	Open reading frame/s
ORT	ornithobacterium rhinotracheitis
P	Polymerase
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction

PhCoV/s	Pheasant coronavirus/es
PL^{pro}	Papin-like proteinase
RBCs	Red blood cells
RBM	Receptor binding motif
RBS	Receptor binding site
RdRp	RNA dependent RNA polymerase
RFLP	Restriction enzyme fragment length polymorphism
RNP	Ribonucleoprotein
Rt-RT-PCR	Real time reverse transcriptase polymerase chain reaction
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-polymerase chain reaction
S	Spike
S1-CTD	S1- C-terminal domain
S1-NTD	S1- N-terminal domain
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SD/1, 2	Subdomain/ 1-2
S-ELISA	Sandwich ELISA
SP	Signal peptide
SPF	Specific pathogen free
ssRNA	Single stranded RNA
TCoV/s	Turkey coronavirus/es
TEM	Transmission electron microscope
TM	Transmembrane
TOC	Tracheal organ culture
UK	United Kingdome
UTR	Untranslated region
VI	Virus isolation
VLP/s	Virus-like particle/s
VN VNT	Virus neutralization Virus neutralization test
vRNP/s	Viral ribonucleoprotein/s

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Chapter (1)

Introduction