**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

Thesis submitted by

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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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# Contents

# 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**<br>**VNT** | Virus neutralization<br>Virus neutralization test |
| **vRNP/s** | Viral ribonucleoprotein/s |

# List of Tables

**Tables of Chapter (3) published paper (1): Serosurveillance and Molecular Characterization of H9N2 Avian Influenza Virus in Lebanon**



**Tables of Chapter (3) published paper (2): Molecular Characterization of VAR2 among IBV Infected poultry in Lebanon**

# List of Figures

**Figures of Chapter (2); Review of Literature**

**Figures of Chapter (3) published paper (1): Serosurveillance and Molecular Characterization of H9N2 Avian Influenza Virus in Lebanon**



**Figures of Chapter (3) published paper (2): Molecular Characterization of VAR2 among IBV Infected poultry in Lebanon**

*Chapter (1)*

# Introduction