# بسم الله الرحمن الرحيم


MONA MAGHRABY

# شبكة المعلومات الجامعية

# التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

# جامعة عين شمس

# التوثيق الإلكتروني والميكروفيلم

# قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

# يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

***MONA MAGHRABY***

Cairo University
Faculty of Veterinary Medicine
Department of Virology

# Rapid methods for detection of Equine Herpes Virus 1 in Arabian Horses in Egypt

**Thesis Presented**
**By**

**Ahmed Mohammed Ahdy Attia**
**(B.V.Sc., Mansoura University, 2005)**
**(M.V.Sc.,Mansoura University,2010)**

**For The Degree of**
**Master in Veterinary Medical Science**
**(Virology)**

**Under supervision of**

**Prof. Dr. Ahmed Abd El-Ghani El- Sanousi**
**Professor of Virology**
**Faculty of Veterinary Medicine**
**Cairo University**

**Prof. Dr. Mohamed Abd El-Hameed Shalaby**
**Professor of Virology**
**Faculty of Veterinary Medicine**
**Cairo University**

**Prof. Dr. Ibrahim Mohamed Farag Diab**
**Researcher professor of Cell Biology**
**National Research Center**

**2021**

# Approval Sheet

This is to approve that Thesis presented by

**Ahmed Mohammed Ahdy attia**

For the degree of MV.Sc. (Virology) has been approved by the examining committee

***Prof. Dr. Samy abd ElSalam Khalil***
Professor of Microbiology and Dean
of faculty of veterinary medicine
alex University

***Prof. Dr- Haitham Mohamed Mahmoud Amer***
Professor and Head of Department of Virology
Faculty of Veterinary Medicine
Cairo University.

***Prof. Dr- Ibrahim Mohamed Farag Diab***
Researcher Professor of Cell Biology
National Research Center
(Supervisor)

***Prof. Dr- Mohamed Abd El-Hamid Shalaby***
Professor 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).

***2021***

Cairo University
Faculty of Veterinary Medicine
Department of Virology

# Supervision Sheet

**Prof. Dr. Ahmed Abd El-Ghani El- Sanousi**
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

**Prof. Dr. Mohamed Abd El-Hameed Shalaby**
Professor of Virology
Faculty of Veterinary Medicine
Cairo University

**Prof. Dr. Ibrahim Mohamed Farag Diab**
Researcher professor of Cell Biology
National Research Center

**(2021)**

**Cairo University**
**Faculty of Veterinary Medicine**
**Department of Virology**

**Name: Ahmed Mohammed Ahdy Attia**
**Title of thesis:" Rapid methods for detection of Equine Herpes Virus type one in Arabian Horses in Egypt"**
**Scientific degree: Master's Degree(M.V.Sc.)**

**Supervisors:**

| | |
|---|---|
| **Prof. Dr. Ahmed Abd El-Ghani El- Sanousi** | **Professor of Virology**<br>**Faculty of Veterinary Medicine**<br>**Cairo University** |
| **Prof. Dr. Mohamed Abd El-Hameed Shalaby** | **Professor of Virology**<br>**Faculty of Veterinary Medicine**<br>**Cairo University** |
| **Prof. Dr. Ibrahim Mohamed Farag Diab** | **Researcher professor of Cell Biology**<br>**National Research Center** |

## Abstract

Equid Herpesvirus 1 (EHV-1) is the most important virus causing pathological disorders in horses. It causes recurrent outbreaks of abortion and neurological disorders with high mortality in Arabian horses in Egypt. As EHV1 is a very contagious disease, rapid and accurate diagnosis is important to broaden our understanding of EHV-1 in the field and implement proper preventive and control measures. Samples were collected from sixty six clinical cases over a period from 2016 to 2019. All samples originated from Arabian horse studs with respiratory, abortigenic and neurological outbreaks in Cairo and Giza governorates. EHV1 was diagnosed preliminarily in these clinical cases by immunohistochemistry using monoclonal antibody against EHV1 glycoprotein B and then by molecular detection using real time PCR which diagnosed EHV-1infection in twenty five samples within short time in less than three hours including DNA preparation. Molecular characterization of glycoprotein B (gB, ORF33) gene was applied for confirmation. Molecular characterization revealed that genetically identical EHV-1 strains were still circulating in Egypt. These strains were closely related to the European EHV-1 strains. Furthermore, EHV-1 sequences from this study showed little or no differences for the amino acid's sequences compared to previously published sequences. This study would be valuable for monitoring of EHV-1 infection in Egypt and determining the gB gene sequence of newly identified EHV-1 field strains which is the most conserved region in the viral DNA and frequently used as a target for diagnostic PCR protocols for the future outbreaks.

**Keywords**: qPCR, EHV-1, glycoprotein B gene (gB, ORF 33), Immunohistochemistry, Abortion, Arabian horses, Egypt.

# *Dedication*

*Dedicated to my Family*

*…Father*

*…Mother*

*….Wife*

*…..My Brothers and*

*Sisters*

# *Acknowledgments*

*All thanks to our merciful God ALLAH who gave me all the graces I enjoy in my life .His magnificent help and compassion gave me the ability and patience to finish this work.*

*I would like to express my gratitude towards* ***Prof.Dr. Ahmed Abd El-Ghani El-Sanousi,*** *professor of virology department, faculty of veterinary medicine Cairo university , for the great support , objective criticism and precious supervision all through the work. Also for this sincere guidance with encouragement during his direct supervision throughout the work.*

*I wish to express my gratefulness and thanks to* ***Prof.Dr. Mohamed Abd El –Hameed Shalaby*** *, professor of virology department, faculty of veterinary medicine Cairo university for, his valuable advices encouragement and support during the whole study.*

*I wish to express my whole gratefulness and special thanks to* ***prof.Dr,Ibrahim Farag.*** *for endless help.*

*Also I would like to express my deepest respect and sincere gratitude to* ***Prof. Dr. Haitham Mohamed Amer,*** *chairman of virology department, faculty of veterinary medicine Cairo university*

*Grateful thanks and sincere gratitude are extended to the Virology Lab members;* ***Dr Bassem Ahmed and Dr.Mahmoud El Gamal*** *for their great efforts and technical support during the whole study and lab work.*

*I would also like to thank* ***prof.DR. Eman Mahfouz*** *and* ***prof.Dr. Hassan Darwish*** *for continuous support and help.*

*Great thanks should be a admitted to* ***prof. Dr Ahmed Abd El Wahed,*** *University of Leipzig and* ***prof. Dr Walid el Azab*** *,Freie Berlin university , for their continuous follow up as well as supplying me with very important reagents and biological materials that helped me in conduction of work.*

*I would also like to thank all my colleagues and all chairmans of National research center and Egyptian agriculture organization for their help.*

*Lastly my cardinal thanks to my beloved Family and faithful friends for their kind patience, sacrifice and acceptance of the long hours I spent in performing this work.*

# Contents

# List of Tables

# List of Figures

# List of Abbreviation

| | |
|---|---|
| A | Adenine |
| AA | Amino Acid |
| ADCC | Antibody-Dependent Cellular Cytotoxicity |
| AGID | Agar Gel Immunodiffusion |
| AHV-1 | Asenine Herpes Virus Type 1 |
| AHV-2 | Asenine Herpes Virus Type 2 |
| AHV-3 | Asenine Herpes Virus Type 3 |
| APC | Antigen Presenting Cells |
| APHIS-USDA | Animal And Plant Health Inspection Service- U.S. DEPARTMENT OF AGRICULTURE |
| BAC | Bacterial Artificial Chromosome |
| BHK | Baby Hamster Kidney |
| BMC | Blood Mononuclear Cells |
| BoHV | Bovine Herpesvirus |
| bp | Base Pair |
| C | Cytosine |
| CAM | Chorioallontoic Membrane |
| CD | Cluster Of Differentiation |
| CF | Complement Fixation |
| CMI | Cell Mediated Immunity |
| CNS | Central Nervous System |
| CSF | Cerebrospinal Fluid |
| CPE | Cytopathic Effect |
| CSPG | Chondroitin Sulfate Proteoglycans |
| Ct | Cycle Threshold |
| CTL | Cytotoxic T Cells |
| CTLp | Cytotoxic T Cells Precursor |
| D | Aspartic Acid |
| DNA | Deoxyribonucleic Acid |
| DNase | Deoxy Ribonuclease Enzyme |
| dpi | Day Post Infection |
| DPOL | DNA Polymerase |
| dsDNA | Double-Stranded DNA |
| E Derm | Equine Derm Cells |
| EAV | Equine Abortion Virus |

| | |
|---|---|
| EBV | Epstein Barr Virus |
| EC | Endothelial Cells |
| ECE | Embryonated Chicken Egg |
| ECM | Extracellular Matrix |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| EEK | Equine Embryonic Kidney |
| EEL | Equine Embryonic Lung |
| EFKI | Equine Fetal Kidney Cells |
| EHM | Equine Herpes Myeloencephlopathy |
| EHV | Equine Herpes Viruse |
| EHV-1 | Equine Herpes Virus Type 1 |
| EHV-2 | Equine Herpes Virus Type 2 |
| EHV-3 | Equine Herpes Virus Type 3 |
| EHV-4 | Equine Herpes Virus Type 4 |
| EHV-5 | Equine Herpes Virus Type 5 |
| EHV-8 | Equine Herpes Virus Type 8 |
| EHV-9 | Equine Herpes Virus Type 9 |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EM | Electron Microscope |
| EMPF | Equine Multinodular Pulmonary |
| ERV | Equine Rhinopneumonitis Virus |
| EVA | Equine Viral Arteritis |
| E% | Amplification Efficiency |
| FA | Fluorescent Antibody |
| FAT | Fluorescent Antibody Technique |
| FAM | 6-Carboxyfluorescein |
| FC | Fragment Crystallizable Region |
| fg | Femto Gram |
| G | Guanine |
| g | G Force |
| gB | Glycoprotein B |
| gC | Glycoprotein C |
| gD | Glycoprotein D |
| gE | Glycoprotein E |
| gG | Glycoprotein G |
| gH | Glycoprotein H |
| GHV-1 | Gazelle Herpes Virus Type 1 |