Cairo University
Faculty of Veterinary Medicine
Department of Virology

Molecular characterization of equine herpes virus from clinically infected horses in Egypt

Thesis submitted

by

Soha Ibrahim Ftouh Mohamed

(B.V.Scs. Fac. Vet .Med ., Menofia Univ. (Sadat branch) ,2005).

Under Supervision of

Prof .Dr. Mohamed Abd El-Hamid Shalaby

Prof. of Virology & Immunology

Faculty of Veterinary Medicine

Cairo University

Dr. Ayman H. El Deeb
Lecturer of Virology
Faculty of Veterinary Medicine
Cairo University

Dr. Sayed A. Hasan Salem
Chief Researcher of virology department
Animal Health Research Institute.
Dokki,Giza

To
Cairo University,

Faculty of Veterinary Medicine,

For The Master Degree in Veterinary Medical Sciences

(Virology)

(2016)

بسم الله الرحمن الرحيم

وَلَوْ أَنَّهُمْ رَضُواْ مَا آتَاهُمُ اللَّهُ وَرَسُولُهُ وَقَالُواْ حَسْبُنَا اللَّهُ سَيُؤْتِينَا اللَّهُ مِن فَضْلِهِ وَرَسُولُهُ إِنَّا إِلَى اللَّهِ رَاغِبُونَ (59)

صدق الله العظيم

سورة التوبة

Supervision sheet

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Name	**Soha Ibrahim Ftouh Mohamed**
Nationality	**Egyptian**
Date of birth	**19/9/1983**
Place of birth	**Menofia**
Specification	**Virology**
Title of thesis	Molecular characterization of equine herpes virus from clinically infected horses in Egypt

Abstract

Equine herpes viruses 1 and 4 are the most important viruses affecting horses of all ages and other members of Equidae family (donkeys and mules) causing extensive economic losses from abortion , neonatal foal death and respiratory diseases. Infection caused by EHV-1 and 4 typically result in establishment of latent infection within the first weeks or months of life with subsequent viral reactivation causing clinical disease and viral shedding during periods of stress.Three hundred sera sample were examined for the presence of specific antibodies against equine herpes virus 1 and 4 in horses, mules and donkeys from 6 governorates in Egypt by indirect ELISA test .Detection of EHV-1 and EHV-4 by nested multiplex PCR and sequence analysis . Isolation of EHV-1 on tissue culture (MDBK cells) and identification by indirect fluorescent antibody technique (IFAT) . Results obtained by ELISA revealed that 234 samples were found positive for the presence of EHV-1 and 4 antibodies with total percentage 78%. One sample was found positive for EHV-1 and three samples for EHV-4 by nested multiplex PCR. One tissue sample from aborted Arabian fetus retrieved EHV-1 cDNA equivalent to 145 bp viral glycoprotein B specific primer

sets using nested multiplex PCR assay . The strain was designated (EHV-1 Egy 2016 glycoprotein B gene partial cds) .Sequence analysis of 145 bp of the amplified fragment for phylogenetic construction revealed 100% compatibility with (Equid herpesvirus 1 isolate CP4 Germany 2016) Gen Bank reference isolate. One tissue sample from aborted Arabian fetus retrieved EHV-4 cDNA equivalent to 206 bp viral glycoprotein B specific primer sets using nested multiplex PCR assay . The strain was designated (EHV-4 Egy 2016 glycoprotein B gene partial cds) .Sequence analysis of 206 bp of the amplified fragment for phylogenetic construction revealed 100% compatibility with (EHV-4 US4 TR2011, EHV-4 TR-EHV4-TCO-11 Turkey 2011 , Equid herpesvirus 4 isolate ER39-67, Equid herpesvirus 4 isolate 3407-77 and Equid herpesvirus 4 isolate 1546-99 Australia 2015 and EHV-4 isolate AQHY12LT2014/1/HM Japan /USA 2014) Gen Bank reference isolates. Virus isolation of EHV-1 was conducted from the positive sample by nested multiplex PCR showing CPE on MDBK tissue culture cells and then the isolate was identified by IFAT .

The PCR technique proved to be more sensitive, rapid and efficient for diagnosis of EHV -1 & 4 infection, while ELISA technique is more sensitive for detection of EHV-1 and 4 antibodies. This study will help in planning for vaccination against Egyptian isolates.

Key words: Arabian horses, Egypt, EHV-1, EHV-4, IFAT, nested multiplex PCR, ELISA.

Dedication

To

The Spirit of My Lovely Sister Nagwa

Acknowledgement

I am thanking at first Allah who gave me everything I have.

Professor .Dr.Mohamed Abd El-Hamid Shalaby main supervisor, I would like to express my great appreciation and thanks for his great interest, never ending source of ideas and support regard to my academic and research during entire period of master program.

Dr. Ayman H . El Deeb, lecturer of virology, I highly appreciate his good and constructive advices during entire period of master program .

Dr. Sayed A. Hasan Salem, I would like to express the deepest appreciation, sincere thanks and gratitude for his persistent ,valuable guidance, encouragement and help during entire period of master program. I never forget his endless support and contribution in academic and research. I highly appreciate his good and constructive advices.

Our Virology department members,I highly appreciate for their continuous support and enthusiastic help, friendly discussions, good advices for academic progress and in the research work, I highly appreciate for their moral and support during my work on thesis .

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