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Faculty of Veterinary Medicine
Department of Virology**

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

Thesis submitted

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بسم الله الرحمن الرحيم

وَلَوْ أَنَّهُمْ رَضُوا مَا آتَاهُمُ اللَّهُ
وَرَسُولُهُ وَقَالُوا حَسْبُنَا اللَّهُ
سَيُؤْتِينَا اللَّهُ مِنْ فَضْلِهِ وَرَسُولُهُ
إِنَّا إِلَى اللَّهِ رَاغِبُونَ (59)

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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

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CONTENTS

Item	Page No.
1. Introduction	1
2. Review of Literature	4
2.1. Historical background of Equine herpes viruses	4
2.2. Economic Importance	7
2.3. The Virus	8
2.3.1. Taxonomy and classification	8
2.3.2. Physical and chemical properties	9
2.3.2.1. Shape and size	9
2.3.2.2. EHV's glycoproteins	9
2.3.2.3. Viral Replication	11
2.3.2.4. The tegument	13
2.3.2.5. The capsid	14
2.3.2.6. The nucleic acid	14
2.3.3. Virus stability	16
2.3.3.1. Effect of temperature	16
2.3.3.2. Effect of PH	16
2.3.3.3. Effect of humidity	16
2.3.3.4. Effect of antibiotics	17
2.3.3.5. Effect of chemical agent	17
2.3.3.6. Effect of field condition	17
2.3.4. Biological properties	17
2.3.4.1. Antigenic properties and antigen relationship	17
2.3.4.2. Virus pathogenesis and tropism	19
2.3.4.3. Haemagglutination character	20
2.3.4.4. Plaque character	20



2.3.4.5.Latent character	20
2.3.5. Propagation and adaptation in laboratory host	21
2.3.5.1.Laboratory animals	21
2.3.5.2. Mice	22
2.3.5.3.Chicken embryo	23
2.3.5. 4.Tissue culture	24
2.4.Host range	26
2.4.1.Horses	26
2.4.2.Ponies	26
2.4.3.Donkeys	27
2.4.4.Zebra	27
2.5. Transmission	28
2.6.Diagnosis	28
2.6.1.Clinical signs	28
2.6.2.Virus isolation	29
2.6.3.Sero-diagnosis	31
2.6.3.1.Complement fixation test (CFT)	31
2.6.3.2.Agar gel precipitation test (AGPT)	32
2.6.3.3.Serum neutralization test (SNT)	32
2.6.3 .4.Enzyme Linked Immunosorbent Assay (ELISA)	33
2.6.3.5.Passive haemagglutination test (PHA) and (HI)	34
2.6.3.6.Flourescent antibody technique (FAT)	35
2.6.4.Polymerase chain reaction (PCR)	35
2.6.5.Sequencing	39
2.7.Natural immunity.	39
3.Material and Methods	41
3.1.Material	41



3.1.1.Sampling and sample preparation	41
3.1.1.1. Serum samples	41
3.1.1.2. Swabs	42
3.1.1.3.Tissues of aborted feti	42
3.1.2.Methods of samples collection and preparation	44
3.1.2.1.Collection and handling of serum samples for serological studies	44
3.1.2.2.Collection of swabs	44
3.1.2.3. Collection of aborted fetal tissues	44
3.2.Detection of the equine herpes virus 1 and 4 antibodies in collected serum samples by ELISA test	45
3.2.1.Reagents and solutions used for indirect ELISA technique	45
3.2.2.Method of detection of EHV-1 and 4 in equines sera using indirect ELISA technique	45
3.3.Detection of the equine herpes virus 1 and 4 DNA using nested multiplex PCR	47
3.3.1.Reagents used for DNA Extraction	47
3.3.2. Method of extraction of viral DNA	47
3.3. 3.Determination of isolated DNA	49
3.3.4. Amplification of EHV DNA using nested multiplex PCR	49
3.3.4.1. Reagents used for nested multiplex PCR	49
3.3.4.2. Method of DNA amplification (PCR)	51
3.4. Gel electrophoresis	51
3.4.1. Chemicals used for electrophoresis	51
3.4.2. Method of identification of PCR products	52
3.5.DNA sequencing	53
3.5.1. Material of DNA sequencing	53
3.5.1.1.Amplified DNA (2 nd nested multiplex –PCR products)	53