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Faculty of Veterinary Medicine
Department of Medicine and Infectious Diseases

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Epidemiological and immunological studies on some viral equine infections causing reproductive problems in Egypt

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Abstract

Studying the prevalence of Equine herpes virus-1(EHV-1), Equine herpes virus-4(EHV-4) and Equine Viral Arthritis (EVA) infection among equines in different governorates of Egypt. Evaluating the indirect Enzyme-Linked Immuno-Sorbent Assay (ELISA) for the diagnosis of them in comparison with the virus isolation. Assessing the efficacy of Polymerase Chain Reaction (PCR) for the diagnosis of the viral infection by using the horses nasopharyngeal swabs isolated virus and the aborted foeti tissues. One hundred eighty two equines of different ages, sexes, localities and breeds showing signs of fever, respiratory manifestations, abortions, ataxia, hind limb paralysis, limb edema, foal depression and death; were examined. The viral isolation on both histopathology and Baby Hamster Kidney (BHK) cell culture, the aborted foeti were histologically examined for the detection of inclusion bodies, the serum samples were collected to detect Immunoglobulin-G specific to EHV-1,EHV-4 and EVA by using ELISA, EHV-4 and EVA were negative .the horses nasopharyngeal swabs isolated virus and the aborted foeti tissues were tested by PCR using specific primers to EHV-1 to confirm the infection with EHV-1.The prevalence of EHV-1 infection in the examined animals were recorded as 4.94%. it was prevalent in Cairo, Giza, Kafr Elsheikh, Monofeia, El Sharkia governorates. The EHV-1 was isolated on the embryonated chicken eggs and the pock lesion was appeared on the chorio-allantoic membranes. The cytopathic effects were also observed on the tissue culture. The Liver of aborted foeti showing necrosis of all hepatic tissue and activation of kuffer cells with heamosidren and the detection of

esinophilic Intranuclear inclusion bodies. Indirect ELISA could detect IgG in all infected equines(N=9) from which EHV-1 isolated. PCR proved the infection with EHV-1 in the aborted foeti (N=3) tissues and gave the similar results by using the horses nasopharyngeal swabs isolated virus where 489 bp PCR products were detected in both. EHV-1 is prevalent in Egypt in different governourates. EHV-1 infection could diagnosed by intranuclear esinophilic inclusion bodies in the aborted foeti tissues. The indirect ELISA could diagnose EHV-1 infection in all ages and sexes groups. PCR on aborted foeti tissues is better for the diagnosis of EHV-1 infection than that on the horses nasopharyngeal swabs isolated virus because it save time and money. the study indicated that there are no EHV-4 and EVA infection found in Egypt.

Keywords: EHV-1, EHV-4 , EVA,ELISA, PCR , diagnosis , Egypt.

DEDICATION

I dedicate my thesis

to my family especially my parents, my wife,

my daughters and my Son

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1-INTRODUCTION

There is a great interest with equine industry all over the world, especially in Egypt and Arabian countries which plays an important role in the national income. Abortion in horses may result from a variety of infectious agents, such as bacteria, viruses or fungi that attack the fetus or its membranes, resulting in fetal death and expulsion.

EHV-l is one of group of alpha-herpes viruses. Both EHV-l and EHV-4 have extensive antigenic cross-reactivity and were previously considered subtypes of the same virus (EHV-l). DNA fingerprinting has demonstrated both the genetic divergence of the two virus species (Radostitis, *et al*. 2007). EHV-1 is an imported pathogen and has been responsible for causing four recognized clinical syndromes in horses throughout the world: upper respiratory disease in young horses, abortions in late pregnant mares, perinatal foal mortality and occasionally neurological disorders (Reed and Toribio, 2004).

Economic losses caused by equine herpes virus -1 is summarized in abortion and loss of fetus, death of horse due to encephalomyeilitis, loss of semen of infected stallion, loss of infected newborn foals and loss of training time and opportunities to perform during convalescence and quarantine. Abortion in affected mares can occur at any time within 1-14 days after the onset of the disease. Most mares abort during the last half of gestation (5-10months). Incidence of abortion is up to 50 percent. The disease

can cause death in horses with a course of disease of 2-15 days. In some cases, the foal will be born alive at term and will die shortly after birth due to infection by the virus. The abortion rate may approach 100% in a herd of susceptible mares. The vaccines that are currently available are the best we have but are of questionable value in preventing abortion, **Amer *et al*. (2011).**

Diagnosis of EHV-1 must be rapid and sensitive so early intervention policies aimed to reduce the virus spread. Routine diagnosis of EHV-1 infection in live animals is usually achieved by virus isolation in SPF embryonated chicken egg, cultured cells from nasopharyngeal secretions or from the tissues of aborted fetus **(Elia *et al*., 2006**). Several rapid and innovative diagnostic techniques based on PCR and enzyme linked immunosorbent assay (ELISA) were applied for the diagnosis of EHV-1 infection **(Ataseven *et al*., 2009).**

Equine Herpesvirus-1 (EHV-1) abortion virus is most often associated with abortions in mares, while Equine Herpesvirus (EHV-4) rhinopneumonitis virus is usually associated with respiratory disease in young horses. Both subtypes have the potential to cause respiratory disease and abortion. Vaccines are available as aids to prevent abortion due to EHV-1 infections. The herpes family of viruses has the capacity to persist in the body of its host in a dormant state as an inapparent carrier after the primary infection. Months or years after the primary infection, the latent herpesvirus may again become manifest with renewed replication and with the potential for initiating new outbreaks of disease in its host as well as susceptible

stable mates. Therefore, it is the existence of these latently infected carrier horses, from which the virus is re-activated by stress-induced circumstances and shed into the environment to infect other individuals, that initiates a new outbreak of the disease **OIE (2015).**

Equine Viral Arteritis virus (EVA) has the potential to cause abortion as well as the more commonly observed contagious respiratory disease and semen-shedding state in infected carrier stallions. No EVA-related abortions were diagnosed at the Animal Health Laboratory, University of Guelph, from 1998 to 2004. By inhalation primarily, also transmission from infected weanlings to broodmare. Mares aborting can transmit virus from aborted fetus, placenta and fluids. This virus can also be spread by anyone handling infective material. Abortion in affected mares can occur at anytime within 1-14 days after the onset of the disease. Most mares abort during the last half of gestation (**5-10 months)**. Incidence of abortion up to 50 percent. The disease can cause death in horses - course of disease from 2-15 days (Afify *et al*. 2013).

The importance of the equine viruses causing reproductive problems forcing us to studying them as summarized in *our research work goals* as follows:

1- Recording the epidemiological data of equine viral diseases causing reproductive problems (equine herpes viruses 1(EHV-1), equine herpes virus 4 (EHV-4) and equine viral arteritis virus (EVA).

2- Identifying the equine viruses causing reproductive problems by the virus isolation and characterization.
3- Investigating the foetal tissues alterations by Gross and histopathological examination of the aborted foeti and their foetal membranes.
4- Screening the infected equines and their contacts by indirect enzyme immunosorbent assay on the collected sera for EHV-1, EHV-4 and EVA.
5- Evaluating polymerase chain reaction on the isolated virus and the aborted foeti tissues for the diagnosis of the equine viral infections causing reproductive problems.

2 - REVIEW OF LITERATURES

2.1.EquineHerpes viruses (EHV) :

2.1.1 Epidemiology:

Foote, *et al*. (2004) indicated that a silent cycle of equine herpesvirus 1 infection has been described following epidemiological studies in unvaccinated mares and foals. In 1997, an inactivated whole virus EHV-1 and EHV-4 vaccine was released commercially in Australia and used on many stud farms. However, it was not known what effect vaccination might have on the cycle of infection of EHV-1. To investigate whether EHV-1 and EHV-4 could be detected in young foals from vaccinated mares. Nasal and blood samples were tested by PCR and ELISA after collection from 237 unvaccinated, unweaned foals and vaccinated and nonvaccinated mares during the breeding season of 2000. EHV-1 and EHV-4 DNA was detected in nasal swab samples from foals as young as age 11 days. These results confirm that EHV-1 and EHV-4 circulate in vaccinated populations of mares and their unweaned, unvaccinated foals. The evidence that the cycle of EHV-1 and EHV-4 infection is continuing and that very young foals are becoming infected should assist stud farms in their management of the threat posed by these viruses.

Borchers, *et al*. (2005) collected a total of 51 sera from a migratory population of Burchell's zebras (Equusburchelli) in the Serengeti National Park (Tanzania) between 1999 and 2001 to assess