Highlighting on viral equine abortion syndrome in some regions in Egypt

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ABSTRACT

Equine herpes virus types 1 and 4 (EHV-1, EHV-4), Equine arteritis virus (EAV) and Equine infectious anemia virus (EIA) are viral causes responsible for significant economic losses in Equine due to abortion and neonatal mortality. In this study, 600 serum samples were collected from horses and donkeys in 6 Egyptian Governorates (Cairo, Alexandria, Giza, Minya, Monufia, and Qalyubia) between 2018 and 2021. Indirect ELISA kits were used for the detection of antibodies against EHV-1, EHV-4, EAV and EIA. Our results revealed that 173 samples out of 600 tested positive for EHV-1 and EHV-4 (28.83%) and 16 samples out of 600 tested positive for EAV (2.67%). However, none of the samples tested positive for EIA. In conclusion, our study revealed that EHV-1 and EHV-4 are one of the most important causes of abortion in Equine and the results of serosurveillance indicated the circulation of EHV-1, EHV-4 in horses and donkeys due to their latency and reactivation from time to time, or due to the horses movement and contacts with other potentially infected horses. Antibodies against EAV due to that percentage of infected horses become persistent infected carriers.

INTRODUCTION

EHV-1, EHV-4 cause economic losses in equine, particularly Arabian horses due to abortion and neonatal mortality (Lunn et al. 2009). EHV-1, EHV-4 have a significant economic impact on the whole equine industry worldwide. EHV-1, EHV-4 cause a variety of diseases, including respiratory problems and abortion (Slater, 2007). EHV-1, EHV-4 are linear double-stranded DNA viruses belong to the family Herpesviridae, subfamily Alphaherpesvirinae and genus Varicellovirus (Davison et al. 2009). Direct contact with EHV-1 infected aborted fetuses, placental tissues, or fomites contaminated by respiratory secretions can cause infection (Reed et al. 2004). EHV-1 and EHV-4 are the most important types (Pavulraj et al. 2021), due to latency, EHV-1, EHV-4...
EIA is caused by Equine Infectious Anemia Virus (EIAV), a lentivirus classified within the *Retroviridae* family, genus *Orthoretrovirinae* ([ICTV], 2017) which infects Equine. EIA has a worldwide distribution ([OIE], 2013a), being one of the eleven Equine diseases requiring compulsory notification to the World Organization for Animal Health ([OIE], 2018). Based on experimental infection of horses with EIAV, EIA can be characterized by fever, diarrhea, lethargy, anemia and thrombocytopenia, associated with a high-level viremia in plasma and abortion. Over time the frequency of disease episodes and the severity of clinical signals typically decrease; thus, persistently infected horses become clinically asymptomatic for EIA indefinitely, reaching the asymptomatic carrier stage and remaining infected for life ([Craigo and Montelaro], 2013). EIAV is primarily transmitted mechanically through infected blood via arthropod vector, most commonly the bite of blood sucking insects of the Diptera order, or by contaminated fomites, which can be transmitted through blood-contaminated instruments like syringes, needles, surgical instruments, and equine accessories ([Mealey], 2007). Transplacental transmission has also been reported ([Gregg and Polejaeva], 2009). ELISA has been approved for diagnosis of EIA, four ELISA kits were approved by the United States Department of Agriculture (USDA) ([Issel and Cook], 1993), but OIE states that positive results should be retested using agar gel immunodiffusion assay (AGID) to confirm diagnosis ([OIE], 2013a).

The current study aims to investigate the circulation of viral causes of abortion, particularly EHV-1, EHV-4, EAV and EIA in equine in Egypt.
2 MATERIALS and METHODS

2.1 Animals

In this study, 600 serum samples were obtained from horses and donkeys in 6 Egyptian Governorates (Cairo, Alexandria, Giza, Minya, Monufia, and Qalyubia) for serosurveillance between 2018 and 2021.

2.2 Collection and handling of serum samples for serological studies

Samples were collected in sterile vacutainer tubes by jugular vein puncture, which then subsequently labelled. These samples were transferred in a clean and dry ice box to the Virology Department at the Animal Health Research Institute (AHRI), El-Dokki, Giza, Egypt. The samples were kept at room temperature for one hour before being stored at 4°C overnight. For serum separation, the tubes were centrifuged at 1500 rpm for ten minutes. Clear non-hemolyzed serum was obtained and stored in sterile screw-capped vials at -20°C.

2.3 Serodiagnosis of EHV-1, EHV-4, EAV and EIA antibodies by indirect ELISA technique

2.3.1 Detection of EHV-1 and EHV-4 antibodies by indirect ELISA

Indirect ELISA commercial kit (IngezimRhinopneumonitis14.HVE.K1®, Ingenasa, Spain) was used for the analysis of 600 serum samples for detection of specific antibodies against EHV-1, EHV-4 as described by the manufacturer. Specific antibodies against the virus in serum samples bound the antigen adsorbed on a solid polystyrene plate. After washing to remove any non-fixed material, immunoglobulins then were detected using a particular anti-equine IgG–peroxidase conjugate. When the substrate solution was introduced to the wells, colorimetric reaction appeared then measured using an ELISA reader (Sanz et al. 1985).

2.3.2 Detection of EAV antibodies by indirect ELISA

Indirect ELISA commercial kit (INGEZIMARTERITIS, Spain) was used for the analysis of serum samples for detection of specific antibodies against EAV as described by the manufacturer. Positive and negative antigen were fixed on a solid polystyrene plate, specific antibodies against the virus in serum samples bound the antigen adsorbed on. After washing to remove any non-fixed material, immunoglobulins then were detected using a particular peroxidase conjugate. When the substrate solution was introduced to the wells, colorimetric reaction appeared then measured using an ELISA reader (Kondo et al. 1998).

2.3.3 Detection of EIA antibodies by indirect ELISA

Indirect ELISA commercial kit (EIAED vet 1114 GB IDvet, France) was used for the analysis of serum samples for detection of specific antibodies against EIA as described by the manufacturer, wells were coated with p26(Gene GAG) recombinant antigen that was fixed on a solid polystyrene plate, specific antibodies against the virus in serum samples bound the antigen adsorbed on forming antibody–antigen complex. After washing to remove any non-fixed material, immunoglobulins then were detected using two Fab (on IgG) or ten Fab (on IgM), the first one bound the immunoglobulins to the microplate and the other bound a peroxidase antigen that used as conjugate. When the substrate solution was introduced to the wells, colorimetric reaction appeared then measured using an ELISA reader. This method is suitable for detection of IgM and IgG antibodies, allow earlier testing of animals and long-term disease surveillance (Piza et al. 2007).

3 RESULTS

By using ELISA test for detection of antibodies against EHV-1, EHV-4, EAV and EIA of 600 horse and donkey in Egypt, our results revealed that 173 samples out of 600 tested positive for EHV-1 and EHV-4 (28.83%) and 16 samples out of 600 tested positive for EAV (2.67%). However, none of the samples tested positive for EIA as was shown in Tab.(1).
Table (1) Percentages of EHV-1, EHV-4, EAV, and EIA specific antibodies detected among horses and donkeys and the total number of positive serum samples in 6 Egyptian governorates by ELISA test

<table>
<thead>
<tr>
<th>Governorate</th>
<th>Total</th>
<th>EHV-1 &amp; 4 Positive (No.)</th>
<th>EAV Positive (No.)</th>
<th>EIA Positive (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairo</td>
<td>60</td>
<td>30 (50%)</td>
<td>6 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Alex.</td>
<td>102</td>
<td>30 (29.41%)</td>
<td>2 (1.96%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Giza</td>
<td>222</td>
<td>50 (22.52%)</td>
<td>6 (2.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Minya</td>
<td>150</td>
<td>60 (40%)</td>
<td>1 (0.67%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Monufia</td>
<td>51</td>
<td>3 (5.8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Qalyubia</td>
<td>15</td>
<td>0 (0%)</td>
<td>1 (6.67%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>173 (28.83%)</td>
<td>16 (2.67%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Based on the findings of this study, it was concluded that EHV-1, EHV-4 are widespread and one of the most common causes of reproductive disease in horses, causing abortion with a high likelihood of fetal mortality (Allen and Murray 2004; Patel and Heldens 2005). EHV-1, EHV-4 are highly contagious and is normally spread through direct contact between infected animals, primarily by infected nasal discharge, uterine discharge, and infected aborted fetuses between infected animals or animal and infected object (Reed et al. 2004).

EAV, being one of the most important reproductive viruses, have a significant economic impact on the equine industry (Timoney and McCollum, 1993). Following natural breeding or artificial insemination, carrier stallions transfer EAV venereally to susceptible mares. Infected mares can then spread the virus to other susceptible groups with whom they come into contact (Timoney et al. 1997). EIA has a worldwide distribution (OIE, 2013a), being one of the eleven Equine diseases requiring compulsory notification to the World Organization for Animal Health (OIE, 2018). EIAV is primarily transmitted mechanically through infected blood via arthropod vector, most commonly the bite of blood-sucking insects of the Diptera order, or by contaminated fomites, which can be transmitted through blood-contaminated instruments like syringes, needles, surgical instruments, and equine accessories (Mealey, 2007). Transplacental transmission has also been reported (Gregg and Polejaeva 2009).

Because of their sensitivity and specificity, ELISA assays are highly recommended and gold standard serological assays for successfully examining the existence of specific antibodies against EHV-1, EHV-4, EAV, and EIA in a large horse population (Lang et al. 2013; Issel and Cook, 1993). ELISA test was used for the serological evidence of silent cycle of EHV-1, EHV-4 infection in mares (Foote et al. 2006). The type specific ELISA is thought to be beneficial for sero-diagnosis and sero-epizootiological studies on EHV-1, EHV-4 infections in both unvaccinated and vaccinated
horses (Yasunaga et al. 2000). So, we used ELISA in our study by collecting 600 serum samples from horses and donkeys from 6 Egyptian governorates for detecting antibodies against EHV-1, EHV-4, our results revealed that 173 horse and donkey out of 600 were positive, the overall percentage 28.83% (173/600) Tab1, while in a recent ELISA based serological study conducted in Egypt during 2016, it was cleared that the overall apparent prevalence rate was 64% (173/270) (Emad et al. 2018). Also, ELISA based serological study was conducted in Egypt during 2015, for detecting antibodies against EHV 1,4 founding overall percentage 62.5% (75/120) (El Sayyad et al. 2015). The results of seroepidemiological investigation revealed the presence of antibody in Arabian horse and donkeys which means that circulation of EHV -1 and 4 is due to that the horses and donkeys having the virus in latent state and it reactivating from time to time, or due to that the horses moving in and out of the premises and having contact with other potentially infected horses and donkeys (Allen, 2002).

In Egypt, there was a little significant information regarding the prevalent EAV in Egyptian horse population. Serological studies indicate that EAV is widely distributed in equine populations around the world (Balasuriya et al. 2013 Balasuriya, 2014).

The seroprevalence of EAV infection can vary among horses of different breeds (Timoney and McCollum, 1993). The OIE recommended that ELISA was used only for serological surveillance studies for EAV, although the VNT is currently the most highly sensitive and specific serodiagnostic test for this virus but it is expensive, labor-intensive, and time-consuming to perform (OIE, 2013c), so we used commercial ELISA in our study for detection of EAV antibodies from 600 horse and donkey, our results revealed that 16 out of 600 were positive, the overall percentage 2.67%(16/600) Tab1. and this result was as was found by (Chung et al. 2013b), but the National Animal Health Monitoring System's Equine survey recorded that only 0.6 percent of the Quarter Horse population was EAV seropositive (NAHMS 2000), and this may due to that neutralising antibodies are discovered one to two weeks after exposure to the virus, peak between two and four months, and last for three years or longer. (Balasuriya et al. 1999; Balasuriya et al. 2002 Balasuriya et al. 2007), it was discovered that sero-positive stallions had developed clinically inapparent equine viral arteritis (EVA). Appearance of neutralising antibodies coincides with the disappearance of virus from the circulation of acutely infected horses (Fukunaga et al. 1981). However, virus can persist in the reproductive tract of the stallion for a variable period despite the presence of high titres of serum neutralising antibodies (Timoney and McCollum, 1993).

Also, in Egypt, there was a little significant information regarding the prevalent EIA in Egyptian horse population. So, we made this serosurveilance by using ELISA test for diagnosis of 600 serum samples from horses and donkeys. In addition, serology involving enzyme-linked immunosorbent assays (ELISA) has also been approved for EIA diagnosis. Four ELISA kits were approved by the United States Department of Agriculture (USDA) and are internationally available for EIA diagnosis. However, the OIE states that a positive result by ELISA should be retested using the AGID to confirm diagnosis since some false-positive results can occur with ELISA (OIE, 2013a). However, in our study, all samples tested by ELISA were negative, Tab1.

CONCLUSION

Based on the data reviewed of this study, it was concluded that EHV-1 and EHV-4 are one of the most important causes of abortion in horses and the results of serosurveillance indicated that circulation of EHV-1 and EHV-4 is due to that the horses and donkeys having the virus in latent state and it reactivating from time to time, or due to that the horses moving in and out of the premises and having contact with other potentially infected horses and donkeys. In Egypt, there was a little significant information regarding the prevalent EAV in Egyptian horse population. Antibodies against
EAV due to that percentage of infected horses become persistent infected carriers. ELISA is highly recommended to successfully examine the presence of specific antibodies against EHV-1, EHV-4, EAV and EIA virus in a large horse population.

REFERENCE
Foote CE, Love DN, Gilkerson JR, Wellington JE, Whalley JM. 2006. EHV-1 and
EHV-4 infection in vaccinated mares and their foals. Aust. Immunology and immunopathology; (41): 41-46.


