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RESEARCH

Detection of equine herpesvirus type 1 and type 4 associated with abortion and respiratory manifestation in horses during 2016 in Cairo, Egypt

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ABSTRACT

Backgrouns*: Equid herpesvirus 1* (EHV-1) and *Equid herpesvirus 4* (EHV-4) are endemic in equine population worldwide. EHV-1 is a major cause of abortion in pregnant mares; also it can cause neurological disorders in horses and respiratory disease in young horses. EHV- 4 is an important equine virus which causes respiratory disease and occasionally abortion.
Methods: the present study, 75 serum samples collected from aborted mares, growing foals and, adult horses suffered from respiratory manifestation were tested using indirect ELISA technique. A total of 33 nasal swabs and 4 vaginal swabs were tested using real time PCR.
Results: Out of 75 tested serum samples, 56 samples were positive for the presence of antibodies against EHVs. EHV-1 DNA was detected in two vaginal swabs, one nasal swab of adult horse and four nasal swabs of growing foals. EHV-4 DNA was detected in one vaginal swab and one nasal swab from growing foal.
Conclusion*:* Indeed, the study reports the infection with two types of EHV and their continuous circulation among Egyptian horses during 2016.

Keywords: Equine herpesviruses (EHVs), Epidemiology, ELISA, Real time PCR.

BACKGROUND

Infection with EHVs causes a serious economic problem in equine industry worldwide; as abortion storm caused by *Equid herpesvirus 1* (EHV-1) especially in breeding farms, also damaging the respiratory tract and lost training time caused by *Equid herpesvirus 4* (EHV-4) infection (Allen and Bryans 1986; Crabb and Studdert 1995).

EHV-1 and EHV-4 are members of *Varicellovirus* genus in *Alphaherpesvirinae* subfamily (Davison *at el.*, 2009). Both types were considered the same virus till 1981 and after using DNA fingerprint analysis, they were considered two distinct viruses (Sabine *et al*., 1981and Studdert *et al.*, 1981).

EHV-1 (equine abortion virus) which is a major cause of abortion in pregnant mares, it can also cause neurological disorders in horses with myeloencephalitis as the main sign (Patel and Heldens, 2005; Lunn *et al.*, 2009). EHV- 4 (equine rhinopneumonitis virus) is an important equine virus which cause respiratory disease and occasionally abortion. In young horses, it is frequently associated with outbreak of acute respiratory disease (Carvalho *et al.*, 2000). Infection by those types of EHV has high morbidity rates as they transmitted easily by inhalation of nasal discharges and by contact with aborted fetus and fetal fluids (Hebia *et al.*, 2007). Since herpesviruses establish latent infection within the host, viruses may shed from asymptomatic animal carriers to susceptible animals leading to spread of the infection in equine population and causing unexpected outbreaks of EHV in closed populations (Welch *et al.*, 1992).

In Egypt, EHV-1 was isolated from aborted fetal organs on CAM of ECE (Hassanien *et al.*, 2002), on BHK cell line (Warda, 2003) and on Vero cell line (Soliman *et al.*, 2008). Also,

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EHV-4 was detected in aborted fetal organs by semi- nested PCR (Amer *et al.*, 2011) and by nested PCR (Al-Shammari *et al.,* 2016).

The aim of this study is detection and characterization of two types of EHVs (1and 4) associated with abortion and respiratory manifestation during 2016 in Cairo, Egypt.

MATERIALS AND METHODS

Clinical samples:

Seventy-five serum samples, thirty-three nasal swabs and four vaginal swabs were collected from aborted mares, growing foals and adult horses suffered from respiratory manifestation in El zahraa stud for Arabian horses during 2016.

EHV IgG detection by Indirect Enzyme linked immunosurbent assay (ELISA):

Detection of specific IgG antibodies to EHV-1 and EHV-4 was done using indirect ELISA technique (INgezim, RINONEUMONITIS). Plates are coated with inactivated EHV antigen, and a monoclonal antibody (MAb) specific for equine IgG was used to detect the presence of equine antibodies.

Molecular detection of EHV-1 and EHV-4 by real time PCR (rPCR):

Extraction of DNA from clinical samples:

Using Allprep DNA/ RNA Mini kit (Qiagen) according to the manufacturer's protocol.

Detection of EHV-1 and EHV-4 by real time PCR:

Detection of EHV-1 and EHV-4 was carried out using rPCR diagnostic kits (Equine herpes virus 1) and (Equine herpes virus 4) genesig® Advanced Kits (Primer design Ltd. ^{TM}), respectively. Briefly, reactions were performed in a total volume of 20µl containing oasig^{TM} 2x q-PCR MasterMix, primer and probe mix (1µl), internal extraction control primer/probe mix (1µl), RNAse/DNAse free water (3µl), and 5µl of template DNA. All reactions were carried out using a real-time PCR machine (StepOne, Applied Biosestyme, USA) with the following cycling parameters; 2 min at 95°C (Enzyme activation), 50 cycles of 10s at 95°C (denaturation), 60s at 60°C (Annealing / extension), and the fluorogenic data was collected during this step through the FAM channel.

RESULTS

Detection of EHV IgG by Indirect ELISA:

Out of 75 tested serum samples, 56 samples were positive for the presence of antibodies against EHVs. 9/10 of aborted mares, 10/12 of adult horses, and 37/53 of growing foals were positive (table 1).

Table 1: Results of analysis of clinical samples by Indirect ELISA and rPCR

Animal type	Serum samples	Results of ELISA	Nasal swabs	Vaginal swabs	Results of rPCR	
		Positive sera			EHV-1	EHV-4
Aborted mares	10	9	——	4	2	1
Adult horses	12	10	13	——	1	——
Growing foals	53	37	20	——	4	1

Detection of EHV-1 and EHV-4 by real time PCR:

Analysis of nasal and vaginal swabs by EHV-1 and EHV-4 rPCR diagnostic kits revealed that out of four vaginal swabs collected from aborted mares, two samples were positive for EHV-1, and one sample was positive for EHV-4. Out of 33 tested nasal swabs, 6 samples were positive (table 1). 1/13 of adult horses and 4/20 of growing foals were positive for EHV-1. One nasal swab of growing foals was positive for EHV-4 (figure1 A and B).

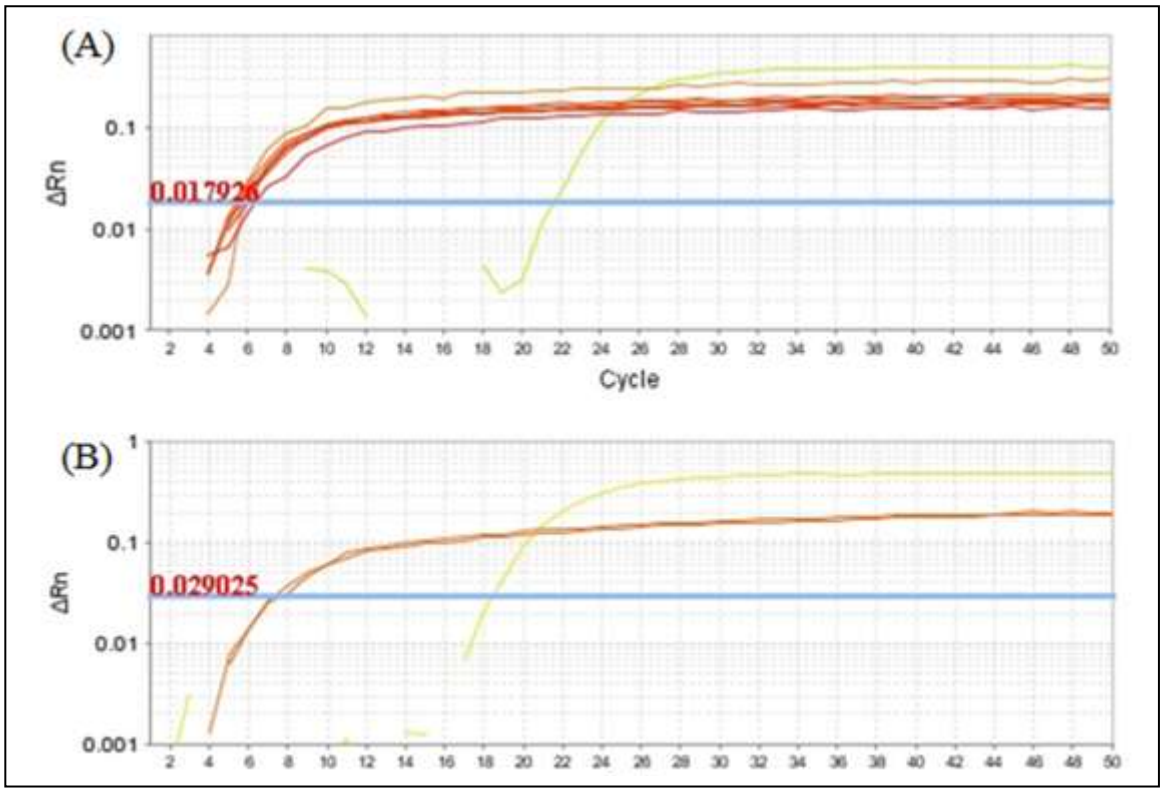

Fig. 1: Amplification curves of positive controls and positive samples of EHV-1 (A) and of EHV-4 (B).

DISCUSSION

EHV type1 and EHV type 4 are endemic in horse population worldwide. Latency and reactivation after exposure to stress factors are important features of EHVs epidemiology, allowing viruses to persist in previously infected horses and subsequently infect other susceptible horses, or lead to abortion in the latently infected horses (Allen *et al.*, 2004). Poor race performance, lost training time, outbreaks of abortion, and neonatal foal mortality are significant causes of economic losses caused by EHV-1and EHV-4 (Elia *et al.*, 2006)**.** EHVs affect the Egyptian Arabian horse farms and cause severe economic losses, as the Arabian horse is one of the most valuable breeds of equine species.

In this study, an investigation has been carried out on clinical samples collected from suspected cases during 2016 for detection of EHV type 1 and type 4 associated with abortion and respiratory disease in Egyptian horses. Testing of serum samples by indirect ELISA technique revealed the presence of antibodies against EHV-1 and EHV-4 among the Egyptian horses. EHV antibodies were present in 56 of 75 collected serum samples; 9/10 of aborted mares, 10/12 of adult horses, and 37/53 of growing foals were positive. However, the utilized ELISA technique

could not discriminate between ratio of the positive serum for EHV-1 and EHV-4. The type specific ELISA technique which enables to differentiate between antibodies against EHV-1 and antibodies against EHV-4 has been used in several serological surveys (Crabb and Studdert, 1993). In Australia, Similar studies conducted by Crabb and Studdert, (1995) and Gilkerson *et al*., (1999b) who estimated the incidence of EHV-4 antibodies in tested mares and foals and found that it was higher than that of EHV-1 antibodies. Also, serological survey carried out in USA reported that the most of tested foals (6-8 months) were seroconversion positive for EHV-1 (Doll and Bryans, 1962, 1963b). In Japan, an epidemiological study carried out and reported that the infection with EHV-1 occurred mainly in winter while EHV-4 infection occurred all over the year (Matsumura *et al*., 1992). Although the utilized ELISA techniques were type specific, they could not differentiate between infected and vaccinated horses.

Since the seroepidemiological studies were given insufficient data, in our study we companied the serological diagnosis with molecular diagnostic technique (Real time PCR). Molecular based technique is more accurate in detection of EHV infection (Carvalho *et al*., 2000). Because of high sensitivity of real time PCR in the detection of viral nucleic acid, it has become the diagnostic technique of choice for several infectious diseases (Lunn *et al*., 2009). The use of fluorescence labelled probes in real-time PCR increase the sensitivity and the specificity. Consequently, real-time PCR for detection of EHVs can differentiate between EHV-1and EHV-4 (Diallo *et al.,* 2006). Also, it can quantify EHVs load in clinical samples, which provide an improvement in the investigation of the epidemiology of EHVs infection (Elia *et al*., 2006). Sensitive and specific real time PCR assays for detection of EHV-1 have been described (Diallo *et al.,* 2006; Elia *et al.,* 2006). Specific real time PCR assay for detection the lytic infection with EHV-4 has been also described (Pusterla *et al.,* 2005).

In the present study, real time PCR diagnostic kits specific for EHV-1 and EHV-4 were used for testing the collected vaginal and nasal swabs. EHV type1 was detected in vaginal swabs and nasal swabs of adult horses and growing foals confirming the ability of this type to cause abortion and respiratory manifestation (Crabb and Studdert, 1995). EHV type 4 was detected in one vaginal swab confirming the incrimination of EHV-4 as a primary cause of abortion (Amer *et al.,* 2011 and Al-Shammari *et al.,* 2016). Also, EHV-4 was detected in nasal swabs collected from growing foals confirming the suggestion of Patel and Heldens (2005) that EHV-4 infection is probably common in foals and start early in life. Infections with EHV-1 and EHV-4 have been documented all over the world within equine species (Smith *et al*., 2003). In Egypt, infections with EHV-1 were recorded (Hassanien *et al.,* 2002, Warda, 2003 and Soliman *et al.,* 2008). Also, infection with EHV-4 was recently recorded (Shammari *et al.,* 2016). In our study, EHV-1 and EHV-4 were detected in collected samples like recorded previously by Amer *et al.,* (2011). In conclusion, the present study reported the continuous circulation of EHV-1 and EHV-4 among Egyptian horses during 2016.

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REFERENCES

Allen, G.P. and Bryans, J.T. (1986). Molecular epizootiology, pathogenesis and prophylaxis of equine herpesvirus 1 infections. Prog. *Vet. Microbiol. Immunol*. 2: 78 – 144.

Allen, G.P., Kydd, J.H., Slater, J.D. and Smith, K.C. (2004). Equid herpesvirus 1 and equid herpesvirus 4 infections. In: Infectious diseases of livestock, ed. Coetzer JAW, Tustin RC, 829– 859. *Oxford University Press, Oxford*.

Al-Shammari, Z.S., Ahmed, B.M., Haroun, M., Afify, A.F., Elsanousi .A.A. and Shalaby, M.A. (2016). A First Molecular Phylogeny of an Egyptian Equine Herpesvirus-4 Strain derived from a Fetal Arabian horse. *J. Vet. Sci. Med. Diagn*., 5:1.

Amer, H. M., Shaltout, A.K., El-Sabagh, I.M., El-Sanousi, A.A. and Shalaby, M. A. (2011). Prevalence of equine herpes viruses 1, 2 and 4 in Arabian horse population in Egypt. *Afr. J. Microbiol. Res*., 5: 4805–4811.

C**arvalho, R., Passos, L.M.F. and Martins, S. (2000).** Development of a differential multiplex PCR assay for equine herpesvirus 1 and 4 as a diagnostic tool. *J. Vet. Med. B*. 47: 351 – 359.

Crabb, B.S. and Studdert, M.J. (1995). Equine herpesvirus 4 (equine rhinopneumonitis virus) and 1 (equine abortion virus). *Adv. Vir. Res.*, 45: 153–190.

Davison, A.J., Eberle, R., Ehlers, B., Hayward, G.S., McGeoch, D.J., Minson, A.C., Pellett, P.E., Roizman, B., Studdert, M.J. and Thiry, E. (2009). The order Herpesvirales. *Arch. Virol.*, 154: 171 – 177.

Diallo, S.I., Hewitson, G., Wright, L., Rodwell, B.J. and Corney, B.G. (2006). Detection of equine herpesvirus type 1 using a real-time polymerase chain reaction. J. *Virol. Meth.*, 131: 92–98.

Doll, E.R. and Bryans, J.T. (1962). Incubation periods for abortion in equine viral rhinopneumonitis. J. Am. Vet. Med. Assoc.; 1413: 351–354.

Doll, E.R. and Bryans, J.T.(1963b). Epizootiology of equine viral rhinopneumonitis. *J. Am. Vet. Med. Assoc*.; 142: 31–37.

Elia, G., Decaro, N., Martella, V., Campolo, M., Desario, C., Lorusso, E., Cirone, F. and Buonavoglia, C. (2006). Detection of equine herpesvirus type 1 by real time PCR. *J. Virol. Meth.*, 133: 70–75.

Gilkerson, J.R., Whalley, J.M., Drummer, H.E., Studdert, M.J. and Love, D.N. (1999b). Epidemiology of EHV-1 and EHV-4 in the mare and foal populations on a Hunter Valley stud farm are: are mares the source of EHV-1 for unweaned foals. *Vet. Microbiol*., 68: 27–34.

Hassanien, M.M., Maysa, H., El-bagoury, F., Magda, A.K., El-Kabbany, M.M.A. and Daoud, M.A. (2002). Trials for isolation and identification of equine herpesvirus abortion in Egypt. *Vet. Med. J. Giza.*, 50: 977-986.

Hebia, I., Fieni, F., Duchamp, G., Destrumelle, S., Pellerin, J.L., Zientara, S., Vautherot, J.F. and Bruyas, J.F. (2007). Potential risk of equine herpes virus 1 (EHV–1) transmission by equine embryo transfer. *Therio.*, 67: 1485 – 1491.

Lunn, D.P., Davis–Poynter, N., Flaminio, M.J., Horohov, D.W., Osterrieder,K., Pusterla, N. and Townsend, H.G. (2009). Equine herpesvirus–1 consensus statement. *J. Vet. Intern. Med.*, 23: 450 – 461.

Matsumura, T.; Sugiura, T.; Imagawa, H.; Fukunaga, Y.and Kamada, M., (1992). Epizootiological aspects of type 1and type 4 equine herpesvirus infections among horse populations. *J. Vet. Med. Sci.;* 54: 207-211.

Patel, J.R. and Heldens, J. (2005). Equine herpesviruses 1 (EHV–1) and 4 (EHV–4) – epidemiology, disease and immunoprophylaxis: a brief review. *Vet. J.*, 170: 14 – 23.

Pusterla, N., Leutenegger, C. M., Wilson, W. D., Watson, J. L., Ferraro, G. L. and Madigan, J. E. (2005). Equine herpesvirus-4 kinetics in peripheral blood leukocytes and

nasopharyngeal secretions in foals using quantitative realtime TaqMan PCR. *J. Vet. Diagn. Invest.*, 17: 578-581

Sabine, M., Robertson, G.R. and Whalley, J.M. (1981). Differentiation of sub–types of equine herpesvirus 1 by restriction endonuclease analysis. *Aust. Vet. J.*, 57: 148 – 149.

Smith, K.C., Blunden, A.S., Whitwell, K.E., Dunn, K.A. and Wales, A.D. (2003).
A survey of equine abortion, stillbirth and neonatal death in the UK from1988 to 1997. *Equine Vet. J.*, 35: 496–501.

Soliman, E.M., Kalad, M.A., Elkbany, M.M., Ebied, E.M., Warda, S.A., Abdelhamid, N.K. and Mohamed, N.S. (2008). Recent isolation and identification of equine Herpes Viral abortion (EHV-1) in Egypt -2007. *9th Vet. Med. Zag. Conference*.

Studdert, M.J., Simpson, T. and Roizman, B. (1981). Differentiation of respiratory and abortogenic isolates of equine herpesvirus 1 by restriction endonucleases. *Sci.*, 214: 562 – 564.

Warda, S.M. (2003). Studies on equine viral abortion problem in Egypt. Ph.D. dissertation, Zagazig University (Benha Branch) Egypt.

Welch, H.M., Bridges, C.G., Lyon, A.M., Griffiths, L. and Edington, N. (1992). Latent equid herpesviruses 1 and 4: detection and distinction using the polymerase chain reaction and co–cultivation from lymphoid tissues. *J. Gen. Virol*., 73: 261 – 268.

Yildirim, Y., Yilmaz, V., and Kirmizigul, A. H. (2015). Equine herpes virus type 1 (EHV-1) and 4 (EHV-4) infections in horses and donkeys in northeastern Turkey. *Iran. J. Vet. Res.*, 16: 341–344.

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