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Highlighting on viral equine abortion syndrome in some regions in Egypt

Soha I. Mohammed*; Abdel Hamid I. Bazid; Momtaz A. Shahein*; Mohammed A. Abo-Elkhair****

*Department of Virology, Animal Health Research Institute, Dokki, Giza: Egypt
**Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Menoufia: 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-1and 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 arelinear 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

Corresponding author: Soha I. Mohammed**;** Department of Virology, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Giza, Egypt.
E-mail address: dr_soha_199@yahoo.com
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enter into a latent state in cells of the lymphoreticular system and neurons in the trigeminal ganglia **(Slater et al. 1994)**. The establishment of lifelong latency in a large proportion of infected animals ensures the survival of herpesviruses in Equine populations and enables the virus to shed sporadically throughout the lifetime of the horse ***(*Gilkerson et al. 2015)** the resistance of mares to abortion could be exhibited if the infection occurred early in pregnancy **(Ali et al. 2020).**

Standard diagnostic serological methods still remain important tools for EHV-1, EHV-4 diagnosis like type-specific *ELISA (***Singh et al. 2001; lang et al. 2013)**. In Egypt, ELISA based serological study conducted for detecting antibodies against EHV-1 and EHV-4, the overall prevalence rate was 64% (173/270***)(*Emad et al. 2018**). Also, another study conducted in Egypt, the overall percentage 62,5% (75/120***) (*El Sayyad et al. 2015***) .**

EAV is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease of horses (**Timoney and McCollum, 1993).** EAV is a small enveloped virus with a positive-sense, single-stranded RNA and belongs to the family *Arteriviridae* (genus *Arterivirus*) in the order *Nidovirales* **(Cavanagh, 1997***)*. Signs include influenza-like sickness in adult horses, abortion in pregnant mares, and interstitial pneumonia in young foals.The virus can spread in many different ways;the respiratory route is the most common.The virus can potentially spread by artificial insemination via the venereal route **(McCollum et al. 1999).** Following natural breeding or artificial insemination, carrier stallions transfer EAV venereally to susceptible mares (**Timoney et al. 1997***).* A variable percentage of stallions (from <10 to 70*%)* acutely infected with EAV can become persistently infected carriers, shedding the virus constantly in semen ***(*Timoney and McCollum, 1993).** The seroprevalence of EAV infection can vary among horses of different breeds (**Timoney and McCollum, 1993).** Serological studies indicated that EAV is widely distributed in Equine population around the world **(Balasuriya, 2014)**. Currently, the OIE recommends that ELISAs are only used for conducting serological surveillance studies. Recently, a new commercial competitive ELISA (EAV antibody test kit, cELISA;VMRD Inc., Pullman, Washington, USA) has become commercially available **(Chung et al. 2013b**), which has been evaluated and validated for the detection of EAV antibodies in equine sera ***(*Chung et al. 2013a).**

EIA is caused by Equine Infectious Anemia Virus (EIAV), a lentivirus classified within the *Retroviridae f*amily, 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 (INGEZIM ARTERITIS, 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

Governorate	Total	EHV-١&٤		EAV		EIA	
		Positive (No.)	Positive (%)	Positive (No.)	Positive (%)	Positive (No.)	Positive (%)
Cairo	60	30	50%	6	10%	0	0%
Alex.	102	30	29.41%	2	1.96%	0	0%
Giza	222	50	22.52%	6	2.7%	0	0%
Minya	150	60	40%	1	0.67%	0	0%
Monufia	51	3	5.8%	0	0%	0	0%
Qalyubia	15	0	0%	1	6.67%	0	0%
Total	600	173	28.83%	16	2.67%	0	0%

DISCUSSION

Based on the findings of this study, it was concluded that EHV-1, EHV-4 are wide spread 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 seropositive 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 serosurveillance 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.**

CONCULSION

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

Ali AA, Refat NA, Algabri NA, Sobh MS. 2020. Fetal lesions of EHV-1 in equine. Anais Da Academia Brasiliera De Ciencias.92.doi:10.1590/00013765202020180837

Allen GP. 2002. Respiratory Infections by Equine Herpesvirus Types 1 and 4. In:Equine Respiratory Diseases. Ed. P. Lekeux, International Veterinary Information Service Ithaca, New York.

Allen G, M. Murray. 2004. Equid herpesvirus 2 and equid herpesvirus 5 infections. In: J.A.W.Coezer,and R.C.Tustin (eds), In fectious Diseases of Livestock, pp.; 860–867. Cape Town: Oxford Press.

Balasuriya UB. 2014. Equine viral arteritis. Veterinary Clinics of North America:Equine Practice ;(30): 543–560.

Balasuriya UB, Go YY, MacLachlan NJ. 2013. Equine Arteritis Virus. Veterinary Microbiology; (167): 93–122.

Balasuriya UB, Heidner HW, Davis NL, Wagner HM, Hullinger PJ, Hedges JF, Williams JC, Johnston RE, Wilson DW, Liu, IK, MacLachlan NJ. 2002. Alphavirus replicon particles expressing the two major envelope proteins of equine arteritis virus induce high level protection against challenge with virulent virus in vaccinated horses. Vaccine; (20):1609–1617.

Balasuriya UB, Snijder EJ, Vandinten LC, Heidner HW, Wilson WD, Hedges JF, Hullinger PJ, MacLachlan NJ. 1999. Equine arteritis virus derived from an infectious cDNA clone is attenuated and genetically stable in infected stallions. Virology; (260):201–208.

Balasuriya UB, Snijder EJ, Heidner HW, Zhang J, Zevenhoven-Dobbe JC, Boone JD, McCollum WH, Timoney, PJ, MacLachlan, NJ. 2007. Development and characterization of an infectious CDNA clone of the virulent bucyrus strain of equine arteritis virus. Journal of General Virology; (88):918–924.

Cavanagh D. 1997. Nidovirales: a new order copring Coronaviridae and Arteriviridae. Arch Virol ;(142): 629–633.

Chung C, Wilson C, Timoney P, Adams E, Adams DS, Chung S, Evermann, JF, Shuck K, Lee, SS, Mcguire TC. 2013a. Comparison of an Improved Competitive enzyme-linked immunosorbent assay with the world organization for animal health-prescribed serum neutralization assay for detection of antibody to equine arteritis virus.Journal of Veterinary Diagnostic Investigation (25):182–188.

Chung C, Wilson C, Timoney P, Balasuriya U, Adams E, Adams DS, Evermann JF, Clavijo A, Shuck K, Rodgers, S, Lee SS, Mcguire TC. 2013b. Validation of an improved competitive enzyme-linked immunosorbent assay to detect equine arteritis virus antibody. Journal of Veterinary Diagnostic Investigation; (25):727–735.

Craigo JK, Montelaro RC. 2013.Lessons in AIDS vaccine development learned from studies of equine infectious anemia virus infection and immunity.Viruses (5):2963–2976.https://doi.org/10.3390/v5122963.

Davison AJ, Eberle R, Ehlers B, Hayward GS, McGeoch DJ, Minson AC, Thiry E. 2009. The order Herpesvirales. Archives of Virology; (154):171–177.

El-Sayyad M, Mohamed F, Momtaz A, Mohamed M, Shahira A & El-Tarabili M. 2015. Detection of Equine Herpes Virus-1in Arabian Horses from different localities in Egypt. AmJ Vet Res; (3): 15-30.

Emad BA, Ahmed Z, Alaa AG, Ahmed E, Raafat M, Sh. 2018. Equine herpes virus type-1 infection: Etiology, epidemiology, pathogenesis, identification and recent diagnosis.Asian J. Epidemiol., (11): 34-45.

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.

Fukunaga Y, Imagawa H, Tabuchi E, Akiyama Y. 1981. Clinical and virological findings on experimental equine viral arteritis in horses. Bulletin of Equine Research Institute; (18):110–118.

Gilkerson JR, KE, Bailey A, Diaz-Méndez, CA, Hartley. 2015.Update on Viral Diseases of the Equine Respiratory Tract. Vet. Clin. North Am. Equine Pract.; (31):91– 104.

Gregg K, Polejaeva I. 2009.Risk of equine infectious anemia virus disease transmission through in vitro embryo production using somatic cell nuclear transfer. Theriogenology 2009;(72):289–299. https://doi.org/10.1016/j.theriogenology..03.009.

International Committee on Taxonomy of Viruses (ICTV)2017: Retroviridae. https://talk.ictvonline.org/taxonomy/.Accessed 09 May 2018.

Issel CJ, Cook RF. 1993. A review of techniques for the serologic diagnosis of equine infectious anemia. J Vet Diagn Investig; (5):137–141.

Kondo T, Fukunaga Y, Sekiguchi K, Sugiura T, Imagawa H. 1998.Enzyme-linked immunosorbent assay for serological survey of equine arteritis virus in racehorses. Journal of Veterinary Medical Science; (60):1043–1045.

Lang A, de Vries M, Feineis S, Muller E, Osterrieder N, Damiani AM. 2013. Development of a peptide ELISA for discrimination between serological responses to equine herpesvirus type 1 and 4. Journal of Virological Methods; (193): 667– 673. https://doi. org/10.1016/j.jviromet..07.044.

Lunn DP, DW, Horohov K, Osterrieder N, Pusterla 2009. EHV-1 Consensus Statement. J. Vet. Intern. Med.; (23):450– 461.

McCollum WH, Timoney PJ, Lee JJW, Habacker PL, Balasuriya UBR, MacLachlan, NJ. 1999.Features of an outbreak of equine viral arteritis on a breeding farm associated with abortion and fatal interstitial pneumonia in neonatal foals. In Proceedings of the Eighth International Conference on Equine Infectious Diseases, Dubai, United Arab Emirates, 1998, 559–560. Edited by U. Wernery JF, Wade J.A. Mumford OR, Kaaden. Newmarket UKR & W Publications.

Mealey RH. 2007. Equine infection anemia. In: Sellon DC, Long M (eds) Equine infectious diseases. Saunders Elsevier, St. Louis, pp; 213–219.

NAHMS. 2000. Equine viral arteritis (EVA) and the U.S. horse industry. http://ageconsearch.umn.edu/bitstream/32749/1/eq98eva1.pdf.

OIE WOfAH. 2013a. Equine Infectious Anemia distribution map. Accessible in URL: http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/ Access date: May 2022.

OIE WOfAH. 2013c. Equine Viral Arteritis distribution map. AccessibleinURL: http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/ Access date: May 2022.

OIE, 2018. OIE manual for terrestial animals, 2018. Equine rhinopneumonitis, http://www.oie.int/standardsetting/terrestrial-manual/ access online/2.05.09_EQUINE_RHINO.pdf (Chapter 2.5.9).

Patel JR, Heldens J. 2005. Equine herpesviruses 1 (EHV-1) and 4 (EHV-4)–epidemiology, disease and immunoprophylaxis: a brief review. Veterinary Journal; (170):14– 23.

Pavulraj S, Eschke K, Theisen J, Westhoff S, Reimers G, Andreotti S, Osterrieder N, Azab W. 2021. Equine Herpesvirus Type 4 (EHV-4) Outbreak in Germany:Virological, Serological, and Molecular Investigations.Pathog. (Basel, Switzerland) 10. doi:10.3390/pathogens10070810.

Piza AST, Pereira ARP, Terreran MT, Mozzer O, Tanuri A, Brandao PE, Richtzenhain L. 2007. Serodiagnosis of equine infectious

anemia by agar gel immunodiffusion and ELISA using a recombinant p26 viral protein expressed in Escherichia Coli as antigen. Preventive Veterinary Medicine; (78): 239-245 : doi:10.1016/ j.prevetmed.2006.10.009.

Reed SM, RE. Toribio 2004. Equine herpesvirus 1 and 4. Vet. Clin. North Am. Equine Pract.; (20):631– 642.

Sanz A, Garcia-Barreno B, Nogal ML, Vin4 uela E, Enjuanes L. 1985. Monoclonal antibodies specific for African swine fever virus proteins. Journal of Virology; (54):199–206.

Singh BK, Yadav MP, Tewari SC. 2001. Neutralizing and complement-fixing monoclonal antibodies as an aid to the diagnosis of equine herpesvirus-1 infection. Veterinary research communications;(25): 675-686.

Slater JD. 2007. Equine herpesvirus.in Equine infectious disease Saunders- Elsevier publisher, Philadelphia.Section II.Chapter.: (13):134-153.

Slater JD, K Borchers AM, Thackray HJ, Field. 1994. The trigeminal ganglion is a location for equine herpesvirus 1 latency and reactivation in the horse. J.Gen. Virol.; (75): 2007– 2016.

Timoney PJ, McCollum WH. 1993. Equine viral arteritis. Vet Clin North Am Equine Pract; (9):295–309.

Timoney PJ, McCollum WH, Vickers ML. 1997. The carrier stallion as a reservoir of equine arteritis virus. Equine Disease Quarterly; 6- 2 .

Yasunaga S, Maeda K, Matsumura T, Sonido T, Kai K. 2000. Application of a type – specific enzyme linked immunosorbent assay for equine herpes virus type 1 and 4 (EHV-1 and EHV-4) to horse population inoculated with inactivated vaccine.J.Vet. Med. Sci.,; 62 (7):687-691.