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Molecular Detection and Phylogenetic Analysis of Equine Herpes Virus-1 in Horses with History or Clinical Signs in Four Provinces of Iran

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Abstract

BACKGROUND: Equine herpes virus-1 (EHV-1) is a major cause of economic loss in horse industry and is well recognized as a cause of abortion, respiratory disease, neurologic disorders and death of neonatal foals.

OBJECTIVES: The aim of this study was to evaluate the frequency of EHV-1 in horses with clinical signs and/or history associated with this virus from four provinces of Iran (Golestan, Tehran, Khuzestan, West Azerbaijan) that have considerable horse population, followed by phylogenetic study of positive cases and compare them with herpes viruses in other parts of the world.

METHODS: Blood samples and nasal swabs were taken from 150 horses from four aforementioned provinces. DNA of samples was extracted and used for detection in real-time PCR TaqMan assay. Finally, phylogenetic trees were designed based on neighbor joining method.

RESULTS: Out of 150 sampled horses, a total of 14 (9.33%) were found to be positive for EHV-1. The results indicated that positive cases of EHV-1 from this study were clustered to herpes virus cases in other parts of the world with a noticeable similarity.

CONCLUSIONS: This study confirmed the presence of EHV-1 in these provinces of Iran, thus consideration should be given to preventive and control programs to prevent dissemination and outbreak of this virus.

KEYWORDS: Equine herpes virus-1, Horse, Molecular detection, Phylogenetic analysis

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Introduction

Herpes virus infections are ubiquitous in equine populations worldwide, among them equine herpes virus-1 (EHV-1) is one of the major causes of economic loss in equine industry (Ataseven, et al. 2009; Pusterla, et al. 2014; Slonska, et al. 2014). EHV-1 has a linear double stranded DNA genome from Alphaherpesvirinae subfamily, genus varicellovirus. Infection with EHV-1 results in contagious respiratory disease, epidemic abortion, neonatal death and myeloencephalopathy (Ataseven, et al. 2009; Pusterla, et al. 2009; Carlson, et al. 2013; Ko, et al. 2013; Hussey, et al. 2014; Vaz, et al. 2016). Upper respiratory disease of foals and adults is associated with EHV-4 or, less commonly, EHV-1(Walter, et al. 2013; Stasiak, et al. 2015; Constable, et al. 2017; Laval, et al. 2017).

The biologic source of such virus originates from a primary lytic infection or the shedding of virus after reactivation of a latent state (Pusterla, et al. 2009; Ma, et al. 2013; Jhonstone, et al. 2016; Laabassi, et al. 2017). Viral latency and reactivation are important features of EHV-1 epidemiology; this virus can establish latent infection in the host, then intermittent recrudescent and viral shedding from asymptomatic carrier to susceptible horses (Ataseven, et al. 2009; Damiani, et al. 2014; Sarkar, et al. 2015; Sarkar, et al. 2016; Oladunni, et al. 2018). Therefore, different techniques have been used to detect and differentiate EHV-1 and there is a clear need for a specific and sensitive technique that allows the rapid diagnosis of clinical disease, as well as surveillance of susceptible populations (Hussey, et al. 2006).

Real-time PCR is a flexible, rapid, sensitive, specific and quantitative method for diagnosis and surveillance of herpes viral diseases (Hussey, et al. 2006; Diallo, et al. 2007; Yilmaz, et al. 2012). Although there are some reports of EHV-1 infection in Iran, this study indicated the presence of EHV-1 in horses with signs associated with this virus. Based on the authors' findings, this is the first study using real-time PCR TaqMan assay on nasal swab and blood samples of horses with clinical signs or clinical history associated with this virus and phylogenetic study in Iran.

This study focuses on estimating the frequency of EHV-1 in nasal swab and blood samples of horses from four provinces of Iran using real-time PCR TaqMan assay and phylogenetic study of positive samples.

Materials and Methods

Samples and sample preparation

Blood samples and nasal swabs were taken from 150 horses (from different sex, breed and age) with clinical signs or history associated with this virus, including respiratory signs (fever, coughing and nasal discharge), neurologic signs (depression, ataxia, recumbency and urinary incontinence) and abortion from different studs of four provinces of Iran that contain large horse population. Provinces include Golestan (north of Iran), Tehran (north of Iran), Khuzestan (south of Iran) and West Azerbaijan (northern west of Iran). The experimental procedures have been carried out in accordance with The Code of EU Directive 2010/63/EU for animal experiments.

Number of horses has been sampled from each province: Golestan: 47, Khuzestan: 45, Tehran: 37 and West Azerbaijan: 21. Sampling was done between December 2015 and December 2016. Five milliliters of blood were collected from jugular vein in EDTA tubes. Nasal swabs were taken from both nostrils then transferred to 1ml virus trans-

port media containing PBS, penicillin 800 IU ml-1, streptomycin 800 µg ml-1 and 0.1% w/v fetal calf serum (OIE, 2015). Samples were immediately transported on ice to Laboratory of Virology in Faculty of Veterinary Medicine at University of Tehran.

The blood samples were centrifuged at 1500 g for 10 min, the buffy coat fraction was removed and stored at -70 ˚C. Nasal swabs with their transport media was stored at -70 ˚C (3). DNA was extracted from 100µl of each whole blood and nasal swab samples using DNA extraction kit (MBST, Iran) by the protocol described by the manufacturer. The quality of extracted DNA was confirmed by the agarose gel electrophoresis.

Reference strain used in this study was purified DNA of EHV-1 strain 89c25 (Kawakami, et al. 1962).

Primers and probes

EHV-1 specific real-time PCR (TaqMan assay) was performed on extracted DNA, isolated from buffy coat fraction and nasal swab solution. The glycoprotein B of Alpha Herpesvirinae was a conserved region and was selected because it contained highly specific sequence that could allow discrimination between the closely related equid herpes viruses EHV-1 and EHV-4 (Wanger et al., 1992).

Primers and probe targeting the glycoprotein B gene of EHV-1 were used from OIE terrestrial, 2015 (OIE, 2015). (Tables 1)

Primers and probe sequence specificity was confirmed by nucleotide – nucleotide Blast search in national reference for biotechnology information (NCBI) database and were synthesized by Sinacolon Ltd. Iran.

The monoplex EHV-1 real-time PCR was performed as a 20µl reaction containing 0.4mM of each dNTPs, 3mM MgCl2, 1 unit TaqDNA polymerase, 0.3µm of each primers and 5 µl of DNA template. The real-time was performed on Rotor-Gene (Qiagene, Germany) machine.

Cycling parameter

Real-time cycling parameters were used for initial denaturation at 95 ˚C for 5 min, the cycling consisted of 40 cycles at 95 ˚C for 15 s and 60 ˚C for 60 s. Results were interpreted based on CT values as follows: CT values below or equal to 35 were considered positive, CT values above 35 were considered negative (Diallo et al., 2006). Four positive samples were sequenced for confirmation of results and phylogenetic study.

Phylogenetic analysis

In order to establish phylogenetic relationship between EHV-1 strains, sequences results of EHV-1 strains obtained from this study were used for phylogenetic analysis using MEGA version 7.0 software.

Statistical analysis

Statistical analysis was performed by SPSS software version 20. Statistical significance was determined using Chi square and Fisher's Exact test. A $p \leq 0.05$ was considered statistically significant.

Table 1. Real-time PCR primers and probe

Primers and probe	Sequences
Forward primer	GGG-GTT-CTT-AAT-TGC-ATT-CAG-ACC
Reverse primer	GTA-GGT-GCG-GTT-AGA-TCT-CAC-AAG
Probe	{FAM}TCT-CCA-ACG-AAC-TCG-CCA-GGC-TGT-ACC{BHQ1}

Primers used for sequence analysis on positive samples described by kirasawa et al.,1993

Results

Out of 150 sampled horses, a total of 14 (9.33%) were found positive for EHV-1, 7 (4.6%) buffy coat samples, 5 (3.33%) nasal swab samples and 2 (1.33%) of both nasal swab and buffy coat samples were found positive for EHV-1. There was not any statistically significant difference between the nasal swab and blood samples with EHV-1 infection ($p>0.05$). Out of 14 positive samples of EHV-1, 10 (71.42%), 1 (7.14%), 3 (21.42%) were found with history or clinical signs of respiratory, abortion and neurologic disease respectively. Out of 47 samples from Golestan, 45 samples from Khuzestan, 37 samples from Tehran and 21 samples from West Azerbaijan, 7 (14.89%), 0 (0%), 4 (10.8%) and 3 (14.28%) were positive for EHV-1 respectively. There was statistically significant difference between EHV-1infected samples from Golestan and Khuzestan. There was no significant association among the other provinces (Table 2).

Sampled horses were divided into 3 different age groups (≤ 5 years, 6–15 years and ≥16 years). Although frequency of infection with EHV-1 was higher in 6–15 years group, there was no significant association among different age groups. Out of 14 positive samples of EHV-1, 9 were taken from crossbred, 1 from KWPN, 1 from Holstein, 3 from Thoroughbred and 1 from Turkmen. There was significant difference between Turkmen and crossbred but there was not any significant difference among other breeds. Out of 14 positive samples for EHV-1, 8 belonged to female horses and 6 belonged to male horses. There was not any significant correlation between the male and female horses infected with EHV-1 (Table 2).

Phylogenetic tree designed based on glycoprotein B gene of EHV-1 by using the real-time PCR sequence, neighbor joining method with bootstrap value equal to 1000 were used. Phylogenetic tree showed that the Iranian EHV-1 strains were homogenous and had a close relationship with the previously reported strains in gene bank (Fig. 1).

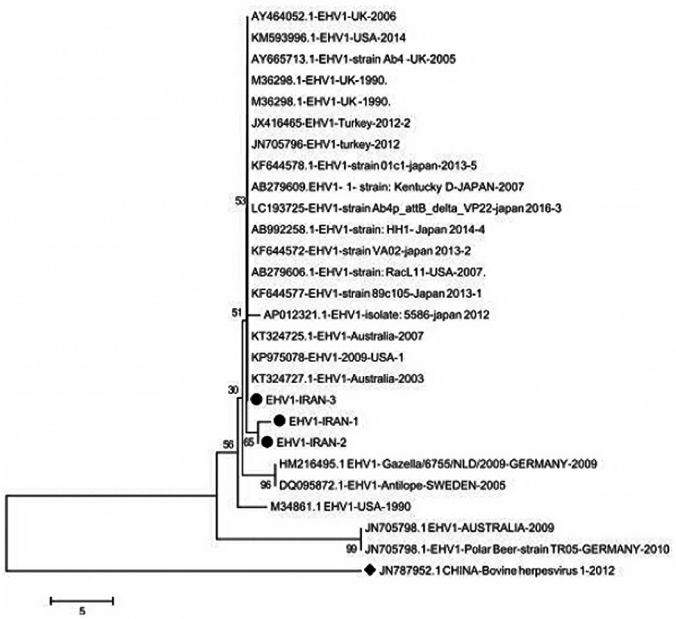

Figure 1. Phylogenetic tree of EHV-1 in Iran. It was generated by neighbor-joining method and Mega 7 software with 1000 bootstrap value. Black circles showed viruses detected in Iran and black diamond showed the out-group strain obtain from gene bank

Table 2. Frequency of infection with EHV-1 based on type of disease, province, age and breeds*.

Type of disease												
Neurologic				Abortion				Respiratory				
C.H		C.S		C.H		C.S		C.H		C.S		
0 0		3 (21.4%)		1 (7.1%)		0 0		9 (64.2%)		1 (7.4%)		
Province												
West Azerbaijan				Khuzestan				Tehran				Golestan			
N		A		N		A		N		A		N		A	
18 (85.7%)		3 (14.2%)^{ab}		45 (100%)		0 0^{b}		33 (89.1%)		4 (10.8%)^{ab}		40 (85.1%)		7 (14.8%)^{a}	
Age															
≥ 16 Years				6 – 15 Years				5 Years≤							
N		A		N		A		N		A					
12 (100%)		0 0^{a}		93 (88.5%)		12 (11.4%)^{a}		31 (93.9%)		2 (6.06%)^{a}					
Breeds															
Thoroughbred				Arab				KWPN				Holstein			
N		A		N		A		N		A		N		A	
14 (87.5%)		2 (12.5%)^{ab}		46 (100%)		0 0^{ab}		15 (93.7%)		1 (6.2%)^{ab}		9 (90%)		1 (10%)^{ab}	
Turkmen				Cross bred											
N		A		N		A									
24 (96%)		1 (4%)^{b}		28 (75.6%)		9 (24.3%)^{a}									
Sex															
Female						Male									
N			A			N			A						
79 (90.7%)			8 (9.19%)^{a}			57 (90.47%)			6 (9.52%)^{a}						

C.S= Clinical sign, C.H= Clinical history, A= Affected, N= Non affected

*The heterogonous letters showed significant statistic differences.

Discussion

Real-time PCR was employed for detection of EHV-1 based on its speed, high sensitivity and specificity (Hussy, et al., 2006; Diallo, et al., 2007; Pusterla, et al., 2009). Samples were taken from different studs in four provinces of Iran. These horses were used for race and jumping competition, breeding and riding. Based on the authors' knowledge, this is the first study to present molecular detection of EHV-1 in nasal swab and blood samples of stud horses using real-time PCR TaqMan assay and phylogenetic study in Iran.

EHV-1 was detected from a total of 7.3% and 4.6% blood sample and nasal swab, respectively. Infection rate of EHV-1 in horses has been investigated worldwide and different results were found based on different regions and different detection methods were used (Ataseven, et al., 2009; Pusterla, et al., 2010; Yilmaz, et al., 2012; Turan, et al., 2012). In this study, some horses were positive only with nasal swab, the others with blood sample and some with both, therefore different kinds of discussion based on their clinical status (clinical signs or history) and type of sample (nasal swab or blood) could be considered.

In a serological survey in Chaharmahal-Bakhtiari, Iran, Momtaz and Hemmatzadeh, (2003) detected 39.08% EHV-1 positive horses. In molecular surveys the prevalence of EHV-1 in Isfahan, Chaharmahal-Bakhtiari and northeast of Iran were 13.2%, 8.1% and 0%, respectively (Sarani, et al., 2012; Tak-

taz, et al., 2015). Variation in detection rate among these studies could be due to climatic condition (moisture, temperature, etc.), frequency of EHV-1 infection in these provinces, immune status of horses, management factors and techniques used to detect this virus (Matsumura, et al., 1992; Gohering, et al., 2006).

The results of this study, like Friday et al., (2000), confirm the absence of significant association between different age groups, however Heninger et al., (2007) and Gohering et al., (2006) reported that, there is significant difference between age groups infected with EHV-1, nevertheless the results show that infection with EHV-1 in the age group of 5–16 years is higher than the other groups. We believe that with increase in age, possibility of EHV-1 infection increases. Basically, the possibility of encountering EHV-1 increases for adult horses used for competition, breeding and training (Friday, et al., 2000; Gohering, et al., 2006; Heninger, et al., 2007; Taktaz, et al., 2015).

The results of this study were similar to Gohering et al., (2006), Lunn et al., (2009) and Taktaz et al.'s, (2015) studies that showed different frequencies of EHV-1 in different breeds of horses, nevertheless there is only a significant difference between two breeds (Turkmen and crossbred);further, there is not any significant difference among other breeds sampled in this study. Higher use of crossbred horses in competitions with stressful condition (poor housing, transportation, gathering a large number of horses) that decrease function of immune system may be associated with higher frequency of EHV-1 infection in these horses.

The results show there is not any significant association between sex and EHV-1 infection. In this category, our results are in agreement with reports of Momtaz and Hemmatzadeh, (2003), Friday et al., (2009) and Taktaz, et al., (2015), on the other hand Gohering et al., 2006 reported sex is a factor in the epidemiology of EHV-1 infection in the Netherlands. Different results between sexes in these studies may be due to differences in rate of exposure to the infection, health status, age, previous vaccination or immune status of the horses sampled in these studies (Momtaz and Hemmatzadeh, 2003; Gohering, et al., 2006; Friday, et al., 2009; Taktaz, et al., 2015). In our study there is no significant association between blood and nasal swab samples of EHV-1 infected, however frequency of EHV-1 in blood was higher than nasal swabs. Results obtained from other parts of the world in this category are different (Brown, et al., 2007; Ataseven, et al., 2009; Turan, et al., 2012; Yilmaz, et al., 2012).

In surveys performed in Turkey, Ataseven et al., (2009), identified frequency of EHV-1 in nasal swabs 30% and in blood 14.3%, Yilmaz et al., (2012), reported frequency of EHV-1 in nasal swabs 13.6% and in blood 20%. Turan et al., (2012) and Brown et al., (2007) reported that analysis of swabs is more sensitive than blood to detect EHV-1 infection.

Higher frequency of EHV-1 in blood samples could be due to the higher tendency of this virus to produce viremia and subsequently formation of latency in PBMC. However, it seems that based on sampling time, type of infection (lytic or latent), sensitivity of laboratory methods and sample size, frequency of this virus in nasal swab and blood samples is variable and could not definitely conclude that frequency of EHV-1 is always higher in blood or nasal swab samples (Brown, et al., 2007; Ataseven, et al., 2009; Turan, et al.,

2012; Yilmaz, et al., 2012). In a survey in Turkey Turan et al., (2012) identified EHV-1 that were homogenous and correlated with European EHV-1. Our results are also agreeable with this survey and EHV-1 obtained from Iran correlated with the EHV-1 obtained from other parts of the world.

Phylogenetic tree showed that the Iranian EHV-1 strains were homogenous and had a close relationship with the previously reported strains in gene bank. Generally, herpes viruses are DNA viruses that use intra nuclear self-proofing system, so the mutation rates in this group of viruses are low, therefore genetic differences among them are low.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Ataseven, V. S., Dağalp, S. B., Güzel, M., Başaran, Z., Tan, M. T., Geraghty, B. (2009). Prevalence of equine herpesvirus-1 and equine herpesvirus-4 infections in equidae species in Turkey as determined by ELISA and multiplex nested PCR. Res Vet Sci, 86(2), 339-344.https://doi.org/10.1016/j.rvsc.2008.06.001.

Brown, J. A., Mapes, S., Ball, B. A., Hodder, A. D., Liu, I. K., Pusterla, N. (2007). Prevalence of equine herpesvirus-1 infection among Thoroughbreds residing on a farm on which the virus was endemic. J Am Vet Med Assoc, 231(4), 577-580.https://doi.org/10.2460/javma.231.4.577. PMID:17696859

Carlson, J. K., Traub-Dargatz, J. L., Lunn, D. P., Morley, P. S., Kohler, A., Kasper, K., ... & Lunn, K. F. (2013). Equine viral respiratory pathogen surveillance at horse shows and sales. J Equine Vet Sci, 33(4), 229-237. .https://doi.org/10.1016/j.jevs.2012.06.006.

Constable PD, Hinchcliff KW, Done SH, et al. (2017). Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats, 11th edition, Elsevier, Melbourne. Australia , p. 1040-1042.

Damiani, A. M., de Vries, M., Reimers, G., Winkler, S., & Osterrieder, N. (2014). A severe equine herpesvirus type 1 (EHV-1) abortion outbreak caused by a neuropathogenic strain at a breeding farm in northern Germany. Vet Microbiol , 172(3-4), 555-562.https://doi.org/10.1016/j.vetmic.2014.06.023. PMID:25042527

Diallo, I. S., Hewitson, G., Wright, L. L., Kelly, M. A., Rodwell, B. J., Corney, B. G. (2007). Multiplex real-time PCR for the detection and differentiation of equid herpesvirus 1(EHV-1) and equid herpesvirus 4 (EHV-4). Vet Microbiol, 123(1), 93-103.https://doi.org/10.1016/j.vetmic.2007.02.004. PMID:17346907

Diallo, I. S., Hewitson, G., Wright, L., Rodwell, B. J., Corney, B. G. (2006). Detection of equine herpesvirus type 1 using a real-time polymerase chain reaction. J Virol Methods, 131(1), 92-98.https://doi.org/10.1016/j.jviromet.2005.07.010.

PMID:16137772

Friday, P. A., Scarratt, W. K., Elvinger, F., Timoney, P. J., & Bonda, A. (2000). Ataxia and paresis with equine herpesvirus type 1 infection in a herd of riding school horses. J Vet Intern Med, 14(2), 197-201.https://doi.org/10.1111/j.1939-1676.2000.tb02236.PMID:10772493

Goehring, L. S., Winden, S. C., Maanen, C. V., Oldruitenborgh-Oosterbaan, M. M. S. (2006). Equine Herpesvirus Type 1-Associated Myeloencephalopathy in The Netherlands: A Four-Year Retrospective Study (1999–2003). J Vet Intern Med, 20(3), 601-607.https://doi.org/10.1111/j.1939-1676.2006.tb02903. PMID:16734096

Henninger, R. W., Reed, S. M., Saville, W. J., Allen, G. P., Hass, G. F., Kohn, C. W., Sofaly, C. (2007). Outbreak of neurologic disease caused by equine Herpesvirus-1 at a university equestrian center. J Vet Intern Med, 21(1), 157-165.https://doi.org/10.1111/j.1939-1676.2007.tb02942.

Hussey, S. B., Clark, R., Lunn, K. F., Breathnach, C., Soboll, G., Whalley, J. M., Lunn, D. P. (2006).

Detection and quantification of equine herpesvirus-1 viremia and nasal shedding by real-time polymerase chain reaction. J Vet Diagn Investig, 18(4), 335-342.https://doi.org/10.1177/104063870601800403.PMID:16921871

Hussey, G. S., Ashton, L. V., Quintana, A. M., Lunn, D. P., Goehring, L. S., Annis, K., & Landolt, G. (2014). Innate immune responses of airway epithelial cells to infection with Equine herpesvirus-1. Vet Microbiol, 170(1-2), 28-38.https://doi.org/10.1016/j.vetmic.2014.01.018.PMID:24560592

Johnstone, S., Barsova, J., Campos, I., & Frampton, A. R. (2016). Equine herpesvirus type 1 modulates inflammatory host immune response genes in equine endothelial cells. Vet Microbiol, 192, 52-59.https://doi.org/10.1016/j.vetmic.2016.06.012.PMID:27527764

Kawakami, Y., Kaji, T., Ishizaki, R., Shimizu, T., Matumoto, M. (1962). Etiologic study on an outbreak of acute respiratory disease among colts due to equine rhinopneumonitis virus. Jpn J Exp Med, 32, 211-229. PMID:14031490

Kirisawa, R., Endo, A., Iwai, H., Kawakami, Y. (1993). Detection and identification of equine herpesvirus-1 and-4 by polymerase chain reaction. Vet Microbiol, 36(1-2), 57-67. https://doi.org/10.1016/0378-1135(93)90128-T.PMID:8236780

Ko, S., Kang, J. G., Yeh, J. Y., Moon, J. S., Choi, G. C., Won, S., & Chae, J. S. (2013). First report on molecular detection of equine upper respiratory infectious viruses in republic of Korea. J Equine Vet Sci, 33(8), 628-636.https://doi.org/10.1016/j.jevs.2012.11.001.

Laabassi, F., Hue, E., Fortier, C., Morilland, E., Legrand, L., Hans, A., & Pronost, S. (2017). Epidemiology and molecular detection of equine herpesviruses in western Algeria in 2011. Vet Microbiol, 207, 205-209.https://doi.org/10.1016/j.vetmic.2017.06.017.PMID:28757025

Laval, K., Van Cleemput, J., Poelaert, K. C., Brown, I. K., & Nauwynck, H. J. (2017). Replication of neurovirulent equine herpesvirus type 1 (EHV-1) in CD172a+ monocytic cells. Comp Immunol Microbiol Infect Dis, 50, 58-62.https://doi.org/10.1016/j.cimid.2016.11.006.PMID:28131380

Lunn, D. P., Davis-Poynter, N., Flaminio, M. J. B. F., Horohov, D. W., Osterrieder, K., Pusterla, N., Townsend, H. G. G. (2009). Equine Herpesvirus-1 consensus statement. J Vet Intern Med, 23(3), 450-461.https://doi.org/10.1111/j.1939-1676.2009.0304.PMID:1964583

Ma, G., Azab, W., & Osterrieder, N. (2013). Equine herpesviruses type 1 (EHV-1) and 4 (EHV-4) masters of co-evolution and a constant threat to equids and beyond. Vet Microbiol, 167(1-2), 123-134.https://doi.org/10.1016/j.vetmic.2013.06.018.PMID:23890672

Matsumura, T., Sugiura, T., Imagawa, H., Fukunaga, Y., & Kamada, M. (1992). Epizootiological aspects of type 1 and type 4 equine herpesvirus infections among horse populations. J Vet Med Sci, 54(2), 207-211.https://doi.org/10.1292/jvms.54.207.PMID:1318750

Momtaz, H ., Hemmatzadeh, F. (2003). A serological survey on equine herpes virus1 and equine herpes virus4 in the horse using ELISA. Pajouhesh & Sazandegi, 59, 63-69.

OIE Terrestrial Manual. (2015). Equine rhinopneumonitis (Equine herpes virus 1 and 4).

Oladunni, F. S., Sarkar, S., Reedy, S., Balasuriya, U. B., Horohov, D. W., & Chambers, T. M. (2018). Absence of relationship between type-I interferon suppression and neuropathogenicity of EHV-1. Vet Immunol Immunopathol, 197, 24-30.https://doi.org/10.1016/j.vetimm.2018.01.007.PMID:29475503

Pusterla, N., Hussey, S. B., Mapes, S., Leutenegger, C. M., Madigan, J. E., Ferraro, G. L., Lunn, D. P. (2009). Comparison of four methods to quantify equid herpesvirus 1 load by real-time polymerase chain reaction in nasal secretions of experimentally and naturally infected horses. J Vet Diagn Iinvestig, 21(6), 836-840. https://doi.org/10.1177/104063870902100611.PMID:19901285

Pusterla, N., Mapes, S., Wilson, W. D. (2010). Prevalence of equine herpesvirus type 1 in trigeminal ganglia and submandibular lymph nodes of equids examined postmortem. Vet Rec, 167(10), 376.http://dx.doi.org/10.1136/vr.c3748.PMID:20817899

Pusterla, N., & Hussey, G. S. (2014). Equine herpesvirus 1 myeloencephalopathy. Vet Clin Equine Pract, 30(3), 489-506.https://doi.org/10.1016/j.cveq.2014.08.006.PMID:25300635

Sarani, A., Mohammadi, G., Mayameei, A., Ak-

bari, M. (2013). Investigation of equine herpesvirus-1 and 4 infections in equine population of Iran by real-time PCR. Human Vet Med, 5(1), 29-33.

Sarkar, S., Balasuriya, U. B., Horohov, D. W., & Chambers, T. M. (2015). Equine herpesvirus-1 suppresses type-I interferon induction in equine endothelial cells. Vet Immunol Immunopathol, 167(3-4), 122-129. https://doi.org/10.1016/j.vetimm.2015.07.015.PMID:26275803

Sarkar, S., Balasuriya, U. B., Horohov, D. W., & Chambers, T. M. (2016). Equine herpesvirus-1 infection disrupts interferon regulatory factor-3 (IRF-3) signaling pathways in equine endothelial cells. Vet Immunol Immunopathol, 173, 1-9. https://doi.org/10.1016/j.vetimm.2016.03.009. PMID:27090619

Słońska, A., Cymerys, J., Godlewski, M. M., Dzieciątkowski, T., Tucholska, A., Chmielewska, A., & Bańbura, M. W. (2014). Equine herpesvirus type 1 (EHV-1)-induced rearrangements of actin filaments in productively infected primary murine neurons. Arch Virol, 159(6), 1341-1349.PMID:24352436

Stasiak, K., Rola, J., Ploszay, G., Socha, W., & Zmudzinski, J. F. (2015). Detection of the neuropathogenic variant of equine herpesvirus 1 associated with abortions in mares in Poland. BMC Vet Res, 11(1), 102. https://doi.org/10.1186/s12917-015-0416-7.PMID:25929692

Taktaz Hafshejani, T., Nekoei, S., Vazirian, B., Doosti, A., Khamesipour, F., Anyanwu, M. U. (2015). Molecular detection of equine herpesvirus types 1 and 4 infection in healthy horses in Isfahan central and Shahrekord southwest regions, Iran. Biomed Res Int, 2015.http://dx.doi.org/10.1155/2015/917854.PMID:26421307

Turan, N., Yildirim, F., Altan, E., Sennazli, G., Gurel, A., Diallo, I., Yilmaz, H. (2012). Molecular and pathological investigations of EHV-1 and EHV-4 infections in horses in Turkey. Res Vet Sci, 93(3), 1504-1507. https://doi.org/10.1016/j.rvsc.2012.01.019.PMID:22401978

Vaz, P. K., Horsington, J., Hartley, C. A., Browning, G. F., Ficorilli, N. P., Studdert, M. J., ... & Devlin, J. M. (2016). Evidence of widespread natural recombination among field isolates of equine herpesvirus 4 but not among field isolates of equine herpesvirus 1.J Gen Virol, 97(Pt 3), 747.PMID: 26691326

Wagner, W. N., Bogdan, J., Haines, D., Townsend, H. G., Misra, V. (1992). Detection of equine herpesvirus and differentiation of equine herpesvirus type 1 from type 4 by the polymerase chain reaction. Can J Microbiol, 38(11), 1193-1196.https://doi.org/10.1139/m92-196.PMID:1335829

Walter, J., Seeh, C., Fey, K., Bleul, U., & Osterrieder, N. (2013). Clinical observations and management of a severe equine herpesvirus type 1 outbreak with abortion and encephalomyelitis. Acta Vet Scand, 55(1), 19.https://doi.org/10.1186/1751-0147-55-19.PMID:23497661

Yilmaz, H., Altan, E., Turan, N., Gurel, A., Haktanir, D., Sonmez, K., Richt, J. A. (2012). First report on the frequency and molecular detection of neuropathogenic EHV-1 in Turkey. J Equine Vet Sci, 32(9), 525-530.https://doi.org/10.1016/j.jevs.2011.12.006.

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تشخیص مولکولی و آنالیز فیلوژنی هرپس ویروس تیپ ۱ تک سمیان در اسب‌های دارای سابقه یا نشانه‌های بالینی در چهار استان ایران

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چکیده

زمینه مطالعه: هرپس ویروس تیپ ۱ تک سمیان یکی از علل مهم خسارتهای اقتصادی در صنعت پرورش اسب است و به عنوان علتی برای سقط، بیماری تنفسی، اختلالات عصبی و مرگ کرهاسبهای نوزاد شناخته شده است.

هدف: هدف از این مطالعه ارزیابی فراوانی هرپس ویروس تیپ ۱ تک سمیان در اسبهای با نشانه های بالینی و یا سابقه بالینی مرتبط با این ویروس در ۴ استان ایران (گلستان، تهران، خوزستان و آذربایجانغربی) که دارای جمعیت اسب قابل توجهی هستند و به دنبال آن مطالعه فیلوژنی موارد مثبت و مقایسه آنها با هرپس ویروسها در سایر مناطق دنیا میباشد.

روش کار: نمونه های خون و سواب بینی از ۱۵۰ راس اسب از ۴ استان فوق الذکر اخذ گردید. DNA نمونهها استخراج شد و با روش real-time PCR TaqMan مورد ارزیابی قرار گرفت. در نهایت بررسی خویشاوندی با ترسیم درخت فیلوژنی بر اساس الگوریتم اتصال همسایه انجام گرفت.

نتایج: از مجموع ۱۵۰ راس اسب نمونه گیری شده، ۱۴ راس (۹/۳۳٪) از نظر هرپس ویروس تیپ ۱ تک سمیان مثبت بودند. نتایج نشان داد که موارد مثبت هرپس ویروس تیپ ۱ تک سمیان حاصل از این مطالعه قرابت قابل توجهی با هرپس ویروسها از سایر مناطق دنیا دارند. .

نتیجه گیری نهایی: مطالعه حاضر وجود این ویروس در استانهای مورد بررسی در ایران را تایید کرد و بنابراین توجه به برنامه‌های پیشگیری و کنترل به منظور جلوگیری از پراکندگی و شیوع این ویروس باید مد نظر قرار گیرد.

واژه‌های کلیدی:

تشخیص مولکولی، هرپس ویروس تیپ ۱ تک سمیان، آنالیز فیلوژنی، اسب

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