

Seasonal, Geographical, Age and Breed Distributions of Equine Viral Arteritis in Iran

Shahin NEJAT¹ Hassan MOMTAZ² Mehrdad YADEGARI¹ Shayan NEJAT³
Farhad SAFARPOUR DEHKORDI⁴ Faham KHAMESIPOUR⁴

¹ Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord - IRAN

² Department of Microbiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord - IRAN

³ Student of Veterinary Medicine, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord - IRAN

⁴ Young Researchers and Elites Club, Shahrekord Branch, Islamic Azad University, Shahrekord - IRAN

KVFD-2014-11934 Received: 10.07.2014 Accepted: 19.10.2014 Published Online: 22.10.2014

Abstract

The aim of this study was to estimate the prevalence of EVA in mare and stallion in various seasons, ages, breeds and regions of Iran. Totally, 470 blood samples which were taken from mares and stallions were tested for presence of EVA antigen and antibodies. Samples were collected in various seasons from various geographical parts of Iran. Blood and serum samples were taken from horses less than 1 year, 1-2 years, 2-3 years and more than 3 years old. Viral RNA and antibodies against EVA were detected in 4.46% and 4.04% of samples, respectively. Results showed that the EVA antigen and antibodies were higher in mare blood samples than stallion. Antibodies and antigen of EVA had the higher incidence in spring season and southern regions of Iran. Also, results showed that there were higher incidences of EVA antigen and antibodies in Standardbred breed and horses less than 1 year old. There were significant differences ($P<0.05$) for the incidence of EVA antigen and antibodies between mares and stallions, less than 1 year old and more than 3 years old horses, spring and winter seasons and finally, southern and northern parts of Iran. This study showed that incidences of Equine viral arteritis antigens and antibodies are related to sex and breed of horses, seasons of samples collection and geographic regions. As far as we know, this study is the first prevalence report of seasonal, geographical, age and breed distributions of EVA in stallions and mares in Iran.

Keywords: Equine viral arteritis, Seasons, Regions, Breeds, Age, Sexual, Iran

İran'da Atların Viral Arteritisi Enfeksiyonunun Mevsimsel, Coğrafi, Yaş ve Cins Yayılımı

Özet

Bu çalışmanın amacı İran'da Atların Viral Arteritisi Enfeksiyonunun (EVA) kısır ve aygırlarda mevsimsel, coğrafi, yaş ve cins yayılımını araştırmaktır. Kısır ve aygırlardan alınan toplam 470 kan örneği EVA antijen ve antikorlarının varlığı yönünden test edildi. Örnekler değişik mevsim ve coğrafi bölgelerden alındı. Kan ve serum örnekleri 1, 1-2, 2-3 ve 3 yaşta daha yaşlı hayvanlardan alındı. Viral RNA ve EVA antikorları sırasıyla %4.46 ve %4.04 örnekte tespit edildi. Elde edilen bulgular EVA antijen ve antikorlarının kısır ve aygırlarda daha fazla oranda bulunduğunu ortaya koydu. EVA antikor ve antijenleri ilkbahar döneminde ve İran'ın güney kesiminde daha fazlaydı. Ayrıca sonuçlar EVA antijen ve antikorlarının Standardbred atlarda ve 1 yaşından daha gençlerde daha fazla olduğunu gösterdi. EVA antijen ve antikorlarının varlığı açısından kısır ve aygırlar arasında, 1 yaşından gençler ile 3 yaşından daha yaşlılar arasında, ilkbahar ile kış mevsimleri arasında ve İran'ın güneyi ile kuzeyi arasında anlamlı farklar ($P<0.05$) saptandı. Bu çalışma Atların Viral Arteritisi antijenleri ve antikorlarının cinsiyet, at cinsi, örnek toplama mevsimi ve coğrafi bölge ile değişiklik gösterdiği tespit edildi. Bilgimiz dahilinde bu çalışma mevsimsel, coğrafi, yaş ve cins göre atlarda EVA yaygınlığını İran'da tespit etmek amacıyla yapılan ilk çalışmadır.

Anahtar sözcükler: Atların Viral Arteritisi, Mevsim, Bölge, Irk, Yaş, Cinsiyet, İran



İletişim (Correspondence)



+98 381 7220836



shndfa@yahoo.com

INTRODUCTION

Equine Viral Arteritis (EVA) is an acute and contagious disease of both horses and donkeys which causes by *Equine Arteritis Virus (EAV)*. It is characterized by fever, edema (especially of the scrotum, prepuce and limbs), nasal discharge, conjunctivitis, and infrequently death in young foals [1-3]. It may also result in abortion of pregnant mares and interstitial pneumonia or pneumoenteritis in young foals. Also, stallions can become persistently infected and shed virus into their semen [4,5].

Several outbreak of EVA have been reported previously from USA [6], UK [7], Spain [8], Denmark [9], South Africa [10] and Australia [11].

The two principal modes of transmission of *EAV* are horizontal, by direct contact with infectious respiratory tract secretions from acutely infected horses, and venereal, through natural breeding or artificial insemination with semen samples of persistently infected stallions [12]. Therefore, determination of the carrier stallion had critical epidemiological importance in the prevention and control of *EAV* infection [13].

Although EVA is a disease almost exclusively of equids, antibodies to *EAV* have been identified in donkeys [10]. Serological surveys have shown that *EAV* infection occurs among horses in North and South America, Europe, Australasia, Africa, and Asia [14] with considerable variation in seroprevalence of *EAV* infection among countries and within equine populations in some countries. There is only one known serotype of *EAV*, but geographically and temporally distinct strains of *EAV* differ in the severity of the clinical disease they induce and in their abortogenic potential [14,15].

The epidemiology and prevalence of EVA in mares and stallions is essentially unknown in Iran. Therefore, this present study was carried out in order to study the seasonal, geographical, age and breed distribution of EVA in mares and stallions blood samples using cell culture, Enzyme Linked Immunosorbent Assay (ELISA) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) assays in Iran.

MATERIAL and METHODS

Samples

From December 2010 and December 2011 in various seasons of the year, blood and serum samples were collected randomly from stallions (n=220) and mares (n=250) (from 3 major breeds of Arab, Standardbred and Thoroughbred). Horses used in this study were evaluated as less than 1, 1 to 2, 2 to 3 and more than 3 years old which were settled in 4 different geographic regions of Iran (north, south, west and east).

The blood samples were collected into clot activator vacuum tubes and centrifuged at 800 × g for 10 min. The separated sera were kept at -20°C before testing. EDTA-blood samples (10 mL) were centrifuged at 18°C (1.400 × g) for 35 min. Buffy coat cells were re-suspended in 4 volumes of sterile 0.2% NaCl to lyse erythrocytes. After 1 min, 7.2% NaCl was added to reconstitute isotonicity. The cells were washed in phosphate-buffered saline and stored at -70°C.

RNA Extraction

The viral RNA was extracted by the guanidium isothiocyanate method. Briefly, 500 µL of 4 M guanidium isothiocyanate, 50 µL of 2 M sodium acetate (pH=4.0), 500 µL of water-saturated phenol, and 100 µL of chloroform were added to the sample, with thorough mixing after the addition of each reagent, and the mixture was left on ice for 15 min. Thereafter, the mixture was microcentrifuged for 20 min at 5°C, and the upper aqueous phase was transferred to a fresh tube. Viral RNA was precipitated with 500 µL of cold isopropanol for 1h at -20°C. After centrifugation, the pellet was resuspended in 150 µL of 4 M guanidium isothiocyanate. The suspension was then precipitated with isopropanol, and the resulting RNA pellet was washed with 75% ethanol, dried, and resuspended in 20 µL of diethylpyrocarbonate- treated water. The RNA samples were kept at -70°C until use.

Oligonucleotide Primers, cDNA Synthesis, and PCR

The oligonucleotide primers used for RT-PCR were from the 3' end of ORF 1b of the *EAV* genome. Their sequences were as those described by other studies [16]:

PEV-10: 5'-GAGGATCCCACTTCATCT-3'

PEV-11: 5'-AATGGTCTGCACTGAGGT-3'

For cDNA synthesis, the reaction mixture was incubated at 70°C for 10 min and was chilled on ice prior to the addition of the reverse transcriptase enzyme and RNasin. RT of *EAV* RNA was carried out at 37°C for 15 min and at 42°C for 90 min in 1× Taq buffer (10× Taq buffer is 500 mM KCl, 100 mM Tris-HCl (pH 8.3), 1% Triton X-100) containing 0.5 mM (each) deoxynucleoside triphosphates and to which 3 µL of A buffer (400 mM Tris-HCl (pH 8.3), 50 mM MgCl₂), 8 U of avian myeloblastosis virus reverse transcriptase (Roche applied sciences), 17 U of RNasin (Fermentas), 4 µL of RNA sample, and 20 pmol of PEV-11 primer were added to a final reaction volume of 30 µL. Thereafter, the mixture was incubated at 95°C for 5 min to inactivate the avian myeloblastosis virus reverse transcriptase, and the following reagents were added: 25 pmol of each sense and antisense primer, 0.15 mM (each) deoxynucleoside triphosphates, 1.5 U of Taq polymerase (Fermentas), and 5 µL of 10× Taq buffer to a final reaction volume of 50 µL. The cDNA was then amplified by 30 successive cycles of denaturation at 95°C for 1 min, primer annealing at 50°C for 1 min, and DNA chain extension at 72°C for 2

min with a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). Twenty µL of final PCR products were run on a 1.5% agarose gel containing ethidium bromide in 1X TBE buffer along with 100 bp DNA ladder (Fermentas, Germany).

Serological Test

Serum samples were tested for antibodies against EAV by the commercial ELISA kit (ID Vet Innovative Diagnostics, France), according to the manufacturer's instructions.

Statistical Analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using the SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA), Chi-square test analyses were performed, and differences were considered significant at values of $P < 0.05$.

RESULTS

Table 1 presents the distribution of EVA antigen and antibody in mares and stallion blood samples. Totally, 21 out of 470 serum samples (4.46%) and 19 out of 470 blood samples (4.04%) were found to be positive for presences of EVA antibody and antigen, respectively. Also, 8 mares

(3.63%) and 13 stallions (5.2%) serum samples were positive for antibodies against EAV. Besides, 7 mares (3.18%) and 12 stallions (4.8%) blood samples were positive for EAV antigen. There were significant differences about $P < 0.05$ for presences of antibodies against virus and viral antigens between mares and stallions.

Table 2 presents the seasonal and geographical distribution of antibodies against EVA and viral antigen in mare and stallion blood samples.

Results revealed that blood and serum samples which were collected from southern regions of Iran and samples which were collected in spring had the highest incidence of antibodies and antigen of EVA in mares and stallions. Statistical analysis were significant for the presences of EVA antibodies and antigens between the samples which were collected in spring with autumn and winter and also, between the samples which were collected from southern parts and northern and western parts of Iran ($P < 0.05$).

Table 3 presents the age and breed distribution of EVA antigen and antibody in mare and stallion blood samples. Totally, the horses less than 1 year old and Standardbred horses had the highest incidence of EVA antigen and antibodies. There were significant differences ($P < 0.05$) for incidences of viral antibodies and antigens between mares and stallions less than 1 year old and mares and stallions more than 3 years old. Also, statistical differences were significant for incidences of viral antigens and antibodies between Standardbred and Thoroughbred breeds ($P < 0.05$).

Table 1. Distribution of EVA antigen and antibody in mare and stallion blood samples

Tablo 1. Kısırak ve aygır kan örneklerinde Atların Viral Arteritisi antijen ve antikorlarının yayılımı

Type and Number of Samples	ELISA (%)	RT-PCR (%)
Stallion (220)	8 (3.63)	7 (3.18)
Mare (250)	13 (5.2)	12 (4.8)
Total (470)	21 (4.46)	19 (4.04)

DISCUSSION

The results of our present study indicated that there were effective seasonal and geographical distributions for incidences of EVA antigens and antibodies against EVA in

Table 2. Seasonal and geographical distribution of EVA antigen and antibody in mare and stallion blood samples

Tablo 2. Kısırak ve aygır kan örneklerinde Atların Viral Arteritisi antijen ve antikorlarının mevsimsel ve coğrafi yayılımı

Type and Number of Samples	Seasonal Distribution							
	Antibody (21 Positive)*				Antigen (19 Positive)**			
	Aut***	Spr	Sum	Wint	Aut	Spr	Sum	Wint
Stallion (220)	2	5	1	-	1	4	1	1
Mare (250)	3	8	2	1	4	6	1	1
Total (470)	5	12	3	1	5	11	2	2
Type and Number of Samples	Geographical Distribution							
	Antibody (21 Positive)*				Antigen (19 Positive)**			
	South	North	East	West	South	North	East	West
Stallion (220)	5	-	2	1	4	-	2	1
Mare (250)	9	1	2	1	8	-	3	1
Total (470)	14	1	4	2	12	-	5	2

* Positive samples based on ELISA methods; ** Positive samples based on RT-PCR method; *** In this table Aut means autumn, Wint means winter, Spr means spring and Sum means summer

Table 3. Age and breeds distribution of EVA antigen and antibody in mare and stallion blood samples**Tablo 3.** Kısırak ve aygır kan örneklerinde Atrların Viral Arteritisi antijen ve antikorlarının yaş ve cinsine göre yayılımı

Type and Number of Samples	Age Distribution							
	Antibody (21 Positive)*				Antigen (19 Positive)**			
	1>	1-2	2-3	3<	1>	1-2	2-3	3<
Stallion (220)	5	-	-	-	2	1	1	-
Mare (250)	14	2	-	-	7	4	2	2
Total (470)	19	2	-	-	9	5	3	2
Type and Number of Samples	Breed Distribution							
	Antibody (21 Positive)*			Antigen (19 Positive)**				
	Standardbred	Thoroughbred	Arab	Standardbred	Thoroughbred	Arab		
Stallion (220)	4	1	2	3	1	2		
Mare (250)	7	3	4	7	2	4		
Total (470)	11	4	6	10	3	6		

* Positive samples based on ELISA methods; ** Positive samples based on RT-PCR method

mares and stallions. Samples which were collected from southern and eastern parts of Iran and also, samples which were collected in spring and summer seasons had the highest range of infection. The main reason for this finding is the fact that temperature and climate may have an effect on the prevalence of antigen and antibodies against EVA.

Spring and summer are breeding equine seasons in many parts of Iran especially southern and eastern regions. Therefore, in these parts and in these seasons, modes of transmission and infection rate become higher than other seasons and regions. The main reason for the highest prevalence of EVA in spring and summer seasons is the fact that during this time climatic events, heat, rain, and thunderstorms, as well as variation of barometric pressure has been changed and may have influence on the autonomic nervous system. These events caused reduction in the levels of animal immunity. Therefore, several infections have been occurred. Also, previous investigation showed that carrier stallions are the natural reservoir of EAV and the virus is maintained in equine populations between breeding seasons [4].

Also, survival of EAV in the environments is temperature dependant; although it may survive only 20-30 min at 56°C and from 2 to 3 day at 37°C, it can survive up to 75 day at 4°C but virus is so sensitive to temperature changes [17]. Therefore, this virus needs moderate constant temperature and especially between 20 to 4°C. After analyzing the average temperatures of these 4 seasons, it has been found that spring and summer had the most constant temperature. Besides, the most constant temperatures have been shown in southern and eastern parts of Iran. Therefore, the high prevalence of antigens and antibodies are seems reasonable in spring and summer seasons and southern and eastern parts of Iran.

Our results showed that there were strong age and

breed distributions for the incidences of EVA antigens and antibodies in mares and stallions in Iran. The horses less than 1 year old followed by, those had 1-2 years old were the highest incidence of EVA antigens and antibodies. Previous studies showed that death in younger than 1 year old foal is one of the main complications of EVA [1-3].

This present study showed that Standardbred followed by, Arab horses had the highest incidences of EVA antigens and antibodies. Antigen and antibodies of EVA were less common in Thoroughbred horses. These findings are in harmony with previous investigations [14,18-20]. Infection is endemic among Standardbred but not Thoroughbred horses in the USA, with 77.5-84.3% of all Standard breeds, but only 0-5.4% of Thoroughbreds being seropositive [14,18,20]. The seroprevalence of EAV infection in Warmblood stallions is also very high in a number of European countries, e.g., 55-93% of Austrian Warmblood stallions were positive for antibodies to EAV [21,22]. Although breed-specific differences might reflect inherent genetic differences that confer resistance to infection, they are more likely reflective of different cultural and management factors within horse populations and breeds. Previous studies have not demonstrated any breed-specific variation in susceptibility to EAV infection or in establishment of the carrier state [23] but our results for the first time showed that EVA antibodies and antigen had the highest incidence in Standardbreds and Arab breeds.

Extensive outbreaks of EVA were reported in North America and Europe [17]. Similarly, EAV infection of horses has been identified in countries including Australia, New Zealand, and South Africa, previously thought to be largely or completely free of the virus. Totally, serological surveys have demonstrated that EAV infection occurs in Europe, Australia, North and South America, Africa, and Asia [1,17]. Japan and Iceland are apparently free of the virus, whereas EAV infection is relatively common in horses in

several European and Asian countries. It seems that, the carrier state that occurs in persistently infected stallions constitutes the natural reservoir of EVA, with carrier stallions venereally transmitted EVA to susceptible mares by natural service or Artificial insemination (AI) [17]. Iran has a long history in breeding and maintaining of horses. Because the high frequency of horse trade with other countries in this area, it seemed so far that EVA infection did not exist.

The results of our study showed that 3.63% of stallion serum samples and 5.2% of mare serum samples were positive for antibodies against EVA. Higher prevalence of antibodies against EVA has been reported previously from Turkey (14.47% by ELISA test) [24]. Other study [3] reported that 51.1% of horses were serologically positive for EVA's antibodies. Another research [25] reported that 8.75% of Tunisian horse sera have antibodies to EVA. The seroprevalence of EVA have been reported previously as 11.3% (Switzerland) [26], 2.3% (UK) [27], 14% (Dutch) [15], 20% (Germany) [28] and 18.6% (USA) [29]. These high differences in prevalence of EVA in blood and serum samples maybe due the facts that type of samples (sex, breed and age of horses), number of samples, methods of sampling, method of experiment, geographical area and even climate of area which samples were collected are different in each investigation.

As far as we know, this present study is the first prevalence report of seasonal, age, breed and geographical distribution of EVA antigen and antibodies in stallions and mares in Iran. It seems that the main cause of prevalence of EVA infection in Iran is lack of diagnosis in the field conditions, and finally lack of programs for control and eradication of the disease by responsible organizations including veterinary organization. Therefore, several investigations should be done on prevalence of this viral disease in Iran. International transportation of carrier stallions or infected semen, play an important role in epidemiology of EAV infection. Therefore, horse transportation should be controlled firmly. More clinical cares should performed on less than 1 year old horses, standard-bred horses and especially on southern and eastern parts of Iran on spring season.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of Biotechnology Research Center and Large Animal Clinic of the Islamic Azad University of Shahrekord for their important technical and clinical support.

REFERENCES

- Horner GW:** Equine viral arteritis control scheme: A brief review with emphasis on laboratory aspects of the scheme in New Zealand. *New Z Vet J*, 52 (2): 82-84, 2004.
- Radostits OM, Gay CC, Blood DC, Hinchcliff KW, Constable PD:** Veterinary Medicine. A Text Book of the Disease of Cattle, Sheep, Pigs, Goats and Horses. 10th ed., Saunders Elsevier, Edingburg, 2007.
- Rola J, Larska M, Rola JG, Belák S, Autorino GL:** Epizootiology and phylogeny of equine arteritis virus in hucul horses. *Vet Microbiol*, 148 (2-4): 402-407, 2011.
- Timoney PJ, McCollum WH, Roberts AW, Murphy TW:** Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. *Res Vet Sci*, 41 (2): 279-280, 1986.
- Wieringa R, De Vries AA, Post SM, Rottier PJ:** Intra and intermolecular disulfide bonds of the gp2b glycoprotein of equine arteritis virus: Relevance for virus assembly and infectivity. *J Virol*, 77 (24): 12996-13004, 2003.
- Timoney PJ, Creekmore L, Meade B, Fly D, Rogers E, King B:** Multi-state occurrence of EVA report of the committee on infectious diseases of horses: Addressing equine viral arteritis in the United States. In, *Proceedings of the One-Hundred Tenth Annual Meeting of the United States Animal Health Association*. Minneapolis, Minnesota: Pat Campbell & Associates and Spectrum Press, 354-362, 2006.
- Camm IS, Thursby-Pelham C:** Equine viral arteritis in Britain. *Vet Rec*, 132 (24): 615, 1993.
- Monreal L, Villatoro AJ, Hooghuis H, Ros I, Timoney PJ:** Clinical features of the 1992 outbreak of equine viral arteritis in Spain. *Equine Vet J*, 27 (4): 301-304, 1995.
- Larsen LE, Storgaard T, Holm E:** Phylogenetic characterization of the g (I) sequences of equine arteritis virus isolated from semen of asymptomatic stallions and fatal cases of equine viral arteritis in Denmark. *Vet Microbiol*, 80 (4): 339-346, 2001.
- Paweska JT, Binns MM, Woods PS, Chirside ED:** A survey for antibodies to equine arteritis virus in donkeys, mules and zebra using virus neutralisation (vn) and enzyme linked immunosorbent assay (ELISA). *Equine Vet J*, 29 (1): 40-43, 1997.
- Huntington PJ, Forman AJ, Ellis PM:** The occurrence of equine arteritis virus in Australia. *Aust Vet J*, 67 (12): 432-435, 1990.
- Balasuriya UB, Maclachlan NJ:** The immune response to equine arteritis virus: Potential lessons for other arteriviruses. *Vet Immunol Immunopathol*, 102 (3): 107-129, 2004.
- Morrell JM, Geraghty RM:** Effective removal of equine arteritis virus from stallion semen. *Equine Vet J*, 38 (3): 224-229, 2006.
- Timoney PJ, McCollum WH:** Equine viral arteritis. *Vet Clin North Am: Equine Pract*, 9 (2): 295-309, 1993.
- Zhang J, Miszczak F, Pronost S, Fortier C, Balasuriya UB, Zientara S, Fortier G, Timoney PJ:** Genetic variation and phylogenetic analysis of 22 French isolates of equine arteritis virus. *Arch Virol*, 152 (11): 1977-1994, 2007.
- St-laurent G, Morin G, Archambault D:** Detection of equine arteritis virus following amplification of structural and nonstructural viral genes by reverse transcription-PCR. *J Clin Microbiol*, 32 (3): 658-665, 1994.
- Holyoak GR, Balasuriya UB, Broaddus CC, Timoney PJ:** Equine viral arteritis: Current status and prevention. *Theriogenol*, 70 (3): 403-414, 2008.
- Timoney PJ, McCollum WH, Murphy TW, Roberts AW, Willard JG, Carswell GD:** The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of virus transmission. *J Reprod Fertil Suppl*, 35 (4): 95-102, 1987.
- Timoney PJ:** Equine viral arteritis: Epidemiology and control. *J Equine Vet Sci*, 8 (1): 54-59, 1988.
- McCollum WH, Bryans JT:** Serological identification of infection by equine arteritis virus in horses of several countries. In, *Proceeding of the 3rd International Conference of Equine Infectious Diseases*. Australia, p. 256-263, 1973.
- Moraillon A, Moraillon R:** Results of an epidemiological investigation on viral arteritis in France and Some other European and African countries. *Ann Rech Vet*, 9 (1): 43-54, 1978.
- Burki FH, Nowotny N:** Objective data plead to suspend importbans for seroreactors against equine arteritis virus except for breeder stallions. *J Appl Anim Res*, 1 (1): 31-42, 1992.
- Timoney PJ, McCollum WH:** Equine viral arteritis: Current clinical and economic significance. In, *Proc AAEP*, 1990.

-
- 24. Yildirim Y, Kirmizigul AH, Tolga Tan M, Gokce E, Irmak K:** Seroprevalence of equine viral arteritis in donkeys in Kars district, Turkey. *J Anim Vet Adv*, 7 (9): 1110-1112, 2008.
- 25. Ghram A, Chabchoub A, Turki I, Boussetta M, Ibn Amor H, Ghorbel A:** Rhinopneumonia and equine viral arteritis: Seroepidemiological study in the northeast of Tunisia. *Arch Inst Pasteur Tunis*, 71 (1-2): 5-12, 1994.
- 26. deBoer GF, Osterhaus AD, van Oirschot JT, Wemmenhove R:** Prevalence of antibodies to equine viruses in the Netherlands. *Vet Q*, 1 (2): 65-74, 1979.
- 27. Glaser AL, De Vries AA, Rottier PJ, Horzinek MC, Colenbrander B:** Equine arteritis virus: A review of clinical features and management aspects. *Vet Quart*, 18 (3): 95-99, 1996.
- 28. Eichhorn W, Heilmann M, Kaaden OR:** Equine viral arteritis with abortions: Serological and virological evidence in Germany. *Zentralbl Veterinarmed B*, 42 (9): 573-576, 1995.
- 29. Hullinger PJ, Gardner IA, Hietala SK, Ferraro GL, Maclachlan NJ:** Seroprevalence of antibodies against equine arteritis virus in horses residing in the United States and imported horses. *J Am Vet Med Assoc*, 219 (7): 946-949, 2001.