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Equine Herpesvirus Type 1 and 4 in Individually Reared Horses in Central and Western Turkey

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Abstract

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The objective of the study was to investigate EHV type 1 and 4 infections serologically in individually reared horses in Turkey.

Equine herpesvirus 1 (EHV1) and 4 comprise two distinct viruses of horses of the Alphaherpesvirinae subfamily. EHV1 is a major cause of abortion, respiratory and neurological disorders in horses. EHV4 is responsible for respiratory disease in foals. In this study, EHV1 and 4 infections were investigated serologically in individually reared horses in Turkey. A total of 188 unvaccinated horses in four provinces were sampled and tested using indirect ELISA. EHV1-specific antibodies were found to be in three out of four provinces as 3.7% (7/188) between 4.5–6.9%. Test results showed that EHV4 is more prevalent than EHV1, the proportion varied among 48.4% and 65.7% in four provinces, 107 out of 188 samples (56.9%) were found to be seropositive. This is the first serological investigation for EHV1 and EHV4 in Turkey.

ELISA, Equine Herpesvirus 1-4, horse, serosurvey, Turkey

EHV-1 and EHV-4 are widespread viral infections of horses in the world and cause important economic losses in the horse industry. There are five distinct herpesviruses known to cause disease in horses: EHV1, 2, 3, 4 and 5. Until 1981, it was thought that EHV 1 and 4 were the same virus, namely EHV-1 (equine rhinopneumonitis and equine abortion virus). As a result of the examination of the restriction endonuclease DNA fingerprint, these viruses comprise two antigenically and genetically distinct viruses (Sabine et al. 1981; Studdert et al.1981) in the Alphaherpesvirinae subfamily. Genomes of EHV 1 and 4 are linear dsDNA molecules, with a 57% G + C content (Darlington and Randall 1963) 145 and 150 kb of length, respectively.

Transmission generally occurs via direct or indirect contact with infectious secrets, foetuses and placentas. Both viruses cause disorders in respiratory, reproductive and nervous systems but most foetal isolates were typed as EHV-1; EHV-4 is mainly recognized as a respiratory system pathogen (Studdert et al. 1984).

It has been indicated that EHV 1 and 4 persist in the trigeminal ganglia, lymphoid tissue of the respiratory system and peripheral lymphocytes of naturally infected horses as viral DNA, and that stress and corticosteroid treatment lead to reactivation of the virus (Welch et al. 1992; Slater et al. 1994).

Herpesviruses are generally not strongly immunogenic, even infected horses could be found as antibody-negative, so pathogenesis of these viruses is very complicated.

Acute respiratory symptoms due to EHV4 include fever, anorexia, nasal and ocular discharge; rhinopneumonitis can develop in the existence of secondary bacterial infection (Studdert 1974). Respiratory system findings due to EHV1 are similar to those produced by EHV-4. Histopathological basis of abortion during EHV1 and EHV4 infections are

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severe vasculitis and thrombosis in the endometrial blood vessels (Edington et al. 1991; Smith et al. 1992). Nearly 95% of the EHV1 abortions occur in the last third of gestation (Allen and Bryans 1986), the infected newborn foals generally die in a few days.

EHV-1 causes a much more severe disease than EHV-4, but seroprevalence and viral diagnosis during field outbreaks studies show that type 1 is not prevalent as type 4 (Crabb and Studdert 1993, 1994; Gilkerson et al. 1994; Van Maanen et al. 2000).

The objective of this study was to investigate the prevalence and proportion of EHV1 and EHV4 infections in horses reared individually at small scale farms in Central and Western Anatolia and to obtain the first data on these infections in studied regions.

Materials and Methods

Sampled animals

This study was conducted in 188 apparently healthy draft horses kept at 170 small scale family farms in four provinces, two in Central Anatolia and two in Aegean Territory (Table 1). The horses used in the study were not vaccinated (EVH1 and EHV4 vaccine) and the age of animals ranged between 9 months and 6 years. The numbers of breeding horses were two at 18 farms and one at 152 farms out of 170. Blood samples were taken from vena saphena and centrifuged at 3 000 *g* for 10 min; serum samples were separated to stock tubes and kept in a freezer (-20 °C) until use.

Serological test (ELISA)

The conventional serological methods like complement fixation and virus neutralisation tests are not capable of distinguishing EHV1 and EHV4 due to cross-reaction (Hartley et al. 2005). An ELISA test was developed recently (Crabb and Studdert 1993; Crabb et al. 1995; Drummer et al. 1995), capable of distinguishing type 1 and 4 and dual infection. In this study, equine herpesvirus type 1 and 4 discriminating ELISA (Enzyme-Linked Immunosorbent Assay) kit (Svanova Biotech AB, Sweden) was used for serological controlling. The test was performed according to the producer's introduction, at the end of the test plates were measured on a 450 nm filter using ELISA reader for determining Optic Density (OD) values. All of the obtained OD values, both controls and samples, were calculated for correction before interpretation of the results.

Results

According to EHV 1/4 ELISA discriminating test, the EHV1-specific antibodies were detected in 7 (3.7%) out of 188 horses, no positive results were determined in the Eskisehir province and very low percentage was detected in Izmir (5.7%) and Afyonkarahisar (6.9%) provinces. The proportion of EHV4-positives were found to be 56.9% (107/188), the highest ratio (65.7%) was detected in the Izmir province (Table 1). A total of 76 samples were determined as seronegative for both viruses, two samples were found to be positive for both infections.

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Table 1. The sampled animals and distribution of EHV1 and 4 infections

Provinces	Number of samples	EHV-1		EHV-4	
		Ab(+)	(%)	Ab(+)	(%)
Izmir	35	2	5.7	23	65.7
Konya	44	2	4.5	27	61.3
Afyonkarahisar	43	3	6.9	25	58.1
Eskisehir	66	-	-	32	48.4
Total	188	7	3.7	107	56.9

Discussion

There are different serological diagnostic methods for determination of EHV1- and EHV4-specific antibodies. Previous serological surveys of EHV1 and EHV4 infections

have employed complement fixation and virus neutralisation tests, but these tests have failed to distinguish two serotypes because of a strong antigenic cross-reactivity. In mid 1990's, the diagnosis was facilitated by the development of the type-specific ELISA using monoclonal antibody (mAb) (Crabb and Studdert 1993; Crabb et al. 1995). Today ELISA is considered as a dependable and preferable method due to its practical advantages and higher sensitivity than provided by other serological tests (Crabb and Studdert 1993; Crabb et al. 1995; Yasunaga et al. 2000; Hartley et al. 2005), and allows new insights to epidemiologic studies.

Vaccination for these viruses has not been applied in the field routinely, except for large herds and race horses in Turkey. The sampled horses in this study were reared individually, 152 out of 188 animals were the only horse at the farm; at 18 farms there were two horses.

The aim of this study was to determine the entity and proportion of EHV1 and EHV4 in the field and considering the risk of transmission in intensive breeding, individually reared horses were preferred. A total of 188 blood serum samples were collected from family-type small farms in two provinces in Central Anatolia and two provinces in the Aegean region in Turkey. Serum samples were controlled using "Equine Herpesvirus 1 and 4 Discriminating Test" and seropositivity for EHV1 and 4 were found to be 3.7% and 56.9%, respectively. The EHV1-specific antibody entity was not determined in samples obtained from Eskisehir and the lowest proportion for type 4 (48.4%) was found to be in the same province. In three other provinces the EHV1 prevalence was determined between 4.5% and 6.9%. According to test results, EHV4 was found to be more prevalent than EHV1; seropositivity was detected as 56.9%, the highest proportion was determined in the Izmir province (65.7%) (Table 1). No significant differences in seroprevalence could be observed between sexes. Detailed health records of the sampled horses were not reached and there is no information whether the sampled animals showed clinical symptoms related to studied infections before or not, but according to information obtained from the farmers, the animals were not vaccinated for EHV1 and EHV4 infections. It is obvious that the obtained values show natural infection.

So far, EHV1 or EHV4 were not studied in Turkey but the DNA fingerprint analysis of the EHV4 isolates shows high genetic stability (Studdert 1983; Welch et al. 1992). Virus isolation and serological field studies have demonstrated that EHV1 and EHV4 infections show wide dissemination in many countries such as Canada (Carman et al. 1997), New Zealand (Jolly et al. 1986), Australia (Gilkerson et al. 1994), the USA (Mumford et al. 1998), Japan (Matsumura et al. 1992) and China (Mason et al. 1989).

EHV1 infection is usually seen in the winter season, the highest incidence of the disease was determined in nearly 3-year-old horses; but no seasonal variation was reported for EHV4, the infection can be found all year long (Matsumura et al. 1992). The sera samples in this study were collected in the summer of 2006, season factor could be a factor in the absence of the clinical findings.

In the USA, Gilkerson et al. (1994) detected 99% seropositivity in mares and foals for EHV4, while the prevalence of EHV1 antibody positive mares and foals was 26.2% and 11.4%, respectively. Similarly, Crabb and Studdert (1993) reported 9% seropositivity for type 1 and 100% for type 4. In another study in unvaccinated horses, Crabb and Studdert (1994) detected all of the horses as positive for EHV4, while 30% were positive for EHV1; the sera were from horses above 2 years of age. Prevalence studies show that EHV4 is much more prevalent than EHV1, as also our study does.

Drummer et al. (1995) studied 33 mares during an outbreak of abortions due to EHV1. The researchers collected sera samples 3, 13 and 67 days after the first abortion and tested the samples using ELISA prepared with recombinant EHV1 antigen. Seroconversion was determined in some aborting mares on day 13, indicating a recent infection; ten seronegative

mares were shown as positive on day 67 and foaled normally; the interpretation was that the infection occurred in late gestation or after foaling.

Due to the latent structure of EHV1 and EHV4 infections (Welch et al. 1992; Slater et al. 1994), the viruses could be reactivated in the presence of factors affecting immunity, such as immunosuppressive therapy, concomitant disease, stress, and pregnancy. On this account, effective precautions like immune-prophylaxis are required for the control of these diseases. Even though inactive vaccines were not found effective for a long time, they reduce the severity of respiratory disorders (Zhang et al. 1998). Live vaccines seem to be more useful in eliciting stimulation on both the cellular and humoral immune systems but carry the risk of latency.

So far there has been no report on seroprevalance of EHV1/4 in Turkey. In a single study in race horses, type 1 was detected in 5 of 36 neonatal dead foals using PCR (Tekelioglu et al. 2006). The obtained values for EHV1 (3.7%) and EHV4 (56.9%) in this study were found to be lower compared to most countries (Crabb and Studdert 1994; Gilkerson et al. 1994) but it is obvious that the infections may create potential health risk. In conclusion, EHV1 and EHV4 infections were investigated serologically for the first time in individually reared horse population in Central and Western Anatolia, and as in most studies, EHV4 was found to be more prevalent. Diagnosis of respiratory and reproductive system infections is difficult due to complex aetiology, EHV 1 and 4 should be taken into consideration. Further investigations to determine the prevalence of two biotypes in different regions is necessary for implementing control strategies.

Equinní Herpesvirus typu 1 a 4 u odděleně chovaných koní ve středním a západním Turecku

Cílem této studie bylo sérologické vyšetření individuálně chovaných koní v Turecku na infekci virem EHV1 a 4. Equinní herpesviry EHV1 a 4 patří k nejvýznamnějším virovým nákazám koní z podčeledi Alphaherpesvirinae. EHV1 je významným původcem virového abortu klisen a onemocnění dýchacího a neurologického aparátu koní. EHV4 způsobuje rhinopneumonii koní. Do studie bylo zahrnuto celkem 188 nevakcinovaných koní ze čtyř provincií, jejichž krev byla sérologicky vyšetřena na přítomnost protilátek proti EHV 1 a 4 nepřímou ELISA metodou. Ve třech ze čtyř provincií byly zjištěny specifické protilátky proti EHV 1 u 3.7 % zvířat (7/188) mezi 4.5–6.9 %. Výsledky prokázaly, že EHV 4 se oproti EHV 1 vyskytuje s vyšší prevalencí pohybující se v rozmezí 48.4 % až 65.7 % u koní ze čtyř provincií a zároveň bylo zjištěno, že z celkového počtu 188 vyšetřených vzorků krve bylo 107 (56.9 %) seropozitivních. Sérologické vyšetření koní na EHV 1 a 4 bylo první studií tohoto druhu v Turecku.

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