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Serological survey of equid herpesvirus 3 infection in Turkish horses

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Abstract: Sera from 1133 Turkish horses were obtained and tested for equid herpesvirus 3 (EHV-3) antibodies using a sero-neutralisation test. The sample consisted of 420 privately owned working horses from 2 regions of Turkey, 428 brood horses from private stud farms and breeding stations, and 285 racehorses from private stud farms. Neutralising antibodies against EHV-3 were found in 287 (25.3%) of the 1133 analysed sera. The highest seroprevalence for EHV-3 was detected in the brood horses (51.2%), followed by the racehorses (10.2%), and the working horses (9.3%). The seroprevalence rates tended to be higher in female horses than in male horses for all 3 horse populations tested ($P < 0.01$). The findings indicate significant differences in EHV-3 seropositivity rates between horses used for breeding and those used for work and racing ($P < 0.001$), suggesting that the intended use of horses may be an important factor in the epidemiological assessment of EHV-3 infection.

Key words: Antibody, equid herpesvirus 3, horse, sero-neutralisation, Turkey

1. Introduction

Equid herpesvirus 3 (EHV-3) causes an acute venereal disease called equine coital exanthema in mares and stallions. EHV-3 is a member of subfamily *Alphaherpesvirinae* within the family *Herpesviridae* (1–3). The infection is recognised by the formation of papules, vesicles, pustules, and ulcers on the genital region of mares and stallions (4,5). EHV-3 is transmitted through coitus via semen and through noncoital examinations via virus-contaminated equipment. Subsequent to primary infection, as in other alphaherpesviruses, the virus can result in a life-long latent infection (2,4). Horses with subclinical and latent infection play an important role in the transmission of the disease (2) as virus reactivation occurs in seropositive animals (5).

The infection was first clinically recognised in Ireland (6,7). Several studies indicated that the infection is widespread in embryo transfer and in horse breeding centres around the world where the prevalence varies from 6%–58% (2,8–10), although there have been limited reports on the prevalence of EHV-3 in working horses (1,11). The purpose of this study was to investigate the seroprevalence of EHV-3 in various horse populations in Turkey, for which there was no information available, in an effort to assess the regional baseline infection/disease prevalence rate and to guide future epidemiological studies.

2. Materials and methods

2.1. Study area and sampled animals

The animal panel consisted of 1133 sera obtained from Turkish horses: 420 privately owned indigenous-bred horses used as pack animals in the Aegean and Mediterranean regions (2012 and 2013), 428 brood horses (Thoroughbred and Haflinger breeds), and 285 racehorses (Thoroughbred and Arabian breeds) from private stud farms and breeding stations affiliated with the Jockey Club of Turkey (2013). The Jockey Club of Turkey is the primary official foundation for breeding of both brood horses and racehorses in Turkey. In the 4 sampling areas for brood horses and racehorses, horse stud farms were common. The fifth area had only one Haflinger stud farm (Table). These brood horses are mated once a year. In the event of pregnancy they are kept under the supervision of a veterinarian at a private stud farm or horse stable affiliated with the Jockey Club of Turkey. All the sera were

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taken randomly from apparently healthy horses that were likely older than 1 year. The sexes of all the horses were recorded. The Laboratory Animal Ethics Committee of Mustafa Kemal University (Hatay, Turkey) approved the study protocol.

2.2. Sero-neutralisation test (SNT)

The reference EHV-3 strain, at a titre of $10^{4.5}$ 50% tissue culture infective doses ($TCID_{50}$)/mL, propagated in equine dermis cell culture was used for the sero-neutralisation test. Serial 2-fold dilutions (from 1:4 to 1:512) of the horse sera prepared with Dulbecco's minimum essential medium in 96-well cell culture microplates were assayed for EHV-3-specific virus-neutralising antibodies as described elsewhere (12). Antibody titres of 1:4 and higher were recorded as positive.

2.3. Statistical analysis

The seroprevalence results for EHV-3 were expressed in percentages. The subjects were divided into 3 groups according to their intended use: brood horses, racehorses, and working horses. The 3 groups were further divided into 2 subgroups according to sex. The brood horses were also grouped according to the stud from which they originated. A chi-square test was used to determine the association between the seroprevalence results of the groups and the subgroups. The overall data analysis was performed using SPSS (ver. 15.0) (SPSS Inc., Chicago, IL, USA).

3. Results

Based on the results of the sero-neutralisation test, the overall seropositivity rate against EHV-3 in the 1133 Turkish horses sampled was 25.3% (Table). Among the 420 privately owned horses in the 2 regions of Turkey, EHV-3 antibodies were found in 39 (9.3%) of the analysed sera samples. Two hundred and nineteen (51.2%) of the 428 brood horse serum samples from the stud farms were positive for EHV-3 antibodies, and specific antibodies against EHV-3 were found in 29 (10.2%) of the 285 sera samples of the racehorses. With regard to the intended use of the horses, the differences in EHV-3 seroprevalence were statistically more significant ($P < 0.001$) in the brood horses compared with the racehorses and the working horses.

The EHV-3 seroprevalence was comparable in female (36.8%) and male (12.3%) horses. Among the 420 privately owned horses from the 2 regions in Turkey, 15 (5.4%) of 276 male horse sera and 24 (16.7%) of 144 female horse

Table. Distribution and seropositivity rates of sera sampled from Turkish horses.

Sampling area no.	Brood horse (n: 428)			Racehorse (n: 285)			Working horse (n: 420)		
	Seropositive % (n)	Sex % (n)		Seropositive % (n)	Sex % (n)		Seropositive % (n)	Sex % (n)	
		♂ (n: 93)	♀ (n: 335)		♂ (n: 161)	♀ (n: 124)		♂ (n: 276)	♀ (n: 144)
1	47.1[a] (49/104)	100.0 (3/3)	45.5 (46/101)	10.3[-] (28/271)	8.7 (13/149)	12.3 (15/122)	12.3[-] (22/178)	9.2 (11/120)	18.9 (11/58)
2	68.7[b] (11/16)	-	68.7 (11/16)	7.1[-] (1/14)	8.3 (1/12)	0.0 (0/2)	7.0[-] (17/242)	2.6 (4/156)	15.1 (13/86)
3	77.9[b] (67/86)	-	77.9 (67/86)	-	-	-	-	-	-
4	41.7[a] (78/187)	35.6 (26/73)	45.6 (52/114)	-	-	-	-	-	-
5*	37.1[a] (13/35)	41.1 (7/17)	33.3 (6/18)	-	-	-	-	-	-
Total % (n)	51.2[A] (219/428)	38.7 (36/93)	54.6 (183/335)	10.2[B] (29/285)	8.7 (14/161)	12.1 (15/124)	9.3[B] (39/420)	5.4 (15/276)	16.7 (24/144)

*Haflinger farm

ab: significant differences in the same column.

AB: significant differences in the same row.

-: Statistically no differences in the same column.

sera tested were positive for EHV-3 antibodies. Of the 428 brood horse serum samples tested from the stud farms, 36 (38.7%) of 93 stallion sera and 183 (54.6%) of brood mare sera tested were positive for EHV-3 antibodies. Of the 285 racehorses sampled, 14 (8.7%) samples from male horses and 15 (12.1%) from female horses tested were positive for EHV-3 antibodies (Table).

Statistically significant differences in the EHV-3 seroprevalence were observed between sexes in brood and working horses ($P < 0.01$), whereas there was no statistically significant difference observed in the EHV-3 seroprevalence in racehorses ($P < 0.05$). The seropositivity rate was higher in the stud farms in the second and third sampling areas compared with the stud farms in the first, fourth, and fifth sampling areas; and this finding was statistically significant ($P < 0.001$).

4. Discussion

Although EHV-3 infection is usually characterised as localised, it causes significant problems for horse breeding by restraining the application of embryo transfer and artificial insemination, as well as temporarily affecting the mating activities of brood horses due the highly contagious nature of the infection (4,10,13). The presence of infection has been reported in Australia, Argentina, Austria, Canada, Denmark, Norway, the US, the UK, Japan, India, and Mongolia (1,2,4,7,8,11,14–16). The seroprevalence of EHV-3 is about 18%–53% around the world (4,7,8). Antibodies specific to EHV-3 were detected in about 27%–48% of horses, without any clinical signs (2,4). In this study the overall seropositivity rate against EHV-3 in the Turkish horses sampled was 25.3%. The seroprevalence rates were higher in brood horses (both mares and stallions) compared with the racehorses and the working horses. Furthermore, the seropositivity rates for EHV-3 infection among the brood and working horse populations tested in this study tended to be higher in female horses than in male horses ($P < 0.01$). These results suggest that EHV-3 prevalence could be affected by the intended use of the horse populations. The higher seroprevalence rate of EHV-3 infection in the breeder horses might be due to the high rate of mating and inspection of the genital organs of these animals. This may increase the likelihood of venereal transmission of the virus, as observed by Pagamjav et al. (11) and Barrandeguy et al. (2,10). However, it is still unknown whether the original source of the infection in working horses is other horses or the environment.

The number of EHV-3 antibody-positive brood horses was much higher in the brood studs in the second and third sampling areas, and the difference was statistically significant ($P < 0.001$). The progression of equine herpesviruses is associated with various factors such as climate conditions, population status, development of management practices, quarantine in infected areas, and other infections (17,18). Disruptions in quarantine and poor management practices at the horse breeding stations and brood stud farms sampled may explain the high rate of seroprevalence in this study.

In conclusion, this serosurvey indicates low circulation of EHV-3 in racehorses and working horses but high circulation in brood horses at stud farms in Turkey. It suggests that the intended use of horses may be an important factor in epidemiological assessments of EHV-3 infection. As there is no vaccine available for EHV-3, and many infections spread rapidly as a result of globalisation, prevention and control strategies for EHV-3 infection should focus on epidemiological screening, development of management practices at stud farms, and strict hygiene practices at breeding stations.

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