

Equine herpes virus type 1 (EHV-1) and 4 (EHV-4) infections in horses and donkeys in northeastern Turkey

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Summary

The herpesviruses infections in equides are caused by five different serotypes of viruses, belonging to family *Herpesviridae*. The goal of this study was to conduct a seroepidemiological investigation of equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4) in horses and donkeys raised in two provinces and their villages in northeastern Turkey. A total of 666 samples from 423 horses and 243 donkeys that were not immunized against these infections were tested with ELISA. While 52.48% of tested horse sera was found to carry specific antibodies to EHV-1, 83.69% of these serums were found to carry specific antibodies to EHV-4. 51. Eighty-five percent of analyzed donkey samples tested positive for EHV-1 and 64.20% of these samples tested positive for EHV-4 antibodies. When the horse and donkey samples were evaluated together, 52.25% were seropositive for EHV-1 and 76.58% were seropositive for EHV-4. This study showed that EHV-1 and EHV-4 infections are quite common in the horses and donkeys being raised in the areas where the study was carried out. In addition, since the area where the study was carried out in the borders of Armenia and Georgia, the high level of seropositive results for these infections leads to the conclusion that we should consider the risk of diseases spreading to neighboring countries. This is the first study to serologically identify EHV-1 and EHV-4 infections in donkeys raised in Turkey.

Key words: Donkey, Equine herpesvirus type-1, Equine herpesvirus type-4, Horse

Introduction

The equine herpesvirus type-1 (EHV-1) and equine herpesvirus type-4 (EHV-4) are in the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family and carry double strand DNA. These viruses are pathogens that affect single-toed ungulates, causing infections of the respiratory system and infections characterized by neurological disorders such as myeloencephalitis, which are responsible for significant economic losses in the equine industry due to sporadic and epidemic outbreaks (Harless and Pusterla, 2006). Horses and donkeys are among the animals affected by this infection. Nasal discharges, direct contact, aerosol contamination, infected feed and contact with contaminated equipment all play a significant role in the spread of both of these viruses (Walker *et al.*, 1998).

Like all other herpesviruses, EHV-1 and EHV-4 remain latent in the trigeminal ganglion or lymphocytes after natural infection (Taouji *et al.*, 2002; Allen *et al.*, 2004). In latently affected animals, the virus begins to spread actively again during stress, birth, weaning, transportation, inoculation and administration of corticosteroids (Foote *et al.*, 2003).

A number of testing methods are used to diagnose these infections. However, due to the cross-reactions that can occur between EHV-1 and EHV-4, type-specific diagnostic methods should be used to distinguish between these infections instead of conventional serological methods such as complement fixation and

virus neutralization (Hartley *et al.*, 2005). Type-specific methods include testing techniques such as polymerase chain reaction (PCR) and type-specific enzyme linked immunosorbent assay (ELISA) (Daly and Doylo, 2003; Tearle *et al.*, 2003; Whalley *et al.*, 2003).

The goal of this study was to identify the seroprevalence of EHV-1 and EHV-4 infections in horses and donkeys raised in the provinces of Kars and Ardahan in northeastern Turkey and to make recommendations for testing and dealing with infections in order to prevent economic losses. This study is significant because it obtained the first data on these infections in horses and donkeys in the area in question, and because it is the first study ever done on these infections in donkeys in Turkey.

Materials and Methods

The animals and area

This study was carried out at small family farms that had one or two horses or donkeys which were 1-7 years old, appeared healthy but were not inoculated, and were located at sites in northeastern Turkey that were above an altitude of 1500 ms and had continental climate. A random method was used to select 267 horses of local breed and 156 donkeys used for transportation and agricultural purposes in the province of Kars. Also, 156 horses of local breed and 87 donkeys were used for the same purposes in Ardahan province. Samples were taken from a total of 423 horses and 243 donkeys (Fig. 1).



Fig. 1: Geographical positioning of the Turkish provinces in which the study was performed

The sera

Blood serum samples were collected by jugular vein puncture into vacuum tubes with clot activator. After clotting at room temperature for 15-30 min and centrifugation at 1500 g at 4°C for 10 min, sera were carefully harvested and stored at -20°C until analysis.

ELISA

A type-specific commercial indirect ELISA kit (Svanovir[®], Svanova AB, Sweden) was used to detect antibodies against the EHV-1 and EHV-4. The test was carried out as recommended by manufacturer. The results were evaluated by reading of plates in ELISA reader at 450 nm.

Statistical analysis

Statistical analysis was carried out with Statistical Package for Social Sciences Software (IBM Corp. Released 2012). Significant differences between EHV-1 and EHV-4 were evaluated using the Chi-square (χ^2). A p-value <0.05 was regarded as significant difference.

The Chi-square (χ^2) test was used to perform a statistical analysis of the significance of difference between the seropositive EHV-1 and EHV-4 values identified in horses, the difference between the seropositive EHV-1 and EHV-4 values identified in donkeys, the difference between the seropositive EHV-1 value in horses and donkeys, and the difference between the seropositive EHV-4 value in horses and donkeys.

Statistical evaluations were also carried out between the two regions where the study was performed. We investigated whether or not there were statistically significant differences between the seropositive EHV-1 and EHV-4 values identified in horses and donkeys in Kars and Ardahan provinces.

Results

ELISA

A total of 666 sera samples were tested for EHV-1 and EHV-4 specific antibodies using ELISA. Overall results revealed that 52.48% (222/423) of the horses sampled and 51.85% (126/243) of the donkeys sampled were EHV-1 seropositive. Seroprevalance of EHV-4 was 83.69% (354/423) in horses and 64.20% (156/243) in donkeys. Specific antibodies against both EHV-1 and EHV-4 in horses and donkeys were found in 49.65% (210/423) and 44.44% (108/243), respectively. A seronegative rate of 13.48% in horses and 41.38% in donkeys was found for the infections in question. These data were shown in Tables 1 and 2.

Statistical analysis

Statistical evaluation revealed that there was a very significant difference ($P < 0.001$) between the EHV-1 and EHV-4 antibody prevalence in horses as well as between the EHV-4 seroprevalance found in horses and donkeys. The difference between the EHV-1 and EHV-4 antibody prevalence values in donkeys was found to be statistically significant ($P < 0.01$).

Statistical analysis was also carried out on the differences in antibody prevalence rates between Kars and Ardahan provinces. A statistically significant difference ($P < 0.001$) was found between the EHV-4 seroprevalance rate in horses in Kars and Ardahan provinces, as well as between the EHV-4 antibody prevalence rate in donkeys in Kars and Ardahan provinces. The difference between EHV-1 seroprevalance values found in donkeys in both regions was found to be statistically significant ($P < 0.05$).

Discussion

The international trading of equines and semen are

Table 1: Distribution of antibody positive samples in horses tested by ELISA

Province	Tested sera No.	EHV-1 (+)	EHV-4 (+)	EHV-1 and 4 (+)	Seronegative
Kars	267	144 (55.17 [*])	237 (90.80)	138 (52.87)	24 (9.20)
Ardahan	156	78 (50)	117 (75)	72 (46.15)	33 (21.15)
Total	423	222 (52.48)	354 (83.69)	210 (49.65)	57 (13.48)

: %

Table 2: Distribution of antibody positive samples in donkeys tested by ELISA

Province	Tested sera No.	EHV-1 (+)	EHV-4 (+)	EHV-1 and 4 (+)	Seronegative
Kars	156	90 (57.69 [*])	117 (75)	84 (53.85)	33 (21.15)
Ardahan	87	36 (41.38)	39 (44.83)	24 (27.59)	36 (41.38)
Total	243	126 (51.85)	156 (64.20)	108 (44.44)	69 (28.40)

: %

restricted due to the contagious diseases. In most countries (Canada, Australia, Sweden) the EHV infections are noticeable diseases, and the control measures include official screening or monitoring, precautions at borders, control of movement inside the country. In present study, a total of 666 sera samples were tested for EHV-1 and EHV-4 specific antibodies. Due to the harsh winter conditions and a wide range of uneven lands in Kars and Ardahan district, horses and donkeys still serve an important role in human life participating in transport and agriculture. Hence, it is crucial to minimize the potential risk of great economic losses resulting from infectious diseases occurring in animals. EHV-1 and EHV-4 infections are widely reported from several countries (New South Wales, Canada, Australia, India, Sweden, and Kyrgyzstan). Seroprevalence rates for EHV-1 and EHV-4 were found 8-85.2% and 95-100%, respectively (Keane *et al.*, 1988; Crabb and Studdert, 1993; Gilkerson *et al.*, 1999; Nordengrahn *et al.*, 1999; Singh *et al.*, 1999; Avci *et al.*, 2014).

In Turkey, Ataseven *et al.* (2009) checked blood serum samples from 290 horses in small private ownerships in three different regions for the existence of EHV-1 and EHV-4 antibodies using the ELISA. The ELISA test revealed that the seropositivity for EHV-1 and EHV-4 was 14.5% and 81.7%, respectively. Ataseven *et al.* (2010) reported EHV-1 and EHV-4 seroprevalence as being 23.2% and 78.0%, respectively by the ELISA conducted on serum of 405 horses collected from five provinces in the East Anatolia region. Similarly, Gur and Yapıcı (2008) reported 3.7% seropositivity for type 1 and 56.9% for type 4 in central and western Turkey.

Few studies have been carried out around the world on the status of EHV-1 and EHV-4 infections in donkeys. It is known that donkeys can become infected with these viruses through close contact with horses. A study performed by Chenchev *et al.* (2011) found the seroprevalence of equine herpesvirus to be 69.7% in donkeys and also determined that antibody titration in the seropositive findings varied between 1:40 and 1:10240.

No study has ever been done on donkeys in Turkey with regard to these infections. This is the first study on EHV-1 and EHV-4 infections in donkeys.

This study revealed an overall seroprevalence rate for EHV-1 of 52.48% for horses and 51.85% for donkeys. Seroprevalence of EHV-4 was 83.69% in horses and 64.20% in donkeys. These percentages are higher than the percentages found by Gur and Yapıcı (2008), Ataseven *et al.* (2009), and Ataseven *et al.* (2010). This situation should be evaluated in view of illegal border transfer of horses in Turkey with Armenia and Georgia. However, the situation in these countries regarding EHV-1 and EHV-4 infections is unknown. Transboundary and emerging diseases are becoming more critical issues, since such infections can rapidly spread throughout an entire region and restrict the international horse trade (Domenech *et al.*, 2006). The reason that the

seroprevalence of both infections is so high in this study is due to a number of factors including weather conditions, high-stress working environments, the existence of latent infections, a continuous flow of animals in and out of these regions, their living conditions and the fact that horses and donkeys are kept together.

It was found that the incidence of EHV-1 infections during winter was the highest among horses of about 3 years of age. On the other hand, EHV-4 is not seasonal but can cause infections all year long (Matsumura *et al.*, 1992). The sera samples in this study were collected at the end of winter and beginning of spring, season could be a factor in the high seroprevalence. Statistically, there was a very significant difference between the EHV-1 and EHV-4 antibody prevalence in horses as well as between the EHV-4 seroprevalence found in horses and donkeys.

Kars and Ardahan provinces constitute a great risk for the occurrence of EHV infections in horses and donkeys since these provinces are placed on the border of Armenia and Georgia. Moreover, a high number of uncontrolled animal movements pose another significant threat factor for the spread of the infection to the other parts of Turkey. The North-Eastern Anatolian region of Turkey shares a border with Armenia, Georgia and control of this border is very difficult given the geographical situation. A control program in this region based on serological testing would promote the prevention of the disease. The preventive policy would also require a well-coordinated effort by the government and horse owners.

In addition, it is clear that animal owners should be informed about infectious diseases and that animal shelters need to be brought up to modern standards.

Consequently, this study demonstrates that EHV-1 and EHV-4 infections were more prevalent in horses and donkeys living in the eastern region of Turkey and, furthermore, that the detectable high seropositivity along the eastern border of Turkey might serve as a risk indicator for the introduction of transboundary diseases.

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