Detection of Antibodies against Equine Herpes Virus-1 and Equine Herpes Virus-4 in Horses in Southeast Anatolia by Indirect Elisa

Oguzhan Avci 1, Sibel Yavru 1, Selim Tokgoz 2 & Mehmet Kale 3

ABSTRACT

Background: Equine herpes viruses are a major cause of severe illness and mortality in domestic horses worldwide. Equine Herpes Virus-1 and Equine Herpes Virus-4 are genetically and antigenically related viruses. Equine Herpes Virus-1 infection is common in horses throughout the world, resulting in abortion and neonatal fetal death, respiratory disease, paresis, sporadic myelitis, myeloencephalopathy, and latent infections. Equine Herpes Virus-4, an important equine viral pathogen, causes respiratory tract disease in horses worldwide. The aim of this study was to investigate the presence of Equine Herpes Virus-1 and Equine Herpes Virus-4 antibodies in domestic horses in Southeast Anatolia.

Materials, Methods & Results: In this study, the blood serum samples of 150 unvaccinated domestic horses were tested for equine herpes viruses including Equine Herpes Virus-1 and Equine Herpes Virus-4 specific antibodies. Blood serum samples were collected from the jugular vein of horses in five different provinces (Adiyaman, Diyarbakir, Gaziantep, Kilis, Sanliurfa) in Southeast Anatolia between November 2011 to January 2012. The presence of the Equine Herpes Virus-1 and Equine Herpes Virus-4 antibodies in the samples was determined with commercially available indirect Enzyme-Linked Immunosorbent Assay (ELISA) kits by using ELISA reader. The optical values of the micro plates were measured at 450 nm. The differences between Equine Herpes Virus-1 and Equine Herpes Virus-4 prevalence were evaluated with chi-square test (Minitab 14.0 Inc., State College, PA, USA). Difference were considered significant when \( P < 0.05 \). Equine Herpes Virus-1 and Equine Herpes Virus-4 specific antibodies were detected as in Adiyaman, Diyarbakir, Gaziantep, Kilis, Sanliurfa as 30% (9/30), 50% (15/30), 0% (0/30), 46.66% (14/30), 46.66% (14/30), 80% (24/30), 73.3% (22/30), 0% (0/30), 83.3% (25/30), 100% (30/30), respectively. Of the serum samples tested, 34.66% (52/150) and 67.33% (101/150) were found to be positive for Equine Herpes Virus-1 and Equine Herpes Virus-4 antibodies, respectively. Thirty horses were determined as seronegative both Equine Herpes Virus-1 and Equine Herpes Virus-4 infections in Gaziantep while 30 samples were found to be seropositive for Equine Herpes Virus-4 in Sanliurfa.

Discussion: Equine Herpes Virus-1 and Equine Herpes Virus-4 belong to the Alphaherpesvirinae subfamily of the Herpesviridae family. Equine Herpes Virus-1 and Equine Herpes Virus-4 have DNA as their genetic material. Equine Herpes Virus-1 and Equine Herpes Virus-4 have a capsid, which displays icosahedral symmetry and is surrounded by a lipid envelope composed of various glycoproteins. Equine Herpes Virus-1 and Equine Herpes Virus-4 infections are common in equine animals in the worldwide. In epidemiological research on Alpha herpes viruses, the detection of Equine Herpes Virus-1 and Equine Herpes Virus-4 specific antibodies is used as an important indicator of the presence of symptomatic carriers in the population. Different laboratory tests can be used for the determination of specific antibodies for mentioned infections. ELISA is usually preferred in diagnosis of herpesviruses infections for its sensitivity and its economic advantages. Vaccination for Equine Herpes Virus-1 and Equine Herpes Virus-4 infections has not been applied in Turkey, so seropositivity results indicated that natural infections. In conclusion, Equine Herpes Virus-1 and Equine Herpes Virus-4 infections are widespread in horses in Southeast Anatolia, and protective measures, including vaccination, should be taken.

Keywords: herpesviruses, equine herpes virus-1, equine herpes virus-4, ELISA.
INTRODUCTION

Worldwide, many mammalian species are susceptible to at least one herpes virus [5]. Herpes viruses, particularly Equine Herpesvirus-1 (EHV-1) and Equine Herpesvirus-4 (EHV-4), cause disease in equine animals (horse, donkey and mule) in the world [1]. EHV-1 and EHV-4 belong to the Alphaherpesvirinae subfamily of the Herpesviridae family [3,15,21]. While infection with EHV-1 results in neurological problems [3], respiratory system disorders, abortion and neonatal death, EHV-4 has effect generally on the respiratory system, but may also lead to abortion [9,18]. Literatures report that epidemics and sporadic cases caused by mixed infection with EHV-1 and EHV-4, involve both the abortion-causing and paralytic form of the viruses [16,22]. The spread of the virus among susceptible animals occurs by means of direct contact, inhalation of aerosols, nasal secret and consumption of contaminated feed [6]. Although various methods are used to diagnosing of herpes infections, type-specific ELISA are also performed for diagnostic purposes [4,11,12,19,20]. Due to latency being a general feature of the herpes viruses, the conduct of routine serological tests bears significance for the control of herpes virus infections [16]. Thus, ELISA is frequently used in the laboratory diagnosis of EHV-1 and EHV-4 as serological test [7,24].

In view of EHV-1 and EHV-4 infections having been reported in Turkey and based on the hypothesis that these infections may be present in Southeast Anatolia, five provinces, known not to have been investigated before for the presence of EHV infections, were selected from this region with an aim to determine the seroprevalence of EHV-1 and EHV-4 infections in horses.

MATERIALS AND METHODS

In this study, blood samples collected 150 horses, unvaccinated against to EHV-1 and EHV-4 and had clinical respiratory disease symptoms, between November 2011 and January 2012 in Adiyaman, Diyarbakir, Gaziantep, Kilis, and Sanliurfa provinces. The 5 mL blood samples were collected from the jugular vein into sterile vacuumed tubes containing a serum separator gel1. The tubes were transferred to the laboratories of the Virology Department of Selcuk University, Faculty of Veterinary Medicine, under cold chain conditions. The sera were extracted by centrifugation at 720 g for 10 min, and were transferred into eppendorf tubes. The serum samples were stored at -80°C until testing. The serum samples were tested for the presence of EHV-1 and EHV-4 specific antibodies using a commercially available indirect ELISA kit (Svanovir, EHV-1/EHV-4)2. The test was performed in accordance with the test procedure provided with the test kit. The optical densities (OD) of the micro plates were measured at 450 nm using an ELISA reader (RT-2100)3.

Statistical Analysis

The differences between the selected provinces for the individual and the total EHV-1 and EHV-4 prevalence were evaluated with chi-square test (Minitab 14.0 Inc., State College, PA, USA). A value of \( P < 0.05 \) was considered to be statistically significant.

Table 1. Distribution of EHV-1 and EHV-4 specific antibodies according to provinces of Southeast Anatolia, Turkey, during the period of November 2011 to January 2012.

<table>
<thead>
<tr>
<th>Province</th>
<th>EHV-1</th>
<th></th>
<th>EHV-4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>%</td>
<td>Positive</td>
</tr>
<tr>
<td>Adiyaman</td>
<td>9</td>
<td>21</td>
<td>30(^a)</td>
<td>24</td>
</tr>
<tr>
<td>Diyarbakir</td>
<td>15</td>
<td>15</td>
<td>50(^a)</td>
<td>22</td>
</tr>
<tr>
<td>Gaziantep</td>
<td>0</td>
<td>30</td>
<td>0(^b)</td>
<td>0</td>
</tr>
<tr>
<td>Kilis</td>
<td>14</td>
<td>16</td>
<td>46.66(^a)</td>
<td>25</td>
</tr>
<tr>
<td>Sanliurfa</td>
<td>14</td>
<td>16</td>
<td>46.66(^a)</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>98</td>
<td>34.66(^b)</td>
<td>101</td>
</tr>
</tbody>
</table>

\( ^a,b,c \) Values marked with different letters in the same column are statistically different (\( P < 0.05 \)). \(^A,B\) The statistical difference between the total prevalence of the viruses is significant (\( P < 0.001 \)).
RESULTS

The distribution of EHV-1 and EHV-4 specific antibodies according to the provinces is presented in Table 1. Of the serum samples tested, 34.66% (52/150) was positive for EHV-1 and 67.33% (101/150) was positive for EHV-4, and the seropositivity rates determined for EHV-1 and EHV-4 differed significantly ($P < 0.001$). Furthermore, the provinces significantly differed from each other for EHV-1 and EHV-4 specific antibodies ($P < 0.05$). Seropositivity rates specific for EHV-1 only, EHV-4 only and both EHV-1 and EHV-4 were ascertained as 11.33% (17/150), 32.66% (49/150), and 23.33% (35/150), respectively.

DISCUSSION

Various serological methods are used to detect specific antibodies produced against EHV-1 and EHV-4 [17]. The complement fixation and virus neutralization tests, which have been used for this purpose in the past, have been replaced by the highly sensitive ELISA, which is based on the use of monoclonal antibodies, and has found common use in veterinary medicine [2,10].

In this study, 34.66% (52/150) and 67.33% (101/150) were determined to be positive for antibodies specific for EHV-1 and EHV-4, respectively, and the difference between the seropositivity rates for the two herpes virus types was statistically significant ($P < 0.001$) [Table 1]. Infections caused by EHV-1 alone, and mixed infections caused by both EHV-1 and EHV-4 have been reported in many countries, including Australia, New Zealand, England, the United States of America, China, Canada and Japan [14,23]. In a previous study conducted in Turkey, 405 blood samples collected from eastern Anatolia horses (Van, Bitlis, Mus, Igdir and Erzurum provinces) detected 94 (23.2%) seropositive for EHV-1 and 316 (78.0%) for EHV-4 [1]. Furthermore, in 188 blood samples collected from clinically healthy horses in different regions of Turkey (İzmir, Konya, Afyonkarahisar and Eskişehir provinces), 7 (3.7%) and 107 (56.9%) were positive for EHV-1 and EHV-4 specific antibodies, respectively [8]. The seropositivity rate determined for EHV-1 in the present study (34.66%, 52/150) was found to be higher than the seropositivity rates previously reported [1,8]. On the other hand, the seropositivity rate determined for EHV-4 in this study (67.33%, 101/150) was observed to be lower than the values indicated in the same literature reports. This result was attributed mainly to the horses sampled in the present study having displayed respiratory symptoms, and thus their bearing a potential risk for herpes virus infection.

Of the blood samples tested in the present study, 17 (11.33%) were seropositive for EHV-1, 49 (32.66%) were seropositive for EHV-4, and 35 (23.33%) were seropositive for both EHV-1 and EHV-4 (Table 1). In previous research carried out in horses, seropositivity rates for EHV-4 was observed to be greater than those detected for EHV-1 [1,8].

In the present study, the EHV-1 and EHV-4 seropositivity rates determined in the five provinces were demonstrated to differ significantly ($P < 0.05$) (Table 1). All of the blood samples taken from Gaziantep province were confirmed to be negative for both EHV-1 and EHV-4 (Table 1). The assessment of the findings obtained in the different provinces demonstrated the province safest in terms of risk for equine herpes virus infection to be Gaziantep, while Sanliurfa presented with an urgent necessity for the implementation of animal health measures, due to the detection of 100% seropositivity for EHV-4.

As the horses included in the present study were not vaccinated against equine herpesviruses, the seropositivity detected in these animals was considered to have arisen from infection. The detected high seropositivity rate was also attributed to the sampling season, among other reasons. The results of the present study have shown that EHV-1 and EHV-4 infections are widespread among horses in Southeast Anatolia, and thus, constitute a health risk for other animal species in the region. It is known that antibodies produced against EHV-1 and EHV-4 do not induce adequate immunity against reinfections and that latent infections result in the continuous shedding of the virus into the environment [13]. Some of the provinces sampled in this study being located along the southeast border of the country and presenting with herpes virus seropositivity also point out to the necessity for measures to be taken for the control of equine herpes virus infection in the region.

CONCLUSION

In conclusion, in view of race horses being raised in Southeast Anatolia, there is a requirement for EHV control strategies to be developed and implemented in the region, as well as for periodical research, covering a broader geographical area, to be conducted with an aim to determine the prevalence of herpes virus infections.

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