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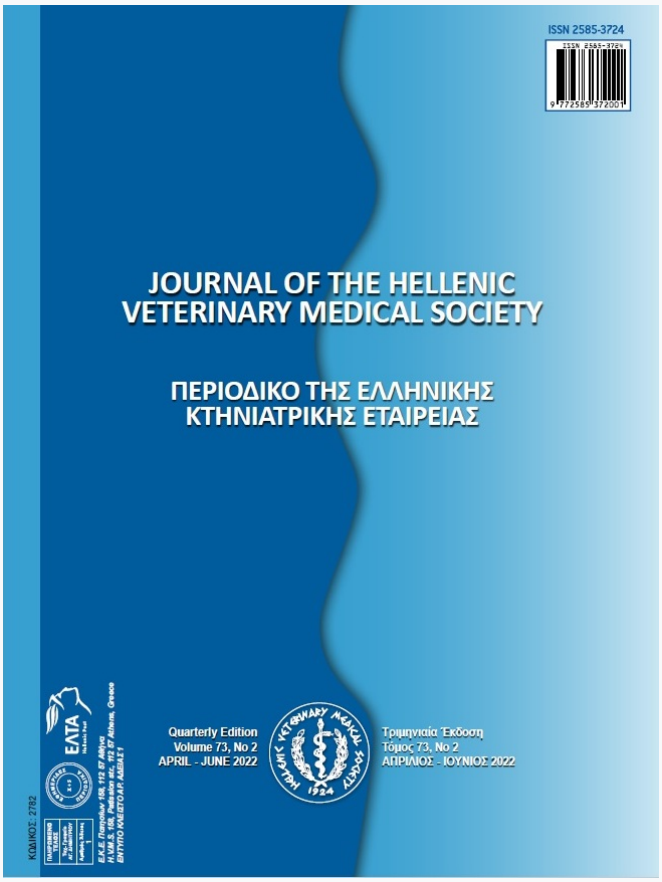

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Research article
Ερευνητικό άρθρο

Equine arteritis virus seroprevalence and risk factors in horses in the inner Aegean and Central Anatolia regions of Turkey

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ABSTRACT: Equine viral arteritis (EVA) is one of the most important respiratory and reproductive diseases of horses. This study aimed to investigate the seroprevalence of equine arteritis virus (EAV) infection and risk factors associated with seropositivity in clinically normal horses (n=262) in the inner Aegean region and Central Anatolia region of Turkey. This cross-sectional study was conducted by collecting sera samples (n = 262) from ambling horses raised in small family-type enterprises (n=65) during the months of February 2014 and February 2017. Epidemiological data were obtained from animal owners during blood sampling. An indirect enzyme-linked immunosorbent assay (ELISA) kit was used to detect specific antibodies against EAV in sera samples. Potential risk factors were determined by univariate logistic regression analysis. According to the test results, 8.40% (95% CI:5.61-12.39) seropositivity was detected in horses. While seropositivity rates by age were 2.47% in animals under 4 years of age, they were determined to be 11.05% in animals aged over 4 years (p=0.02). There was a statistically significant correlation between age, participation in races and festivals, precautions taken against rodents and insects at enterprises and EAV infection seropositivity ($p<0.05$). The findings obtained from this study are important in terms of informing breeders about the presence of this infection, taking infection-related biosecurity measures, and fighting the disease.

Keywords: Equine arterit virus,horses,seroprevalance, risk factors, Turkey

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INTRODUCTION

Equine viral arteritis (EVA) is a viral disease characterized by disorders of the reproductive and respiratory systems of horses and is economically important for the horse industry due to its contagious nature. The disease agent, equine arteritis virus (EAV), was renamed Alphaarterivirus equid and is included in the Alphaarterivirus genus of the *Equarterivirinae* sub-family in the *Arteriviridae* family. The EAV genome consists of positive-polarity, single-stranded RNA, and the virus is enveloped (Amat et al., 2016; Cruz-Lopez et al., 2017).

The transmission of EAV between horses occurs by the respiratory and veneral routes. Mares infected at the last stages of pregnancy can give birth to infected foals. Stallions can carry the virus for years and transmit the virus through natural mating or artificial insemination. Furthermore, viruses can be transmitted through caregivers and the materials used (Collins et al., 1987; Guthrie et al., 2003; Holyoak et al., 2008; McFadden et al., 2013). After an incubation period of 3 to 14 days, the disease can be asymptomatic or severe depending on the animal's age, immune status, care and nutrition conditions. EVA may cause abortions in mares, death of newborn foals, and sperm disorders due to edema and hyperthermia in stallions. Fever, loss of appetite, edema, conjunctivitis, and pneumonia can usually be observed in infected animals (Cole et al., 1986; Del Piero et al., 1997; Cruz-Lopez et al., 2017).

Serological studies demonstrate that EAV infection is spread worldwide, especially in domestic horse populations in North and South America, Europe, Australia, Africa, and Asia. However, countries such as Iceland, Japan, and New Zealand are free from the disease (McFadden et al., 2013; Balasuriya, 2014). EAV seroprevalence may vary between countries and between horses of different breeds and ages in the same country. Although there is limited information on the disease seroprevalence in Turkey, the studies determined that the seroprevalence rate varied between 5-23.4% (Yılmaz et al., 1996; Bulut et al., 2012; Gür et al., 2018; Gür et al., 2019).

There are little data on potential risk factors associated with EAV seropositivity in horses. Previous studies have identified age, sex, breed, and high horse density as important risk factors for EAV seropositivity (McCollum and Swerczek, 1978; Nejat et al., 2015; Cruz et al., 2016). It is important to identify risk factors for the control and prevention strategies of EAV infection. As far as we know, our study is the first study that investigated risk factors associated with the infection in Turkey. The aims of this study were to investigate the seroprevalence of EAV infection and risk factors associated with the infection in ambling horses raised by people in small family-type enterprises in theinnerAegeanand Central Anatolia regions of Turkey.

MATERIAL AND METHODS

Study area

This study was conducted in the inner Aegean re-

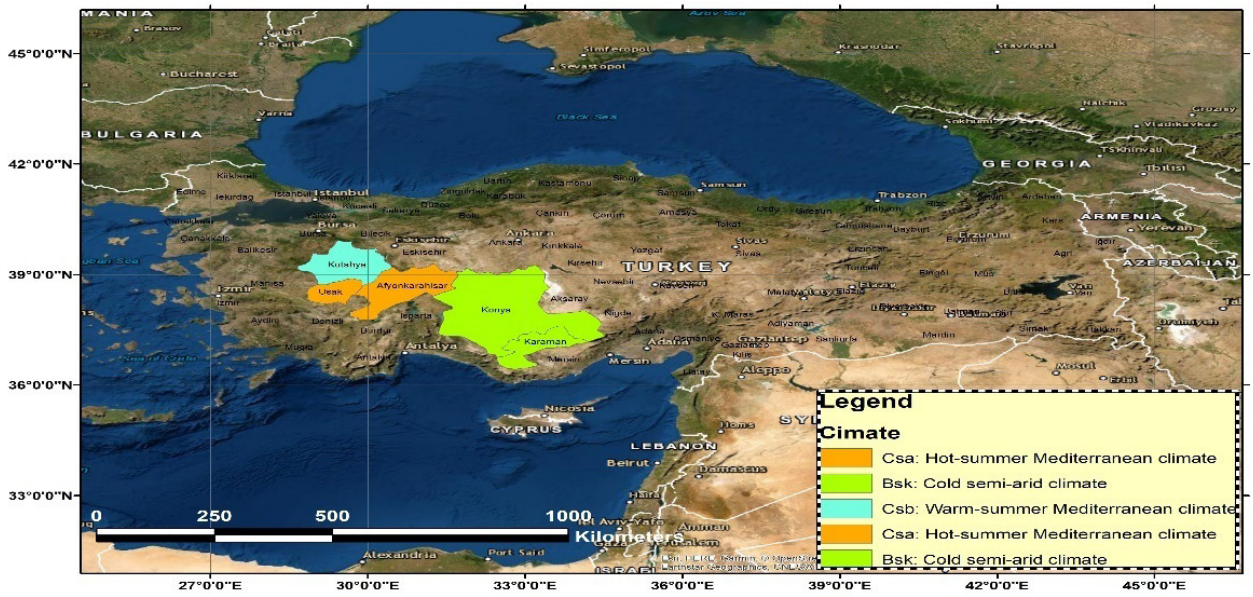

Figure 1. Study area

gion, including Afyonkarahisar, Kütahya, and Uşak-Provinces, and the Central Anatolia region, including Karaman and Konya Provinces (Figure 1). In the study area, according to the Köppen climate classification, Kütahya province exhibits (Csb: Warm-summer Mediterranean climate), Afyonkarahisar and Uşak provinces exhibit (Csa: Hot-summer Mediterranean climate), and Karaman and Konya provinces exhibit (Bsk: Cold semi-arid climate) characteristics. The provinces do not have a coast, and their altitude varies between 900-1039 m (GDM, 2018). There were approximately 3700 equidae animals in the study area (MAF, 2020).

Sample collection and serological test

Although the detailed health records of animals were not kept, horses were not vaccinated against equine pathogens, and no clinical findings were detected in terms of the disease during sample collection.There were no quarantine stables in sampled enterprises. Furthermore, enterprises owners reported that they did not apply isolation procedures. There was no clustering, except for a few stud farms, especially in the Central Anatolia region. Some stud farms have buffer zones, and some stud farms have paddocks which were next to public roads. According to enterprises' report, horses were monitored for signs of illness for two weeks after they returned to the enterprise from festivals or races.

The sample size was calculated based on a 95% confidence interval and a desired relative precision of 5% by considering the prevalence rate determined in the study previously conducted in Turkey (Bulut et al., 2012 (23.4%) (Dean et al., 2013). Blood samples were collected from a total of 262 horses from 65 different enterprises using the random sampling method in the study area. For the test, blood samples taken from the *vena jugularis* into sterile vacuum silicone tubes were brought to the laboratory at 4^0 C and centrifuged at 3000 rpm for 10 min after overnight storage at room temperature. The sera obtained were taken into stock tubes and stored at -20^0 C until they were subjected to testing. A commercial indirect ELISA test kit (Indirect Equine Viral Arteritis, ID Vet, France) was used in the study. The ELISA test was conducted following the procedure reported by the manufacturer. To obtain optical density (OD) data, measurements were performed at 450 nm, and OD values were calculated.

Risk factors and statistical analysis

Questionnaires were applied to the enterprise owners during blood collection to determine risk factors in terms of EAV seroprevalence. The epidemiological data obtained were entered into Microsoft Excel spreadsheets. Explanatory variables included provinces, climate (Bsk/Csa/Csb), animal's age group (≤4 years/ >4 years), sex (male/female), animal's participation in races-festivals (yes/no), measures against rodents and insects applied in the enterprise (yes/no).

Descriptive statistics were performed to determine the individual seroprevalence rate based on potential factors examined in epidemiological data and the corresponding rates of seropositive animals. The association between the occurrence of EAV infection and potential risk factors was firstly examined by univariate analysis using the chi-square ($\chi 2$) test. Data were analyzed using the R program (R Core Team, 2018). The odds ratio (OR) was calculated for each risk factor for EAV seropositive animals. In all the analyses, confidence levels at 95% were calculated, and a level of $p<0.05$ was used for a statistical significance level.

RESULTS

The mean age of horses in the study population was 6.4 years. Of the 262 sera tested, 22 samples were detected to be seropositive (Table 1). Seropositivity was detected in 14 of the 65 enterprises examined. Enterprise-based EAV infection rates varied between 0.00-53.50%. The overall apparent seroprevalence at the individual level was determined to be 8.40% (95% CI:5.61-12.39).

Six variables were associated with EAV seropositivity in univariate logistic regression analysis. The difference between seropositivity and climate classification was found to be significant (χ^2:6.78, p=0.03). However, no significant difference was observed according to sex distribution ($\chi 2$:2.87, p=0.09). In the model, the EAV seroprevalence was higher in animals older than 4 years than animals ≤4 years (OR: 0.20, 95% CI: 0.04-0.89), in horses participating in races and festivals compared to those not participating in ambling horse races and festivals (javelin game) (OR: 0.12, 95% CI: 0.01-0.97), in enterprises without control practices compared to enterprises with rodent and insect control practices (OR: 0.28, 95% CI: 0.08-0.98). The estimated OR and the corresponding 95% confidence interval, the values containing the models of the variables are presented in Table 1.

DISCUSSION

The EVA seroprevalence in the study area was de-

Table 1. Explanatory variables (potential risk factors) investigated in a cross-sectional study for antibodies against EAV

Factor	Categories	Positive	Negative	Percentage %	χ2	p -value	OR	95%CI	p-value
Province	Afyonkarahisar *	3	71	4.05					
	Kutahya	1	31	3.13			1.31	0.13-13.09	0.81
	Usak	1	22	4.35	7.12	0.12	0.92	0.09-9.39	0.95
	Konya	12	75	13.79			0.26	0.07-0.97	0.04
	Karaman	5	41	10.87			0.34	0.07-1.52	0.16
Climate	Csb *	1	31	3.13					
	Csa	4	93	4.12	6.78	0.03	0.75	0.08-6.96	0.80
	Bsk	17	116	12.78			0.22	0.02-1.71	0.14
Age	≤4 years *	2	79	2.47	5.35	0.02			
	> 4 years	20	161	11.05			0.20	0.04-0.89	0.03
Sex	Female*	19	166	10.27	2.87	0.09			
	Male	3	74	3.90			2.82	0.81-9.83	0.10
Outings to competitions/ festival	No*	1	65	1.52	5.43	0.01			
	Yes	21	175	10.71			0.12	0.01-0.97	0.04
Measures against rodentsand insects	Yes*	3	86	3.37	4.42	0.03			
	No	19	154	10.98			0.28	0.08-0.98	0.04

*Referance
OR: Odds ratio
CI: Confidence interval

tected to be 8.40% (95% CI:5.61-12.39). Our seroprevalence estimation is similar to the seroprevalence study results previously conducted in Turkey by Yılmaz et al. (1996) 5%, Kırmızıgül et al. (2007) 8.75%, Turan et al. (2007) 14.2%, Hasan (2008) 7.5%, Gür et al. (2015) 11.3%, and Acar et al. (2016) 10.8%. Bulut et al. (2012) found a high seroprevalence of 23.4%. This may, possibly, originate from the researchers' field of study, the inclusion of horses older than 3 years in the study, and the number of samples used in the study (Rola et al., 2011).

In the positive enterprises in the study, the intra-enterprise seropositivity rate varied between 20-53.50%. This rate is consistent with the previous study reporting that the intra-enterprise seropositivity rate varied between 30-60% (Cruz et al., 2016). It is thought that high seropositivity in positive enterprises may be associated with the transmission of the virus by the carrier stallions in the enterprise.

The results of our study are similar to the results of the studies carried out worldwide, in Austria (10.9%) (Kölbl et al., 1991), Tunisia (8.75%) (Ghram et al., 1994), and Hungary (8.33%) (Szeredi et al., 2005). On the contrary, the results of the studies performed in France (15.2%) (Moraillon and Moraillon, 1978), the Netherlands (14%) (de Boer et al., 1979), the United Kingdom (18.5%) (Newton et al., 1999), and Spain (16.8%) (Cruz et al., 2016) are higher than the results of our study. These differences between studies can be attributed to many factors such as different cultural and management systems specific to the country in general, biosecurity measures, the region where the study was conducted, housing conditions, insufficient information about the disease, the number of animals, the age of sampled animals, sample size, the analysis method used, and not conducting screening tests at certain intervals.

The results of the conducted risk factor analysis highlighted some potential existing and previously unknown risk factors for individual seropositivity against EVA. In our study, it was determined that sex was not a potential risk factor for EAV infection, although a higher rate of seropositivity was obtained in mares than stallions.The observed higher seropositivity rate in mares can be explained by the number of sampled mares. The number of sampled mares (n= 185) in this study was higher than number of sampled stallions (n = 77). Previous studies have found a higher rate of seropositivity in mares compared to stallions, which is consistent with our study results (Rola et al., 2011; Cruz et al., 2016). Furthermore, in a study conducted in Iran, sex was defined as a potential risk factor for EAV infection, and it was reported that infection was observed at a high rate in mares when the age groups under 1 year of age and over

3 years of age were evaluated (Nejat et al., 2015). The difference between the studies may be due to the climatic conditions of the region where samples were collected, the number of samples, the analysis method, and the evaluation of results according to age groups. There was a significant correlation between the animals' age profile and the risk of EAV infection in our study (χ2:5.35, p=0.02). Previous studies have demonstrated that age is a risk factor associated with EAV infection (GimenoSuarep et al., 2011; Rola et al., 2011; Cruz et al., 2016). Considering that horses need only one encounter with EAV throughout their life to become seropositive, our study is consistent with the high rate of EAV seropositivity due to the increasing rate of infection as the age increases (Cruz et al., 2016).

Concerning the climate of the locations where the horse enterprises sampled in our study are situated, EAV was more common in the cold semi-arid climate (Bsk) in the Central Anatolia region. Although climate has not been recognized yet as a potential risk factor for the transmission of EAV infection, the locations of enterprises in the Central Anatolia region with this climate are places where the temperature is lower than all of them during the summer months. This may be associated with the resistance of EAV to environmental conditions because the virus can remain infectious for 2 to 3 days at 37 °C and for 75 days at 4 °C depending on the ambient temperature (Holyoak et al., 2008). Differences also emerge in the management of horses depending on climatic conditions. While horses in enterprises situated in a location with a colder climate tend to be kept in larger groups and indoors, horses tend to be kept outside in paddocks in places with a milder climate. Moreover, a study focusing on horse diseases reported that the density of animals in the enterprise increased the risk of transmission of infectious diseases (Glass and Barnes, 2013). The number of animals susceptible to infection in large enterprises is higher than in small enterprises, which may contributing maintenance of infection, and increasing transmission rate of the infection within the enterprises. Therefore, it would be beneficial to take measures for the prevention and control of EVA regarding the detection of possible carrier stallions or acutely infected horses in high density horse holdings, and to review the biosecurity practices of the enterprise.

Another risk factor examined in our study was participation in races and festivals. In Turkey, ambling horses were used in transportation in the past century, and competitions have started to be held with these horses over time. Horse breeding is carried out for ambling horse races, javelin game, and festivals in different regions of Turkey (Yuceer et al., 2016). The inner Aegean and Central Anatolia regions where the study was conducted are regions where horse competitions and festivals are held intensively. In our study, EVA seroprevalence was determined to be higher in horses participating in races and festivals. This can be explained by the spread of the infection due to the transmission of the virus from infected horses to susceptible horses as a result of horses coming together during races and festivals.

In our study, EAV seroprevalence was lower in enterprises applying preventive measures against insects and rodents than enterprises not applying such measures. It is thought that this may be due to the fact that enterprise owners applying preventive measures against insects and rodents are more concerned about infectious diseases and take the necessary biosecurity measures due to their awareness of high standard business management practices.

A limitation to this study is that it is a cross-sectional design, which has limited ability to confirm temporality of the risk factors for EAV infection. Furthermore, the study used a EAV specific antibody ELISA testing kit, thus this test cannot establish whether the test-positive animalis due to the virus retention, or due to adaptive immunity from previous exposure to the infection. In the future, a study is needed to establish if clinical EAV occurs in Turkey in order to determine the long-term effects of EAV infection of prevention and control measures.

CONCLUSIONS

In this study, potential risk factors associated with EAV seropositivity were identified. Subclinical horses and persistently infected asymptomatic stallions play an active role in the spread of EAV infection. It is important to implement biosecurity practices in an enterprise to maintain animal health and welfare through an outbreak of contagious disease. It will be beneficial for horse breeders to increase biosecurity measures at their facilities, to identify acutely infected horses and carriers, and to routinely perform EAV tests before bringing newly purchased horses to an enterprise. It will be beneficial to conduct more studies on EAV in the other regions of Turkey to evaluate the impact of potential risk factors associated with EAV seropositivity identified in this study.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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