Serological Investigation of Equine Viral Arteritis Infection in Donkeys and Horses in the Eastern Anatolia Region

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Geliş Tarihi: 18.05.2018 **Kabul Tarihi: 25.11.2018**

Abstract: In this study, equine viral arteritis (EVA) infection was serologically investigated in adult horses (n=193) and donkeys (n=227) in five different localizations in Elazig and Tunceli provinces, Eastern Anatolia Region. As result of indirect ELISA, positivity in horses was 15% (29/193) in total, ranged from 6.5% and 24.3%. The average value for donkeys was 8.3% (19/227), the rates varied between 2.4% and 14.2%. The mean age of the sampled horses and donkeys was 7.4 and 13.2, respectively, while the mean age of the seropositives was 7.1 and 11.8. Statistical analysis showed no correlation in the donkeys between sex and infection exposure, but there was a significant correlation in gender in horses. This is the first report on EVA in the studied provinces. Considering focused animal population and sampling criteria, determining of positivity in all the studied localizations shows that the infection has been potential for the spread in the future.
Keywords: *Donkey, East Anatolia, Equine viral arteritis, Horse, Turkey.*

Doğu Anadolu Bölgesinde At ve Eşeklerde Equine Viral Arteritis Efeksiyonunun Serolojik Olarak Araştırılması

Özet: Bu çalışmada Doğu Anadolu Bölgesi'ndeki Elazığ ve Tunceli illerinde 5 farklı lokalizasyonda yetişkin ve klinik olarak normal olan atlarda (n=193) ve eşeklerde (n=227), equine viral arterit (EVA) enfeksiyonu serolojik olarak araştırılmıştır. İndirekt ELISA sonucuna göre atlarda %6.5 ile %24.3 arasında değişen oranlarda pozitiflik bulundu, toplamda ise 193 örnekten 29'unda (%15) seropozitiflik tespit edildi. Eşekler için ortalama değer %8.3 (19/227) olarak bulunmuş olup, oranların %2.4 ile %14.2 arasında değiştiği görüldü. At ve eşeklerin yaş ortalamaları sırasıyla 7.4 ve 13.2'dir, pozitiflerin ise yaş ortalamaları 7.1 ve 11.8'dir. İstatistik analizinde cinsiyete ve enfeksiyona maruz kalma arasında eşeklerde fark yoktu, fakat aygırlarda anlamlı bir bağıntı olduğu belirlendi. Bu çalışma, örneklenen illerde EVA ile ilgili ilk araştırmadır. Hedef alınan hayvan populasyonu ve örnekleme kriterleri göz önüne alındığında, çalışılan tüm yerlerde pozitifliğin tespit edilmiş olması, gelecekte enfeksiyonun yayılma potansiyelinin olduğunu göstermektedir.
Anahtar Kelimeler: *At, Eşek, Doğu Anadolu, Equine viral arteritis, Türkiye.*

Introduction

Equine Viral Arteritis (EVA) usually causes asymptomatic disease, severe clinical disorders also occur during outbreaks. Main hosts are horses, donkeys, mules and zebras, (Paweska and Barnard, 1993; Paweska et al., 1997; Balasuriya, 2014), alpacas are also reactive to the virus (Weber et al., 2006). All equine species thought to be susceptible to the infection (Balasuriya, 2014; Paweska et al., 1997).

Equine Arteritis Virus (EAV) is classified in family *Arteriviridae*, in order of Nidovirales (Snijder and Meulenberg, 1998; Cavanagh, 1999). Agent is an enveloped, single stranded, positive sense RNA virus. EAV has one serotype, but small variations in the field isolates result with prognostic diversity (Murphy et al., 1992; Stadejek et al., 1999, 2006). The agent can transmit through respiratory and venereal tracts. As a general feature of the infection, asymptomatic carrier status seen in about 30 to 60% of stallions for as long as 6-7 years (Timoney et al., 1986, 1987). The duration of virus spread in mare is limited with only to acute phase (Timoney and McCollum, 1985). On this account, only semen and stallions that were tested and negativity proved ones allowed to do international trade (Metcalf, 2001). Mortality can be seen in foals with digestive and respiratory system disorders (Del Piero et al., 1997). Deaths are rare in adults, but reproductive system problems are common. Despite its widespread distribution, occasionally emerging epidemics characterized by abortion storms and deaths in young animals (Eichhorn et al.,

1995; Holyoak et al., 2008; Timoney and McCollum, 1988).

After first identification in the US in 1953, EVA was well investigated in horses (Doll et al., 1957). Generally, up to 20% seropositivity have been detected in various countries. The highest value (73%) reported in Australia (Huntington et al., 1990). Despite the low antibody (Ab) rate in the United Kingdom, infection continues to be a problem (Newton et al., 1999), as in many other countries (Pronost et al., 2010). Infection rates increase with age and seropositivity is higher in males (Rola et al., 2011). The pathogenetic features of the infection have been well described only in horses (Del Piero et al., 1997; Campos et al., 2014; Timoney et al., 1986; Vairo et al., 2014). The first serological evidence in the donkey was determined in the samples collected from South Africa between 1989 and 1992 by Paweska and Barnard (1993). The susceptibility of donkeys has been described by many researchers (McCollum et al., 1995; Paweska et al., 1995; Ramina et al., 1999; Stadejek et al., 2006). Experimental studies and field investigations showed that the pathogenic features of the infection were not exactly the same, but were almost identical to those in horses (McCollum et al., 1995; Paweska et al., 1996, 1997). Current serological data are limited comparing horses. Paweska and Barnard (1993) found 17% positivity in 734 donkeys in South Africa and reported 30% rate in some localization. Serological studies in Turkey carried out mainly in horses and reported proportions are usually less than 10% (Hasan, 2008; Kirmizigul et al., 2007, 2009; Un et al., 2014; Yildirim et al., 2008; Yilmaz et al., 1996). Slightly higher rates were reported in a few studies; 10.8% (22/204) (Acar et al., 2016), 14.3% (9/63) (Turan et al., 2007) and 16% (57/346) (Marenzoni et al., 2013). Bulut et al. (2012) have reported the highest value as 23.4% (89/380) so far, which was determined in the Central Anatolia.

The aim of this study was to investigate EAV infection serologically in donkey and horses, to determine and compare the presence and rates of infections in these species, and to obtain the first data in Elazig and Tunceli provinces.

Materials and Methods

Sampled animals: Blood serum samples were collected between March 2009 and August 2010 from the small private farms in the rural areas of Elazig and Tunceli provinces. Total of 91 horses and 131 donkeys sampled from Elazig province; Central villages, Palu and Maden districts. From Tunceli, a total of 102 horse and 96 donkey samples were collected from the Pertek and Hozat districts. A total of 193 horses and 227 donkeys were sampled at five locations (Table 1). Depending on the preferred breeding pattern, nearly all the donkeys were female (92.5%, 210/227). The gender of the horses (n=193) was almost equal (99 females and 94 male). The ages of horses were between 1 and 16 years old. There was only one foal in two-month-old (Figure 1).

The sampled donkey's age was ranged from 2 to 30, there was only one 4-month old donkey. (Figure 2). The mean age of the donkeys was 13.2 and that of the horses was 7.1. The number of horses and donkeys sampled in this study was less than 3 in each farm. Dual species breeding was never been found on the same farm.

During sampling, age, sex and clinical examination findings of the animals were noted. All animals were clinically normal. Detailed health record does not obtain but according to the anamnesis, some young foals were told to have experienced clinical disorders but abort cases were very rare. Blood samples were drawn from Vena Jugularis into vacutainer tubes containing silicone, transferred to the laboratory at cold chain. The blood samples were centrifuged at 3000xg for 10 minutes. Subsequently, serum fractions separated and then stored at -20^{o}C until testing.

Enzyme Linked Immunosorbent Assay (ELISA): An indirect ELISA (ID Vet, France) was preferred for EAV-specific antibody controls due to its high sensitivity and specificity (Kondo et al., 1998). The sera samples were tested according to the manufacturer's instructions.

Results

ELISA results: EAV specific antibodies were detected in all studied locations and in both species. At horses, values ranged from 6.5% to 24.3%. It was found that, the highest value was found in Maden/Elazig (24.3%), but the average proportions in Elazig (14.3%, 13/91) and Tunceli (15.7%, 16/102) was close to each other. Out of 193 horses, 29 (15%) were seropositive for EVA infection.

Comparing horse, lower rates were determined in donkeys. The lowest value was detected at central Elazig (2.4%), the mean value of this province was 5.3% (7/131). In the Tunceli province, 11.1% and 14.3% rates were detected in the two districts, with an average of 12.5% (12/96). The mean seropositivity of positive donkey was 8.3% (19/227) (Table 1). According to the sex distribution of Ab positivity, no significant difference was observed in horses (f-16.1%/m-13.9%) and donkeys (f-8.5%/m-5.8%) (Table 2).

Table 1. The location and number of the sampled horses and donkeys and EVA test results.

No	Localisation	Horse			Donkey		
		Sample no	(Ab+)	(%)	Sample no	(Ab+)	(%)
1	**Elazig/Center**	31	2	6.5	41	1	2.4
2	**Elazig/Palu**	23	2	8.7	44	4	9.1
3	**Elazig/Maden**	37	9	24.3	46	2	4.3
4	**Tunceli/Pertek**	34	6	17.6	54	6	11.1
5	**Tunceli/Hozat**	68	10	14.7	42	6	14.3
	Total	193	29	15	227	19	8.4

Table 2. Distribution of EAV Ab positivity by sex in horse and donkey.

Sex	Horse			Donkey		
	Sample no	(Ab+)	(%)	Sample no	(Ab+)	(%)
Female	99	16	16.1	210	18	8.5
Male	94	13	13.9	17	1	5.8
Total	193	29	15	227	19	8.4

The ages of the all sampled horses ranged from 1 to 16 years. Most of them were between 5 and 8 years old (average 7.4). Mean age of Ab positives was 7.1 years (Figure 1).

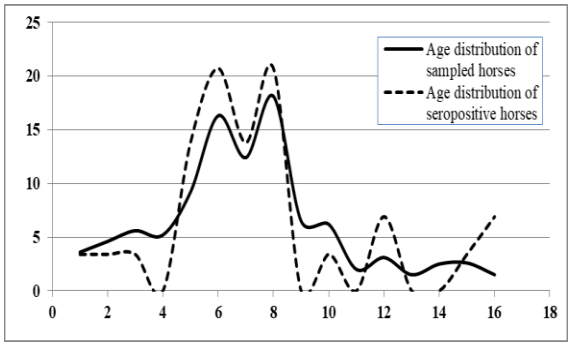

Figure 1: Age distribution of all horses and EVA positives (%).

The sampled donkeys were between four months old and 30 years of age. The majority of donkeys were between 10 and 15 years old. The mean age of all sampled donkeys was 13.2 while ab positives was 11.8. (Figure 2).

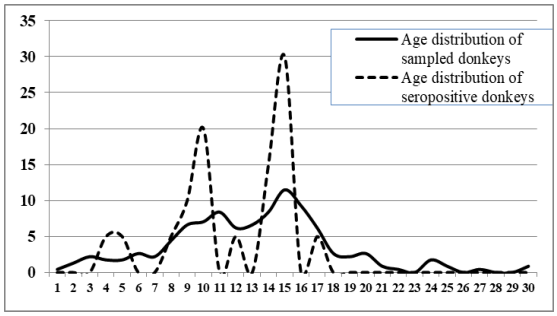

Figure 2: Age distribution of all donkeys and EVA seropositives (%).

Statistical analysis: According to chi-square analysis; there was no statistically significant difference between sex and exposure to infection in donkeys (P>0.05). However, correlation between sex and viral exposure was detected in horses, seropositivity rate was higher in stallion (P<0.05).

Discussion

In this study, blood samples were collected from small private family enterprises in five counties at two provinces to determine the spread of EVA in rural areas. According to the test results, the rates are almost equal in both horses and donkeys in two localisations, Palu/Elazig (8.7-9.1%) and Hozat/Tunceli (14.7-14.3%). The rates were slightly higher in horses raised in the central district of Elazig (6.5-2.4%) and Tunceli/Pertek (17.6-11.1%). The biggest inconsistency was detected in the Maden district of Elazig, the ratios were 24.3% at horses and 4.3% at donkeys (Table 1). The incidence observed in this study in horses (15%) was similar or slightly higher than previous reports in the near regions (Hasan 2008; Kirmizigul et al., 2007; Yildirim et al., 2008; Yilmaz et al., 1996). However, the data on donkeys (8.3%) was lower than that reported by Yildirim et al. (2008) (14.47%) in Kars province. According to age distribution, seropositive horses were generally between 5 and 8 years old (av. 7.1), and donkeys were 10 and 15 years old (mean 11.8). Positivity was found to be in accordance with age distribution. Infection was seen most frequently in horses between 4 and 9 and between 6 and 16 in donkeys. The gender of the sampled horses was very close, and there was no significant difference in the sex of the positives (13.9% male-16.1% female). The distribution of

positivity by sex was 5.8% (1/17) for male donkeys and 8.5% (18/210) for female donkeys. These data suggested that gender of donkeys did not associate with seropositivity for EAV.

Studies conducted in Turkey have mostly focused in Marmara, Eastern Anatolia and Central Anatolia regions. The seropositivity reported in the Northeastern Anatolia has ranged from 5% to 9.5% (Kirmizigul et al., 2007; Yilmaz et al., 1996). In addition, 400 horse and 76 donkey samples were negative at the same region (Kirmizigul et al., 2009). Gur et al., (2015) determined an incidence of 11.3% (14/124) in Van. Un et al. (2014) have reported a seropositivity of 8.4% (32/379) in Sanliurfa province. Serologic investigations in the Marmara region were almost similar; 7.5% (Hasan, 2008), 14.3% (9/63) (Turan et al., 2007), 16% (57/346) (Marenzoni et al., 2013). There were a few study carried out in Central Anatolia; Acar et al. have reported a seropositivity of 10.8% (22/204) while Bulut at al. (2012) have detected the peak value of 23.4%). Viral antigen was isolated from horses with respiratory system disorders and the isolates showed close relation to the virulent strain Bucyrus of the North American origin (Ataseven et al., 2013). Except for Iceland and Japan (Kondo et al., 1998), infection has spread worldwide (Timoney and McCollum, 1988). EAV occasionally causes an outbreak characterized with abortion and newborn deaths (Newton et al., 1999; Pronost et al., 2010). Various incidences have been reported in different countries around the world; 55.1% (Rola et al., 2011) in Poland, 15% in Mongolia (Pagamjaw et al., 2011) to 73% in Australia (Huntington et al., 1990).

In the light of the current literature, the pathogenesis of the infection appears to be the same in the studied species. However, severity of the disease differs depending on the variant viruses (Del Piero et al., 1997; Eichhorn et al., 1995; McCollum et al., 1995; Murphy et al., 1992). Ramina et al. (1999), identified the virus in semen of both donkey and horses in Italy. In an experimental infection with KY-84 strain, moderate and severe clinical findings have observed in horses while mild prognosis in donkeys (McCollum et al., 1995). Paweska et al. (1995) isolated the agent in semen of an antibody positive donkey stallion in South Africa. Stadejek et al. (2006) determined a genetically new EAV genotype from naturally infected donkeys and found 60 to 70% of the nucleotide identity of the reference virus. In another study, researchers suggested that the new type evolved from original South African asinine strain via propagation in different host species, especially draw attention at the possible role of donkeys (Paweska et al., 1995). Recently, a new European has isolate (08P178, EU-1 clade) was reported. Disorders were limited to respiratory and alimentary systems (Vairo et al., 2014). Generally, data on pathogenetic characteristics are less in donkey than in horses.

Serological research reports give there are up to 20% positivity in Standard-bred horses and donkeys in many parts of the world. The infection appears to increase in general in the world (Holyoak et al., 2008; Huntington et al., 1990; Pagamjaw et al., 2011; Rola et al., 2011). From time to time, the infection turns out to be serious outbreaks. The studies in our country show that approximately one in every 10 animals was exposed to the virus, but the clinical status of the infection is unknown due to the absence of detailed health records. Since the economic prospects of ordinary horse and donkey breeding at as family-type, small farm in rural areas was accepted as insignificant, preventive health practices are not being carried out and adequate attention to diagnosis and treatment is not carefully shown to these animals. Horses and donkeys are still indispensable instruments of sustainable rural agriculture. Breeding purposes are different; standard-bred are used for transportation, as a pet, a companion and pack animal. Donkeys are also more multifunctional animals. They are quite preferential by many aspects like low breeding costs, mood temperament and tough structure under field conditions. However, due to the increase in agricultural mechanization, the need for standard-bred horses and donkeys has been reducing. The number of horses, donkeys and mules has decreased by 36.4%, 48.6% and 43.9% respectively in the last decade in Turkey. According to TUIK 2017 data; there are 120.000 horse, 152.000 donkey and 38.000 mule. The reduction is likely to continue.

The first detection of the EVA infection was in 1996 in Turkey (Yilmaz et al., 1996). There is no data on previous history due to the lack of retrospective studies. The main advantage of the studied population in this study is a considerably low viral transmission possibility by no co-breeding of sensitive species in any of the studied farms. In addition, the donkeys are mostly located in mountainous areas; this fact can explain lower infection rates. Nevertheless, it was saw that the both species exposed to the virus in all studied districts. The first data was obtained on EVA in these provinces. Even though, the determined rates are consistent with previous studies, it was higher than expected considering the sampled population profile. When time distribution of the serological data from all over the Turkey is considered, it appears that there has been a slight increase in the last decade, and it has potential to widespread in the upcoming years, and standard-bred horses and donkeys may create a risk for pure racehorses. It is

necessary to determine the actual profile of the infection by conducting virus isolation and countrywide surveillance as a preliminary basis for the implementation of preventive practices.

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