

Serological investigation of equine viral arteritis in donkeys in eastern and south-eastern Anatolia regions of Turkey

Sibel Gür¹, Bünyamin İrehan², Metin Gürçay³, Turhan Turan⁴

¹Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Virology, Afyonkarahisar, Turkey

²Veterinary Control Institute Elazığ, Virology Laboratory, Elazığ, Turkey

³Bingöl University, Faculty of Veterinary Medicine, Department of Virology, Bingöl, Turkey

⁴Cumhuriyet University, Faculty of Veterinary Medicine, Department of Virology, Sivas, Turkey

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Abstract

Equine arteritis virus is classified in the *Arteriviridae* family and causes reproductive and respiratory disorders. The host spectrum includes many species of the *Equidae* family. Horses, donkeys and mules are the most sensitive species. The infection was serologically investigated in adult donkeys on small private family type enterprises in eastern and south-eastern Anatolia in this study. A total of 1,532 samples were collected from 28 different locations in 6 different provinces in these two regions. The number of donkeys sampled from each farm was between 1 and 3. Serum samples were tested by indirect enzyme-linked immunosorbent assay (ELISA). As a result of sero-controls, 53 animals were positive (3.45%). The presence of infection was determined in all the provinces; Elazığ (7%, 17/241), Tunceli (2.4%, 3/122), Van (2.9%, 10/342), Bitlis (4.6%, 5/107), Şırnak (2.7%, 12/440) and Siirt (2.1%, 6/280). Seropositivity was detected in 22 of the 28 locations. In this study, data were obtained from a significant number of animals for the first time in these regions. Although the values were not high, the findings revealed the presence of infection in the majority of the investigated sites. Despite the fact that the incidence was not high in donkeys probably due to restricted management conditions, the incidence may increase over time and may pose a risk for thoroughbred horses unless necessary measures are taken.

Equus asinus, seroprevalence, viral infection

Equine viral arteritis (EVA) is one of serious infections that can affect horses. Donkeys, mules and alpacas are also susceptible to natural infection as well as the main host (Paweska and Barnard 1993; Paweska et al. 1996; Paweska et al. 1997; Weber et al. 2006).

The equine arteritis virus (EAV) is an enveloped, single stranded virus. The genome is of positive sense ribonucleic acid (RNA), which is classified in the *Arteriviridae* in the *Nidovirales* order (Cavanagh et al. 1994). The EVA virus has only one serotype (Westcott et al. 1995). There are limited antigenic variations among certain isolates but the severity of disorders shows alterations according to strain (Murphy et al. 1992; Stadejek et al. 1999). These differences in prognosis and abortion rates have been observed in outbreaks (Timoney et al. 1987b; Eichhorn et al. 1995; McCollum et al. 1995; Wood et al. 1995; Del Piero et al. 1997).

The most important mode of transmission is via the respiratory route, in both natural and experimental infections (Guthrie et al. 2003). Nevertheless, sexually transmitted infections from imported stallions are important for spread between farms and countries (Metcalf 2001; Holyoak et al. 2008). Clinical signs may vary in relation to age, strain and dose of the virus, individual factors, environmental conditions, herd size and intensity (Vaala et al. 1992; Timoney and McCollum 1993; MacLachlan et al. 1996; Paweska et al. 1996). After an incubation period of 3 to 14 days, fever, nasal and ocular injuries,

Address for correspondence:

Sibel Gür
Department of Virology
Faculty of Veterinary Medicine
Afyon Kocatepe University
03200, ANS Campus, Afyonkarahisar, Turkey

Phone: +90 272 2281312
E-mail: sibelgur@aku.edu.tr
<http://actavet.vfu.cz>

rhinitis, conjunctivitis, cough, diarrhoea, ataxia, urticaria, respiratory distress, oedema in orbita, limbs, scrotum, prepuce and mammary gland can be observed (Vaala et al. 1992; Del Piero et al. 1997; Szeredi et al. 2005; Holyoak et al. 2008).

The presence of EAV infection has been reported in horse populations all over the world since its first description in the United States in 1953 (Doll et al. 1957). Seroprevalence values are quite variable based on the country, race and management conditions. Japan and Iceland are virus-free (Kondo et al. 1998), contrary to the remaining parts of the continents (Timoney and McCollum 1988). Seroprevalence is generally less than 20% (Eichhorn et al. 1995; McCollum et al. 1995; Hullinger et al. 2001; Cruz et al. 2016). The incidence of EVA has been increasing especially in the last two decades (Glaser et al. 1997; Rola et al. 2011). According to the latest reports, the virus is in circulation in different parts of the world like Poland (Rola et al. 2011), Algeria (Laabassi et al. 2014), France (Amat et al. 2016), Spain (Cruz-Lopez et al. 2017), Serbia (Lazić et al. 2017) and Chile (Rivas et al. 2017). There is limited information about Turkey. Specific antibody rates vary between 5% (Yılmaz et al. 1996) and 14.4% (Yıldırım et al. 2008) in horses and donkeys.

The aim of this study was to serologically investigate EAV infection in donkeys to reveal a realistic profile by controlling relatively extensive numbers of animals in private small farms in eastern and south-eastern Anatolia.

Materials and Methods

Sampled animals

Donkeys are used as draft animals and often breed with ruminant species on farms in rural areas. The average number of donkeys is 1 to 3 per farm. Clinically healthy adult (≥ 1 year old) donkeys were randomly selected for sampling. Mostly female animals were available on farms depending on management practices. Of the six studied provinces, four were located in the East, and two were in the southeast Anatolia region (Siirt and Şırnak). Although detailed health records of animals were not kept, animal owners reported that some symptoms that may be associated with EVA and abortus cases were encountered from time to time. Blood samples were collected by venipuncture from vena jugularis into tubes containing silicone, and transported to the laboratory at 4 °C. After centrifugation at 3000 rpm for 10 min, sera samples were separated into stock tubes and stored at -20 °C until testing.

Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA is used to detect EVA virus specific antibodies due to its high sensitivity and specificity (Kondo et al. 1998). The test was carried out according to the manufacturer's instructions (Indirect Equine Viral Arteritis, ID Vet, France). At the end of the test, the plates were read in 450 nm and the obtained values were calculated.

Results

This study was carried out in six provinces located in east Anatolia ($n = 4$) and southeast Anatolia ($n = 2$) regions. Elazığ, Tunceli, Van and Bitlis are located in the east Anatolia region. The samples from Elazığ were collected from 6 localities; the central villages (0/24), Maden (1/51), Karakoçan (1/40), Alacakaya (3/56), Sivrice (8/40) and Baskil (4/30). Total seropositivity was found to be 7% (17/241). In the Tunceli province, 4 locations were selected; Central (1/40), Hozat (1/32), Mazgirt (0/24) and Pertek (1/26). In total, of 122 samples, three were detected as seropositive (2.4%) (Table 1).

A total of 342 donkeys were sampled in the Van province; Center (3/42), Bahçesaray (0/92), Erciş (1/96), Gürpınar (1/20) and Muradiye (5/92), and 2.9% were detected as positive. The smallest number of samples were collected from Bitlis, Center (3/92) and the Hizan county (2/15).

The Şırnak and Siirt provinces are in the southeast Anatolia region. A total of 440 donkeys were sampled in Şırnak. In total, 2.7% of the animals were positive in the province and its districts of Center (1/100), Cizre (1/49), İdil (5/195), Uludere (2/50) and Silopi (3/46). The

lowest percentage was detected in Siirt at 2.1% (6/280) and its districts, namely Center (3/92), Baykan (0/20), Şirvan (0/38), Kurtalan (1/34), Eruh (2/46) and Aydınlar (0/50).

Of the 1.532 donkeys in total, 53 were found to be seropositive for EAV infection. Of the 28 locations, all samples were found to be negative in only 6 districts. The percentages ranged from 1% to 20% in the other 22 locations.

Discussion

Pathogenetic studies are mostly focused on horses. Donkeys are at least as sensitive as horses and the pathogenesis is the same (McCullum et al. 1995; Paweska et al. 1995; Paweska et al. 1996). We know that the incidence of EVA infection is higher in stallions due to the long duration of the carrier state (Rola et al. 2011). Antigen positivity can be detected for up to six years and more after acute infection. Some stallions can shed the virus throughout their lifetimes (Timoney et al. 1986; Timoney et al. 1987a,b). However, in an EVA epidemic in Kentucky in 1985, affected mares were screened regularly and all of them were identified as antigen negative soon after the acute stage (Timoney et al. 1987b). An effective control of EVA infection depends on the detection of persistently infected asymptomatic stallions. Field studies show that the incidence increases with age. Rates are higher in males than in females. Standardbred horses are at a greater risk than purebred horses due to prophylactic applications and management factors (Timoney and McCullum 1988; Cruz-Lopez et al. 2017).

There are several studies on EAV infection in donkeys around the world. Paweska and Barnard (1993) found 17% positivity in donkeys in South Africa; in some regions 30% rates have been detected. An experimental study carried out with horses and donkeys (Paweska et al. 1996) reported that donkeys were more susceptible to the infection compared to horses and that their seroprevalence was higher. Furthermore, researchers have noted that the infection in donkeys is prolonged. According to past experiences, uncontrolled importation of commercial stallions is the most effective factor in triggering an epidemic (Wood et al. 1995). Similarly, using uncontrolled semen for artificial insemination has the ability to transmit infection between countries (Metcalf 2001). Therefore, many countries have introduced compulsory control for the semen and stallion trade.

The history of EVA in Turkey is unknown due to the absence of retrospective studies. Serological evidence has been available since 1996. In the first report, 5% (5/100) positivity was detected in horses (Yılmaz et al. 1996). Another study reported values of 9.5% (19/200) and 8% (16/200) in the Kars and Ardahan provinces (northeastern Anatolia), respectively (Kırmızıgül et al. 2007). Hasan (2008) did not isolate any of the 252 horses from seven stud farms in the Marmara region despite a seropositivity of 7.5%. Only two serological studies have been carried out regarding donkeys which manifested 14.4% (11/76) (Yıldırım et al. 2008) and 8.4% (19/227) (Gür et al. 2018). A single molecular study has been carried out in which the open reading frame (ORF) 1b gene was analysed from samples from nine positive horses with respiratory disorders in four different regions of Turkey. There were minor differences between TR-EVA strains (94.7%). These strains were found to be closely related to strains of North American origin (vaccines and virulent strains of Bucyrus) (Ataseven et al. 2013).

In this study, 7% and 2.4% of the samples collected from animals in the Elazığ and Tunceli provinces, respectively, were positive. The two highest values (13% and 20%) were determined in the Elazığ province. These adjacent provinces are located in the eastern Anatolia region. The other four provinces are also border neighbours. The obtained values were quite similar to each other (Van 2.9%, Şırnak 2.7%, Siirt 2.1% and Bitlis 4.6%). In total, the rate (3.4%) was the lowest in Turkey so far (Table 1). All of the samples were negative in six of the 28 locations in total. Seropositivity was below 5% in 15 districts, whereas

Table 1. The numbers and locations of sampled donkey and EVA test results.

Provinces	Boroughs	No of samples	EAV		No of samples	Total EAV	
			Ab(+)	%		Ab(+)	%
Elazığ 38°67'43 -39°22'32	Central	24	0	0	241	17	7.05
	Maden	51	1	1.9			
	Karakoçan	40	1	2.5			
	Alacakaya	56	3	0			
	Sivrice	40	8	20			
Baskil	30	4	13.3				
Tunceli 39°09'92 -39°54'35	Central	40	1	2.5	122	3	2.45
	Hozat	32	1	3.1			
	Mazgirt	24	0	0			
	Pertek	26	1	3.8			
Van 38°49'45 -43°38'32	Central	42	3	7.1	342	10	2.92
	Bahçeşaray	92	0	0			
	Erciş	96	1	1			
	Gürpınar	20	1	5			
	Muradiye	92	5	5.4			
Bitlis 42°10'95 -38°40'05	Central	92	3	3.2	107	5	4.67
	Hizan	15	2	13.3			
Şırnak 37°51'89 -42°45'37	Central	100	1	1	440	12	2.72
	Cizre	49	1	2			
	İdil	195	5	2.5			
	Uludere	50	2	4			
	Silopi	46	3	6.5			
Siirt 37°93'262 -41°94'0	Central	92	3	3.2	280	6	2.14
	Baykan	20	0	0			
	Şirvan	38	0	0			
	Kurtalan	34	1	2.9			
	Eruh	46	2	4.3			
	Aydınlar	50	0	0			
Total					1.532	53	3.45

EVA - equine viral arteritis; EAV - equine arteritis virus

the rate was between 5% and 10% in three localities, and rates between 10% and 15% were observed in two districts. Previously, 11.3% (14/124) rates were detected in horses in the Van province (Gür et al. 2015). Gür et al. (2018), reported 14.3% (13/91) in Elazığ and 15.7% (16/102) in Tunceli provinces in horses. Additionally, in Elazığ and Tunceli provinces, 5.3% (7/131) and 12.5% (12/96) were determined in donkeys, respectively. Data related to the infection were obtained for the first time in the Bitlis, Şırnak and Siirt provinces. In these small provinces which are mostly located in a mountainous terrain, traditional small scale private farms are still very common. The number of donkeys has decreased dramatically in the last few decades as a result of agricultural mechanization, but they are still preferred in daily farm work in rural areas in the county. Routine vaccination has not been applied to any infectious disease in donkeys. Special importance is not given

to their reproduction due to their low economic value. No preference was made regarding sex in the sampling, however, the majority of the animals were female and most of the farms had no stallion. A limited number of donkey stallions are commonly used in most villages for reproduction. The farms are not very close to each other and none of them had horses. Separate breeding of donkeys from horses may reduce the possibility of cross-species transmission. Disadvantageous factors for in-contact transmission such as intensive breeding, mixed-sex and dual breeding with horses were not deemed important in the studied population. However, the common use of stallions appears to be a noticeable risk factor for sexual transmission. No EVA epidemic has generated concern in Turkey so far despite the presence of the infection in most of the controlled localisations. Regional problems may remain unidentified or unreported especially in donkeys and standardbred horses. A strict control of imported semen contributes significantly to prevention with other prophylactic applications on stud and thoroughbred farms.

In conclusion, the lowest rates in the country were determined in this study probably as a consequence of focused race and preferred breeding conditions. However, the widespread presence of the disease suggests that EVA holds a potential risk for the future. Therefore, it is necessary to obtain extensive field data for other regions of the country and implement preventive studies also for private farms which are still an important part of our agriculture.

Conflict of Interest

The authors declare that they have no commercial or financial competing interests.

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