An investigation of equine infectious anaemia infection in the Central Anatolia region of Turkey

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ABSTRACT

In this study, 162 horses, 80 donkeys and 51 mule serum samples were collected in Konya city. Additionally, 64 horse serum samples from Ankara and 49 samples from Kayseri city were included in the study. A total of 406 serum samples were examined by agar gel immunodiffusion (AGID) and enzyme-linked immunosorbent assay (ELISA) for antibody to equine infectious anaemia virus (EIAV) and no positive result was detected.

Key words: donkey, equine infectious anaemia, horse, mule, Turkey.

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INTRODUCTION

Equine infectious anaemia (EIA) is a chronic disease of all members of equidae, including horses, donkeys, mules, ponies and zebra . It is characterised by periodical fever, anaemia, thrombocytopaenia and leukopaenia[4,10]. Equine infectious anaemia virus infection is of considerable importance for the equine industry[16]. The prevalence of infection varies throughout the world and depends on the density of the horse population, the proportion of carrier animals, the insect vector population, and control activities in the particular area[27]. EIA has a worldwide distribution and has been diagnosed on all continents except Antarctica. The virus is endemic to the Americas, parts of Europe, the Middle and Far East, Russia and South Africa[1,7,11]. Transmission occurs mechanically by transfer of blood from an infected horse. In nature, spread of the virus is most likely *via* interrupted feeding of bloodsucking horseflies on a clinically ill horse and then on susceptible horses, or from the use of contaminated needles[22]. Most naturally occurring outbreaks of acute and subacute cases of this disease develop during the late summer and early autumn months in the temperate zone. This coincides with the peak of the biting insect population, especially bloodsucking horseflies, deer flies (tabanids), stable flies (*Stomoxys* spp.), mosquitoes and possibly midges[12,17,24,26]. Biting insects, such as deer flies, horse flies and stable flies rather than mosquitoes, are generally considered to play a primary role in mechanical transmission of the EIA virus[20]. The fly population of the Mediterranean and south-eastern Anatolian regions of Turkey and animal movement increases in summer; therefore, the disease could spread quickly and easily in Turkey.

Once a horse is infected with EIA virus, its blood remains infectious for the remainder of its life. This means that the horse is a viraemic carrier and can potentially transmit the infection to other susceptible animals[8,34]. The most important factor for prevention of EIAV infection is separation of infected from healthy animals. Thus, the animals should be tested periodically with serological tests and seropositive animals should be considered as persistently infected even if no obvious clinical signs are seen, and these animals should be isolated from healthy animals[18]. The most useful serological tests for screening of EIAV infection are AGID and ELISA[8,13]. The AGID test, widely known as Coggin's test, has been approved by United States Department of Agriculture (USDA) for diagnosis of EIAV infection, but many researchers state that ELISA is more sensitive than AGID[2,25].

The health statement for European Union (EU) intra-Community movement requires all horses to be clinically examined and declared healthy prior to movement. The requirement for health certificates and clinical examinations prior to movement does, however, not apply to the movement of registered horses under the Tripartite Agreement (TPA) between the United Kingdom, Republic of Ireland and France[11]. The EIA status of most of the horse, donkey and mule populations in Turkey is largely unknown as surveillance is not carried out. Usually only a relatively few horses (mainly racing horses and horses on large farms) are closely monitored and possibly tested for EIA. In this study, serum samples were collected from privately owned horses, donkeys and mules. The purpose of the present study was to investigate the prevalence of EIA antibodies in horses, donkeys, and mules in the Central Anatolia region of Turkey.

MATERIALS AND METHODS

The Central Anatolia region is predominantly an agricultural region and has many working horses belonging to villagers, who breed horses in small studs for carrying their belongings. The total number of horses, donkeys and mules in the region is about 41 000[29].

In this study, the Simple Random Sampling technique of the Regional Sampling method was used[35]. Random sampling points were selected in three provinces in the Central Anatolia region. Sample units close to these points were then determined, and the selected animals were considered representative of the population. Blood samples were collected from 275 horses, 80 donkeys and 51 mules older than 3 years without clinical signs of the disease (Table 1). Blood samples were placed in clot activator vacuum tubes and centrifugated at 3000 rpm for 10 minutes. The separated serum samples were heat-inactivated at 56°C for 30 minutes before testing. Commercial AGID kits were obtained from VMRD Inc. (Pulman, WA, USA), and the test was carried according to the manufacturer's instructions. The agar was prepared as a 1 % solution of noble agar in 14.5 mℓ borate buffer (pH 8.6); 15 mℓ of it was then transferred to plastic Petri dishes. The test pattern consisted of six peripheral wells around a centre well: 50 $\mu\ell$ of antigen was placed in the centre well, reference positive serum

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(50 $\mu\ell$) was added to one peripheral well, and the other five wells were used for test serums (50 $\mu\ell$). The Petri dishes were then incubated for 24–48 hours at room temperature to form the precipitin line that is characteristic of a positive sample, whereafter the results were recorded. Suspect samples were retested by AGID. A commercial ELISA (Viral Antigen Inc., Memphis, TN, USA) test kit was also used as a different technique. The results were evaluated spectrophotometrically at 450 nm adsorbance.

RESULTS

Two hundred and fifty owners from the main cities (Konya, Ankara, Kayseri) of the Central Anatolia region agreed to cooperate in the study. A total of 406 animals (275 horses, 80 donkeys and 51 mules) was tested. All horses, donkeys and mules were negative for antibodies to EIAV.

Table 1: **Distribution of serum samples of horses, donkeys and mules from 3 cities in the Central Anatolia region of Turkey.**

Animals	Central Anatolia			Total
	Konya	Ankara	Kayseri	
Horse	162	64	49	275
Donkey	80	–	–	80
Mule	51	–	–	51
Total	293	64	49	406

DISCUSSION

EIA infection is prevalent in all parts of the world and it is of importance for the equine industry[26,28]. EIA is a notifiable disease in Turkey and there are official rules and regulations about EIA infection in Turkey[23].

Besides the several serological tests for the diagnosis of the infection, AGID and ELISA have been reported as the most sensitive tests and they have been used by many researchers[4,13]. AGID tests and ELISAs are accurate, reliable tests for the detection of EIA in horses, except for animals in the early stages of infection and foals of infected dams[28,32]. Confirmation of a clinical diagnosis of EIA was hampered until 1970 by the lack of a simple, reliable diagnostic test. The successful adaptation of the AGID test for the diagnosis of EIA by Coggins and Norcross[6] provided a reliable and economical diagnostic tool. Additionally, the ELISA has been successfully used for determination of antibodies against EIAV by many researchers, and a good correlation between two tests was found[22,33].

Donkeys and mules have been traditionally used for transport as have horses in Turkey and these animals are kept by local people and are generally unregistered. In previous studies[3,5,36] evaluating EIA prevalence in horses in Turkey using the AGID test, and in horses, donkeys and mules using both the AGID and ELISA, no seropositivity for EIA was documented. In the current study, the prevalence of antibodies against EIA was studied in horses, donkeys and mules only from the Central Anatolia region using both AGID and ELISA. In total, 406 serum samples were collected in three cities (Table 1), and none of them was positive. This situation shows that the disease is not present or that it might be present but could not be detected because of the low sampling numbers or because the immune response of those animals had not yet been mounted. The virus titre, however, is higher in horses with clinical signs and the risk of transmission is higher from these animals than the carrier animals with lower titres[19]. High plasma viraemia levels characterise the first disease episode, which is usually referred to as the acute stage of equine infectious anaemia[9]. Donkeys and mules infected with two strains of EIAV had significantly lower amounts of plasma-associated virus and/or viral nucleic acid levels than similarly infected horse or pony controls during the critical early stages following infection[10,31]. As was concluded in this study, researchers in previous studies[3,5,15,22,30,33,36,37] reported that no seropositivity was detected in horses, donkeys and mules with no clinical signs and they attributed this to the health of all animals or that the animals were in the early stages of acute EIA. Asymptomatic and suspected animals may be detected by using a more sensitive technique, such as polymerase chain reaction (PCR), to test for viral nucleic acids in blood or tissues[14,21].

CONCLUSION

In this research, besides the horse sera, donkey and mule samples were tested because these animals also play a role as carriers. Regions with high density horse, donkey and mule populations and sampling units close to these regions were selected, with a homogenous distribution of animals in the selected regions. It is concluded that EIA is not present the Central Anatolia region of Turkey and that EIA offers no potential risks for the horse-breeding and racing industry in this part Turkey.

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