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RESEARCH ARTICLE

Crimean Congo Hemorrhagic Fever Virus-Specific Antibody Detection in Equids

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) virus infection is a tick-borne zoonotic disease that is endemic in a large region of the world and is a potentially fatal disease, especially threatening human health. One of the main indicators that the disease is endemic in a region is the presence of antibodies specific to CCHFV in animal populations. Many animal species may carry CCHFV asymptomatically and hence participate to the disease's transmission cycle. Serological studies have shown that equids are important to the survival of CCHFV in nature. Nevertheless, need for more studies on this subject in Türkiye. For this study, blood samples were taken from 11 horses and 86 donkeys of different sex and variable ages in the provinces of Afyonkarahisar and Burdur. The presence of CCHFV-specific antibodies in the blood serum of these animals, which are held by the breeders for a variety of purposes, was studied. The double-antigen ELISA test method, which is a fast and reliable method, was used for the detection of specific antibodies. As a result, a total of 51.54% (50/97) seropositivity was detected. Findings indicate that equidae may play an important role as a reservoir in the epidemiology of CCHFV.

Keywords: Crimean Congo Hemorrhagic Fever, Donkey, ELISA, Equide, Seroprevalence

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Tek Tırnaklı Hayvanlarda Kırım Kongo Kanamalı Ateşi Virusu'na Spesifik Antikorların Tespiti

ÖZ

Kırım Kongo kanamalı ateşi (KKKA) virus enfeksiyonu, dünyanın geniş bir bölgesinde endemik olan ve özellikle insan sağlığını tehdit eden, potansiyel olarak ölümcül bir hastalık olan kene kaynaklı zoonotik bir hastalıktır. Hastalığın bir bölgede endemik olduğunun ana göstergelerinden biri, hayvan popülasyonlarında KKKAV'ye özgü antikorların varlığıdır. Birçok hayvan türü KKKAV'yi asemptomatik olarak taşıyabilir ve bu nedenle hastalığın bulaşma döngüsüne katılabilir. Serolojik çalışmalar, doğada KKKAV' nin hayatta kalması için tek tırnaklıların önemli olduğunu göstermiştir. Ancak Türkiye'de bu konuda daha fazla çalışmaya ihtiyaç vardır. Araştırma için Afyonkarahisar ve Burdur illerinde çeşitli cinsiyet ve yaştaki 11 at ve 86 eşekten kan örnekleri alındı. Yetiştiriciler tarafından çeşitli amaçlarla tutulan bu hayvanların kan serumlarında KKKV'ye özgü antikorların varlığı araştırıldı. Spesifik antikorların tespiti için hızlı ve güvenilir bir yöntem olan çift antijenli ELISA test yöntemi kullanıldı. Sonuç olarak toplamda %51.54 (50/97) seropozitiflik tespit edildi. Bulgular, tek tırnaklı hayvanların KKKA'nın epidemiyolojisinde rezervuar olarak önemli bir rol oynayabileceğini göstermektedir.

Anahtar Kelimeler: Kırım Kongo Kanamalı Ateşi, Eşek, ELISA, Equide, Seroprevalans

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INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is an enveloped, segmented, and negative-sense single-stranded RNA virus in the *Orthonairovirus* genus in the *Nairoviridae* family within the order *Bunyavirales* (Lombe et al. 2021, Sana et al. 2022). The CCHFV is a tick-borne virus that was named for the geographical places where it was first characterized in the Crimea (1944) and recognized in the Congo (1969) (Bente et al. 2013). CCHFV is the causative agent of *Crimean-Congo hemorrhagic fever* (CCHF) in humans. (Blanco-Penedo et al. 2021). CCHF, is a zoonotic arboviral disease that poses a great threat to public health therefore according to the World Health Organization (WHO), CCHFV is the most predominant causative agent of viral hemorrhagic fever outbreaks worldwide (Sorvillo et al. 2020). Humans and a wide range of other animal species are at risk of contracting CCHFV (Ali et al. 2019, Ali et al. 2021, Kaba 2022). It has been reported that CCHFV can be transmitted to humans through a tick bite, contact with blood, body fluids, or tissues of a viremic animal or human (Bente et al. 2013). In addition, it has been stated that infection can occur in humans through contact with raw or undercooked meat after slaughter and through the consumption of contaminated meat (Fazlalipour et al. 2016, Mostafavi et al. 2017). In humans, acute CCHF is characterized by fever, chills, muscular discomfort, headache, nausea and vomiting, stomach ache, and joint pain. Ecchymosis and bleeding from the mucous membranes of the nose and vagina can worsen the condition in extreme situations. The fatality rate from CCHF ranges between 9% and 50% on average (Hawman and Feldmann 2018). Although it is life-threatening for humans, it generally does not cause illness in animals that carry it asymptomatically. However, domestic and wild animals play a crucial role in the circulation of the virus into new areas by carrying transovarially infected mature ticks (Spengler et al. 2016).

It has been reported that CCHF has spread to a wide geography as far as Africa, Eastern Europe, the Middle East, and Central and South Asia (Sana et al. 2022). In Türkiye, CCHF cases have also emerged in western regions as well as in Central Anatolia and the Black Sea region in recent years (Elaldı 2004, Simsek et al. 2018). More than 30 different species of ticks from various genera carry the virus in places where CCHF is endemic (Shahid et al. 2021). The CCHFV is maintained as a reservoir and spreads by the bites of *Ixodid* (hard) ticks, particularly those belonging to the genus *Hyalomma*. Tick species have been reported in the Western Mediterranean regions of Afyonkarahisar and Burdur in Türkiye (Bakırcı 2009, Eser 2012). Among these identified species, there are also tick species that are effective in the transmission of CCHFV (Gargili et al. 2017).

In previous studies, it has been reported that antibodies against CCHF have been detected in many domestic and wild animals, including cattle, goats, sheep, horses, pigs, camels, donkeys, mice, dogs, rabbits, and ostriches (Mangombi et al. 2020, Spengler et al. 2016, Pak 1972). The presence of CCHFV antibodies in a diverse host spectrum indicate that CCHFV is endemic in these areas and will continue to circulate in nature for many years (Ceianu et al. 2012). Thus, testing for specific antibodies against CCHFV by serological tests is crucial for establishing the fact of current or prior viral infection. This is the first research ever conducted on equids in Türkiye, and the results provide crucial baseline information on the prevalence of CCHF in the region. This study is expected to provide current data on the detection of antibodies against CCHF in equidae animals such as horses *(Equus caballus*) and donkeys (*Equus asinus*) bred for various purposes.

MATERIAL and METHOD

Animals

Samples were obtained for this investigation from 11 horses and 86 donkeys used as mounts in rural parts of Türkiye's Western Mediterranean region (Figure 1), which were used for transportation in livestock and daily works. Anamnesis information for asymptomatic animals was collected from their owners during sampling. All procedures were approved by the Animal Ethics Committee (AEC) Burdur Mehmet Akif University, Türkiye (102/912, 25.05.22).

Sample Preparation

Vena jugularis was used to collect blood samples into a sterile, vacuumed kaolin tube. It was transported to the virology laboratory under cold chain conditions for analysis on the same day. Serum samples were transferred to sterile tubes after being centrifuged at 3000 rpm for 10 minutes at room temperature. All samples were stored at -20°C until analyzed.

ELISA Assay

The detection of antibodies specific to CCHFV in a total of 97 serum samples was investigated according to the commercial ELISA kit (ID Screen® CCHF Double Antigen Multi-species, Grabels, France) procedure. The diagnostic sensitivity and specificity of the ELISA kit are %96.8-99.8 and %99.8-100, respectively (Sas et al. 2018). In summary, after adding 50 µl of dilution buffer to all wells, 30 µl of negative and positive controls were placed in 2 wells. 30 µl serum samples were distributed to the remaining wells.

Then, the incubation conditions and procedures stated in the test procedure were followed. Following the validation controls, the data were measured in an ELISA microplate reader at 450 nm and interpreted based on the optical density (OD) values. OD values less than or equal to 30% were defined as negative, whereas those greater than 30% were regarded as positive. The detection of CCHFV-specific antibodies in collected serum samples was recorded.

Figure 1: Distribution of the samples according to the provinces from which they were obtained and some tick species reported to be present in these provinces

RESULTS

The distribution of the OD values obtained after the analysis is shown in Figure 2. Forty-six out of a total of 86 donkey serum samples were positive for the presence of the antibody.

A total of fifty animals found to be seropositive, including 33 females and just 17 males. Four horses were found to be seropositive out of a total of 11 serum samples. Three of them found to be seropositive were female, whereas just one was male. It is found that the average age of male donkeys was 8.8 years and that of females was 5.2 years, whereas the average age of male horses was 6.9 years and that of females was 5.2 years. Briefly, the median age of seropositive female animals was lower than that of seronegative male animals, and the seroprevalence of female animals was greater than that of male animals. Overall, 51.54% (50/97) of the animals tested positive for the presence of the antibody (Table 1).

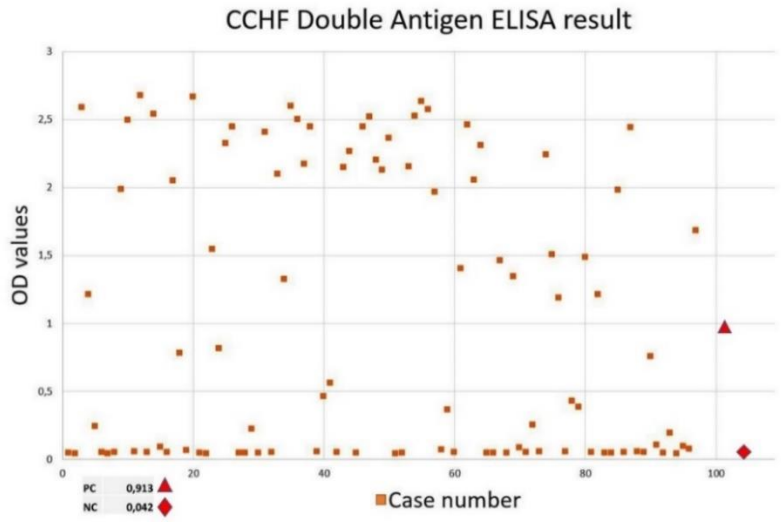

Figure 2: OD values distrubitons. PC: Positive control, NC: Negative control.

Table 1. Distribution of test results by animal species, sex, and age.

	Donkey		Horse	
	Male	Female	Male	Female
Age (Mean)	8.8	5.2	6.9	5.2
Total number of samples	31	55	5	6
CCHF positive numbers	16	30	1	3
CCHF negative numbers	15	25	4	3
Rate of seroprevalance (%)	51.61	54.54	20.0	50.0

DISCUSSION

Human actions, including increasing deforestation, invasion of natural animal habitats, and climate change, dramatically enhance the possibility for exposure and spillover of novel zoonotic diseases like CCHF. Newer research has also put an emphasis on the dangers of epidemics and pandemics because of their potential effects on human health (Aydin et al. 2020, Karamese et al. 2020). When healthcare authorities like the World Health Organization (WHO) include zoonotic diseases as CCHFV that have caused outbreaks on their lists, it demonstrates the disease's significance for human and animal health (Gilbride et al. 2021).

This study is the first to report on the prevalence of CCHF in equids, located in Türkiye's Western Mediterranean region. According to the findings of this investigation, a total of 51.54% (50/97) were found to be seropositive, including 53.48% (46/86) donkeys and just 36.36% (4/11) horses (Table 1). Recently, in northern Türkiye, Albayrak et al. (2012) reported an antibody prevalence of 66% in sheep and 85% in goats. In the Marmara region, Tuncer et al. (2014) also reported the seroprevalence of CCHFV was 13% in cattle, 31.8% in sheep, and 66% in goats. Despite the lack of comparable data from Türkiye, only a small number of studies were undertaken on the other species. Among them, there is no study on equids about CCHF seroprevalence in Türkiye. On the other hand, an investigation conducted in Senegal, indicated that the CCHF seroprevalence rate was high in horses but low in donkeys, supporting the results of the current study (Mangombi et al. 2020). It was also noted that positive animals had a higher mean age than negative animals. Even though this research shows that horses have a high seroprevalence, which seems to match the results of other studies, we think that the small number of samples may be misleading. However, high levels of antibodies were detected in donkeys in this study. We concluded that the significant seroprevalence we found was most likely to be attributed to the length of time these animals lived and how long the antibodies lasted in their bodies. When average age is considered, the animals with positive are younger than the animals with negative, according to this data. Since young animals are preferred over old animals in terms of energy and endurance in daily work, the chance of viral exposure increases. We believe that high antibody positivity in young animals is related to this scenario. Donkeys exposed to tick bites in rural settings are regarded to be possible sources of CCHF in people (Lwande et al. 2012). Therefore, it has been suggested that animals such as horses and donkeys play an important role in the epidemiology of the disease (Ibrahim et al. 2015). There is also a high potential of tick species transfer from animals to people in the areas where the samples were taken, *Hyalomma marginatum*, *Rhipicephalus bursa*, and so on (Figure 1). The fact that animals used as transportation in rural areas of these regions are likely to be exposed to ticks shows that these animals will play a big role in how CCHFV spreads.

While studies of the virus are commonly conducted in high-risk populations or places, the real prevalence of the disease remains unclear. Despite the fact that Türkiye was infection-free before the 2000s, it has become the global "epicenter" of the disease, with over a hundred new cases in recent years (Ozturk et al. 2017). In Türkiye, particularly in the Black Sea and Central Anatolia regions, CCHF is prevalent (Leblebicioglu et al. 2016). Despite this, new cases have been reported in the western regions in recent years due to changing climatic conditions, movement and trade of infected livestock, and migratory bird routes (Elaldı 2004, Öztürk et al. 2017, Simsek et al. 2018). Due to the rising human population in the Eastern Mediterranean region (Al-Abri et al. 2017), the favorable circumstances for vectors, and the migration of animals and wild birds transporting infected ticks from Syria, Iraq, and Iran, CCHFV has become endemic in the southwest of Türkiye. In the study areas, equids are commonly used for everyday labor due to the relative ease of access to land, which in turn increases the likelihood of vector-host contact. This investigation determined that there were a high specific antibodies in equidae in the Western Mediterranean region. The foregoing findings support the hypothesis that CCHF may be spreading in these places.

In many parts of the world, the domestication of donkeys, mules, and horses was crucial to the progress of civilisation. These animals have served numerous purposes for humans throughout history, including transportation, race, warfare, entertainment, friendship, labor, and sport. They are still in use for work today, especially in countries with low and middling incomes and in places with steep hills. In Türkiye, these animals are still used as riding animals, draught animals in agricultural operations, sources of power for wheeled vehicles, and pack animals. They carry firewood, water, grain, hay, and a multitude of other goods over short distances. They escort sheep flocks and carry the equipment of shepherds, and a weak newborn lamb that cannot follow the flock in rural areas (Yılmaz and Wilson, 2013). These mentioned events are very common in rural areas, which increases the chance of being exposed to vectors like ticks. Thus, it is crucial to study infectious agents that can be detected in or transmitted by these animals, for the benefit of both animal and human health (Alkan et al. 2013, Aydin et al. 2020, Gilbride et al. 2021; Timurkan et al. 2019). The reason that the seroprevalence of CCHFV infection is so high in this study is due to a number of factors, including hot weather conditions, global warming, the existence of asymptomatic animals, a continuous movement of hosts in and out of these regions, their living conditions, and the fact that horses or donkeys are kept together with other livestock. The foregoing data provide support to the hypothesis that equids contribute significantly to the maintenance of high CCHFV prevalence despite the fact that they likely do not act as direct sources of viral transmission in the same way that viraemic livestock do.

It was found that the incidence of CCHFV infection during spring-summer was the highest among samples animals. On the other hand, CCHFV is considered to be seasonal but can cause infections because of reservoir animals (Spengler et al. 2016). The sera samples in this study were collected at spring and summer seasons, and this could be a factor in the high seroprevalence because of the high activity of ticks. In eastern Türkiye, CCHF cases are often reported between June and August. However, western Türkiye has a longer tick season because of the warmer and milder weather compared to the east. This results in a seasonal pattern of vector incidence, watt the peak occurring between April and November. The western Türkiye region has recently become endemic for CCHF. Therefore, it indicates that the natural foci of CCHFV may spread further to other provinces in western Türkiye. Furthermore, it is crucial to take a variety of anti-epidemic actions targeted at reducing epizootic activity and boosting population immunity to CCHFV. Even though most people in the Western Mediterranean provinces live in cities, they mostly have their own farms where they grow their requirements of vegetables and fruits. Although the residents of the region do not farm, there is an increase in agricultural activity throughout the spring and summer months for this reason. This condition also has a high potential for enhancing the interaction of tick and host (Sorvillo et al 2020). For future seroprevalence studies that will be done in both rural and urban areas, like the provinces of Afyonkarahisar and Burdur, it may be helpful to think about the routines, ways of life, and interactions of the rural area. Additionally, it is clear that animal owners should be informed about such arboviral diseases and that they need to learn how to deal with vectors in such rural areas. Ticks, in particular, act as both mechanical and biological vectors. They allow pathogens to spread, which leads to re-transmission, and so they keep infections with them through all of their life stages. Increased CCHFV instances and recent environmental changes, such as global warming, that favored larger tick numbers, suggest that public health concerns regarding tick-borne illnesses will become more urgent in the western areas of Türkiye.

CONCLUSION

In endemic regions, CCHF is a threat to anyone who works closely with animals, including animal herders, livestock workers, and those who work in slaughterhouses. Individuals working in healthcare in endemic regions are at risk of contracting an infectious disease due to their unprotected contact with patients' blood and other bodily fluids. Travelers and locals alike who come into contact with hosts in endemic areas are at risk. According to the results of the study, CCHFV has the potential to function in equidae as a reservoir. We believe that the findings will aid in future research to manage the disease from a global public health perspective.

Ethical Permission: All procedures were approved by the Animal Ethics Committee (AEC) at Burdur Mehmet Akif University, Turkey (No: 102 / 912).

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