Epidemiological Investigation of Crimean-Congo Hemorrhagic Fever infection in Cattle in some provinces of Turkey

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Geliş Tarihi / Received: 18.01.2018, **Kabul Tarihi** / Accepted: 06.05.2018

Abstract: Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic disease which causes lethal hemorrhagic fever in humans. The purpose of this study was to investigate the CCHF infection status in cattle in the Central Anatolia region and the central-west part of the Aegean region of Turkey. For this purpose, EDTA whole blood (n = 329) and sera (n =329) samples were obtained from epidemiologically independent herds (n =94) during the months of March 2016 and September 2017. The exposure status to CCHF was determined using ELISA for detection of CCHF virus (CCHFV) specific IgG antibodies in cattle sera samples. Real-time reverse-transcriptase PCR was used to detect viral RNA in EDTA whole blood samples. The CCHFV-specific IgG antibodies were detected in 4 out of 329 animals, accounting for 1.2% prevalence rate. CCHFV RNA was not detected in EDTA whole blood samples. The low seroprevalence suggests only sporadic introduction of CCHFV. Further epidemiological studies are needed to determine the distribution of CCHFV infection in livestock in Turkey.

Key words: Crimean-Congo hemorrhagic fever, Epidemiology, Turkey, Cattle, ELISA, Real-time RT-PCR

Türkiye'nin bazı İllerindeki Sığırlarda Kırım Kongo Kanamalı Ateş Enfeksiyonunun Epidemiyolojik Araştırılması

Özet: Kırım-Kongo Kanamalı Ateşi (KKKA) insanlarda öldürücü hemorajik ateşe neden olan zoonotik bir hastalıktır. Bu çalışmanın amacı Türkiye'nin İç Anadolu ve Orta Batı Ege Bölgelerindeki sığırlarda KKKA enfeksiyon durumunu araştırmaktı. Bu amaçla Mart 2016 ve Eylül 2017 tarihleri arasında epidemiyolojik olarak birbirinden bağımsız işletmelerden (n=94) EDTA'lı tam kan (n = 329) ve serum (n = 329) örnekleri toplandı. KKKA'ne maruziyet durumu, sığır serum örneklerinde KKKA virusuna (KKKAV) spesifik lgG antikorlarının ELISA ile tespit edilmesi ile belirlenmiştir. Real time reverse transkripsiyon PCR yöntemi, EDTA'lı tam kan örneklerinde viral RNA varlığını tespit etmek için kullanılmıştır. Üç yüz yirmi dokuz hayvanın, dördünde KKKAV spesifik lgG antikoru tespit edildi ve %1.2 prevalans oranı hesaplandı. EDTA'lı tam kan örneklerinde KKKAV RNA'sı tespit edilememiştir. Düşük seroprevelans oranı sporadik KKKAV vakalarının olduğunu düşündürmektedir. Türkiye'deki çiftlik hayvanlarında KKKAV enfeksiyonunun dağılımını tespit etmek için daha fazla epidemiyolojik çalışmalara ihtiyaç vardır.

Anahtar kelimler: Kırım Kongo Kanamalı Ateşi, Epidemiyoloji, Türkiye, Sığır, ELISA, Real-time RT-PCR

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic disease that can cause serious hemorrhagic disease in humans. CCHF is characterised by fever, weakness, myalgia and hemorrhagic signs with case fatality rates ranging from 5% to 80% [5, 37, 39]. Crimean-Congo hemorrhagic fever virus (CCHFV), the causative agent of CCHF, is a single-stranded RNA virus and belongs to the *Nairovirus* genus of the *Bunyaviridae* family [5].

CCHFV is primarily transmitted by *Hyalomma* spp. ticks [35]. The virus can also be transmitted horizontally and vertically within the tick population [11]. Wild (giraffe, rhinoceros, eland, kudu, buffalo and zebra) and domestic animals (cattle, sheep, goats, horses, donkeys and camels) can be infected and play a role in the spread of virus [25]. Viremia lasting up to 2 weeks can be observed in infected animals, but they do not show clinical signs. However, seroconversion can be observed in infected animals [30].

CCHFV infections have been reported in Sub-Saharan Africa, Asia, the Middle East and Southeastern Europe [5, 9, 13]. CCHF was first reported in the Tokat Province in Turkey in 2002, and more than 9700 human cases have been reported from 2002 to 2016 [19].

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Detection of CCHFV-specific antibodies in the animal population especially cattle can successfully be used as indicator for the presence or absence of CCHFV in an area [22, 31, 35]. Because *Hyalomma* tick species, which are both vectors and reservoirs of CCHFV are generally found in cattle, but can also cause infestation in sheep and goats [15]. Information about CCHFV infection in animals in Turkey is very limited. Therefore, the aim of this study was to investigate the CCHFV infection status in cattle in Central Anatolia Region and central-west part of the Aegean region of Turkey.

Materials and Methods

Study Area

This study was conducted during the months of March 2016 and September 2017 in the Afyonkarahisar Province in the central-west part of the Aegean region and in the Konya and Aksaray Provinces in the Central Anatolia region of Turkey (Fig. 1). These regions have continental climate characterised by hot and dry summer. The elevation of the Afyonkarahisar, Konya and Aksaray Provinces are 1034 m, 1031 m and 980 m, respectively. The annual rainfall in the studied provinces varies between 322 mm (Konya Province) and 439 mm (Afyonkarahisar Province) (Turkish State Meteorological Service). The number of herds in the Afyonkarahisar, Konya and Aksaray Provinces was 38993, 55565 and 17225, respectively (Turkish Statistical Institute, 2016).

Sample collection

EDTA whole blood (n =329) and sera (n = 329) samples from cattle were collected from 94 epidemiologically independent herds in the Afyonkarahisar, Konya and Aksaray Provinces (Table 1). On average, three to four cattle per herds were sampled randomly. All samples were collected from cattle older than 12 months, which were grazing frequently on common pastures. No clinical signs of disease were observed in the sampled animals at the sampling time. Buffy coat cells were obtained from EDTA whole blood samples by centrifugation at 2200 rpm for 10 minutes, and used for RNA extraction.

Figure 1. Location of investigated regions in Turkey.

Serological analysis

The CCHFV-specific IgG antibodies in the sera samples were detected using an adapted ELISA method with a commercial kit (Vectorbest, Novosibirsk, Russia) with modifications including the use of goat anti-bovine IgG-HRP conjugate (Southern Biotech, Birmingham, USA) [23, 28]. The reported sensitivity and specificity of the adapted ELISA method were 98% and 99%, respectively [23].

Sera samples were diluted 1:100 in dilution buffer (by manufacturer), and were incubated for 1 h at 37°C. After the wash step, goat anti-bovine IgG-HRP conjugate (Southern Biotech, Birmingham, USA) diluted 1:6.000 in conjugate dilution buffer (by manufacturer) was added to each well. Tetramethylbenzidine (Sigma-aldrich, USA) solution was added to each well after incubation period of 30 min at 37°C. The reaction was stopped with

H2SO4, and optical density of each well was read at 450 nm (reference wavelength 620 nm) filter using a spectrophotometer (Bio-Tek Instruments Inc., Winooski, Vt.) All samples were run in duplicate.

RNA extraction and real-time RT-PCR

RNA extraction was carried out from the buffy coat cells from EDTA whole blood samples using QIAamp Cador Pathogen Mini Kit (Qiagen, Hilden, Germany). Real-time RT-PCR was performed with primers and probe designed by Wölfel et al. [36] that amplified nucleotides 181-bp region near the 5′-end of the S segment of CCHFV. One step real-time RT-PCR was performed with the QuantiFast Probe RT-PCR plus Kit (Qiagen, Hilden, Germany). Amplification was carried out in a Rotor-Gene Q (Qiagen, Hilden, Germany) with the following conditions: reverse transcription step of 20 min at 50 °C and 5 min at 95 °C, followed by 45 cycles at 94 °C for 15 sec, 59 °C for 30 sec. A set of synthetic oligonucleotides described by Atkinson et al. [3] was used as the positive control, whereas nuclease-free water was used as the negative control for the analyses. The optimization of the assay was carried out by using both positive and negative controls.

Statistical analysis

The confidence interval was calculated using GraphPad InStat version 3.10 (GraphPad Software, San Diego, CA, USA).

Results

Seroepidemiological study

The result of this study showed that 4 out of 329 sera samples were positive for CCHFV specific antibodies (1.2% prevalence; 95% CI: 0.4%-3.2%) (Table 1). Ages of the seropositive cattle were higher than 4 years old. On the province basis, the highest antibody prevalence (3.4%) was found in the Afyonkarahisar Province whereas no CCHFV-specific antibodies were found in the cattle in the Konya and Aksaray Provinces (Table 1).

Table 1. The seroprevalence of CCHFV infection in the study area

Province	No. examined herds	No. positive herds	CCHFV seroprevalence (%)
Afyonkarahisar	35	2	3.4% (4/118)
Aksaray	25	-	- (0/89)
Konya	34	-	- (0/122)
Total	**94**	**2**	**1.2% (4/329)**

Detection of CCHFV RNA by real-time RT-PCR

CCHFV RNA was not detected in the buffy coat cells from EDTA whole blood samples.

Discussion

Crimean-Congo hemorrhagic fever has been reported in many regions of Asia, the Middle East, Sub-Saharan Africa and South-eastern Europe [5, 9, 13]. The endemic presence of CCHFV in Turkey has already been reported [17, 19, 32].

The first CCHF cases were reported in Turkey in 2002, in the region of Tokat Province, which is located in northern Turkey. After the first cases, CCHF occurred in the regions of the Black Sea and northern parts of the Central Anatolia [19]. Furthermore, CCHFV infection has been reported in non-endemic areas of the Turkey [27, 33]. Afyonkarahisar, Konya and Aksaray Provinces are not located in endemic areas of the Turkey, but the presence of CCHFV infection in the Afyonkarahisar Province was confirmed by existence of clinical human cases in 2008 [34].

In Turkey, most of the studies were conducted to determine the presence of CCHFV in ticks [1, 12, 26, 32, 38]. There are no sufficient data concerning the animal infection with the CCHFV in Turkey. Detection of CCHFV specific immunoglobulins in domestic animals is important indicators for the circulation of CCHFV in a region and risk for human infection [29]. Furthermore, it has been reported that following infection CCHFV specific IgM titers decline to undetectable levels in about sixteen weeks, while IgG titers remain detectable for at

least 5 years [21]. This study therefore investigated the prevalence of CCHFV-specific IgG antibodies in cattle. CCHFV infection status in cattle in Central Anatolia Region and central-west part of the Aegean region of Turkey has not been investigated to the best of found knowledge.

CCHFV specific antibodies were found in cattle in two herds in the Afyonkarahisar Province. No animals were tested positive for CCHFV-specific antibodies in the Konya and Aksaray Provinces. This result can be explained by the number of sampled cattle and the low seroprevalence of CCHF in these provinces. Furthermore, human CCHF cases were reported in the Afyonkarahisar Province whereas no human CCHF cases were reported in the Konya and Aksaray Provinces [34]. Previous studies have been reported a strong association between the appearance of human CCHF cases and seropositivity in the livestock [30].

In this study ages of the seropositive cattle were higher than 4 years old. This finding is consistent with the previous studies reported that probability of exposure of cattle to infected tick in the pasture increases with age [4, 10]. In the present study, seropositive cattle were residing in two herds; it might be an isolated local CCHFV circulation in that area.

Reported seroprevalence of CCHFV in cattle were 1% in the Giza governorate in central Egypt [14], 4.74% in 10 regions of Albania (Has, Kavaje, Kukes, Berat, Kolonje, Pogradec, Rreshen, Korce (Bulgarec/Qatrom) and Gjirokastra) [21], 6.7% in the Maharashtra region of India [24], 6.8% in the Southern Khorasan region of Iran [20], 13% in the Marmara region of Turkey [33], 17.3% in the Vardar region of Republic of Macedonia [23], 31% in the Malishevë municipality in Kosovo [8] and 71% in the Aytos municipality in Bulgaria [4]. The result of this study showed that overall 1.2% (4/329) of the cattle was positive for lgG antibodies to CCHFV. These different serological results can be explained by the competent vector distribution, host preference of tick vectors, climate and environmental changes, detection method, sample size and management conditions [16].

CCHFV infected animals do not show clinical signs but they have a viremic phase lasting up to 7-15 days [30, 35]. There have been few studies on the status of CCHFV infection in animals in Turkey [2, 18, 33]. Albayrak et al. [2] found CCHFV RNA in the blood of small ruminants in northern Turkey. A previous study has detected viral antigens in the blood of cattle in the Marmara region, a non-endemic region, of Turkey [33]. However, in this study CCHFV RNA was not detected in EDTA whole blood samples. This result suggests that sampled animals were not viremic at the time of sampling. It has also been suggested that there is no active circulation of the virus in the investigated regions. Members of the genus *Hyalomma* spp. ticks are the principal vectors of CCHFV [6]. Environmental factors such as temperature, humidity, precipitation and altitude have a significant effect on tick activities and can thus alter the incidences CCHFV infection [7]. Most of the CCHF cases have been reported in the northern part of the country and middle Black Sea region indicating that CCHF is endemic in that region of Turkey [17, 32]. Afyonkarahisar, Konya and Aksaray Provinces are not located in endemic areas of the Turkey. Thus, it can be speculated that environmental factors in the sampled area are not suitable for the spread of the CCHFV infection.

In conclusion, the results of this study indicate that seroprevalence of CCHFV was low in cattle in the investigated regions. Prevalence of CCHFV infection may change depending on the location of the prevalence study, and geographical and environmental factors that affect the abundance of tick population. Therefore, further epidemiological studies are needed to determine distribution of CCHFV in domestic animals in the whole country.

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