RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 10: 1358-1363 (October 2023)

Crimean-Congo Hemorrhagic Fever Virus Infection in Domestic Ruminants in Van Province, a Non-endemic Region in Turkey

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10.18805/IJAR.BF-1601

ABSTRACT

Background: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne viral disease of humans that is most widespread in Africa, Asia, the Middle East and Southeast Europe. The disease has continued to be a health problem in Turkey and other endemic countries in recent years. CCHF virus does not cause clinical disease in animal hosts but develops an antibody response and a transient viraemia. The aim of this study is to evaluate the status of CCHF infection serologically and virologically in livestock in the Van region, a non-endemic area in Turkey.

Methods: A total of 491 whole blood and 491 serum specimens were collected from cattle, sheep and goats in different locations of Van province in Turkey from May 2020 to October 2021. To determine the CCHFV-specific antibody, all sera from animals were tested with a double-Ag ELISA-Ab kit. An RT-PCR assay was performed on total leucocyte samples for the virological study to detect the presence of European lineage-1 CCHFV nucleic acids and viraemic animals.

Result: The prevalence of CCHFV-specific antibodies in animals was found to be 36.4%. The seroprevalence values detected were 11.6%, 64.45% and 81.63% in cattle, sheep and goats, respectively. In the RT-PCR assay, 1.22% (6/491) of the tested animals were found to be viraemic at the time of sampling.

Key words: Crimean-congo hemorrhagic fever, ELISA, RT-PCR, Ruminant.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) infection is a tick-borne viral disease that causes mild fever and severe hemorrhagic fever with non-specific symptoms and sometimes progresses to fatal bleeding in humans but is asymptomatic in infected animals. The mortality rate in cases reported between countries varies between 5 and 50% in humans (Whitehouse, 2004; Tuncer *et al.,* 2014).

The etiological agent known as *Crimean-Congo hemorrhagic fever virus* (CCHFV) has an enveloped, negative-sense single-stranded RNA genome in the *Orthonairovirus* genus, which belongs to the *Nairovirdae* family. The viral genome consists of three segments; small (S), medium (M) and large (L), which encode the viral nucleocapsid (N), envelope glycoproteins (G_N and G_C) and RNA-dependent RNA polymerase (RdRp), respectively (Carter *et al.,* 2012; Garrison *et al.,* 2020).

CCHFV is widely distributed in some countries in Africa, Asia, the Middle East, eastern Europe and more recently in south-western Europe (Yılmaz *et al.,* 2009; Chinikar *et al.,* 2010; Mahzounieh *et al.,* 2012; Tuncer *et al.,* 2014; Ozan and Ozkul, 2020). Based on the prevalence of infectious diseases and the one health concept, when the disease is evaluated on the basis of countries; among the five categories, Turkey is in the first category with Iran, Iraq and Pakistan. In countries in this category, human cases are reported annually through surveillance systems and the infection is endemic (Spengler *et al.,* 2018).

The CCHF infection in humans was first detected clinically in Turkey in 2002 and a definitive diagnosis was made in 2003. More than 10,000 confirmed cases have been reported in Turkey since 2002, with a relatively low mortality rate (4.6%) compared to other countries. In recent years, the number of suspected CCHF-infected cases in humans and the incidence rate have decreased. About 2/3 of the cases were seen among rural farmers and housewives (Vatansever *et al.,* 2007; Ergonul *et al.,* 2009).

The main vectors and reservoirs of the CCHFV are ticks. The life cycle of the virus in nature is maintained between ticks-vertebrates-ticks because it is possible for the virus to be transmitted between ticks transovarially, transstadially

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How to cite this article: Babaoglu, A.R., Oguz Ertas, F., Orunç Kilinç, Ö., Çetin, N. and Aslan, L. (2023). Crimean-Congo Hemorrhagic Fever Virus Infection in Domestic Ruminants in Van Province, a Non-endemic Region in Turkey. Indian Journal of Animal Research. 57(10): 1358-1363. doi: 10.18805/IJAR.BF-1601.

Submitted: 22-10-2022 **Accepted:** 09-01-2023 **Online:** 09-02-2023

and vertically. Livestock serve as a transient reservoir for CCHFV, but do not show clinical signs (Kasi *et al*., 2020). Ticks of the genus *Hyalomma marginatum* are accepted as the primary vectors of the CCHFV for numerous wild and domestic vertebrates, including cattle, sheep and goats. The prevalence of human cases has proven to be closely related to vector and reservoir distribution. Asymptomatic CCHF infection is common in many vertebrate species, both wild and domestic. As it is currently known, domestic and wild animals serve as indicator animals for the circulation of CCHFV in the environment (Nalca and Whitehouse, 2007; Ergonul, 2012; Schuster *et al.,* 2016; Spengler *et al.,* 2016).

For the control and prevention of infectious diseases, it is very important among the effective factors to reveal epidemiologically dynamic information about the reservoir and circulation of the agent. There is limited data about the origin and current status of CCHFV in animals in Turkey. The present study was designed to reveal the epidemiological dynamic information on CCHF infection in three ruminant species in the Van region, a non-endemic region in Turkey.

MATERIALS AND METHODS

Sampling

The study was carried out in the province of Van, located in the Eastern Anatolia region of Turkey (Fig 1). A total of 491 whole blood and 491 blood serum were collected from three ruminant species, including 276 cattle, 166 sheep and 49 goats (Table 1). Sampling was made from ruminants over six months old that were bred by the public in different districts of Van province, especially in the districts near the Iranian border, between May 2020 and October 2021. Serological and virological studies of the samples were carried out in the laboratory of the Department of Virology at Van Yuzuncu Yil University, Faculty of Veterinary Medicine, in Turkey.

Serological study

To determine the CCHFV-specific antibody, all 491 serum samples of animals taken into tubes were centrifuged at 2000 rpm and the sera transferred to the stock tubes were inactivated at 56°C for 30 minutes in order to inactivate indigenous complement and kept at -80°C until testing. Sera samples were tested with a commercial double-Ag ELISA-Ab kit (IDVet, France) reported and validated in the previous study using the same commercial test kit (Sas *et al.,* 2018) to detect specific antibodies against the CCHFV nucleoprotein. The ELISA assay was performed according to the manufacturer's instructions.

Virological study

Viral RNA isolation was performed on a total of 491 whole blood samples taken into tubes containing EDTA using a universal blood RNA extraction kit for purification of genomic RNA (EURx, Poland, Cat. no. E3598) as indicated in the manufacturer's recommendation. To detect the presence of European lineage 1 CCHFV nucleic acids and viraemic animals, a one-step RT-PCR kit (Grisp, Portugal, Ref. GK64.0100) and S-segment partial sequence encoding primers were used in the conventional reverse transcription polymerase chain reaction (RT-PCR) assay as described previously (Burt *et al.,* 1998). Briefly, sense and antisense primers (5′-TGGACACCTTCACAAACTC-3′ and 5′-GACAAATTCCCTGCACCA-3′) targeting the S segment of the viral genome to amplify a 536-bp-length PCR product were used in a RT-PCR assay.

RESULTS AND DISCUSSION

CCHFV antibodies detection

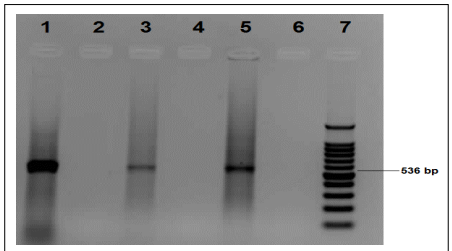
All 491 serum samples, comprising 276 cattle, 166 sheep and 49 goats, were examined. Specific antibodies against CCHFV were detected in all three of the ruminant species tested. As a result of double-Ag ELISA, the seropositivity rate was determined in cattle, sheep and goats as 11.6% (32/276), 64.45% (107/166) and 81.63% (40/49), respectively. Considering all the animals included in the study, the seropositivity rate was found to be 36.45% (179/491) (Table 1).

CCHFV nucleic acid detection

All 491 whole blood samples were taken for a RT-PCR assay for CCHFV nucleic acid detection. As a result of the testing with specific primers based on the S gene, 1.22% (6/491) positivity was detected in the tested animals (Table 1). Agarose gel photographs of the obtained amplicons are shown in Fig 2.



Fig 1: Geographical map of Turkey: The red circle shows location of Van province in the Eastern Anatolia region of Turkey, where the three ruminant samples were collected.



Line 1: Positive control; Line 2: Negative control; Line 3 and 5: Positive samples; Line 4 and 6: Negative samples; Line 7: 100 bp DNA ladder.

Fig 2: The amplified fragment (536 bp) of CCHFV in the RT-PCR assay is shown on 1.5% agarose gel.

Annual cases from CCHFV-endemic countries have been reported during the past few decades (Mardani and Namazee, 2017). During the past 8 years, cases have been decreasing in Turkey, but the incidence of CCHF remains the highest worldwide, with 371 confirmed cases reported in 2016 (Cosgun *et al.,* 2017). With the spread of CCHFV to wide geographic regions, changes in climatic conditions and no approved effective and specific treatment or vaccine for diseases, the virus is considered a public health risk for many regions (Mertens *et al.,* 2013). Although the serological data on the presence of the CCHF infection in humans in Turkey has been proven for a long time (Swanpoel, 1994), this disease was first reported clinically in 2002 in the province of Tokat (WHO, 2008). In the following years, the number of cases increased and more than 9,700 human cases were reported between 2002 and 2016 (Leblebicioglu *et al.,* 2016). This disease has been seen as a health problem in Turkey for the last decade. It has been shown that the infection tends to spread in non-endemic regions other than those defined as endemic (Tuncer *et al.,* 2014).

Hyalomma ticks and various animal species (domestic and wild vertebrates) are known as the natural vector and reservoir of the virus; however, the role of wild and domestic animals as asymptomatic reservoirs in the endemic transmission cycle of the virus has been demonstrated. In vertebrate species, the asymptomatic viraemia period can last up to 7-15 days (Hoogstraal, 1979; Ergönül, 2006). It has also been proven that the distribution and prevalence of human cases are closely related to vector and reservoir distribution (Ergonul, 2012). Serological and virological studies reveal that many wild and domestic animals, such as cattle, sheep and goats, are susceptible to the CCHF virus. These animals are thought to serve as a replicative host to the virus, in addition to developing an antibody response and transient viraemia to the CCHFV (Whitehouse, 2004) and isolation of the virus from livestock and small mammals has been reported in previous studies (Hoogstraal, 1979; Whitehouse, 2004; Ergönül, 2006). Until now, in the Van province, there has been one virological tick survey study and virus detection in tick species (Dinçer *et al.,* 2017), but no studies on infection in domestic and/or wild animals. The presence of antibodies against the CCHFV in animals is the best indicator of the presence of the virus in that region and the risk of disease in humans (Tuncer *et al.,* 2014; Mertens *et al.,* 2015; Spengler *et al.,* 2016; Whithouse, 2004).

A serological survey of a total of 491 blood serums in the present study revealed that 36.45% of the ruminant species tested were exposed to CCHFV infection. Goats had the highest seroprevalence rate (81.63%), followed by sheep (64.4%) and cattle (11.6%). Between ruminant species, the difference in seroprevalence values was significant. The statistical differences between the three ruminant species may be related to the susceptibility of the animal species, the distribution of vector ticks and pasture use. A possible reason for the higher prevalence of infection in ovine ruminants is thought to be continuous pasture throughout the season.

Similar studies (Hassanein *et al.,* 1997; Mohamed *et al.,* 2008; Telmadarraiy *et al.,* 2009; Williams *et al.,* 2000; Sas *et al.,* 2017) reported that the prevalence of CCHFV antibodies in small ruminants was consistent with our serological results and confirmed that it was higher than in cattle. To compare the results, data similar to this study from Turkey; Tuncer *et al.* reported the seropositivity rate of CCHFV infection in domestic animals as 33.1% in their study conducted in the Marmara region of Turkey, which is considered non-endemic for CCHF (2014). In the endemic region, the seroprevalence of the virus has been reported as 17% in cattle, 37% in sheep (Kirbas *et al.,* 2010), 79% in cattle (Vatansever *et al.,* 2007), 66% in sheep and 85% in goats (Albayrak *et al.,* 2012). In a recent study, the antibody rate in cattle was reported as 1.2% in three different provinces (Şevik, 2018). The evaluation of these results together shows that CCHF may have different epidemiological patterns in animals between endemic and non-endemic areas.

In different countries, different seroprevalence rates have been reported in domestic animals. In a study conducted in Bulgaria, a high seroprevalence rate (18.4%) was determined for the virus in ruminants in many regions where there were no human cases (Christova *et al.,* 2018). Similar seroprevalence rates exist for Balkan countries: Greece has 25% (Papa *et al.,* 2014), Kosovo has 18.4% (Fajs *et al.,* 2014), the Republic of Macedonia has 14.6% (Mertens *et al.,* 2015) and Albania has the lowest seroprevalence rate of 4.7% (Lugaj and Bërxhol, 2014). The CCHFV seroprevalence rate has been reported to be 57.7% in Niger (Mariner *et al.,* 1995), 39.3% in Iran (Telmadarraiy *et al.,* 2009) and 3.13% in Egypt (Mohamed *et al.,* 2008). The seropositivity rate of 36.45% in the present study is compared with some previous studies (Vatansever *et al.,* 2007; Kirbas *et al.,* 2010; Albayrak *et al.,* 2012; Tuncer *et al.,* 2014; Şevik, 2018) in Turkey, indicating an intermediate prevalence of infection in the population tested. In addition, different seroprevalence rates between provinces in the same country show the effect of microclimate conditions on the epidemiology of the infection.

In the present study, using the RT-PCR technique for CCHFV nucleic acid detection, six out of 491 blood samples were found to be positive. In the 2017 tick study in the mentioned region (Dinçer *et al.,* 2017), the presence of the virus was shown in 52.2% of the tick pools tested. According to the findings of this study, the identification of 1.5% (6/491) of the tested animals as viraemic indicates active virus circulation in the Van province, which is considered a

Table 1: Distribution of the ruminant samples by ruminant species, CCHFV-Ab detection and viral nucleic acid detection in tested animals.

Ruminant species	Blood samples	CCFHV-Ab		RT-PCR
		Positive	Seroprevalans (%)	Positive
Cattle	276	32	11.6	-
Sheep	166	107	64.45	5
Goat	49	40	81.63	1
Total	491	179	36.45	6

non-endemic area. In Turkey, there are many studies on the transmission of infection from endemic to non-endemic regions in humans (Boluk *et al.,* 2009; Ertugrul *et al.,* 2009; Hekimoglu *et al.,* 2012). In a study conducted in Erzurum, a neighboring province of Van, in 2014, the seroprevalence of infection in humans was found to be 1.3% (Yagci-Caglayik *et al.,* 2014). In addition, in a study conducted in the Van province, the serological presence of CCHFV in humans was reported at 14.4% (Bayram *et al.,* 2017). Despite this active virus circulation, the low incidence of human cases in the region may be due to unidentified rural cases without fatalities or a lack of sufficient data or studies.

CONCLUSION

As a result, this study evaluating the prevalence of CCHFV in three ruminant species in the Van region shows that infection is widespread in cattle, sheep and goats, even in non-endemic areas. High seroprevalence rates and viraemic animals found in farm animals could lead to future endemics in rural areas, as well as an increased risk of infection for humans in close contact with infected animals. It is emphasized to conduct a risk assessment for public health and conduct similar studies in non-endemic regions and with wild animals that may contribute to the creation of epidemiological dynamic data and the determination of effective control measures in the region.

ACKNOWLEDGEMENT

The authors thank Prof. Dr. Aykut Ozkul, who kindly shared the CCHFV RNA positive control for this study.

Funding

The current study was financially supported by the scientific research projects coordination unit of Van Yuzuncu Yil University with project ID: TYD-2020-8900.

Ethical statement

The study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval date: 29/08/2019; Decision no: 2019/08) and Ministry of Agriculture and Forestry of the Republic of Turkey (Approval date and no: 07/05/2019-1401211).

Conflict of interest

The authors declared that there is no conflict of interest.

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