

DOI 10.24425/pjvs.2020.135809

*Short communication*

# **Molecular detection of crimean-congo hemorrhagic fever virus (CCHFV) in tick samples but not in blood and milk samples of domestic ruminant species (cattle, sheep and goat) in northern Turkey**

**T. Özüpak<sup>1</sup>, H. Albayrak<sup>2</sup>**

<sup>1</sup>Department of Fish Diseases Laboratory, Veterinary Control Institute, 55200, Yeşildere Neighborhood, Atatürk Boulevard Alaçam Street, 55200 Atakum /Samsun, Turkey

<sup>2</sup>Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, 55139, Samsun, Turkey

## **Abstract**

Crimean Congo Hemorrhagic Fever (CCHF) is an important disease. The objective of this study was to investigate the presence / prevalence of CCHFV in tick and milk and blood samples of domestic ruminant (cattle, sheep and goat) in Resadiye town of Tokat province, where the disease is endemic. Although no virus RNA was found from whole blood and milk samples, it was detected in 10 of 78 (12.8%) tick pools. Viral loads ranged from 4.8x10<sup>4</sup> copies/ml to 2.66x10<sup>9</sup> copies/ml. Out of 171 serum samples examined, 113 (66.1%) were found to be positive for CCHFV. In conclusion, it was revealed that the prevalence of CCHFV was more common in small ruminants than in cattle. It is an important result in terms of public health that virus cannot be detected. The detection of virus RNA in tick samples shows that CCHFV is still endemic in domestic animals.

**Key words:** Crimean Congo Hemorrhagic Fever Virus, domestic ruminant, milk, tick

## Introduction

Crimean Congo Hemorrhagic Fever is a zoonotic disease caused by one of the viruses causing hemorrhagic fever syndromes (Ergönül 2006). CCHFV is the *Orthonairovirus* strain of *Nairoviridae* (Garrison et al. 2020). The virus is transmitted to humans either by bites of Ixodid ticks or by contact with infected blood or tissues from patients or viremic animals. Ixodid ticks, especially the species *Hyalomma marginatum*, are the main vector and also natural reservoir of CCHFV. These ticks feed on various domestic (e.g., cattle, sheep, goats) and wild animals which play an essential role in the amplification and spread of the virus (Bente et al. 2013). Many studies on the vector tick species, seroepidemiology and CCHF virus detection, have been conducted in the endemic regions of the world (Albayrak et al. 2012).

## Materials and Methods

### Tick processing, whole blood, milk and serum samples

A total of 335 ticks were collected between March and July of 2015 from 42 sheep (42 pools, 221 ticks in total) and 36 goat (36 pools, 114 ticks in total) grazing in Resadiye town of northern Turkey. No ticks were collected from cattle due to the intensive use of antiparasitic drugs. The ticks were collected directly from the animals. After identification using standard keys, ticks were stored at  $-80^{\circ}\text{C}$  until testing for the presence of CCHF viral RNA. A total of 171 whole blood, milk and serum samples were collected from the same animals and cattle.

### RNA extraction, real time RT-PCR assays and enzyme-linked immunosorbent assay (ELISA)

Viral RNA was extracted from 350  $\mu\text{l}$  of tick pool supernatant, whole blood and milk samples by using the MagNA Pure LC RNA Isolation Kit III (Roche, Mannheim, Germany) and they were stored at  $-80^{\circ}\text{C}$ . All samples were tested by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) using a set of primers and probe. The primers and probe were described previously (Ozan and Ozkul 2020). All serum samples were tested for the presence of specific IgG antibodies against CCHFV by commercial ELISA kit (Vectorbest, Novosibirsk, Russia) with minor modification.

## Results

### Tick species and distribution

A total of 335 adult ticks were collected from 42 sheep (42 pools, 221 ticks in total) and 36 goat (36 pools, 114 ticks in total) in Resadiye town of Turkey. Four tick species were identified and the most abundant were *Hyalomma marginatum* 36.4% (122/335), *Hyalomma detritum* 29.0% (97/335), *Rhipicephalus bursa* 22.1% (74/335). *Dermacentor marginatus* represented 12.5% (42/335) of the total number of ticks. *H. marginatum* was found in all flocks and herds in the region.

### CCHFV nucleic acid and antibody detection

In 113 (66,1%) of a total of 171 blood serums collected from 7 villages in Resadiye town of Tokat province, virus antibodies were found in 25 (38.5%) of 65 cattle blood serums, 35 (83.3%) of 42 sheep blood serums, and 53 (82.8%) of 64 goat blood serums. According to rRT PCR results, virus RNA was not found in any of the 171 plasma and milk samples collected from cattle, sheep and goats. Virus RNA was found in 10 (12.8%) of the tick homogenisates obtained from ticks collected from 78 sheep and goats.

## Discussion

The presence of CCHF is known in many countries in Europe, Asia and Africa. Since 2002, the presence of CCHFV infections has been documented in endemic regions in Turkey (Albayrak et al. 2012). Although it has been reported that the virus is not transmitted vertically in sheep, intensive passive antibody transfer has been reported with colostrum (Gonzalez et al. 1998). On the contrary, in a study conducted by using antigen ELISA test method, Akdeniz (2010) found antigen positivity in sheep milk with a rate of 7.3% and in cow milk with a rate of 0.96%. In both humans and animals, CCHFV has been found from blood with many methods in viremia phase (Albayrak et al. 2012). In this study, it was not possible to detect virus RNA in both blood and milk. The fact that the virus was not found in both sample pairs brings to mind that the reason was about sampling time. The fact that although viremia lasts short in animals, the virus amount in blood increases up to 4.7log LD50/ml shows that there is still a high risk in contamination (Gonzalez et al. 1998). In the present study, seropositivity rates were found as 38.5% in cattle, as 83.3% in sheep and as 82.8% in goats. In a great majority of the studies conducted in Turkey and the world, seropositivity rate in small ruminants was found to be higher when compared with cattle.

Similar results were found in the present study. When these results are brought together, it is thought that it will be more reasonable to talk about the time of being exposed to the virus rather than a kind of sensitivity to CCHFV. Similarly, the fact that high amounts of viruses are found in ticks poses a great risk for animal keepers, animal breeders, rural residents, slaughterhouse workers and animal health workers. For this reason, care should be taken for the protection of animals from external parasites by emphasizing tick control in animals, especially in small ruminants, which have been neglected until now, and routine controls should be implemented within a specific program.

### Acknowledgements

This study was supported by Ondokuz Mayıs University Scientific Research Projects Commission with the project number PYO.VET.1904.15.009.

### References

- Akdeniz G (2010) Investigation of Crimean-Congo hemorrhagic fever virus antigen in raw cow and sheep milk. Cumhuriyet Üni. Medical School, Sivas, Master Thesis: 47-50.
- Albayrak H, Ozan E, Kurt M (2012) Serosurvey and molecular detection of Crimean-Congo hemorrhagic fever virus (CCHFV) in northern Turkey. Trop Anim Health Prod 44: 1667-1671.
- Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M (2013) Crimean-Congo hemorrhagic fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antivir Res 100: 159-189.
- Ergönül Ö (2006) Crimean-Congo haemorrhagic fever. Lancet Infect Dis 6: 203-214.
- Garrison et al (2020) ICTV Virus Taxonomy Profile: Nairoviridae, Journal of General Virology 101: 798-799
- Gonzalez JP, Camicas JL, Cornet CP, Wilson ML (1998) Biological and clinical responses of West African sheep to Crimean-Congo haemorrhagic fever virus experimental infection. Res Virol 149: 445-455.
- Ozan E, Ozkul A (2020) Investigation of Crimean-Congo hemorrhagic fever virus in ruminant species slaughtered in several endemic provinces in Turkey. Archives of Virology 165:1759-1767.