

## Serological data suggest the spread of Crimean-Congo hemorrhagic fever virus in domestic animals in Kosovo - a short communication

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### ABSTRACT

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus that causes silent infections of livestock animals and severe illness in humans. Circulation of CCHFV among animals within a geographic region can be detected by the presence of antibodies to the virus. In Kosovo, human cases have occurred most frequently in the Malisheve region, but have not been seen in Vushtrri. The study was carried out in 2018 in Malisheve and Vushtrri to determine the prevalence of antibodies to CCHFV in domestic animals in Malisheve and Vushtrri, and if there is evidence that the virus is becoming more widely distributed within Kosovo. Blood samples were collected from 385 animals (285 cattle, 87 sheep and 13 goats), from 33 locations and 58 farms in total. Sera were tested using enzyme-linked immunosorbent assay (ELISA) for anti-CCHFV antibodies (IgM/IgG). The seroprevalence of antibodies in Malisheve was 24.7% (59 out of 239) (CI 19.65-30.52), while in Vushtrri the seroprevalence of antibodies was 4.8% (7 out of 146) (CI 2.34-9.57). This is the first record of seropositive livestock in the Vushtrri region, suggesting that the virus is spreading in Kosovo through the movement of infected animals. Public health and veterinary state institutions should therefore start an integrated surveillance program with a concept of "One Health", to control and prevent the spread of the virus.

**Key words:** Crimean-Congo hemorrhagic fever; zoonosis; tick-borne virus; One Health concept

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### Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a viral tick-borne disease with serious consequences in humans. The virus belongs to the order *Bunyavirales*, family *Nairoviridae*, genus *Orthonairovirus* (INTERNATIONAL COMMITTEE ON TAXONOMY OF VIRUSES, 2016). Human cases have been reported in Southeast Europe, such as in Albania, Bulgaria, Kosovo, and in the last two decades in Turkey (EPISOUTH, 2008). In Kosovo, over 305 people

have been diagnosed with CCHF since 1954, with a lethality rate of 21%. Most infected persons were associated with animal farming (NATIONAL INSTITUTE OF PUBLIC HEALTH, 2016). CCHF poses a risk for farmers and other agricultural workers, veterinarians, laboratory staff, and medical and military personnel (PAPA et al., 2010).

CCHFV circulates in an enzootic cycle between ticks and different animals in a geographical zone,

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and the risk of human infection correlates with the presence of infected tick species and the number of people bitten by infected ticks (HOOGSTRAAL, 1979; ESTRADA-PENA et al., 2012). Serological testing for anti-CCHFV antibodies in animals provides reliable epidemiological data, which can help to detect virus circulation in a new geographical zone. Determination of seroprevalence in animals can be followed by monitoring for the presence/absence of competent tick species, detection of viral RNA, and identification of the viral strain. CCHFV has been found in at least 31 tick species, including several genera of the *Ixodidae* family (ERGONUL et al., 2004). In Kosovo and Albania, viral RNA has been detected in two tick species: *Hyalomma marginatum*, the vector of highly pathogenic strains (Clades V/Europe 1) and *Rhipicephalus bursa*, which transmits less pathogenic or non-pathogenic strains (Clades VI/Europe 2) (SHERIFI et al., 2014; SHERIFI et al., 2018; PAPA et al., 2017).

Infected animals develop a transient viremia without signs of disease. The identification of mammalian hosts has therefore been based largely on the detection of virus-specific antibodies in serum collected from livestock (BENTE et al., 2013). In humans, detection of anti-CCHFV IgM antibodies is possible from approximately one week after the onset of disease until four months past infection. During the course of CCHF infection, IgM antibodies are rapidly followed by IgG responses. Anti-CCHFV IgG antibodies remain detectable for at least five years (EMMERICH et al., 2021), while in cattle CCHFV IgG antibodies persist for a long time, which supports seropositivity being significantly associated with age (ZOUAGHI et al., 2021). Some previous seroprevalence studies in Kosovo have shown different rates of infection. In 2013, 43% of tested cattle in Malisheve municipality were seropositive, while a survey in 2008 found a seropositivity of cattle of 4% and sheep of 3% (SHERIFI et al., 2015; TARAKU et al., 2018). This difference in the rate of infections in animals, with higher numbers in 2013 than in 2008, may be related to the increase in the number of infected ticks in 2012 (SHERIFI et al., 2014).

The aim of this study was to survey the seroprevalence of CCHF in animals in two regions

of Kosovo, respectively Malisheve municipality, which is known as an endemic area, and Vushtrri municipality, which is a non-endemic area of CCHF in humans.

### Materials and methods

This study was conducted in 2018 in two municipalities of Kosovo, Malisheve, which is known as an endemic area, and Vushtrri a non-endemic area of CCHF. The definition of the Malisheve municipality as an endemic area of CCHF is related to the occurrence of cases in humans, while the municipality of Vushtrri is a non-endemic area because so far, no cases of CCHF in humans have occurred. In Malisheve municipality blood samples were collected from animals from 19 locations: Malisheve, Senik, Banje, Dragobil, Drenoc, Jancisht, Astrazup, Pillacic, Panorc, Bubl, Mlecan, Llapceve, Kijeve, Gajrak, Marali, Bubavec, Rud, Shkoze and Lladroc. In Vushtrri municipality blood samples were collected from 16 locations: Vushtrri, Novolan, Maxhunaj, Duboc, Druar, Grace, Bivolak, Dumnice e Eperme, Dumnice e Mesme, Dumnice e Poshtme, Galice, Mihaliq, Becuk, Kolle, Stanoc and Shalc.

The blood samples were collected in accordance with the basic ethical principles, for the purpose of diagnosis. Blood samples were obtained from 285 cattle, 87 sheep, and 13 goats (all included animals were older than one year). In Malisheve, 185 samples were taken from 37 cattle farms, and 54 samples were taken from 6 sheep farms. In Vushtrri, 100 samples were obtained from 21 cattle farms, and 33 samples from sheep and 13 from goats were obtained from 10 sheep and goat farms. 5-10 ml blood samples were obtained from each animal by jugular venipuncture, and sera were separated at the laboratory and kept at -20 °C until analysis. Sample data were recorded regarding location, ownership, ear tag number, age, and breed.

All collected sera were tested using a competitive enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions, ID Screen® CCHF Double Antigen Multi-species ELISA (Innovative Diagnostics IDVet® company, Grabels, France) for detection of CCHFV antibodies (IgM/IgG) in the plasma

or serum from cattle, sheep and goats, or other susceptible organisms. The diagnostic sensitivity of this kit is reported to be 98.9% (CI (95%): 96.8-99.8%) and the diagnostic specificity 100% (CI (95%): 99.8-100%). The OD for each sample was read at a wavelength of 450 nm. The results of the anti-CCHFV antibody detection were calculated as a percentage of the positive or negative control, respectively, as indicated by the manufacturer.

## Results

Of the 239 tested animals in Malisheve, 24.7% (59 out of 239) were positive for anti-CCHFV (IgM/IgG) antibodies (CI 19.65-30.52), while of 146 tested animals in Vushtrri, 4.8% (7 out of 146) were positive (CI 2.34-9.57), as shown in Table 1.

Table 1. Results of samples analyzed for Crimean-Congo hemorrhagic fever virus (CCHFV) in animals in Kosovo, 2018

Municipality	Malisheve			Vushtrri			
Location	No. of animal tested	CCHF positive	%	Location	No. of animal tested	CCHF positive	%
Malisheve	25	0	0	Vushtrri	10	0	0
Astrazup	22	5	22.7	Druar	9	1	11.1
Dragobil	17	3	17.6	Duboc	2	0	0
Drenoc	8	2	25.0	Maxhunaj	1	0	0
Banje	36	6	16.6	Stanoc	25	2	8.0
Bubavec	10	0	0	Novolan	9	0	0
Rud	22	10	45.4	Shalc	9	1	11.1
Marali	2	0	0	Grace	5	0	0
Gajrak	8	6	75.0	Bivolak	13	0	0
Bubl	8	7	87.5	Becuk	4	0	0
Llapceve	7	1	14.8	Kolle	4	0	0
Pllacic	16	2	12.5	Mihaliq	5	1	20.0
Kijeve	2	0	0	Galice	5	0	0
Mlecan	5	2	40.0	Dumnice E	15	0	0
Panorce	10	4	40.0	Dumnice M	20	1	5.0
Shkoze	10	6	60.0	Dumnice P	10	1	10.0
Jancisht	16	3	18.7	-	-	-	-
Lladroc	8	0	0	-	-	-	-
Senik	7	2	28.5	-	-	-	-
Total	239	59	24.7	Total	146	7	4.8

Of the 19 locations in Malisheve municipality, 14 were positive, with the highest infection rate in the village of Bubl 87.5% (7/8) followed by Gajrak 75.0% (6/8), Shkoze 60.0% (6/10), Rud 45.4% (10/22), Panorce 40.0% (4/10), Mlecan 40.0% (2/5), Senik 28.5% (2/7), Drenoc 25.0% (2/8), Astrazup 22.7% (5/22), while in Jancisht 18.7% (3/16), Dragobil 17.6% (3/17), Banje 16.6 (6/36), Llapceve 14.8% (1/7) and Pllacic 12.5% (2/16) a lower seroprevalence of CCHFV antibodies was found. Anti-CCHFV antibodies were not detected

in animals in 5 locations: Malisheve, Bubavec, Kijeve, Lladroc and Marali.

In Vushtrri municipality, antibodies were detected in 6 out of 16 locations. The highest infection rate was found in Mihaliq 20.0% (1/5), Shalc 11.1% (1/9), Druar 11.1% (1/9), Dumnice e Poshtme 10.0% (1/10), Stanoc 8.0% (2/25) and Dumnice e Mesme 5.0% (1/20). Ten locations were negative: Vushtrri, Duboc, Maxhunaj, Novolan, Grace, Bivolak, Becuk, Kolle, Galice and Dumnice e Eperme (Fig. 1).

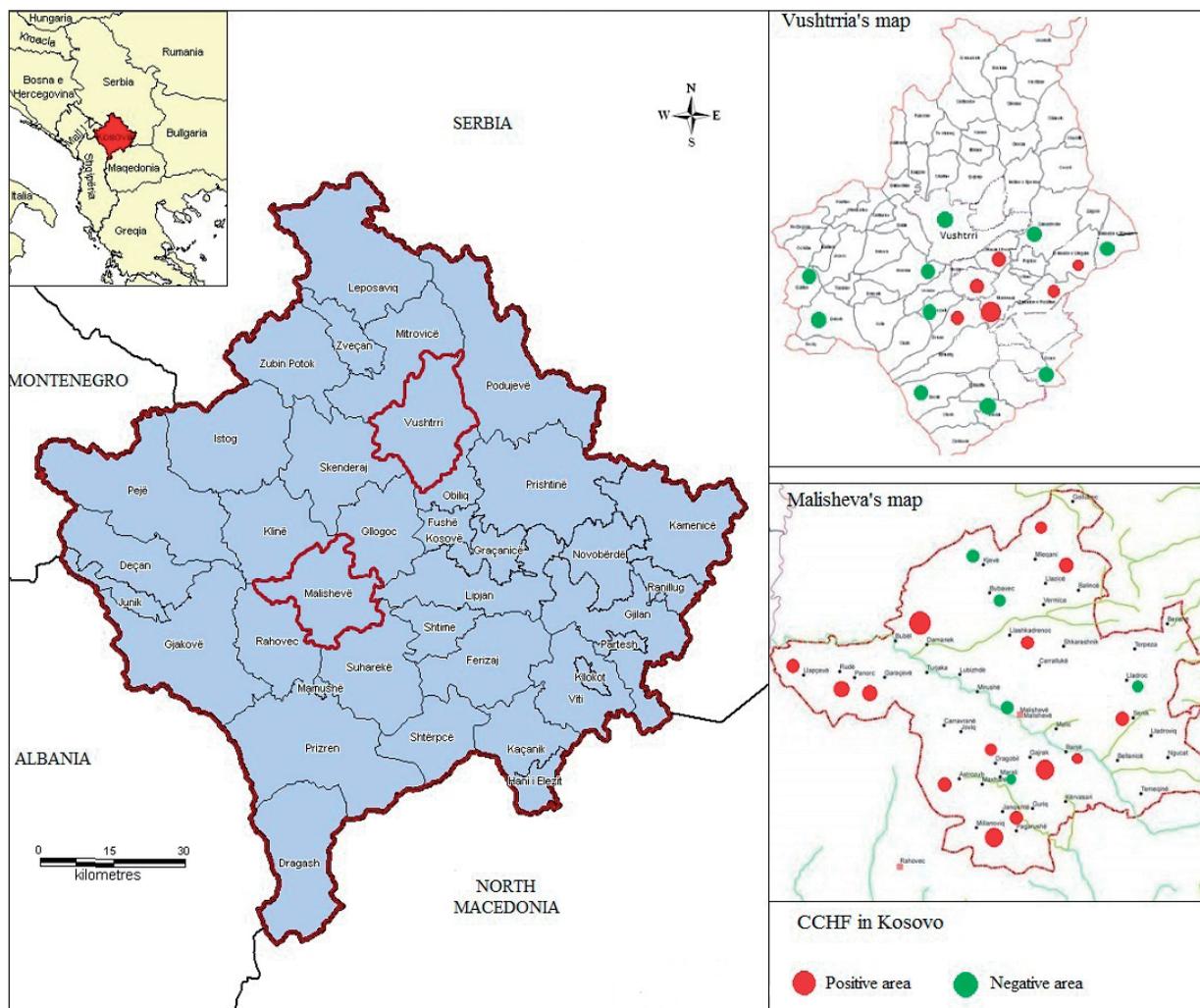


Fig. 1. Crimean-Congo hemorrhagic fever virus (CCHFV) in animals in the municipalities of Malisheve and Vushtrri.

Age is an important factor since 80% of positive animals were 6 to 10 years old, followed by 15% of them 3 to 6 years old, and only 5% of positive animals were 1 to 3 years old. While infections in young animals (1-3 year-old) occurred in the municipality of Malisheve, in the municipality of Vushtrri infections were most common in animals aged between 6 to 10 years.

### Discussion

In this study, the serological screening of animals in Malisheve, known as an endemic region, compared with Vushtrri, as a non-endemic

region was undertaken for the first time. The presence of CCHFV antibodies in cattle, sheep and goats was found with an overall seroprevalence of 24.7% (59/239) in Malisheve, and 4.8% (7/146) in Vushtrri, respectively.

The last outbreak of CCHF in humans in the Malisheve district was recorded in 2013, when 11 people died, where most of them had a history of livestock farming activities (NATIONAL INSTITUTE OF PUBLIC HEALTH, 2016). Disease outbreaks in the Malisheve district and neighboring locations (Kline, Suhareke, and Rahovec) have occurred every 3 to 5 years,

respectively in 2001, 2005, 2010 and 2013, with high mortality rates. The overall prevalence of 24.7% of anti-CCHFV antibodies in domestic animals in Malisheve municipality was therefore not surprising, especially considering the presence and distribution of the tick species *Hyalomma marginatum* in this region. Preliminary studies have shown that 98% of ticks collected in this region are *H. marginatum*, with a prevalence of CCHFV RNA of up to 8%. In some hyperendemic zones or foci, such as Bubl, the prevalence of viral RNA in ticks is as high as 30%, which corresponds to seropositivity of domestic animals as high as 87.5% (SHERIFI et al., 2014).

In contrast to the Malisheve district, the Vushtrri municipality in northern Kosovo showed a low infection rate of animals, with seropositivity of only 4.8%. However, this represents the first detection of CCHFV infection of domestic farm animals in this area, suggesting the possibility that CCHFV is spreading from one region to others in Kosovo, through the trade and movement of infected animals, as well as the possible transport of infected ticks by migratory birds. The regular monitoring of CCHFV infection in domestic animals and ticks should therefore be undertaken. A program for treatment of domestic farm animals with acaricides was implemented in the municipality of Malisheve in 2014, together with education for population awareness of protective measures against tick bites and the prevention of CCHF. The future control and prevention of CCHF in Kosovo should be based on an integrated program of surveillance of animals, ticks and humans, in which veterinary and human public health institutions and entomologists work together under the One Health concept.

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**ZHABARI, Z., B. XHEKAJ: Nalaz serološkog testiranja upućuje na širenje virusa krimsko-kongoanske hemoragijske groznice u domaćih životinja na Kosovu - kratko priopćenje. Vet. arhiv 92, 155-160, 2022.**

#### SAŽETAK

Virus krimsko-kongoanske hemoragijske groznice (CCHFV) se prenosi krpeljima i uzrokuje latentne infekcije u stoke te teže oblike bolesti u ljudi. Cirkulaciju CCHFV-a među životinjama unutar jednoga geografskog područja može biti otkrivena prisutnošću protutijela na virus. Na Kosovu se slučajevi bolesti u ljudi događaju mnogo češće u području grada Mališeva, dok ih u području Vučitrna nije bilo. Budući da ima dokaza o znatnijem širenju virusa na Kosovu, ovo je istraživanje provedeno 2018. s ciljem utvrđivanja prevalencije protutijela na CCHFV u domaćih životinja u području gradova Mališevo i Vučitrna. Uzorci krvi prikupljeni su od 385 životinja (285 goveda, 87 ovaca i 13 koza), s 33 lokacije i 58 farmi. Serumi su pretraženi testom (ELISA) za protutijela anti-CCHFV (IgM/IgG). Seroprevalencija protutijela u Mališevu bila je 24,7 % (59 od 239 životinja) (CI 19,65 – 30,52), dok je u Vučitrnu seroprevalencija protutijela bila 4,8 % (7 od 146) (CI 2,34 – 9,57). Ovo je prvi zabilježeni slučaj seropozitivnosti u stoke na području Vučitrna, što upućuje na to da se virus na Kosovu širi kretanjima zaraženih životinja. Stoga bi javnozdravstvene i veterinarske državne institucije trebale uspostaviti jedinstven sustav nadzora u okviru koncepta *Jedno zdravlje*, kako bi se širenje virusa kontroliralo i spriječilo.

**Ključne riječi:** krimsko-kongoanska hemoragijska groznica; zoonoza; virus prenosiv krpeljima; koncept Jedno zdravlje

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