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Short Communication

A Survey of Crimean-Congo Hemorrhagic Fever in Livestock in Republic of Kosova

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is endemic in Kosova. The aim of our study was to detect the IgG and IgM antibodies to CCHF virus in cattle and sheep in endemic and non-endemic areas of Kosova. In total sera of 172 cattle and 95 sheep were tested by the Indirect Immunofluorescence Assay (IIA) for quantitative determination of antibodies to CCHF virus. Seven of 172 cattle sera (4.07%) and three of 95 sheep sera (3.16%) tested positive for IgG, but IgM were detected in the serum of only one sheep. The results of this study confirmed the fact that CCHF is endemic in four municipalities of Kosova and that there is a risk of the spread of viruses to other non-endemic regions through the movement of infected animals.

Keywords: *Crimean - Congo hemorrhagic fever virus (CCHFV), Cattle, Sheep, IgG, IgM*

Kosova Cumhuriyeti'nde Kırım Kongo Kanamalı Ateşi Üzerine Çiftlik Hayvanlarında Bir Araştırma

Özet

Kırım Kongo Kanamalı Ateşi (KKKA) Kosova'da endemiktir. Bu çalışmanın amacı endemik ve endemik olmayan Kosova bölgelerinde sığır ve koyunlarda KKKA virusuna karşı IgG ve IgM antikorlarını belirlemektir. Toplam 172 sığır ve 95 koyun İndirek İmmunoflorasan Tekniği ile KKKA virusuna karşı antikorların kantitatif tespiti amacıyla kullanılmıştır. 172 sığırın 70'i (%4.07) ve 95 koyunun 3'ü (%3.16) IgG pozitif olarak tespit edilirken IgM sadece 1 koyun serumunda belirlenmiştir. Bu çalışmanın sonuçları Kosova'nın 4 belediyesinde KKKA'nın endemik olduğunu doğrulamış ve enfekte hayvanların taşınması ile endemik olmayan bölgelere yayılım riskinin bulunduğunu göstermiştir.

Anahtar sözcükler: *Kırım Kongo kanamalı ateşi virusu (KKKAV), Sığır, Koyun, IgG, IgM*

INTRODUCTION

The causative agent of CCHF is a member of the Bunyaviridae family, genus Nairovirus. The disease is transmitted to humans through tick bites, by crushing infected ticks or by nosocomial contact with blood of infected animals or humans. Humans are the only known hosts of CCHF virus: the disease is manifested as an acute febrile illness followed by hemorrhagic syndrome with mortality rates of up to 50% [1]. The humans at greatest risk of infection are agricultural workers, veterinarians, abattoir workers, and other persons in close contact with animals and ticks [2]. The circulation of the virus in nature is enzootic, tick - vertebrate - tick, and *Hyalomma* ticks are considered to be the most important transmitters and source of the virus, determining distribution worldwide [2-5]. A wide variety of domestic animals and birds (cattle, sheep, goats and ostriches), as well as small wild mammals (hares and hedgehogs) can become infected with the virus, and these infections are usually asymptomatic and subclinical [6,7]. Livestock and other hosts can transmit CCHFV to humans during the viremic period [6].

CCHF is endemic in some parts of Europe, Asia and Africa. In recent years, cases of human infection have increased, and have been reported from different countries. The disease has emerged or re-emerged in Turkey, Kosova, Bulgaria, Albania and Greece. This has been attributed to mild winters and to the disruption of agricultural activities,

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both accounting for an increased tick population, as well as to the migration or transportation of tick-infested birds or animals [8,9].

Outbreaks of CCHF in Kosova usually occur in spring and summer with a reported human fatality rate up to 25.5% [10]. In 2010, 2265 patients were reported to have tick bites, with 141 suspected cases of CCHF, 29 cases were confirmed using the PCR technique, and among these, there were 8 deaths. Approximately the same number of persons with tick bites and infected patients were reported in 2013 (Report of National Institute of Public Health in Kosova, 2013). CCHF in Kosova is present in 50% of the territory with common characteristics of altitude, climate, low bush, fragmented agricultural land and the presence of the *Hyalomma* tick. Hyper-endemic zones are in the central and south west of Kosova: Malisheve, Kline, Rahovec and Suhareke. *Hyalomma marginatum* dominates in the endemic municipalities, with 90.2% versus 24.3% in the non-endemic regions. 3.6% of ticks tested positive with CCHFV by RT-PCR [11]. In 2012 a sero-prevalence of the healthy human population was 4.0% (range 0-9.3%) and in cattle 18.4%, mostly in endemic areas of Kosova [10].

The aim of this study was to detect the IgG and IgM antibodies to CCHF virus in cattle and sheep and to monitor the movement of sero-positive animals from endemic to non-endemic regions in the Republic of Kosova.

MATERIAL and METHODS

Kosova is located in the Balkans in Southeastern Europe, with continental and Mediterranean climates. It has a land area totaling 10,908 km^2 and a human population of around 2 million (Agency of Statistics of the Republic of Kosova, www.ask.rks-gov.net). Kosova has around 250.000 cattle and 120.000 sheep (Kosova Food and Veterinary Agency).

The study was conducted in CCHF-endemic regions that includes four municipalities in the central and southwest parts of Kosova, namely Malisheve, Suhareke, Prizren and Rahovec *(Fig. 1)*, and in non-endemic regions including four municipalities (Prishtina, Gjilan, Mitrovice and Peja). Sera were collected in the summer of 2008 from 267 domestic animals, 172 from cattle and 95 from sheep. Identification of the specimens included location, ownership and date of collection. Animals registered in the system of the identification unit at Kosova Food and Veterinary Agency were checked in 2014 for their movements around Kosova in the years 2010-2014. Sera were tested using the IIA for quantitative determination of IgG and IgM antibodies to CCHF virus in the Institute of Tropical Medicine "Bernhard Nocht", Hamburg, Germany. IgG and IgM antibodies to CCHF virus were detected using acetone-fixed Vero cells infected with CCHF virus (strain ArD39554, GenBank accession number DQ211641). Cultivation of the virus was carried out in a BSL 4 laboratory. Serum samples were tested in twofold steps starting at a dilution of 1:40. Cell smears were routinely counterstained with anti-CCHF nucleocapsid monoclonal antibody A4 [12] using Rhodamine-anti-mouse as a secondary antibody [2]. A chi-square test was used to determine the presence of antibodies in cattle and sheep and to establish whether there was a significant difference between endemic and non-endemic regions.

RESULTS

The study has shown that 10 from 267 tested animals (3.75%) tested positive for the presence of IgG and IgM antibodies to CCHFV. Seven of 172 (4.07%) cattle and three of 95 (3.16%) sheep sera tested positive. Nine animals were positive for IgG antibody to CCHF virus, the serum of only one sheep tested positive for IgM. The region is a factor that influences the presence of antibodies in cattle and sheep. In endemic regions, 6.67% tested positive, as opposed to 0.76% in non-endemic regions. This difference was statistically significant, $\chi 2$ (1) =6.464, P=0.011 *(Table 1)*.

Nine samples from 135 tested animals or 90% of positive cases were detected in endemic regions (Prizren, Suhareke, Rahovec and Malisheve). We estimated with 95% confidence that the proportion of ruminants in endemic regions carrying the antibodies against CCHF in sera was between 2.40% and 10.93%. One cattle serum of

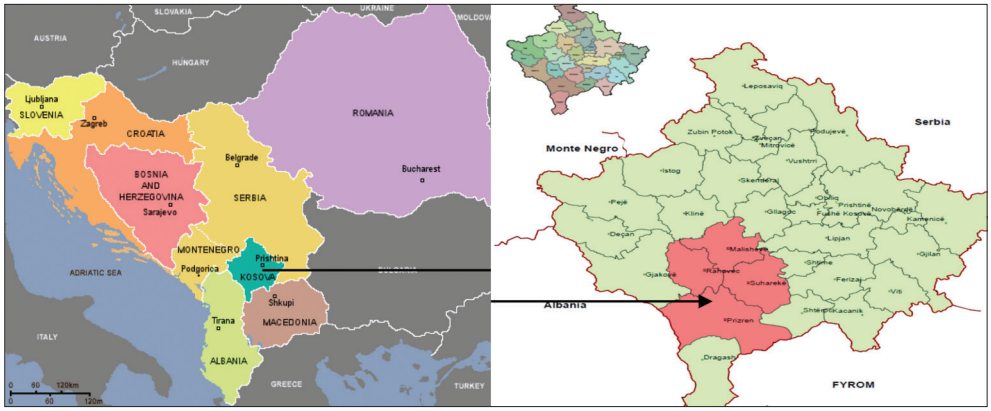

Fig 1. Map of Southeast Europe and Republic of Kosova - endemic regions of CCHF

Şekil 1. Güneydoğu Avrupa ve Kosova Cumhuriyeti Haritası - KKKA endemik bölgeler

Table 1. *Sero-prevalence of antibodies to CCHF virus in cattle and sheep in Kosova*
Tablo 1. *Kosova'da sığırlar ve koyunlarda KKKA virüsüne karşı antikor Sero-prevalansı*

Animals/Zone	Nr. of Samples	IgG Positive	IgM Positive	% Positive 95% CI	Negative Animals	Negative Animals (%)	X^2	P Value
Cattle	172	7	0	4.07 (1.09-7.05)	165	95.93	/	/
Sheep	95	2	1	3.16 (0.42-6.74)	92	96.84	/	/
Total	267	9	1	3.75 (1.45-6.04)	257	96.25	/	/
Endemic	135	8	1	6.67 (2.40-10.93)	126	93.33	6.464	0.011
Non endemic	132	1	0	0.76 (0.74-2.26)	131	99.24	/	/
Total	267	9	1	3.75 (1.45-6.04)	257	96.25	/	/

132 tested animals tested positive from the non-endemic region (Mitrovice). 29 of 267 (10.8%) tested animals were moved from endemic to non-endemic regions, where there was one positive detection of IgG to CCHFV.

DISCUSSION

In Kosova there are no data about humans infected directly by contact with animals. 54% of the humans infected and diagnosed with CCHF have evidence of being bitten by a tick, only 4% of patients were infected via contact from human to human and around 40% of infections are of unknown etiology [13]. The livestock husbandry in Kosovo is such that some households in rural areas keep livestock for family needs, where they often slaughter animals at home and may have direct contact with the blood of infected animals. Even field veterinarians during meat inspection and treatment of animals can be infected through contact with the blood of infected animals. A number of individuals became infected while removing ticks by hand from animals and crushing them. According to the Clinical Center for Infectious Diseases, the largest number of patients with CCHF is to be found in the municipalites of Malisheve with 65%, Kline with 11.5% and Rahovec, Prizren and Suhareke with a total of 15%. This study has confirmed that the vast majority of sera-positive animals are located in the endemic municipalities. This is an indication that the CCHF virus circulates in endemic municipalities, with the highest prevalence of CCHF virus found in *Hyalomma marginatum* ticks in the municipalities of Malisheva (8.6%) and Klina (4%) [11]. The fact that the domestic animal population is kept partially in pasture contributes to increase the tick population. Furthermore the fragmentation of agricultural land and the presence of wild animals are playing an influential role in increasing the tick population as they host the immature ticks (larvae and nymphs) and act as a reservoir for the viruses.

There is a need for further studies in order to identify which wild animals play an important role in hosting immature ticks *Hyalomma marginatum* in endemic regions in Kosova.

It is important to note the fact that in Kosova it is a common practice to trade domestic animals in open animal markets that are present in every municipality. In our study, 11% of animals tested from endemic regions had moved through trading in non-endemic regions and in one case we found one cow IgG seropositive for the CCHF virus. In the study conducted by Sherifi et al.[11], a tick with CCHF virus was found and removed from a cow which had been moved from an endemic municipality to a non-endemic municipality. This practice of trading and moving domestic animals with ticks infected with CCHF virus may present a great risk for the spread of the CCHF virus to other regions, where so far no reports of human infections have been received.

The results of this study are important for the Kosova national authorities that are responsible for controlling and monitoring the CCHF in Kosova. To prevent the spread of the CCHF virus during spring and summer seasons from endemic municipalities to non-endemic municipalities, it is important to treat domestic animals with Acaricides prior to taking them to the animal market for trading.

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