



Research Article

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## Seroepidemiology of CCHF in Domestic Animals in Endemic Areas in Albania

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### Abstract

Crimean-Congo hemorrhagic fever (CCHF) is an arboviral zoonotic infection which is endemic in some areas of the country. The aim of this study was to assess the seroprevalence of CCHFV in previous and recent endemic areas of the country. This cross-sectional serologic study was conducted in period 2010-2014 by the Institute of Public Health in Tirana, Albania. The survey included 152 sera specimen of cattle which were randomly collected from seven districts in Albania over the period 2010-2014. All samples were collected randomly from seven districts in Albania and were examined for anti-CCHFV IgG. Of the total of 152 samples examined, 102 (67.1%) were positive to IgG ELISA. The highest positivity rate was among cows (88.3%) and in recent endemic areas of Has and Kukes, 89.7% and 82.9% respectively. In recent endemic areas humans can potentially contract the disease as compared to other areas of the country.

**Keywords:** Crimean-Congo hemorrhagic fever, domestic animals, seroprevalence

### 1. Introduction

Crimean-Congo haemorrhagic fever (CCHF) is the most widespread, tick-borne viral disease affecting humans. The disease is endemic in many regions, such as Africa, Asia, Eastern Europe, and the Middle East. The known distribution of CCHFV covers the greatest geographic range of any tick-borne virus and there are reports of viral isolation and/or disease from more than 30 countries across four regions: Africa (Democratic Republic of Congo, Uganda, Mauritania, Nigeria, South Africa, Senegal, Sudan), Asia (China, Kazakhstan, Tajikistan, Uzbekistan, Afghanistan, Pakistan,

India), Europe (Russia, Bulgaria, Kosovo, Turkey, Greece, Spain), and the Middle East (Iraq, Iran, Kuwait, Saudi Arabia, Oman, United Arab Emirates (UAE)) (1). The geographic distribution of CCHF coincides with that of ixodid ticks, particularly those of the genus *Hyalomma*. In Europe, *Hyalomma marginatum* is the main CCHFV vector, while *Hyalomma asiaticum* appears to be the principal vector in Asia. In 2006, *H. marginatum* was detected for the first time in the Netherlands and in southern Germany (2,3). Ixodid ticks, especially those of the genus *Hyalomma*, are both a reservoir and a vector for the virus. The CCHF virus (CCHFV), a Nairovirus of the family Bunyaviridae, has been isolated from 30 species of ixodid tick (3). Numerous domestic and wild animals, such as cattle, goats, sheep, small mammals, rodents, and birds, in which the infection is mainly asymptomatic, serve as amplifying hosts for the virus (4). There is no specific treatment or vaccine against CCHF and it is considered an emerging arboviral zoonotic disease in many countries, possibly due to increased vector bionomics and climate change. Recently, the incidence of CCHF has increased rapidly in the countries of the World Health Organization Eastern Mediterranean Region (WHO EMR), with sporadic human cases and outbreaks of CCHF being reported from a number of countries in the region. Despite the rapidly growing incidence of the disease, there are currently no accurate data on the burden of the disease in the region due to the different surveillance systems for CCHF used in these countries. Moreover, there is no definitive preventive and control strategy for CCHF owing to the fact that many aspects of the disease, such as the maintenance and transmission of the virus and the pathogenesis of the disease in humans, remain poorly understood (5). Considering the zoonotic nature and public health importance the aim of this study was to assess the seroprevalence of CCHFV in previous and recent endemic areas of the country.

## 2. Material and Methods

**Blood specimen collection:** The survey included 152 sera specimen of cattle which were randomly collected from seven districts in Albania over the period 2010-2014. These localities include (Berat, Elbasan, Gjirokaster, Gramsh, Has, Kukes, Skrapar).

Sterile vacutainers were used to draw the blood from jugular veins and were left to clot. Sera were separated afterwards and stored at -20°C until used.

**Laboratory examination:** Adaptation of a commercial CCHFV ELISA and IFA.

ELISA commercial test for identifying of specific CCHFV antibodies in human sera (Vector Best, Novosibirsk, Russia) was adapted for testing sera from sheep and goats. All washing steps were performed with PBS-Tween20. Sera were diluted 1:100 in dilution buffer (90% SDB- and 10 % SPSSD-buffer of the manufacturer). Diluted sera (100  $\mu$ l/well) were incubated for 1 h at 37°C. After washing the plates, 100  $\mu$ l/well of rabbit-anti-sheep-IgG-HRP conjugate (Southern Biotech, Birmingham, AL, USA) or rabbit-anti-goat-IgGHRP conjugate (Southern Biotech) were added. Both were diluted 1:6000 in conjugate dilution buffer (by manufacturer) and incubated for 30 min at 37°C. After washing the plates, 100  $\mu$ l tetramethylbenzidine (TMB, Bio-Rad, Munich, Germany) solution was added per well, and the reaction was stopped with H<sub>2</sub>SO<sub>4</sub> after 10 min. The extinction was measured at  $k = 450$  nm (reference wavelength 620 nm). Serum samples with OD-values (background reaction subtracted) of 0.5 and lower were defined as 'negative', whereas serum samples with OD-values of 0.6 and 0.7 were defined as 'inconclusive' and serum samples with OD-values of 0.8 and higher were defined as 'positive'. (6)

**Statistical analysis:** data were analyzed using SPSS software. Chi-square test was used to test the differences between proportions. Statistical significance was set for  $p$  value  $\leq 0.05$ .

## 3. Results & Discussion

Distribution of domestic animals by district is shown in table 1. As noted, most of domestic animals belong to district of Has (38.2%) followed by district of Kukes (27%) which are recent endemic areas, with a significant difference as compared to other district ( $p < 0.01$ ).

Cows account for 50.7% of animals, followed by sheep (29.6), bovine (17.1%) and goats (2.6%), ( $p < 0.01$ ).

**Table 1.** Distribution of domestic animals by district

District	Type of domestic animals				Total n (%)
	Bovine	Cow	Goat	Sheep	
Berat	0	1	0	13	14 (9.2%)
Elbasan	3	0	0	0	3 (2.0%)
Gjirokaster	5	0	0	13	18 (11.8%)
Gramsh	7	0	0	0	7 (4.6%)
Has	0	58	0	0	58 (38.2%)
Kukes	0	18	4	19	41 (27.0%)
Skrapar	11	0	0	0	11 (7.2%)
Total n (%)	26 (17.1%)	77 (50.7%)	4 (2.6%)	45 (29.6%)	152

The serologic results are shown in table 2.

According to ELISA serology the majority of specimens (67.1%) resulted positive for IgG to CCHF [95%CI 59.3 – 74.1]. A very small number of specimens (3.3%) resulted cut-off.

**Table 2.** ELISA IgG serologic results

ELISA IgG	N	%	95%CI
Cut-off	5	3.3%	1.42 – 7.48
Negative	45	29.6%	22.9 – 37.3
Positive	102	67.1%	59.3 – 74.1
Total	152	100.0%	

Table 3 presents the serologic result by type of animal. The highest positivity rate was among cows (88.3%), followed by sheep (55.6%), bovine (30.8%) and only one case among goats (25%),  $p < 0.01$ .

**Table 3.** Serologic result by type of animal

Domestic animals	Total specimens	Cut-off	Negative	Positive	Prevalence (%) of positive specimens
Bovine	26	0	18	8	8 (30.8%)
Cow	77	3	6	68	68 (88.3%)
Goat	4	0	3	1	1 (25%)
Sheep	45	2	18	25	25 (55.6%)

**Table 4.** Serologic result by district

District	Total specimens	Cut-off	Negative	Prevalence (%) of positive specimens
Berat	14	1	5	8 (57.1%)
Elbasan	3	0	3	0
Gjirokaster	18	0	17	1 (5.6%)
Gramsh	7	0	4	3 (42.9%)
Has	58	3	3	52 (89.7%)
Kukes	41	1	6	34 (82.9%)
Skrapar	11	0	7	4 (36.4%)
Total	152	5 (3.3%)	45 (29.6%)	102 (67.1%)

As noted from the above table the recent endemic areas of Has and Kukes show the highest positivity rate, 89.7% and 82.9% respectively, with a significant difference to other districts ( $p < 0.01$ ). Crimean-Congo hemorrhagic fever (CCHF) is a well-known infectious nosology in Albania since its first report in 1974; however, CCHF primary serological studies have been commenced since 1969. For this cross sectional study, we have tested the blood samples of previous and recent endemic areas of the country.

From our results we have different values in different areas. We found the presence of infection (antibodies) in animals in areas where cases with hemorrhagic fever were observed more than one to two decades ago, where since then has not been observed cases with hemorrhagic fever in humans. This phenomenon is observed in districts other than Has and Kukës. This indicates that the infection in these areas is still present and we think it can become active. We think that these results should be a signal especially for human service which should take strong observation in these areas and in equivocal cases should immediately take appropriate measures. The results of our study are similar with other studies conducted in Albania regarding the seroprevalence of CCHFV in cattle (6,7,8). In Kosovo the presence of the CCHF antibodies was found in 14% of livestock, and in 32.6% of sheep (9).

Countries like Turkey and other Balkan countries with the exception of Greece have recorded circulation of CCHF strains among animal hosts, ticks, and humans, and have established CCHF endemicity (10). The wide outbreaks that occurred in Turkey were preceded by several decades of serologic evidence of a zoonotic CCHF focus. Livestock originated from endemic zones resulted 14% seropositive on CCHF virus, only sheep were 32.6% seropositive (11).

The establishment and maintenance of a CCHF endemic focus requires an environment favoring an efficient contact between competent ticks and animal hosts with relatively high prevalence of infection. Heterogeneity in infection levels may occur even within endemic foci, as a result of variable climate and environmental suitability for ticks and animal hosts

Over recent decades, alterations in natural ecosystems, intensive agriculture, global warming, and the exponential increase of movement of people for any cause have provided the framework for the expansion of several vector-borne diseases globally, from endemic areas to neighboring nonendemic areas as well as to distant continents. In animal that were infected the virus was amplified to a high level which was sufficiently enough to transmit to the tick vectors, as shown in former investigational surveys which employed cattle inoculation. Seroconversion was detected among infected cattle due to development of a viremia of a low-tire (12).

In the phase of viremia cattle shed the virus and vulnerable humans can contract the disease through bites of infected ticks (13). The outcome of these studies highlight the significant contribution of cattle in the transmission of the infection.

This finding suggested that cattle may play an important role in the epidemiology of the disease. On the contrary, a high rate of lethality from CCHFV infection is observed among humans which reaches up to 30%.

#### 4. Conclusions

The results of this survey suggest that in recent endemic areas humans can potentially contract the disease as compared to other areas of the country. Occupational exposure risk should be reduced among professionals handling with animals by implementing the measures of protection and avoiding risky behaviors (14). Doctors and other medical staff ought to think about this infection in individuals with signs and symptoms similar to CCHFV. Mapping of ticks population in the country and promotion are of paramount importance to prevent future CCHF outbreaks.

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