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Serological Survey of Crimean-Congo Hemorrhagic Fever Virus in Kolonje-Erseke, Albania

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Abstract

Crimean-Congo Hemorrhagic Fever (CCHF) is a tick-borne viral zoonosis which occurs widely in Africa, Eastern Europe, and Asia within the distribution range of ticks of the genus *Hyalomma*. The CCHF virus (CCHFV) belongs to the *Nairovirus* genus (family *Bunyaviridae)* and causes a severe disease in humans, with a reported mortality rate of 3–30%. The geographic range of CCHFV is the most extensive of the medically significant tick-borne viruses. The aim of this study was to examine the distribution of CCHFV among the cattle, sheep and goats in Kolonje-Erseke region of Albania. This survey was carried out in 2013. Blood samples were taken from the jugular vein of 54 cattles, 29 sheep and 9 goats. The samples were immediately taken to the laboratory and their serum separated by centrifugation with 3500 rpm in 10 minutes. The sera were kept in the Faculty of Veterinary Medicine, Agricultural University of Tirana, at -20°C until analysis. They were tested with an immunological method using indirect ELISA at Friedrich-Loeffler-Institute (FLI), Greifswald Germany. Through this technique it was possible to identify CCHFV-specific IgG antibodies in serum samples of infected animals. From these results we had an indication about the prevalence of CCHF infection respectively, 7,4% in cattles, 96,5% in sheep and 88.8% in goats. This study can clearly confirms the presence of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) in a high level 64,2% in livestock in Kolonje-Erseke region of Albania.

Keywords: CCHFV, *Hyalomma*, Indirect ELISA, IgG antibodies, FLI

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tickborne disease caused by CCHF virus (CCHFV) of the genus *Nairovirus* in the family *Bunyaviridae*. The CCHFV infection is transmitted to humans by tick bites, handling of ticks, exposure to blood or tissues of viremic livestock, or direct contact with blood and bodily fluids of infected patients [1]. Crimean Congo hemorrhagic fever (CCHF) is endemic to Africa, the Balkans, the Middle East, and parts of Asia within the distribution range of ticks of the genus *Hyalomma*. [2] A disease named Crimean hemorrhagic fever was first observed in the Crimean Peninsula in 1944, and the causative agent which was isolated in 1967, was found to be identical to Congo virus isolated in 1956 from a febrile child in the Belgian Congo (now Democratic Republic of Congo–DRC), hence the names Crimean and Congo are used in combination [3, 4, 5, 6]. By 1979, the virus was known to occur in many countries in Eastern Europe and Asia. Case fatality rates recorded in Eastern Europe and Asia varied from 15% to 40%, but in Africa only 1/15 known human infections had been fatal. Nevertheless, suggestions that African strains of the virus were less pathogenic for humans than Eurasian strains were rejected on the grounds that observations had been too limited [7]. CCHF virus has been isolated from at least 30 species of ticks, but in most instances there is no proof that the ticks are capable of serving as vectors since virus isolated from engorged ticks may merely have been present in the blood meal obtained from a viraemic host [2, 8, 9, 10, 11]. Nevertheless, the ability to transmit infection has

been demonstrated for ixodid ticks of several genera, and transovarial transmission of the virus from adult females to the succeeding generation of larval ticks has been shown to occur in a few members of the *Hyalomma*, *Dermacentor*, and *Rhipicephalus* genera. However, the coincidence in distribution of CCHF virus and *Hyalomma* ticks implies that members of this genus are the most important vectors of the virus [2, 12]. The status of CCHFV-specific antibodies in the animal population of a region is a good indicator for the presence or absence of CCHFV in the respective area [13, 30].

2. Material and Methods

2.1 Sera from cattle, sheep and goats

This study was carried out in the Kolonje-Erseke region of Albania. Kolonje District was one of the thirty-six districts of Albania that is now part of Korçe County. It had a population of 14,318[2]. The district had an area of 805 km². It is in the south-east of the country, and its capital was Erseke. It is bounded by Korçe to the north, Greece to the east including the regional units of Kastoria and Ioannina. The town of Erseke is built at the foot of mount Gramos, Albania's fourth-highest mountain with a peak at 2,525 m above sea level. The geographic features of Kolonje district are presented in table 1.

In this study, blood samples were collected from cattle, sheep and goats by veterinarias, in 2013. Blood samples were taken from the jugular vein of 54 cattle, 29 sheep, and 9 goats. The data of serum samples are presented in table 2.

The samples were immediately taken to the laboratory and their serum were separated by centrifugation at 3500 rpm for 10 minutes. Each blood sample was stored at -20°C in the Faculty of Veterinary Medicine, Agricultural University of Tirana, until analysis. The collected sera from cattle, sheep and goats were immunologically tested by using the indirect ELISA assay at Friedrich-Loeffler-Institute (FLI), Greifswald Germany.

Table 1. The geographic features.

The study area	Altitude	Latitude	Longitude	Temp-Max	Temp-Min
Kolonje	1016 m	$40^0$20’14,38” N	$20^0$40’46,09” E	15.0 ^{0}C	5.6 ^{0}C

Table 2. Collected serum samples.

Region/Location (village, farm)	*Number*	*Animal species CT-cattle*	*Date of sample Collection (Day/Month/Year)*	*Gender M-male/ F-female*	*Housing S-stable/ P-pasture*	*Tick defense Measures D-defense/ND-no defense*
Kolonje-Erseke	54	Cattle	16/05/2013	F-female	P-pasture	ND-no defense
Kolonje-Erseke	29	Sheep	30/05/2013	F-female	P-pasture	ND-no defense
Kolonje-Erseke	09	Goats	30/05/2013	F-female	P-pasture	ND-no defense

2.2 Indirect ELISA

IgG and IgM antibodies are detectable from about 7 days after onset of disease in humans. Specific IgM declines to undetectable levels by 4 months postinfection, but IgG remains detectable for at least 5 years. All collected sera were sent to Friedrich-Loeffler-Institute (FLI) in Greifswald, Germany in November 2013. The indirect ELISA was used for the detection of IgG antibodies in the serum samples. Briefly, the following ELISA protocol was used. A recombinant Nucleocapsid (N-) protein of CCHFV was used as antigen. It was added half of the wells of a 96-well microtiter plate, were it adhere to the plastic through charge interactions. A solution of skim milk was used for blocking all free binding sides and to

reduce background reactions. Each serum samples was added to two wells without the N-protein. In case CCHFV-specific antibodies were in a serum sample, they bind to the N-protein. All unspecific antibodies were washed away. As a secondary antibody a peroxidase labelled bovine specific conjugate was added to each well.

This conjugate formed antibody complexes with the CCHFV-specific antibodies of the serum sample. For the detection of this complex, a substrate for the peroxidase was added. The substrate changes color upon reaction with the enzyme and shows therewith, that CCHFVspecific antibodies are in the serum samples which have bound to the N-protein. The higher the concentration of the primary antibody present in the serum, the stronger the color change. A spectrometer was used to give quantitative values for color strength. Data were analyzed with SPSS, v. 19. We used chi square testing for the comparison of variables in the analysis.

3. Results and Discussion

A total of 92 serum samples from cattle, sheep and goats were tested with an immunological methods using indirect ELISA at Friedrich-Loeffler-Institute (FLI), Greifswald Germany. Through this technique it was possible to identify CCHFV-specific IgG antibodies in serum samples of infected animals. The data presented in table 3 indicates the presence of CCHFV in Kolonje-Erseke. From this results we had an indication about the antibody prevalence of CCHF infection respectively, 7,4% in cattle, 96,5% in sheep and 88.8% in goats. The chi-square test was used for comparison of results between cattle, sheep and goats. In this analysis *p* Values>0.01 (p=0.451) was considered no-significant at the 0.01 level.

Table 3. The results obtained from indirect ELISA.

Region/Location (village)	Serum sample tested (Final result)				
	Animal species	**Total samples**	**Positive samples**	**Negative**	**Aantibody prevalence**
Kolonje-Erseke	Cattle	54	4	50	7.4%
Kolonje-Erseke	Sheep	29	28	1	96.5%
Kolonje-Erseke	Goats	09	8	1	88.8%
Total		**92**	**40**	**52**	**64.23%**

From this study is clear the higher antibody prevalence in sheep (96.5%) than in goats (88.8) and cattle (7.4%). We have to underlined that our results are resembles with the results of the other outhers. Evidence of CCHFV infection (IgG positive) was also found in a follow-up study of livestock in Iraq respectively 443/769 (57.6%) in sheep, 279/562 (49.6%) in Goats and 122/411 (29.3%) in cattle [28] and in Iran respectively 277/728 (38%) sheep, 49/135 (36%) goats and 23/130 (18%) [29]. Detection of CCHFV antibodies in domestic animals has been important in providing initial evidence of circulating virus and in localizing CCHFV foci and increased risk for human infection [14, 15, 16]. In Kolonje-Erseke areas are documented facts with human CCHF cases, and the prevalence of this infection was high (96.5%) not only in sheep but in goats and cattle too. Domestic animal species are often implicated in CCHFV transmission when human CCHF cases are detected. Sheep have been recognized as very important CCHFV reservoirs in certain endemic regions, and have been epidemiologically linked to human cases on several occasions [17, 18, 19, 20]. In Uzbekistan, three CCHF cases were described in persons involved in the handling of tissue from a cow [21]. Similarly, the first patient in an epizootic of CCHFV in Mauritania became ill shortly after butchering a goat [22]. As such, increased CCHFV IgG seropositivity in livestock often parallels reports of CCHF cases in humans with exposure to livestock (e.g., slaughterers, butchers, and farmers), particularly in those who handle blood and organs from infected livestock [23]. Abiotic variation by season, country, and region is reported in CCHFV seroprevalence studies. Studies in

Turkmenistan (then Turkmen Soviet Socialist Republic [SSR]) reported an increase in CCHFV seropositive domestic animal species during the summer season, and found large variations between regions and individual farms (seropositivity range 5.9%–32%) [24]. A subset of biotic factors determining domestic animal CCHFV seroprevalence were investigated in Senegalese sheep by Wilson et al., who reported that the sex of the animal did not affect antibody prevalence [25]. Geographic variation of CCHFV seroprevalence in domestic animals within a single country has also been reported in several studies [26]. Ecological factors in Kolonje-Erseke district are very suitable for the life cycle development of ticks. These factors are more favorable to the presence of ticks due to uncultivated lands, the presence of stones and shrubby, high level of rainfall and not too low temperatures in the winter months. These ecological and climatic factors can maintain the larvar stage prepared for the following period. It should be emphasized the fight against ticks has not been active and accomplished in all areas. In areas with a high prevalence of CCHF infection for instance in Kolonje-Erseke district, the methods for ticks destruction are not implemented in programmed order.

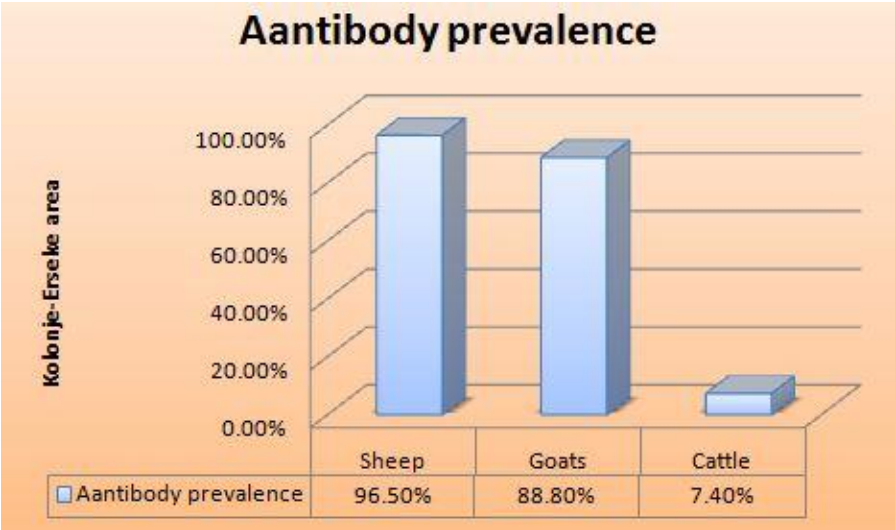

Figure 1. The prevalence of CCHFV-specific IgG antibodies, in sheep, goats and cattle from Kolonje-Erseke area.

Small ruminants are suitable as indicator animals for seroepidemiological CCHFV monitoring studies to determine the presence or absence of CCHFV in a given region. [13]. This is surprising as observations support the idea that cattle are the preferential feeding host for adult *H. marginatum* ticks [27]. It should also be pointed out that in Vrepcke area, a few years ago there were serious infection cases in humans. The deficient control of domestic animal from the veterinary specialists is associated with the high prevalence of CCHF infection. Further studies are recommended, especially if we consider a fact that Kolonje-Erseke area is a remote area from other areas infected where the spread of *Hyalomma* ticks is difficult someway. Infected birds migration can be considered as one of the main factors influencing to the CCHF infection spread in this remote area.

4. Conclusions

The scientific data presented in this study indicated that CCHF virus is present in Kolonje-Erseke of Albania. The results demonstrate a widespread infection among sheep, goats and cattle with the CCHF virus. Higher infection rates in livestock may lead to future outbreaks in rural areas. Our findings indicate that the risk of importing emerging infectious diseases along with live animals poses a serious risk to public health. Consequently, detailed risk-based surveillance is necessary to understand the complete scenario of CCHFV prevalence in livestock in Vrepcke because

Hyalomma tick species, the primary vectors of CCHF, are present on animals here. In addition, a survey among at-risk human populations is also needed. Findings from these surveillance activities would help institute more diagnostic facilities and risk-based surveillance and assist in developing a preparedness plan at the human–animal interface. We think that it is recommended that further studies be carried out on additional livestock, high-risk groups of humans, and ticks, to characterize in detail the CCHF virus status in Kolonje-Erseke. CCHFV is widely distributed, circulates in numerous vertebrate species, and can be transmitted to humans in several ways. Serosurveillance of animals will continue to be an essential tool for monitoring levels of endemic transmission and for investigating areas where CCHFV is not known to circulate.

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