

A Seroepidemiological Survey of Crimean-Congo Hemorrhagic Fever Virus among Goats and Sheep in Lezhe-Torovica Province, Albania

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Abstract:

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic vector-borne viral disease with a case fatality rate of 2-50% in human. CCHFV is classified within the *Nairovirus* genus in the *Bunyaviridae* family. The virus was found in over 30 countries, including Albania. A broad spectrum of animals can be infected by CCHFV without showing clinical symptoms. The virus can be transmitted mainly through direct contact with blood or tissues from infected livestock or through bites of *Hyalomma* ticks. The aim of this study was to examine the distribution of CCHFV among sheep and goats in Lezha-Torovica region of Albania. This survey was carried out in 2013. Blood samples were taken from the jugular vein of 9 sheep and 10 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 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 prevalence was much higher in goats than this infection in animals is obviously different, in goats is higher than in sheep, respectively 90% and 22%. It is recommended that further studies be carried out on additional livestock, high-risk groups of humans, and ticks, to determine the CCHF disease status in Lezha-Torovica.

Keywords: CCHFV, Nairovirus, Bunyaviridae, Indirect ELISA, IgG

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is an acute zoonotic infection caused by the CCHF virus, a member of the genus *Nairovirus* of the family *Bunyaviridae*. Serological investigations revealed that numerous wild and domestic vertebrates, including cattle, sheep and goats, are susceptible to the CCHF virus [5]. Although high fever, malaise, severe headache, gastrointestinal symptoms, hemorrhage and a fatality rate of 5-50% are observed in infected humans, the virus generally produces no disease conditions in animal hosts. Animals developing transient viremia and an antibody response to the CCHF virus. They function as amplifying hosts for the agent. The virus is transmitted to humans mainly through the bite of an infected tick or by direct contact

with the blood and tissues of viremic livestock or human patients [4, 5]. The virus follows tick-vertebrate-tick cycles in nature. The virus circulates by transovarial as well as transstadial transmission in the tick population [5]. Due to this, ticks are classified not only as vector but also as reservoir for CCHF virus [2]. *Hyalomma* ticks, particularly *H. marginatum*, are accepted as the main vector for the CCHF virus, although in more than 30 tick species the virus has been detected [1, 5]. A wide variety of domestic animals (e.g. sheep, cows, goats and ostriches) as well as large wild herbivores, hares and hedgehogs, can become infected with the virus, and those infections are usually asymptomatic [3]. CCHF

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virus is endemic in some parts of Africa, Asia, the Middle East, and south-eastern Europe.

2. Material and Methods

2.1 Sera from sheep and goats

This study was carried out in Torovuca in the province of Lezha, in north-western of Albania at altitude 41°5'40" north, 41°35' south, 38°08' west and 20°10'03" east. Lezha has a geographical area of approximately 1588.4 m², and is situated with the

Adriatic Sea to its western with a coastline length of 38 km, to the north with the district of Shkodra and to the south with the district of Kurbin. The province enjoys a moderate, continental climate, with average temperatures of 15°C. Annual rainfall averages from 1,200 to 1,700 mm. In this study, blood samples were collected from sheep and goats by veterinarians, in 2013. Blood samples were taken from the jugular vein of 9 sheep, and 10 goats. The data of serum samples are presented in table 1.

Table 1. Collected serum samples.

Region/Location	Number of samples	Animal species	Date of sample Collection (Day/Month/Year)	Gender	Housing	Tick defense Measures
Lezha-Torovica	9	SH	16/04/2013	F-female	P-pasture	ND-no defense
Lezha-Torovica	10	GO	16/04/2013	F-female	P-pasture	ND-no defense

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 sheep and goats were immunologically tested by using the indirect ELISA assay at Friedrich-Loeffler-Institute (FLI), Greifswald Germany.

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 post-infection, 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-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 CCHFV-specific 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

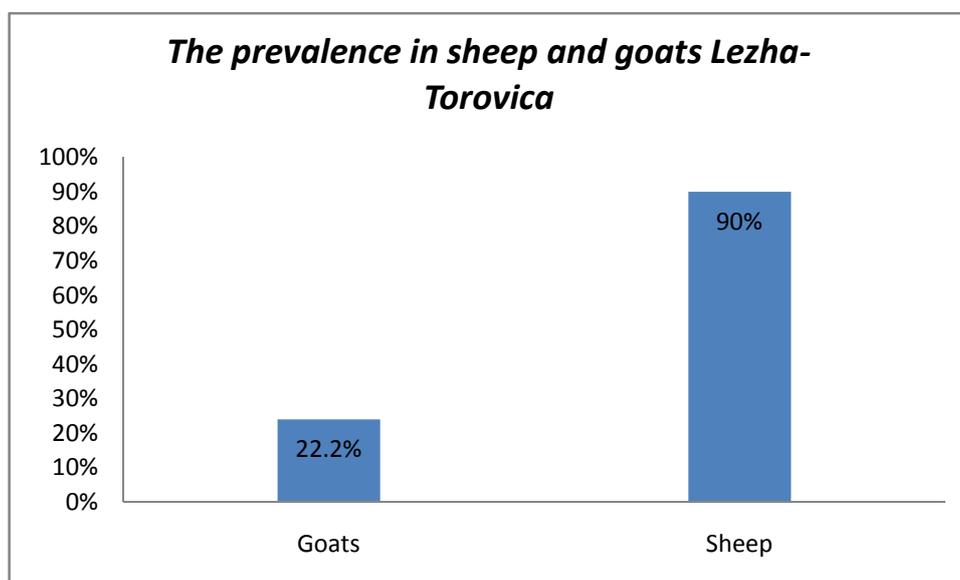
A total of 19 serum samples from sheep and goats were tested with indirect ELISA at FLI Germany. By this technique it was possible to demonstrate the presence of CCHFV-specific antibodies in sera. The data presented in table 2 indicates the presence of CCHFV in Lezha-Torovica. The antibody prevalence is obviously different in sheep and goat. The prevalence in goats was 90% and is much higher than in sheep (22%). The chi-square test was used for comparison of results for sheep and goat. In this analysis p Values > 0.01 ($p=0.655$) was considered non-significant at the 0.01 level.

Table 2. The results obtained from indirect ELISA.

Region/Location (village)	Serum sample tested (Final result)				
	Animal species	Total samples	Positive sample	Negative	Antibody prevalence
Lezha-Torovica	Sheep	9	2	7	22.2%
Lezha-Torovica	Goats	10	9	1	90%
TOTAL		19	11	8	57.89%

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 Lezha-Torovica. It is also suggested that a

similar study should be performed in other northern neighboring provinces (Shkodra), to identify the epidemiological features of CCHF virus in the other provinces bordering the Adriatic Sea

**Figure 2.** The prevalence of anti CCHF IgG, in sheep and goats from Lezha-Torovica region.

From these preliminary results, we draw attention not only to human service but also to veterinarians. These services should undertake measures to combat ticks in animals, as they are constant risk for human infection. Additionally, a powerful propaganda should become with the animal owners to these areas for the recognition and the danger of this infection.

4. Conclusion

The scientific data presented in this study indicated that CCHF virus exists in Lezha Albania and that humans are at risk to be infected with the virus. The results demonstrate a widespread infection among sheep and goats with the CCHF virus. Higher infection rates in livestock may lead to future outbreaks in rural areas. It is recommended that livestock attendants and slaughter house workers should consider strict hygienic measures when handling tick-infested livestock or their associated

products. The residents have to be aware of the risk of being infected with CCHFV as a result of consumption of uncooked animal-derived products. Physicians and medical health workers in Lezha should consider this virus in their efforts to diagnose the disease in patients with clinical presentations compatible with those of CCHF.

Future surveillance program for CCHFV should be extended to include other susceptible animals such as cattle. Similar studies in other non-endemic areas and wild animals may be helpful to create an epidemiological risk map.

5. References

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