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SEROLOGICAL SURVEY OF CCHFV IN CATTLE IN 10 REGIONS OF ALBANIA

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic vector-born viral disease with a case fatality rate of 2-50% in human. CCHFV is classified within the *Nairovirus* genus in the *Bunyaviridae* family. Its causative agent is a negative-sense, single-stranded (ss) RNA genome containing S (small), M (medium), and L (large) segments which encode for the nucleocapsid protein (NP), the envelope glycoproteins G1 and G2 and RNA-dependent RNA-polymerase, respectively. The virus can be transmitted mainly through direct contact with blood or tissues from infected livestock or through bites of *Hyalomma* ticks. The present survey was carried out using samples collected in 2013. The aim of this study was to examine the distribution of CCHFV among cattle in 10 regions of Albania (Has, Kavaje, Kukes, Berat, Kolonje, Pogradec, Rreshen, Korce (Bulgarec/Qatrom) and Gjirokastra). The samples were tested with an immunological method using indirect ELISA at Friedrich-Loeffler-Institut (FLI), Greifswald Germany. Through this technique it was possible to identify in 16 out of 337 serum samples from cattle CCHFV-specific IgG antibodies. These results demonstrate the presence of CCHFV in livestock and therewith the presence of CCHFV in Albania.

KEYWORDS: CCHFV, (ss) RNA, Nairovirus, Bunyaviridae, Indirect ELISA, IgG

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a severe hemorrhagic disease with a case fatality rate of up to 50%. The virus causing the disease is tick-borne and belongs to the family *Bunyaviridae*, genus *Nairovirus* [Donets et al., 1977; Ellis et al., 1981; Martin et al., 1985]. Like other *Nairoviruses*, CCHF virus is an enveloped single stranded negative-sense RNA virus and its tripartite genome consists of a small (S), a medium (M) and a large (L) segment which encode for the nucleocapsid protein (NP), the envelope glycoproteins G1 and G2 and a RNA-dependent RNA-polymerase, respectively [Marriott et al., 1992; Marriott et al., 1994]. The virus is transmitted to humans through the bite of infected ticks or by direct contact with blood or tissues from viremic animals or humans [Camicas et al., 1994; Ergönül, 2006]. Infected humans can spread the disease via close contacts which may result in community outbreaks and nosocomial infections [Burney et al., 1980; van Eeden et al., 1985; Mardani, 2001; Jamil et al., 2005]. The potential human to human transmission along with the high lethality rates, the fears that the virus could be used as a bioterrorism agent and the increase of the incidence and geographic range of the Crimean-Congo hemorrhagic fever make the virus an important human pathogen. Like other tick borne zoonotic agents, CCHF virus circulates in nature in an enzootic tick-vertebrate-tick cycle. CCHF virus is transmitted by ticks of the genus *Hyalomma* and in particular by *Hyalomma marginatum marginatum.*

Ticks of the genus *Hyalomma* serve as vectors and reservoir of the CCHF virus and the geographic distribution of the disease coincide with the global distribution of *Hyalomma* ticks [Charrel et al., 2004; Whitehouse, 2004; Vorou et al., 2007]. The virus is reported in over 30 countries of Africa (Democratic Republic of Congo, Uganda, Mauritania, Nigeria, S. Africa, Senegal, ect), Southeast Europe (Russia, Bulgaria, Kosovo, Turkey, Greece, ect), the Middle East (Iraq, Iran, Saudi Arabia, Oman) and Asia (China, Kazakhstan, Tajikistan, Uzbekistan, Pakistan) [Morikawa et al., 2007]. In this regard, the geographical distribution of CCHF virus is the greatest among all tick-borne viruses. CCHF virus has been isolated from adult ticks of the genus *Hyalomma* in the '60s and transovarial and transstadial transmissions have been already suggested since viral isolates have been also found in field collected eggs and unfed immature stages of *H. marginatum* [Watts et al., 1988]. CCHF virus has been also isolated in laboratory from other tick genera e.g. *Rhipicephalus, Ornithoros, Boophilus, Dermacentor* and *Ixodes spp.*

MATERIALS AND METHOD

Materials

Sera from Cattle

In animals, CCHFV infections do not cause clinical signs. We collected sera from (Has, Kavaje, Kukes, Berat, Kolonje, Pogradec, Rreshen, Korce and Gjirokastra) area. Blood was taken from the jugular vein by vakutanier and was left for two hours to coagulate. The samples were immediately taken to the laboratory and their serum was 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 were immunologically tested by using the indirect ELISA at Friedrich-Loeffler-Institut (FLI), Greifswald Germany. The data of serum samples are presented in table 1.

Table 1: The Collected Serum Samples from Respective Areas

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
Has-Fejzo	50	Cattle	05/05/2013	F-female	P-pasture	ND-no defense
Kavaje	54	Cattle	16/05/2013	F-female	P-pasture	ND-no defense
Kukes-Caje	11	Cattle	16/05/2013	F-female	P-pasture	ND-no defense
Berat-Terpan	50	Cattle	15/04/2013	F-female	P-pasture	ND-no defense
Kolonje-Erseke	54	Cattle	16/05/2013	F-female	P-pasture	ND-no defense
Pogradec-Leshnice	6	Cattle	08/05/2013	F-female	P-pasture	ND-no defense
Rreshen	40	Cattle	17/04/2013	F-female	P-pasture	ND-no defense
Korce-Bulgarec	10	Cattle	08/05/2013	F-female	P-pasture	ND-no defense
Korce-Qatrom	10	Cattle	08/05/2013	F-female	P-pasture	ND-no defense
Gjirokastra-Picar	50	Cattle	17/04/2013	F-Female	P-Pasture	ND-no defense
Total	**337**	**Cattle**		**F-Female**	**P-Pasture**	**ND-No Defense**

METHOD

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 FLI in November 2013. The indirect ELISA was used for the detection of IgG antibodies in the serum samples (Mertens et al. 2015). 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 sample was added to two wells with and two 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 primary antibodies in the serum, the stronger the change of color. A spectrometer was used to give quantitative values for color strength.

RESULTS AND DISCUSSIONS

A total of 337 serum samples were tested using an indirect ELISA at FLI, in order to identify the presence of CCHFV-specific IgG antibodies. The data presented in the table below indicate for the first time CCHFV infections in cattle in different areas of Albania. Beside the formerly known areas where CCHF cases were observed in humans regularly we found also animal infections in regions with either no or long past history (more than 10 years ago) of CCHFV infections in humans.

Table 2: The Results Obtained from Indirect ELISA Assay

Region/Location (Village)	Serum Sample Tested (Final Result)				
	Total Samples	Positive Sample	Equivocal	Negative	Prevalence (%) of Positive Sample
Has-Fejzaj	50	7	1	42	16.67%
Kavaje	54	0	0	54	0%
Kukes-Çaje	11	0	0	11	0%
Berat-Terpan	50	2	3	45	4.4%
Kolonje-Erseke	54	4	0	50	8%
Pogradec-Leshnice	6	0	0	6	0%
Rreshen	40	1	1	38	2.6%
Korce-Bulgarec	10	0	0	10	0%
Korce-Qatrom	10	0	0	10	0%
Gjirokastra-Picar	50	1	1	48	2.1%
Total	**337**	**15**	**6**	**316**	**4.74%**

In areas with documented human CCHF cases, the prevalence of this infection in cattle is high (e.g. has 16% and Kolonje-Ersekeke-Kolonje 7%). In other areas for example in Gjirokastra-Picar and Rreshen where no human CCHF cases were reported the prevalence in cattle is low (2%). The finding of antibody positive cattle in areas where no or only long ago human cases were reported, is indicative for either for a recent spread of CCHFV or -more likely- that there is an yet undetected established virus circulation in these areas. In order to prevent human cases in future, public health protection measures like improved CCHFV surveillance, reduction of CCHFV exposure risks, e.g. by vector intervention and increased public awareness programs should be initiated..

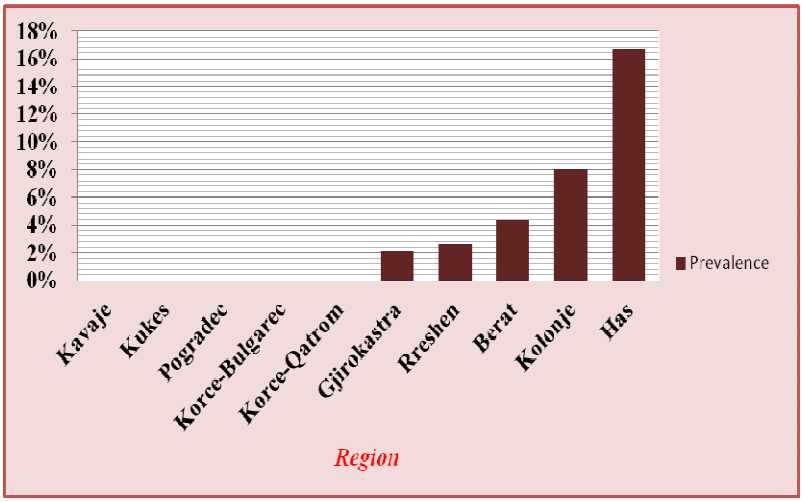

Figure 1: The Prevalence of CCHFV in Different Countries of Albania from Indirect-Elisa Results

We also emphasize that the here described animal infection are mainly found in outdoor leisure suburbs which have very favorable conditions for infestation with ticks of the genus Hyalomma. As shown in Table no.1, no protective measures against ticks were used. From these preliminary results, we draw attention not only to human service but also to human veterinary. 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

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