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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 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 aim of this study was to examine the distribution of CCHFV among cattle of 10 regions in Albania respectively in (Has, Kavaje, Kukes, Berat, Kolonje, Pogradec, Rreshen, Korce (Bulgarec/Qatrom) and Gjirokastra). The data taken from this study indicates for the presence of CCHFV Crimean-Congo Hemorrhagic Fever virus in the country. The serums were conserved in -20°C and tested with immunological methods using indirect ELISA assay in Friedrich-Loeffler Institute (FLI), Greifswald Germany. Through this technique it was possible to identified IgG antibodies in infected serum samples. From these results we had an indication about the presence of IgG antibodies in 15 blood samples from 337 cattle in total. These results can clearly proved the presence of CCHFV in livestock in Albania.

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

INTRODUCTION

CCHF is a severe hemorrhagic fever with a case fatality rate of up to 50%. The virus that causes the disease is a tick-borne virus belonging to the family Bunyaviridae, genus Nairo virus [Donets et al., 1977; Ellis et al., 1981; Martin et al., 1985]. Like other nairo viruses, CCHF virus is an enveloped single stranded negative-sense RNA virus and its tripartite genome consists of a small (S), a medium (M) and large (L) segment which encode for the nucleocapsid protein (NP), the envelope glycoproteins G1 and G2 and an RNA-dependent 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 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 mortality 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. Humans are being infected mainly through direct contact with blood or tissues from infected livestock or through tick bites. CCHF virus is transmitted by *Hyalomma* genus ticks and in particular by *Hyalomma marginatum marginatum.*

Ticks of the genus *Hyalomma* serve indeed 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 than 30 countries in 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; Koutis, 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 *Hyalomma* genus ticks 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*, respectively [Watts et al., 1988]. CCHF virus has been also isolated in laboratory from other tick genera eg. *Rhipicephalus, Ornithoros, Boophilus, Dermacentor* and *Ixodes spp.*

MATERIALS AND METHOD

Materials

Sera from Cattle

In animal CCHF disease does not cause clinical signs. We collected sera from (Has, Kavaje, Kukes, Berat, Kolonje, Pogradec, Rreshen, Korce and Gjirokastra) area where we had indication about the presence of CCHFV in humans. Blood was taken from the jugular vein by vakutanier and was left for two hours to coagulate. Then the serum was taken by centrifugation (15 minutes at 3000 rfm / rotate for minute) and was preserved in freezing in -20 degrees. The data of serum samples are presented in the below table.

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

All the collected sera were sent to Friedrich Loeffler Institute in Greifswald, Germany in November 2013. We used the indirect ELISA assays for the detection of IgG antibodies in infected serum samples. IgG and IgM antibodies are detectable by ELISA assay from about 7 days after the onset of disease. Specific IgM declines to undetectable levels by 4 months post-infection, but IgG remains detectable for at least 5 years.

The serum were analyzed by indirect ELISA using the following protocol. The steps of "indirect" ELISA follows the mechanism below:

- A buffered solution of the antigen to be tested for is added to each well of a *microtiter plate*, where it is given time to adhere to the plastic through charge interactions.
- A solution of nonreacting protein, such as *bovine serum albumin* or *casein*, is added to well (usually 96-well plates) any plastic surface in the well that remains uncoated by the antigen.
- The *primary antibody* is added, which binds specifically to the test antigen coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
- A *secondary antibody* is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody. In other cases, as in the diagram to the left, the primary antibody itself is conjugated to the enzyme.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows the secondary antibody has bound to primary antibody, which strongly implies the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in research.
- The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. Within common-sense limitations, the enzyme can go on producing color indefinitely, but the more primary antibody is present in the donor serum, the more secondary antibody + enzyme will bind, and the faster the color will develop. A major disadvantage of the indirect ELISA is the method of antigen immobilization is not specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface.

RESULTS AND DISCUSSIONS

A total of 363 serum samples were tested with immunological methods using indirect ELISA assay in Friedrich-Loeffler Institute (FLI), Germany. Through this technique it was possible to identified the presence of IgG antibodies in infected sera. The data presented in the table below indicates the presence of Crimean Congo Hemorrhagic Fever Virus in different areas of our country. These data can clearly proved for the first time the CCHF infection in cattle in many areas of our country. We have identified the infection in animals in regions which have no history of this infection in humans or have been many years ago, besides the traditional areas, where cases of this infection observed in humans were almost every year. This shows not only the spread of the infection, but at the same time, shows that in these areas there is a vector, and a virus present. The prevalence of infection in animals is obviously different in different areas. In traditional areas with the presence of CCHFV in humans, the prevalence of this infection in cattle is high for example in Has-Fejzaj is 16.67% and in Kolonje-Ersekeke-Kolonje is 8%, while in other areas for example in Gjirokastra-Picar and Rreshen where there was no evidence for the presence of CCHFV in humans, in cattle this infection is present in respectively in 2.1% and 2.6%.

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%**

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 10 years ago, where since then has not been observed cases with hemorrhagic fever in humans. This phenomenon is observed in Berat-Terpan area. 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 same assessment should be in other areas too, such as Gjirokastra and Rreshen regions. From the results of this study we should mentioned the presence of CCHFV infection mainly in mountain areas with different altitudes from sea level, whereas in areas such as Kavaja, Korca and Pogradec where the serum samples were taken from field areas in cattle, the antibodies were not present.

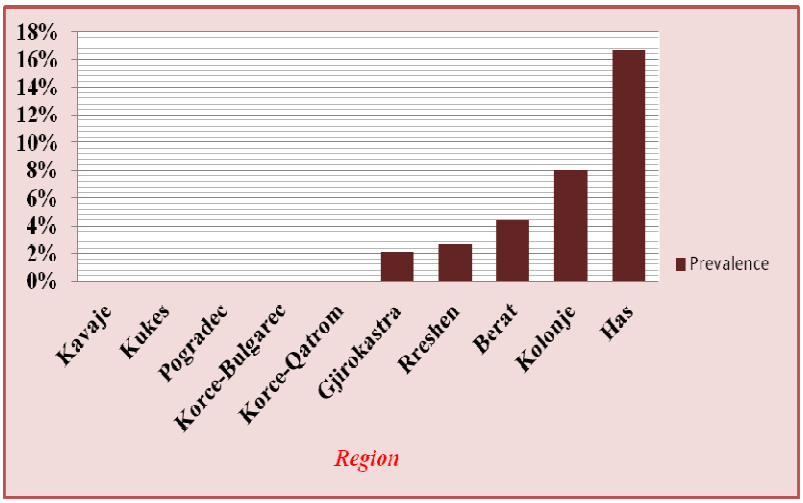

Figure 1: The Prevalence of CCHFV in Different Countries of Albania from Indirect-Elisa Results

We also emphasize that this infection is found mainly in female animals, which are kept in outdoor leisure suburbs wich creates very favorable conditions for infestation from the genus of Hyaloma tick. As shown in Table 1, to these animals are not taken protective measures against ticks.

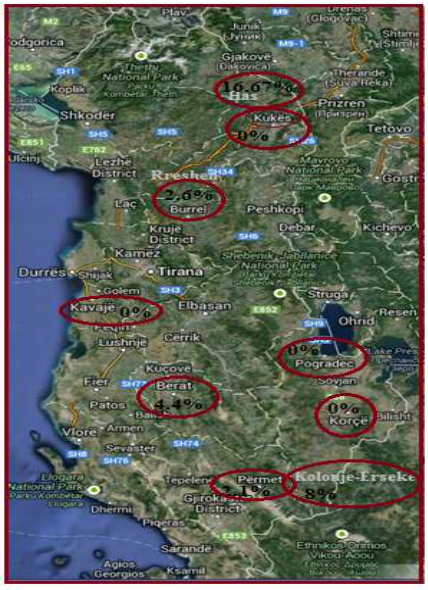

Figure 2: Positive Prevalence Presented Respectively in the Albanian Cities Map

From these preliminary results, we draw attention not only to human service but also to human veterinary too. 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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