Sheep as the Hosts of the CCHF and Tick in Kosovo

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Abstract

Crimean-Congo Hemorrhagic Fever (CCHFF) virus which cause danger disease in country of Balkan. But, this disease is none in Asia, Africa, South Evrope in Kosova, Albania, Macedonia, Greece, Bulgaria, and Serbia. There strong evidence that the Kosova sheep appear to play a role as virus and tick-vector host. There are evidences for presence of the tick genus *Hyalomma*, *Rhipicephalus, Ixodes*, and *Haemophisalis*. We were collected and identify 202 ticks from 2 municipality Prishtina and Malisheva area sheep. There is confirmed that the genus *Hyalomma* as the principal vector of CCHFV in endemic area of Malisheva municipality wih 7%, and *Rhipicephalus* as the principal vector of CCHFV non-endemic areas of Pristine with 90 %. We also confirm that the high presence of tick in endemic area Malisheve species *Hyalomma* (7%), *Ixodes* (2,5%), *Haemophisalis* (0,5%) and *Rhipicephalus* (90%) in non-endemic Pristine area. We also tested sheep from 5 municipalities as Peja, Gjakova, Junik, Prishtina, and Hani Elezit. It was tested 137 blood samples of sheep with the immunological ELISA method. Specific IgG antibody were detected in all sampled municipality, and detected high prevalence in municipality Gjakova 100% and Prishtina 83,33%,falowed with Peja 25 %, Hani Elezit 12 % and Junik 10%. In basis of result which we receive, we can conclude that the CCHFV is present and circulate in sheep of Kosova municipality in border of Albania, Serbia, Montenegro and Macedonia.

Keywords: Virus, CCHF, Sheep, Tick, Kosovo

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is tick-borne virus disease with high fatality. The causative agent is the CCHF genuses Orthonairovirus, family Nairoviridae, order Bunyavirales, the most tick-borne virus. Cases have been reported in Africa, Asia, Middle East, and South-Easte Europe (1,3,4,5). Significant incidence of disease increase in the last decades in southern-east Europe in Kosova (1,2,6,9,10) Table 1., with endemic and non-endemic zone in central and south part, with dry climate, hot summers and cold winter, abundant rainfall, low bushes, and density farming. These conditions provide an ideal of ecosystem condition for epidemiology of CCHF, special for main vector of CCHFV tick *Hyalomma*, *Rhipicephalus*, *Ixodes, Haemophisalis* genus (1,6,12,13) Table 2, Fig 3. The aim of the study was the determining the presence and prevalence of CCHF in sheep of 5 municipality (Peja, Gjakova, Juniku, Hani Elezit, Prishtina), which are in the border of 4 neighbor country; Albania, Serbia, Macedonia, Montenegro Fig.1.To investigate one connection with presence of CCHFV in Kosova and neighbor country (1,3,4,6,7,8,10,13,14). We tested 137 serums of blood samples, from sheep of non-endemic municipality, Peja, Gjakova, Junnik, Hani Elezit, and Prishtina.

We investigate possible of relation between presence of CCHFV in the 5 municipalities of Kosova which are in border of 4 neighbor countries of Kosova, and prevalence of the present cases in Kosova sheep, and cases in neighbor countries,where cases have been recorded (1,3,6). Kosova sheep was used as the host of vector tick, the collection of tick was taken 3 locality in 2 municipalities Malisheve (Dragobil village) and Prishtina (Graqanica, Hijalin).

Matherial and Methods

Investigation was done during 2012-2014 in 5 municipality of Kosova sheep which a in border of the counties; Albania, Macedonia, Serbia and Motenegro, Peja, Gjakova, Junik, Hanii Elezit, Prishtina. Serum and tick were collected during summer of 2012-2014 fom 137 sheep. Animals were not in the system of the identification unit of Kosova Food and Veterinary Agency. All collect sera were tested using the ELISA for quality and quantity determination IgG and IgM antibody to CCHFV in the Frirch-Loefler Institute (FLI) Greiswald, Germany. Samples with a positive or inclusive result in the in-house ELISA were rerun in a sheep adapted commercial CCHFV-IgG-ELISA. In cases of divergent results samples were tested in a sheep adapted commercial CCHFV-IgG-IFA (Evrooimun Lubeck, Germany) for final confirmation. Where 202 tick were collected from sheep in central area where of Kosova, where cases of CCHFV. Ticks are both environmental reservoir and vector for virus, carrying it from wild animals to domestic animals and human. Data suggest that domestic animals, especially sheep should be considered one of principal hosts of adult tick. Identification of tick genus was done with sepal methods for classification tick, using guide (Estrada Pena, A.Bouattour, J.L. Camicas & A .R.Walker 2004) (Fig 3). For discovery and determine of tick, we used properties which are: anus, mouth apparatus, cuticulla, abdomen, part of abdomen, tramp, genital apparatus, legs, palp, spiracule. Identification determination of tick was done in Agricultural Fakulty of Pristine.

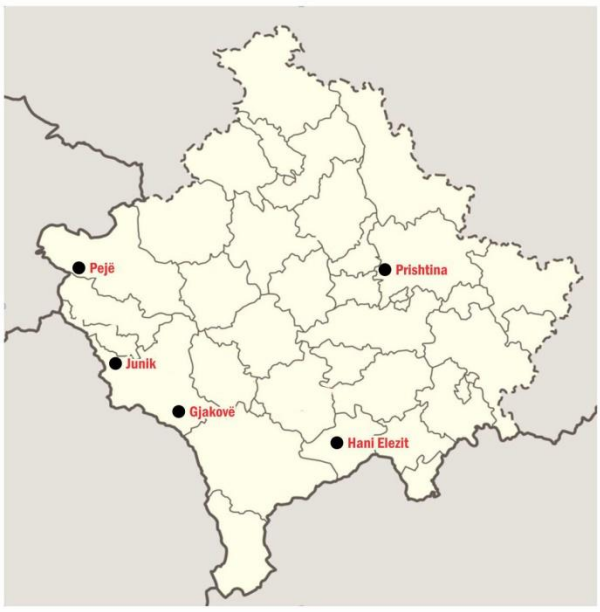


Fig - 1

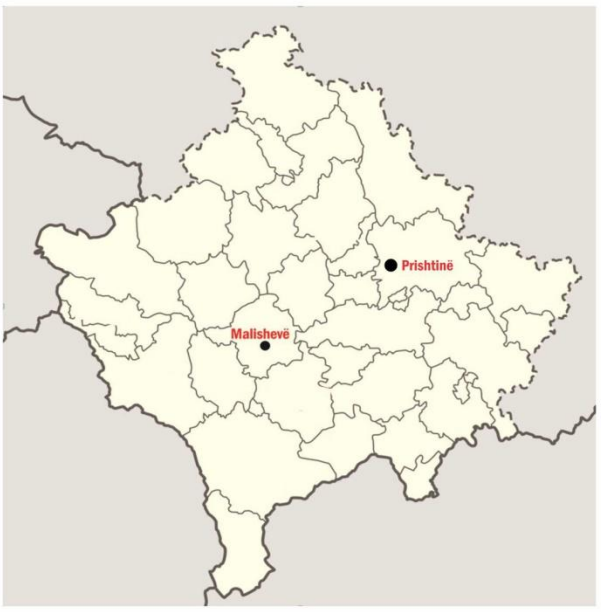


Fig - 2

Results

The first description of CCHFV from Kosova a reported for cases in the municipality Suhareka in the village Nishor and in village Ciflik in municipality Hani Elezit on the border with Macedonia (1). There is strong evidence for the sporadic cases have continuously recorded on this area of Balkan Peninsula since 1952 (1). Overall prevalence is comparable to other endemic countries in the Balkan region, Bulgaria 2,8%, Greece 4,2% and Turkey 2,3%, and notably lower than in earlier reports from Turkey 10-19,6%'.

In this investigation we tested 173 sheep from 5 municipality of Kosova ((Peja, Gjakova, Junik, H.Eleezit and Prishtina) they are in border of Montenegro, Albania, Macedonia and Serbia. In Peja 8 sheep, Gjakova 21, Junik 28, Prishtina 30, Hani Elezit 50. However, in 5 area of Kosova the infection on human in very low compering with endemic municipality of Malisheve 43,63% and Rahovec 25,25% of human cases, but in sheep of municipality in border of Albania-Gjakova was 100%, with Serbia, was Prishtina 83,33%, Montenegro, Peja 25%, Macedonia, H.Eleziit 12%.

Cases of CCHFV in sheep of Kosova in border with Albania and Macedonia a in corelacion with cases non in literature. The prevalence of IgG special antibody among sheep in Gjakova is extraordinary high.

The results of our study clearly show an overlap between the incidence rate of CCHFV in Kosova, sereoprevalenc in healthy human population and the sereoprevprvalenc in endemic area in sheep. From ecological point of view, the results are consistent with the ecological niches present in Kosova area. Sereoprevalence is highest in areas with low vegetation and high density of farming.

Whereas, our investigation of 202 sample of genus of tick present in Kosova show that (Table 2) (Fig 3) presence of species *Hyalomma* 29, species *Rhipicephalus* 137 cases, species *Ixodes* 13 cases and *Haemophisalis* 2 species, from total number of 202 collect ticks. But our results show that the in non-endemic area Prishtina is present genus *Rhipicephalus* with 137 cases from 202 tick collect in to area of Kosova (Table 2). This result is in connection with data from literature (1).

Table 2: Results from commercial CCHFV-IgG-IFA in sheeps.

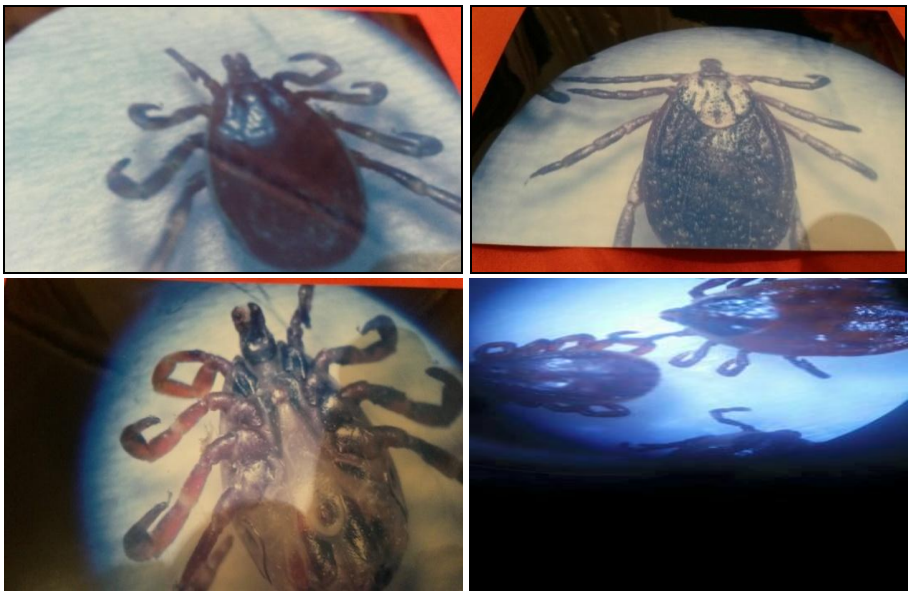
Region	Specie	Total Number	No of Positive Cases	Percentage
Prishtinë	Sheep	30	25	**83.33**
Hani Elezit	Sheep	50	6	**12.00**
Junik	Sheep	28	3	**10.71**
Gjakove	Sheep	21	21	**100.00**
Peje	Sheep	8	2	**25.00**
Total	Sheep	137	57	**41.61**

The data obtained during ticks typing is presented in the table below. The typing is done not only on the basis of the specie but also sex.

Data obtained during the typing of collected ticks

Nr.	Region where are collected	Total number of ticks	Type and sex of Ticks						
			Hyalomma		*Rhipicephalus*		Others		
							Ixodes	*Hemophisalis*	
			M	F	M	F	M	F	Without defining. sex
1	Prishtine (Graçanicë Hajkobill)	158	-	-	83	74			1
2	Malishevë (Malishevë, Astrazum)	44	18	11	-	-	5	8	2
	Total	**202**	**18**	**11**	**83**	**74**	**5**	**8**	**3**

Fig. 3 Ticks unde stereomicroscope identification



Discussion

Our result of investigation has confirmed that the majority of the seropositive animals located in border of municipality with Albania, Gjakova with 100%, fallowing Prishtina with 83,33 %, in border with Serbia, Peja with 25% in border with Montenegro, Hani Elezit with 12% in border with Macedonia, and Junik with 10%. Thereby prevalence in Gjakova municipality in border with Albania and neighbor endemic municipality Malisheve and Rahovec, is comparable with the data of the literature (1,2). This data is in connection with rates presence of the CCHFV in Albania and neighbor municipality Malisheve and Rahovec (1,2), since the overall sereoprevalenc rate in both countries is present. Overall sereoprevalenc is comparable to other endemic countries in Balkan region, Bulgaria 2,8%, Greece 4.2% and Turkey 2,3 % and notably lower than in earlier reports from Turkey (10-19.6%). Thereby, sereoprevalence in the hyper-endemic areas in Kosova is comparable to the report from Turkey. Since the sereoprevalenc rates in both counties are comparable.

Our investigation suggests that there is a high rate of unapparent infections in Kosova. From ecological point of view, the results are consistent with the ecological niches present in Kosova. This result is confortable also with result of investigation in cattle. Sereoprevalence in Kosova is generally consistent with cumulative incidence of CCHF from 1999 to 2013(1) in different municipality, with highest incidence of disease (1). These results suggest that CCHFV could have been present in the sheep in Kosova region in the past and has not been recognized. Of note are also neighbor municipality with endemic municipality are in correlation with high sereoprevalent cases as the Gjakova municipality with neighbor municipality Malisheva and Rahovec. There is an indication that CCHFV circulate in endemic and non-endemic area with highest prevalence of CCHFV in endemic municipality. From 173 sheep was examined serum of blood with animal IgG enzim-linked immunosorbent assay (ELISA) at FLI Grecifswald, Germany the presence of CCHF specific IgG of high prevalence of CCHF infection in Gjakova 100%, neighbor of Malisheva and Rahovec municipality, Prishtina with 83,33%,neghber with Malisheva municipality, Peja with 25%, H.Elezit wiith 12% and Juniku with 10 % of prevalence. It is important to emphasis that the Gjakova is loced near Hasi region in Kukes (Albania), and the circulation of the CCHFV is related with presence of CCHFV among sheep in Has-Albania. We state that the differences between Kosova and Albania is related with the number of the sheep examined in Kosova is higher than in Albani. We make this comparable as the climate condition of the mating season are more or les equal.

There is a need for further investigation in order to identify which wild animals play an important rol in hosting virus and tick as vector. Furthermore, from sheep of endemic municipality Malisheve, and non-endemic municipality Prishtina we collected 202 sample of tick for the investigation the species of tick present in Kosova (Fig.3). From literature (1,2) is non that the tick genus *Hyalomma*, *Rhipicephalus, Ixodes,* and *Hemophisalis* are the principal vector of CCHFV in Kosova. Sheep should be considering the principle host of adult tick-the vectors of the virus. For identification and specification of tick we used the guide of Estrada-Pena (2004). {Fig.3). Our investigation show that the genus *Hyalomma* (7,0%), *Ixodes* (2,5%) and *Hemophisalis* with (0,5%), are prevalent in endemic area, and in non-endemic area Pishtina prevalent is genus *Rhipicephalus* with (90%). This result are overlap with literature (1,2). The results of our study clearly show an overlap between the cases rates of tick and CCHFV. From an ecological point of view, the results are consistent with the ecological niches present in Kosova. The results of this study are important for the authority of Kosova that are responsible for monitoring the CCHFV in Kosova.

Conclusions

Crimean-Congo hemorrhagic fever (CCHF) is an acute, tick borne disease often associated with hemorrhagic presentations and high case fatality rate. Kosovo result highly endemic area for CCHF in sheep under ELISA examination. Our result of investigation in Kosovo showed prevalence 41.61% in total of examined sheep. ELISA results have confirmed that the majority of the seropositive animals located in border of municipality with Albania in Gjakova region, where all sheep result positive (100%). Following Prishtina with 83,33 %, in border with Serbia, Peja with 25% in border with Montenegro and Hani i Elezit with 12% in border with Macedonia. The lowest seroprevalence in the sheep was recorded in Junik with 10% of examined samples. The study confirmed that the genus *Hyalomma* as the principal vector of CCHFV in endemic area wih 7%, and *Rhipicephalus* as the principal vector of CCHFV non-endemic areas with 90 %. This study confirm that the high presence of tick in endemic area species *Hyalomma* (7%), *Ixodes* (2,5%), *Haemophisalis* (0,5%) and *Rhipicephalus* (90%) in non-endemic area.

Acknowledgments

We thank FLI Greifswald, Germany for their hard work in making this project.We are grateful to Prof. Dr Martin H. GROSCHUP for his help is doing work in this project. We also thank to Dr K.Sherifi for his help in identification and classification of tick.

References

1. Avsic-zupanac T (2007) Epidemiology of Carimean-Congo hemorrhagic fever in the Balkans. In; Ergonul O., Whitehous CA, editors CRIMEAN-CONGO hemorrhagic fever; A Global Perspectiv Dordrecht, Springer, 328.

2. Antoniadis, A. et al (1988) Crimean-Congo hemorrhagic fever in Greec in 1st International Siumposum on Hantaviruses and CCHF, Halkidiki, Greec.

3, Christova, I. (2006) Epidemiology of CCHFV In Bulgaria (Personal communication).

4. Drosten C., et al. (2002) CCHF in Kosova, J. Clin microbbiol 40:1122-1123.

5.. Eltari,E., et al (1988)Crimean-Congo Hemorrhgic feverin Albania, In 1st International Symposium Hantaviruses and CCHF ,Halkidiki, Greece p.34

6. Gligic, A., et al. (1977) The first isolation of the CCHFV in Yugoslavia, vojnosanitar pregd, endemic and non-endemic Bulgarian location.J vectr Borne Dis 50(4)265-220

8. Heneberg, D. at al.(1968) CCHFV in Yugoslavia, vojnosanit pregl 25; 181-184.

9. Hodgstrall H. (1977) The epidemiology of tick-born CCHF in Asia, Europ, and Africa. J.Med. Entomol. 15 (4) 307-417.

10. Humolli, F. at al. (2014) et al. (2014) Prevalence of CCHF in human population, livestock and tick in Kosova PLOS one Thenth ANNIVERSARY

11. Levi, V. (1972) seasonalll activity of thetick of the family Ixodidae in focus of Crimean hemorrhagic fever in Paradijak region.Savremena Med.23; 44-50.

12. Papadopulos, O., et al (1980) CCHF in Greece; isolation of the virus from Rhificephalus bursa tick and a preliminary serological surwei. In Vesnjak-Hirijan .Arboviruses in the Mediteran countries. Gustaf Ficher. Studgard.

13. Papa, A., et al. (2002) CCHF in Albania. Eur J. Clin. Miicrobiol. Infect disease 21; 603-606

14. Papa, A., et al (2004) CCHF in Bulgaria. Emerg Ifect Dis 10; 1465-1467.