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Article

Crimean–Congo Hemorrhagic Fever in Kosovo: Detection of *Orthonairovirus haemorrhagiae* in Ticks from Non-Endemic Areas

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Simple Summary

Crimean–Congo hemorrhagic fever (CCHF) is a severe zoonotic tick-borne disease caused by *Orthonairovirus haemorrhagiae*, with widespread distribution across Asia, Africa, the Middle East, and Europe, including the Balkans. In Kosovo, several endemic hotspots have previously been identified in the central and south-western parts of the country. To investigate possible viral circulation in non-endemic areas of eastern Kosovo, 745 ticks were collected throughout the years 2017–2019 from seven locations in the municipalities of Prishtina, Gjilan, and Kamenica. Viral RNA was detected in seven ticks (0.94%), of whom six were *Rhipicephalus bursa* (three in Hajvali/Prishtina and three in Busavat/Kamenica) and one was *Hyalomma marginatum* (tick collected in Bresalc village, Gjilan municipality), associated with a human CCHF case reported in a dairy farmer in 2018. These findings confirm the circulation of the virus in non-endemic regions of Kosovo and emphasize the importance of enhanced surveillance and targeted tick control measures.

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Abstract

Crimean–Congo hemorrhagic fever (CCHF) is a severe tick-borne zoonotic disease associated with high case fatality rates in humans and represents a significant public health threat in endemic areas of Kosovo. However, data on viral circulation in ticks in non-endemic regions remain limited. CCHF is caused by *Orthonairovirus haemorrhagiae*, formerly known as the Crimean–Congo hemorrhagic fever virus (CCHFV). In the present study, 745 ticks were collected from domestic animals and the environment throughout the years 2017–2019 in seven locations of non-endemic regions in the eastern part of Kosovo, respectively Prishtina, Gjilan and Kamenica municipalities. The ticks after species identification were analyzed using RT-PCR for detection of viral RNA of CCHFV. Seven ticks (0.94%)

tested positive, including six *Rhipicephalus bursa* ticks, three of them collected from grazing cattle and sheep in the village Hajvali (Prishtina municipality) and three others in the village Busavat (Kamenica municipality) from grazing cattle, while one positive sample was detected in a *Hyalomma marginatum* tick collected from a grazing dairy cow in Bresalc village (Gjilan municipality). The virus detected in *Rh. bursa* is considered to be consistent with the *Aigia virus,* formerly classified as CCHFV Clade VI/Europe 2 or the AP92-like lineage, which has generally been associated with a lower pathogenic potential and the absence of reported human cases in these areas. The first reported human CCHF case in a non-endemic area occurred in the Bresalc locality (Gjilan Municipality) in June 2018 in a dairy farmer. This occurrence coincided with the detection of *Orthonairovirus haemorrhagiae* RNA in one *Hyalomma marginatum* tick among 36 sampled ticks collected from the same farm, following clinical and laboratory confirmation of CCHF in infected patients. Consequently, this finding indicated the circulation of CCHFV in this area. These results demonstrated active circulation of CCHFV in tick populations in regions previously considered non-endemic and highlighted a potential public health risk associated with pathogenic strains. Genomic and tick surveillance, combined with targeted vector control measures and public awareness, are essential for reducing CCHFV transmission and preventing human infections.

Keywords: Crimean–Congo hemorrhagic fever; *Orthonairovirus*; ticks; *Rhipicephalus bursa*; *Hyalomma marginatum*; Surveillance; Kosovo

1. Introduction

Crimean–Congo hemorrhagic fever is a severe tick-borne zoonotic disease associated with high case-fatality rates of up to 40%. The disease is widely distributed across Africa, Asia, the Middle East, and South-Eastern Europe, including the Balkan region [1,2]. In humans, CCHF is caused by an *Orthonairovirus* primarily transmitted by *Hyalomma* ticks, which act as vectors and reservoirs of the virus. Wild and domestic animals may become infected and develop transient viremia without exhibiting clinical manifestations, thereby acting as amplifying hosts [3]. Domestic ruminants, including cattle, sheep, and goats, play a role in the transmission cycle by infecting feeding ticks and serve as valuable sentinels for public health surveillance, since increased animal seroprevalence frequently precedes the occurrence of human cases [4]. In recent years, the distribution of *Hyalomma* ticks has expanded to different countries due to climate change and the movement of infested migratory birds, increasing the risk of CCHF virus spreading to previously nonaffected regions [5–10]. CCHF occurs sporadically or is endemic in some regions in the Balkans and Asian countries such as Turkey, Bulgaria, Kosovo, Albania, and Greece [11]. Between 1944 and 2025, over 2000 CCHF cases were reported in Europe, mainly in southern and eastern regions. The highest numbers of human cases were recorded in Bulgaria (1623), Kosovo (339) and Albania (146) [2]. According to recent national surveillance data, the Asian part of Turkey reports the highest number of CCHF cases between the years 2011 and 2024, a total of 11,811 laboratory confirmed cases, with an average of about 844 cases annually and a case fatality rate of 4.7% [12]. In 2023 and 2024, five human CCHF cases were reported in the neighboring country, North Macedonia. Genetic analysis of the virus strain showed that it clustered with regional strains belonging to Europe 1 (Clades V) and was most closely related to CCHFV strains previously identified in Kosovo [13,14].

In Kosovo, between 1995 and 2019, 245 cases of CCHF in humans have been registered, of which 64 have died (case fatality rate 23.7%), while most of the infected patients were farmers [15,16]. The last CCHF outbreak in Kosovo occurred in 2013, where out of 26 infected people, 11 of them ended fatally [17]. Kosovo is considered an important CCHF virus hotspot and a relevant setting for studying virus ecology as well as for future vaccine and therapeutic research [18]. Kosovo's climate is influenced by a continental air mass, leading to cold winters, hot and dry summers, and mild transitional seasons, these conditions are suitable for *Hyalomma* ticks. The detection of CCHFV has been confirmed in three tick species in Kosovo. *H. marginatum*, *Rh. bursa* and *I. ricinus. H. marginatum* remains the main vector and carrier of the highly pathogenic virus strain, grouped in Clades V/Europa 1 (Kosova Hoti) [19]. CCHF virus detected in tick species of *Rh. bursa* seems to be the vector of a mild pathogenic or non-pathogenic strain for humans, grouped in Clades VI/Europa II (like AP92), newly named *Aigai* virus (AIGV) or *Orthonairovirus parahaemorrhagiae* [20]. Of 1102 tested ticks in the year 2012, 40 (3.6%) were CCHF viral RNA-positive, belonging to *H. marginatum* (29 positive), *Rh. bursa* (10 positive), and *I. ricinus* (one positive). CCHFV was detected in *H. marginatum* from Malisheve and Kline municipalities, whereas CCHFV was detected in *Rh. bursa* ticks originated from Prishtina municipality. CCHFV was detected only in one *I. ricinus* in the Kline municipality [21]. The distribution of competent tick vectors is determined by the range of their vertebrate hosts and the environmental conditions necessary for its development to adulthood. Within a given area, tick population density may vary annually depending on host availability, climatic conditions, vegetation changes, and other factors influencing molting success and reproductive output of ticks [22]. Considering that CCHF occurs in limited endemic regions of the country, this study aimed to examine the presence of the virus in ticks collected from the non-endemic regions in the eastern part of Kosovo, namely in Prishtina, Gjilan and Kamenica municipalities and to assess the risk of spread of infected ticks from endemic areas to non-endemic regions.

2. Material and Methods

2.1. Location and Tick Sample Collection

Tick samples were collected from seven locations in the municipalities of Gjilan, Prishtina, and Kamenica, which are characterized by a predominantly continental climate with warm summers, moderate cold winters and rainfall throughout the year, especially during spring and autumn. The relief of these municipalities mainly consists of plains, hills, and mountainous terrain. Prishtina is largely situated in the Kosovo plain with gently rolling hills, whereas Gjilan and Kamenica are characterized by more hilly and mountainous landscapes suitable for agriculture and livestock grazing with average elevations of 600 to 800 m. Sampling sites were selected randomly in collaboration with local veterinarians and animal owners, based on reported tick infestations in grazing animals. Environmental sampling sites using the flagging method were also selected randomly.

In total, 745 tick specimens were collected. Ticks were collected using two methods: manual removal using forceps from domestic animals (cattle and sheep) to collect *Hyalomma marginatum* (Figure 1) and *Rhipicephalus bursa*. Flagging method to collect *Ixodes ricinus* and *Dermacentor marginatus* from vegetation using a white flag (1 m $\times$ 1 m). All samples were collected between May and July from 2017 to 2019. During fieldwork, standard personal protective equipment was used, including latex gloves, protective goggles, and masks. The sampled ticks were placed in 2 mL tubes and stored at -80 °C in a deep freezer at the Laboratory of Parasitology, Faculty of Agriculture and Veterinary, University of Prishtina. The genus and species identification of ticks were carried out using a stereomicroscope, following standard taxonomic identification keys [23].

Figure 1. *Hyalomma marginatum* ticks in the udder region of grazing cattle.

2.2. Tick Collection and Detection of CCHFV in H. marginatum Ticks Associated with the First Report of CCHF in a Dairy Farmer from Non-Endemic Areas

During the second week of May 2018, while tick sampling was being conducted in non-endemic locations of Gjilan municipality, a severe CCHF case was reported in a dairy cattle farmer from the village of Bresalc. This case was epidemiologically monitored by the National Institute of Public Health, including laboratory findings, where the diagnosis of CCHF was confirmed. Two weeks later in the same dairy farm, 36 tick samples were subsequently collected and placed in 2 mL tubes for further testing.

2.3. RNA/DNA Extraction from Ticks

Individual ticks were transferred into 2 mL Eppendorf tubes containing three to five stainless steel beads (7 mm; Qiagen, Hilden, Germany) and pre-cooled in liquid nitrogen. The tubes were then processed using a TissueLyser LT (Qiagen) equipped with a −20 °C cooled rotor at 50 Hz for 2–5 min to achieve mechanical disruption and homogenization. In some cases, repeated freezing and lysis cycles were required to ensure complete homogenization, as tissue disruption was not always uniform. Samples were resuspended in 300–500 µL of phosphate-buffered saline (PBS), depending on tissue size, supplemented with 500 IU/mL penicillin, 10% fetal bovine serum, and 500 µg/mL amphotericin B, followed by centrifugation at 2000× *g*. Viral RNA/DNA extraction was then performed using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions.

2.4. Reverse Transcription-PCR (RT-PCR) for the Detection of Viral RNA of CCHFV

The extracts of up to 5 ticks were pooled to reduce testing costs, following standard surveillance protocols for tick-borne pathogens. Pools testing positive were subsequently de-pooled and individual ticks re-tested to identify CCHFV-positive specimens. For the initial RT-PCR screening, 2 µL of RNA from up to five individual tick extracts were pooled (10 µL total), resulting in 149 pools for CCHFV detection. Only pools testing positive for CCHFV were further analyzed through individual tick PCR assays. Detection of CCHFV (Europe 1, Clade V; Europe 2, Clade VI or Aigai virus) was performed using the RealStar® CCHFV RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) according to the manufacturer's protocol. All amplification and sequence analysis were performed using a Bio-Rad CFX96® system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

3. Results

From a total of 745 ticks, four species were identified: *H. marginatum*, *Rh. bursa*, *I. ricinus, and D. marginatus.* Among the ticks, *H. marginatum* was the predominant species, accounting for 392 (52.6%) of the total sampled ticks from domestic animals. The second most prevalent species was *Rh. bursa* with 245 specimens (32.8%), followed by *I. ricinus* with 63 (8.4%), while *D. marginatus* represented the lowest frequency with 45 specimens (6%). The distribution of tick species varied among the investigated locations. A total of 83 ticks were collected using the flagging method, comprising 38 *I. ricinus* and 45 *D. marginatus*. All specimens tested negative for CCHFV. In the Municipality of Gjilan, Cernice village, 91 ticks were collected, of which 78 belonged to *H. marginatum* and 13 to *Rh. bursa*. No positive ticks for CCHF virus were detected in this locality. In Bresalc village, 36 ticks were sampled from one dairy farm, consisting mainly of *H. marginatum* (35) and one *I. ricinus*. Molecular testing revealed one positive sample for CCHF viral RNA in *H. marginatum*, representing 2.7% positivity in this locality. In Livoç village, a total of 167 ticks were identified, including 84 *H. marginatum*, 38 *I. ricinus*, and 45 *D. marginatus*. All ticks from this locality were negative for CCHF viral RNA.

In Hajvali (Prishtina), 239 ticks were collected (226 *Rh. bursa*, 13 *H. marginatum*). Three ticks (1.25%) tested positive for CCHFV, all from adult *Rh. bursa* ticks, in two female ticks collected from two individual cattle and one male tick collected from one sheep, confirming circulation of this species, as well as a potential vector of CCHFV in the area.

In Kamenica municipality, tick sampling was conducted in three locations, namely in Shipashnicë, Busavat, and Bratillocë. In Shipashnicë, 27 ticks were found, including 25 *H. marginatum* and two *I. ricinus*, and all samples tested negative for CCHF viral RNA. In Bratillocë, 35 ticks were sampled, dominated by *H. marginatum* (33) and two *I. ricinus* ticks, with no positive findings for CCHFV. The highest number of sampled ticks in Kamenica municipality was collected in the village of Busavat, where 150 ticks were collected and analyzed. Of these, 124 belonged to *H. marginatum*, six to *Rh. bursa*, and 20 to *I. ricinus*. Importantly, for the first time in this locality, three tick samples (2%) tested positive for CCHF viral RNA. The virus was detected only in *Rh. bursa* adult ticks, in two female and one male tick collected from three individual cattle. This finding highlighted the epidemiological significance of this locality as a potential source of CCHFV circulation and suggests the involvement of *Rh. bursa* in maintaining the virus in this area and its spreading through the movement of domestic animals.

Out of seven investigated locations, CCHFV was detected in three, suggesting its circulation in non-endemic areas. Overall, of 745 tested tick samples, seven (0.94%) were CCHFV positive. CCHFV was detected in six *Rh. bursa*, whereas one positive sample was detected in *H. marginatum*. The virus was not detected in *I. ricinus* and *D. marginatus*. The predominance of positive findings in *Rh. bursa* suggested that this species may play an important role in the ecology and transmission of CCHFV in Kosovo in non-endemic areas (Table 1 and Figure 2).

Table 1. Detection of CCHF viral RNA in ticks from non-endemic regions, 2017–2019.

Municipality	Village	Nr. Ticks	*Hyalomma marginatum*	*Rhipicephalus bursa*	*Ixodes ricinus*	*Dermacentor marginatus*	CCHFV
Gjilan	Cernicë	91	78	13	/	/	Negative
	Bresalc	36	35	/	1	/	1 positive *H. marg.*
	Livoç	167	84	/	38	45	Negative

Table 1. *Cont.*

Municipality	Village	Nr. Ticks	*Hyalomma marginatum*	*Rhipicephalus bursa*	*Ixodes ricinus*	*Dermacentor marginatus*	CCHFV
Prishtina	Hajvali	239	13	226	/	/	3 positive *Rh. bursa*
	Shipashnicë	27	25	/	2	/	Negative
Kamenica	Busavat	150	124	6	20	/	3 positive *Rh. bursa*
	Bratillocë	35	33	/	2	/	Negative
Total		745	392	245	63	45	7 positive

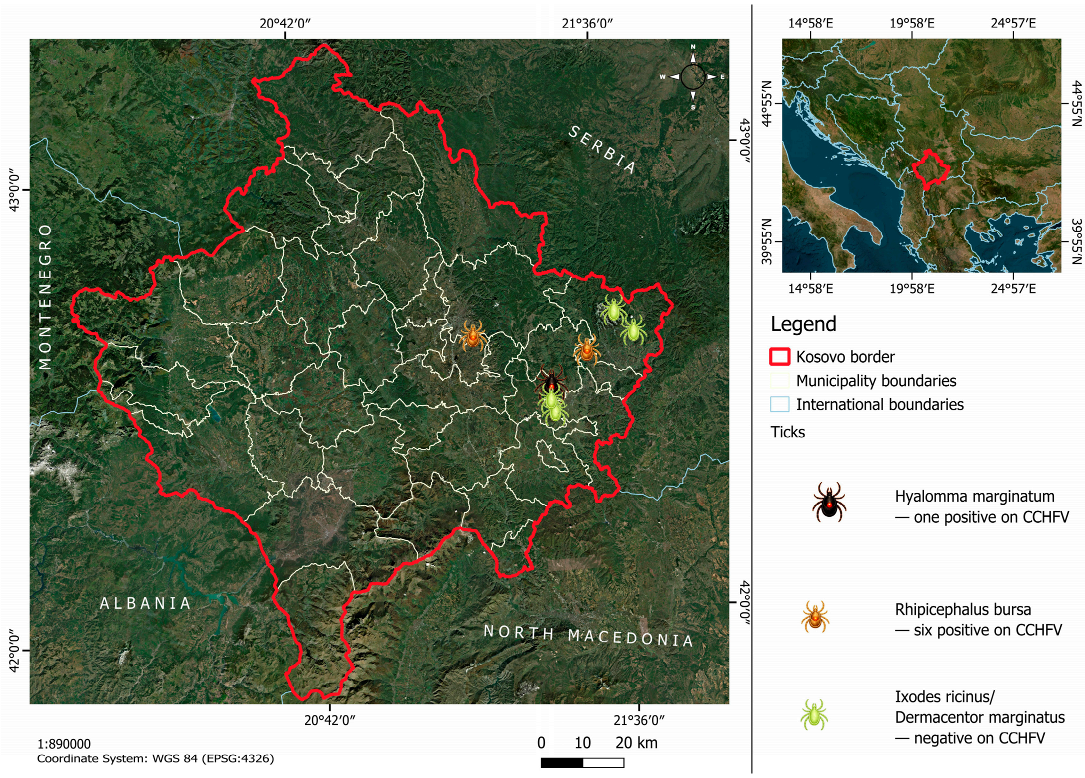

Figure 2. The map of tick distribution and detection of CCHFV in non-endemic regions. Color coding indicates tick species and CCHFV detection status. *Hyalomma marginatum* (black with a red dot) indicates a vector associated with highly pathogenic CCHFV strains, whereas *Rhipicephalus bursa* (brown-orange) indicates a vector associated with low-pathogenicity or non-pathogenic CCHFV strains. CCHFV was not detected in *Ixodes ricinus* or *Dermacentor marginatus* (yellow).

Detection of CCHFV in H. marginatum Ticks Associated with the First Report of CCHF in a Dairy Farmer from Non-Endemic Areas

In the second week of May 2018, the first case of CCHF was reported in a dairy farmer in the locality Bresalc, Gjilan municipality, previously known as a non-endemic area. Two weeks after receiving the information from the Clinic of Infectious Diseases at the University Clinical Centre of Kosovo in Prishtina regarding the clinical and laboratory confirmation of the CCHF case, we collected 36 ticks from the dairy grazing cattle, 35 of them were identified as *H. marginatum*. In one *H. marginatum* male tick, CCHF viral RNA was detected by RT-PCR, indicating that the farmer had been infected through contact with the infected tick. Following discussions with the patient's family, it was confirmed that the infected farmer had continuously removed ticks from the cattle with his bare hands, believing that this area is non-endemic for CCHF and therefore posed no risk. The patient's first visit to a physician during the pre-hemorrhagic phase (fever, dizziness, weakness and headache) was not considered suspected for CCHF by physicians at the first investigation. Four days later, after the appearance of hemorrhagic signs (hematemesis and melena), and upon the recommendation of an infectious disease specialist, the patient was transferred to the Clinic for Infectious Diseases of the University Clinical Centre in Prishtina. Immediately after hospitalization at the clinic, the patient was confirmed positive for the presence of the virus and treatment was done according to standard protocols. The patient has survived the CCHF disease.

4. Discussion

The present study confirms the presence of CCHFV in ticks collected from the non-endemic regions of Kosovo from 2017 to 2019, in the municipalities of Prishtina, Gjilan and Kamenica. However, in May 2018, a confirmed human case was reported in a dairy cattle farmer from the village of Bresalc in Gjilan, marking the first detection of the virus in ticks from this locality. In the study, out of 745 ticks tested by RT-PCR, seven (0.94%) were detected positive for the virus. The majority of positive samples were found in *Rh. bursa* ($n = 6$), with three positives from Hajvali (Prishtina municipality) and three from Busavat (Kamenica municipality). Additionally, one positive *H. marginatum* tick, sampled from a grazing cow in Bresalc (Gjilan), was infected with the virus. A 2012 surveillance study documented higher CCHFV prevalence in *Rh. bursa* in the same Hajvali locality (10/131, 7.6%) compared to our 2017–2019 findings (1.25%), suggesting annual or multi-annual fluctuations in viral prevalence [21]. These findings indicate the circulation of the virus in tick populations within these localities, even in the absence of clinically reported human cases. Molecular studies showed that CCHFV strains detected in *Rh. bursa* is of low pathogenicity or non-pathogenic, clustering within Clade VI, Europe 2, closely related to the AP92-like strain previously identified in Greece and recently named Aigai virus. The absence or rarity of human CCHF cases in areas where infected *Rh. bursa* ticks predominate may be associated with the lower pathogenicity of these viral strains [20]. There are limited reports of human infections associated with this virus strain and the have mostly been presented with mild clinical manifestations or subclinical infections [24].

The first report of a CCHF case in a dairy farm in 2018 in a non-endemic region was associated with the detection of CCHFV in *H. marginatum* ticks collected from the dairy cows, which confirms the circulation of a highly pathogenic virus strain in this region. A similar infection scenario was observed in endemic areas, where, in 2014, a farmer from Senik village, Malisheve, was infected with CCHF after removing ticks from animals. Among a total of nine ticks collected from a dairy cow, one tick tested positive for CCHFV [25]. Phylogenetic analyses from previous studies have shown that CCHFV strains detected in *H. marginatum* ticks belong to Clade V (Europe 1/Kosovo Hoti), which are highly pathogenic for humans and are causing severe disease in humans with a high lethality rate. The distribution of *H. marginatum* in eastern Kosovo, including Prishtina, Gjilan and Kamenica, with increasing numbers of infested livestock, especially in grazing cattle, is evidence that this invasive tick species is increasing in number and expanding geographically. Some hotspots in the municipality of Malisheva, Rahovece, Klina and Suhareka (central and south-western part of Kosovo) were known as endemic areas for CCHF in humans, with major outbreaks recorded in the past. The last outbreak was recorded in 2013, in which, from 26 infected persons, 11 have died, predominantly people engaged in agricultural and livestock-related activities [15]. This outbreak was strongly associated with the high abundance of *H. marginatum* infected ticks, which constitutes over 90% of the tick population in certain areas, with an infection rate from 1 to 10%, in addition to infectious ticks in some endemic hot spots, the prevalence reached up to 30% [21].

CCHFV circulates in an enzootic cycle between ticks and various vertebrate hosts. Human infection risk depends on virus prevalence in tick populations and the frequency of tick exposure. In Kosovo, periodic outbreaks have been recorded in endemic regions, typically occurring every 3–5 years in the past, registered from 1995 to 2013, likely reflecting natural enzootic cycles and fluctuating viral prevalence in ticks. A significant risk factor for virus spreading is the movement of animals (livestock and wild animals) or through migratory birds. The environmental and climatic conditions, as well as the presence of different numbers of animals in a specific area, may create a favorable milieu for tick survival and reproduction, which has recently shown increased prevalence of *H. marginatum* in the Gjilan municipality with 67% compared to the study in 2012 with 52.2% [21]. From 2014 to 2019, Kosovo reported a considerably lower number of CCHF cases compared to other previous years. This CCHF reduction in cases may be attributed to the implementation of control measures by various national institutions. Since 2013, Kosovo has established an inter-ministerial committee for prevention and control of CCHF, led by the Ministry of Health and involving relevant institutions such as the National Institute of Public Health, Clinic for Infectious Diseases, as well as the Veterinary, Environmental and Agricultural Institutions. In addition, these institutions have received support from international projects, including the "German Biosecurity Programme", which has facilitated the implementation of the national action plan, particularly tick surveillance activities. Key preventive measures of the action plan were focused on increasing public awareness, acaricide treatment of livestock again ticks, strengthening diagnostic capacity, and increasing of biosecurity levels. Since 2014, a livestock acaricide treatment program has been partially implemented in some endemic regions.

The observed reduction in the number of CCHF cases in Kosovo may also be partly attributed to decreased human–tick contact. In recent years, the increased use of modern mechanized agricultural technologies has reduced the need for manual works on agricultural land. In contrast to earlier periods, when agricultural activities were predominantly performed by hand and a larger proportion of the rural population spent extended time in fields, the frequency of exposure to tick infested environments was considerably higher. The education of farmers on avoiding the manual removal of ticks has also contributed in reducing the number of CCHF cases. Educational programs for farmers and individuals at risk should continue consistently to prevent human cases, together with integrated surveillance of ticks, animals, and humans under the concept of "One Health" in both endemic and non-endemic regions of Kosovo.

5. Conclusions

This study confirms the CCHFV circulation in ticks in non-endemic regions in the eastern part of Kosovo (Prishtinë, Gjilan and Kamenicë). The detection of CCHFV was confirmed in two tick species, namely in *Rh. bursa* ticks, which are vectors and carriers of low or non-pathogenic strain (Aigai virus), found in Hajvali (Prishtine) and Busavat (Kamenice), while a highly pathogenic strain was detected in one *H. marginatum* tick from Bresalc (Gjilan). This finding is associated with the first report of a CCHF case in a dairy farmer. Considering the epidemiological situation of CCHF in Kosovo, continuous surveillance and coordinated action plans by national public health institutions are essential for monitoring CCHFV circulation and implementing effective prevention and control strategies. Ongoing studies should focus on livestock, wildlife, and tick populations to better define endemic areas and assess human infection risk. The development of a wide national risk map for CCHF would significantly support public health decision making and preventive strategies.

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Institutional Review Board Statement: The University of Prishtina regulates research ethics through the Regulation on the Work and Procedures of the Ethics Committee for Scientific Research. According to this Regulation, the Ethics Committee reviews research activities involving humans and animals when the proposed procedures raise ethical concerns or involve interventions that may affect the welfare, integrity, or rights of research subjects. The research in question involved the collection of ticks from domestic animals for epidemiological and scientific purposes. Furthermore, tick removal from domestic animals is considered a routine veterinary and husbandry practice. Consequently, it is not regarded as an invasive experimental procedure requiring ethical review or approval. Based on the provisions of the University's Regulation, the collection and removal of ticks from domestic animals as conducted in this study did not require review or approval by the Ethics Committee for Scientific Research of the University of Prishtina.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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