Journal of Zoonotic Diseases
https://jzd.tabrizu.ac.ir/
Published by the University of Tabriz
Online ISSN: 2717-2910

The first serological detection and risk factors analysis of tick-borne Crimean-Congo hemorrhagic fever virus among sheep in Algeria

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Article type:
Original article

Keywords:
Algeria
Antibodies
Crimean Congo Hemorrhagic Fever
Sheep
Zoonotic

Article history:
Received:
August 18, 2024
Revised:
September 6, 2024
Accepted:
September 27, 2024
Available online:
October 19, 2024

Abstract

The Crimean-Congo hemorrhagic fever (CCHF) is one of the zoonotic arboviral diseases transmitted by ticks. It is endemic in several parts of the world, including some African countries. This study was carried out to determine the possible circulation of the CCHF virus in Algeria. To this end, the study was carried out in several regions of northeastern Algeria, in which the sheep species was particularly targeted because of its importance in the epidemiology of the disease. Blood samples were collected from 276 sheep between September and November 2023, and the obtained sera were analyzed using an Enzyme-Linked Immunosorbent Assay (ELISA) to detect the presence of anti-CCHF virus antibodies. Region, age, sex, livestock farming type, and farm management system were analyzed as potential risk factors using a Chi-square ($\chi 2$) test and a multivariate regression analysis. The results revealed an overall prevalence rate of 39.13 %, suggesting the exposure of the sheep population to the CCHF virus, and hence the circulation of the virus throughout the study region. Region, age, and livestock farming type were determined to be potential risk factors associated with exposure to the CCHF virus. This is the first study to report the circulation of the CCHF virus among the Algerian sheep population. Further studies should be carried out to better understand CCHF epidemiology in the country.

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https://doi.org/10.22034/jzd.2024.18672
https://jzd.tabrizu.ac.ir/article_18672.html
Cite this article: Bennia S.E.G., Maamcha B.Z., Tiar G., Frissou N., Degui D., Rezoug F., Bouzid H., and Lamara A. The first serological detection and risk factors analysis of tick-borne Crimean-Congo Hemorrhagic Fever virus among sheep in Algeria. Journal of Zoonotic Diseases, 2025, 9 (2): 752-761

Introduction

The CCHF is one of the serious arboviral diseases transmitted by ticks (1), which is caused by a virus belonging to the Orthonairovirus genus of the Nairoviridae family (2). It was first described in 1944 in Crimea, before being isolated in Congo in 1956 (3). The disease is now widespread in several countries in Europe, Asia, and Africa (4), with approximately 50 countries currently recognized as endemic (5). Although the presence of CCHF is regularly reported in some countries, it is only occasionally described in others (6), and has sometimes been reported only after epidemiological investigations (7, 8). In recent years, the number of human cases has continuously increased, with mortality rates sometimes reaching 50 % during epidemics (9), posing a potential threat to health, especially in the absence of a specific treatment or an approved vaccine (10). However, the epidemiological data remain uncertain (11). The CCHF virus has been isolated from various tick species, although ticks of the *Hyalomma* genus are recognized as the main reservoirs and vectors of the disease (12).

The presence of the CCHF virus has also been documented in a wide range of domestic and wild animal species (2), which are asymptomatic reservoir hosts and sometimes amplifiers (6, 11). While little is known about the epidemiological situation of the CCHF virus in certain North African countries, it seems that Algeria is still being classified among the areas free from the disease, as no indigenous cases have been reported. However, infection with the CCHF virus has been reported in *Hyalomma aegyptium* ticks, and recently in dromedaries (13, 14). In addition, the infection has been reported in certain Algerian-neighboring North-African countries such as in Morocco, where infected ticks associated with migratory bird species have been reported (15), and also in Tunisia in a tick from a dromedary (16). Furthermore, there is serological evidence of infection by CCHF virus in humans and certain livestock species reported in Tunisia (17).

Although many domestic and wild animals, including livestock, can be infected with the CCHF virus, small ruminants are considered the most suitable domestic indicators of viral circulation during sero-epidemiological surveys (18). Therefore, we aimed to investigate the possible presence and circulation of the CCHF virus in the sheep population in Algeria, and to identify the associated risk factors.

Materials and methods

Study region

A cross-sectional study was conducted between September and November 2023 to determine the presence of anti-CCHF virus antibodies among sheep populations in different regions of northeastern Algeria. Different regions, with special geographical and bioclimatic diversity, situated in the regions of Bordj Bou Arreridj (36° 04′ 00″ N, 4° 46′ 00″ E), Mila (36° 26′ 59″ N, 6° 15′ 51″ E), Constantine (36° 17′ 00″ N, 6° 37′ 00″ E), Annaba (36°54′00″ N,7°46′00″ E), and El Taref (36° 46′ 02″ N, 8° 18′ 50″ E) constituted the geographical regions targeted by the study. The overall study area extends for 14 487 km^2, and it is characterized by mountainous terrain, high plains, and coastal plains, exhibiting a variety of bioclimatic zones ranging from humid to semi-arid (Figure 1).

Sampling and data collection

A random sample was drawn from the different study zones. The minimum sample size was determined according to Thrusfield and Brown's sample size formula (19), based on an expected prevalence of 18.1 % (20), with a 95 % confidence level and an absolute precision of 5 %. Accordingly, a total of 276 sheep blood samples were randomly collected from the following study regions: Bordj Bou Arreridj (n=92), Mila (n=51), Constantine (n=47), Annaba (n=52), and El Tarf (n=34). To avoid introducing age bias, sheep over four years of age were excluded from this study because they represent a minority compared to the whole population, which is dominated by young sheep for

economic reasons. The blood samples were taken from each animal in pre-identified vacutainer tubes, and then stored at a temperature of +4 °C, before being transported to the laboratory. After centrifugation at 3000 rpm for 5 min, the obtained serum was placed in pre-labeled Eppendorf tubes and systematically stored at a temperature of -20 °C until use. In addition, through a questionnaire, specific data were collected from each farm concerning the farm management system (intensive/semi-intensive), livestock farming type (cattle and sheep farms versus sheep-only farms), and age of the animals.

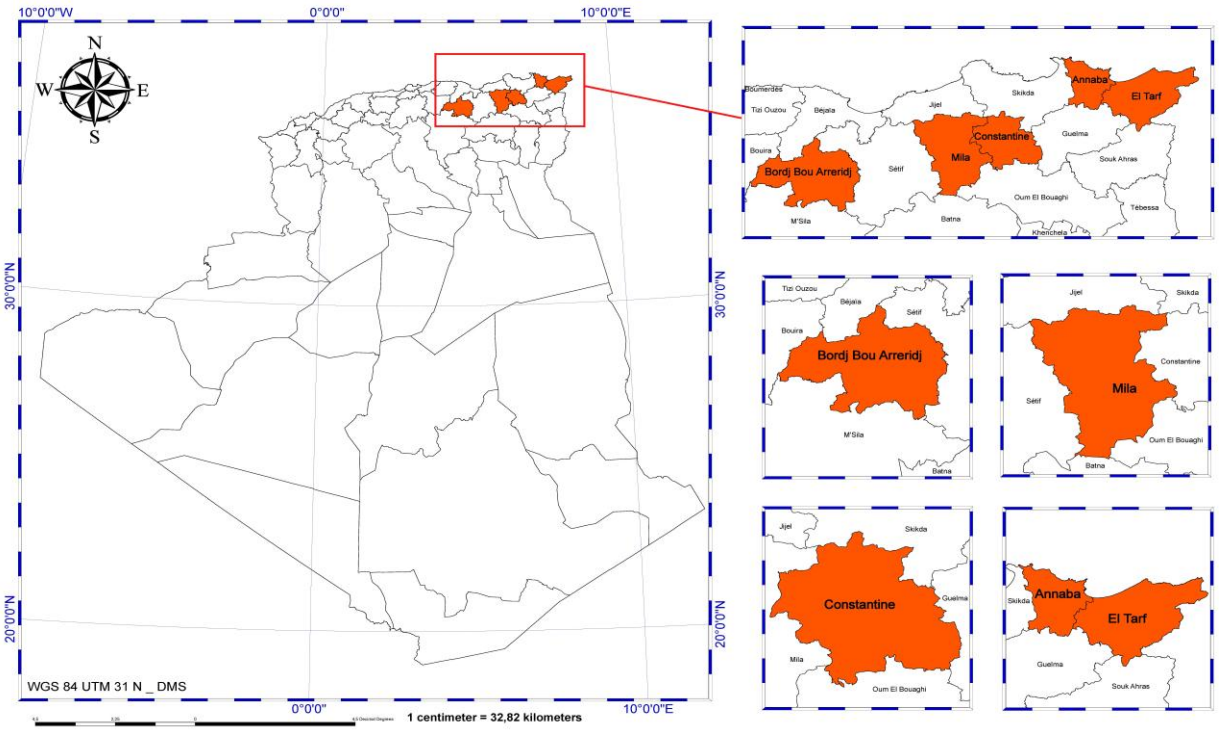

Fig 1. Study Regions

Serological test

To determine the presence anti-CCHF virus antibodies, the serum samples were tested using the Enzyme-Linked Immunosorbent Assay (ELISA) kit, IDScreen®, CCHF Double Antigen Multispecies, (IDVet, Grabels, France) following the procedures defined by the manufacturer (21). The results were interpreted based on the optical density (OD), read at 450 nm using an automated ELISA microplate reader (BioTek ELx800GIDX, USA) and ID SOFT™ software. For each sample, the positivity percentage (S/P%) was determined using the following formula: Optical density ratio of the sample (OD sample)/ the optical density of the positive control (ODpc) × 100. Samples with S/P% values higher than 30 % were considered positive.

Statistical Analyses

The seroprevalence was determined with a 95 % confidence interval (CI). For risk factor analysis, an initial univariate analysis of data was performed to select the independent variables associated with the seropositivity of the CCHF virus using chi-square ($\chi 2$) tests, with a level of significance set at $p< 0.05$.

A multivariate logistic regression model was then used to evaluate the strength of the association between the risk of CCHF virus infection and the potential risk factors. Odds ratios (OR) and CIs set at 95% were also calculated. The level of significance was set at $p< 0.05$. The statistical analyses were performed using the IBM SPSS statistics software, version 27.0.

Results

From a total of 276 serum samples collected from sheep coming from five regions situated in northeastern Algeria, ELISA test results revealed 108 seropositive cases for anti-CCHF virus antibodies, representing a global seroprevalence of 39.13 % (95 % CI: 33.34–44.92 %), with rates ranging from 19.23 % to 57.61 % depending on the study region. The results of the univariate analysis (Table 1) showed a marked difference between the prevalence rates recorded in the different study regions ($p< 0.001$). In addition, the animals aged 2–4 years showed a statistically significantly higher prevalence rate than the younger group. A similar trend was observed in the context of livestock farming type and the sex of the animals, where a statistically significantly higher prevalence ($p< 0.001$) was detected in the mixed livestock farms compared to the sheep-only farms, as well as among females compared to males ($p< 0.001$). Furthermore, although the prevalence of CCHF observed in the semi-intensive farms 41.04 % (95 % CI: 34.62–47.46 %) was numerically higher than that of the intensive farms 29.78 % (95 % CI: 16.21–43.35 %), no statistically significant influence of the farm management system was found (p=0.15).

Table 1. Univariate Analysis of Potential Risk Factors Associated with CCHF Virus Seropositivity

	Variable	N. of collected sera	Serum positive for CCHF Virus	Prevalence % (95% CI)	χ^2	*P*-value
Region	Annaba	52	10	19.23 (47.32-67.90)	25.641	< 0.001
	Bordj Bou Arreridj	92	53	57.61 (8.15-30.31)		
	Mila	51	16	31.37 (18.19-44.55)		
	Constantine	47	20	42.55 (27.88-57.23)		
	El Tarf	34	9	26.47 (10.85-42.10)		
Age	[1-2]	147	30	20.40 (13.81-27)	46.285	< 0.001
	[3-4]	129	78	60.46 (51.91-69.01)		
Sex	Male	55	10	18.18 (07.65-28.70)	25.641	< 0.001
	Female	221	98	44.34 (37.74-50.09)		
Farm management system	Intensive	47	14	29.78 (16.21-43.35)	2.076	0.15
	Semi- intensive	229	94	41.04 (34.62-47.46)		
Livestock farming type	Sheep-only Farms	192	60	31.25 (24.63-37.86)	16.448	< 0.001
	Mixed-livestock Farms	84	48	57.14 (46.33-67.94)		

The results of the multivariate logistic regression analysis highlighted three potential risk factors associated with CCHF seropositivity among sheep, namely the region, age, and livestock farming type

(Table 2). Animals from the region of Bordj Bou Arreridj had a higher risk of infection (OR 5.192, 95 % CI 2.042-13.201, $p< 0.001$) than those from the other study regions. Similarly, the risk of infection was strongly associated with age (OR 6.161, 95 % CI 3.271–11.606, $p< 0.001$). Sheep aged 3–4 years had a higher risk of infection than those aged 1–2 years. As for the livestock farming type, the presence of cattle increased the risk of infection (OR 2.845, 95 % CI 1.54–5.258, $p< 0.001$). Sheep raised on mixed farms containing cattle had a higher risk of being seropositive than those raised on sheep-only farms.

Table 2. Multivariate Analysis of the Association between Risk Factors and the Risk of CCHF Virus Infection

Risk Factors		Odds Ratio (95% CI)	*p*-value
Region	Annaba	Reference [a]	
	Bordj Bou Arreridj	5.192 (2.042-13.201)	< 0.001
	Mila	0.921 (0.321-2.644)	0.879
	Constantine	2.242 (0.796-6.312)	0.126
	El Tarf	0.85 (0.264-2.74)	0.786
Age	[1-2]	Reference	
	[3-4]	6.161 (3.271-11.606)	< 0.001
Sex	Male	Reference	
	Female	1.78 (0.761-4.166)	0.184
Livestock farming type	Sheep-only farms	Reference	
	Mixed-livestock farms	2.845 (1.54-5.258)	< 0.001

[a] Reference group

Discussion

Although the presence of CCHF has been documented in certain African regions, no data are currently available regarding the epidemiological situation of this disease in Algeria, with the exception of two studies suggesting the circulation of the virus in certain north Saharan regions among ticks (13) and dromedaries (14).

In endemic zones, domestic animals are known for their potential role in CCHF epidemiology (22). In addition to the infection's hidden symptomatology, they contribute to the maintenance and transmission of the CCHF virus, especially since they also act as amplifying hosts (23, 24). For this reason, small ruminants, among which sheep are considered sentinel hosts, make it possible to detect the CCHF virus circulation during sero-epidemiological investigations (18, 25), mainly in new geographical areas (26).

This study is the first to document the presence of anti-CCHF virus antibodies among the sheep population in Algeria. In fact, an overall prevalence rate of 39.13 % (95 % CI: 54.9–64.7 %) was found, suggesting the exposure of the sheep population to the CCHF virus, which constitutes initial evidence of the active circulation of the virus in the country. This study showed relatively high prevalence rates recorded among the animals in the targeted study region, ranging from 19.23 % to 57.61 %. Consequently, this suggests that the CCHF virus is widely spread within the entire study area and appears to be more widespread over a large part of the country, given that serological and molecular evidence was reported in the northern Saharan regions of the country, namely the regions of Laghouat, Biskra, El Oued, Touggourt, and Ourgla (13, 14).

The overall prevalence reported in this study is similar to that reported in Kosovo 41.61 % (27),

Turkey 39.6 % (28), and Senegal 38.42 % (29) and significantly higher than those observed in Mauritania 16 % (30), Niger 3 % (31), and Tunisia 6.2 % (32). Prevalence rates of 85.71 %, 74 %, 76.9 %, and 57.6 % were also reported in Turkey, Bulgari, Iran, and Iraq, respectively (33-36). In addition, seroprevalence variations between the different study regions were demonstrated by the serological results of this study, which are similar to those reported in various countries (25, 37). Indeed, a high seroprevalence of 57.61 % was observed in Bordj Bou Arreridj, in contrast to other study regions.

Based on the multivariate regression analysis of risk factors associated with CCHF seropositivity, sheep from the Bordj Bou Arreridj region were 5.1 times more likely to be CCHF seropositive than those from other study regions (OR 5.192, $p< 0.001$). This difference may partly be explained by the fact that the infection can be limited by space or sporadic over time (38).

Overall, the CCHF virus seroprevalence rate among sheep varies considerably from one country to another and often from one region to another, which is attributable to the epidemiological characteristics specific to each region (39) and the endemic nature of the CCHF virus. Moreover, several factors may influence seroprevalence rates, particularly the sampling method (20), the sample size, the density of the animals in the study area, the geographical and climatic diversity, the vector species diversity and their abundance, as well as the presence of various potential vector host species (40, 20). Added to this are the management system of livestock (41), and the effectiveness of prevention and control measures which also stand as influential factors (42).

Multivariate analysis revealed that age had the strongest association with CCHF seropositivity; sheep aged 3–4 years were 6.1 times more likely to be seropositive for CCHF than younger ones (OR 6.161, $p< 0.001$). These results are congruent with certain observations reporting a relationship between CCHF virus prevalence and animal age (30, 43); prevalence is significantly higher among older animals than younger ones, which is seemingly attributable to the additional age factor (6, 30), to the higher probability of degree and exposure time to CCHF virus, to infected ticks (44), and the infection susceptibility relative to younger animals (24, 37). However, despite the absence of clinical symptoms among the sheep, serology has been positive for several years (6). Furthermore, previous studies have concluded that sex is not an influential factor in the CCHF virus prevalence among sheep (20, 24, 30, 37), which goes in line with the results of this study.

The livestock farming type is seemingly also one of the factors that is associated with CCHF seropositivity on sheep farms. The possibility of being CCHF seropositive was 2.8 times higher in sheep raised on mixed livestock farms which include cattle than the ones raised in sheep-only farms (OR: 2.845, $p< 0.001$). This may be attributed to cattle, which represent the main host of *Hyalomma* genus ticks that constitute the main vector and reservoir of the CCHF virus (12), with a predominance of these ticks among cattle compared to sheep (45, 46). Consequently, their presence in mixed livestock farms may increase the risk of sheep infection by these ticks and hence the risk of exposure to the CCHF virus.

In previous studies, the farm management system was suggested as one of the risk factors that can play a decisive role in CCHF seropositivity (37). Indeed, animals from semi-intensive farms are highly exposed to ticks (47), as they are less susceptible to correct sanitary prophylactic measures, particularly anti-parasitic treatment. In addition, the possible interactions that frequently occur in grazing areas with other infected herds (37) or possibly with wild fauna contribute to the spread of the disease to other animals (41). However, in this study, despite the fact that the seroprevalence rate recorded in semi-intensive farms (41.04 %) is numerically higher than that recorded in intensive farms (29.78 %), this difference was not statistically significant ($p=0.15$). This can be linked to the

inadequacy of the samples taken from the intensive farms, or probably to new tick-bearing animals that were introduced into the farms, or to the possible failure of antiparasitic treatments.

Despite the absence of direct economic impacts of the CCHF virus infections on farm animals (48), their importance as a human disease cannot be ignored. Moreover, despite CCHF being endemic in Africa, the epidemiological situation remains poorly understood (8).

Conclusion

This is the first study to document the circulation of the CCHF virus among sheep population in Algeria. The results emphasize the importance of sheep in CCHF epidemiology and, consequently, their crucial role in sero-epidemiological investigations. In addition, the risk factor analysis revealed that the region, age, and livestock farming type affected the seroprevalence of the virus. Further studies should focus on providing a clear understanding of the epidemiology of this disease in Algeria and other North African countries.

Acknowledgment

The authors of this paper express their utmost gratitude to Dr. Salim Djemouai, Dr Nacer Logzit, and Dr. Mohamed Laoubi, whose contributions have greatly helped in conducting and shaping this research work.

Ethical approval

Not applicable

Conflict of interest statement

There is no conflict of interest.

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