



Seroepidemiological Study of Crimean-Congo Hemorrhagic Fever (CCHF) in Small Ruminants in Thi-Qar Governorate, Southern Iraq, 2023

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Abstract | This study was conducted to evaluate the sero-epidemiological status of CCHF infection in small ruminants after a recent human outbreak in Thi-Qar Governorates, southern-Iraq in 2023. The present study used 304 small ruminants (sheep and goats), focusing on tick infestation status, age, and sex of animals. Serum samples were obtained from sheep (n=242) and goats (n=62) located in rural and urban areas around recent focal human cases of CCHF infection in Thi-Qar, Iraq, and then screened using ELISA test (the "ID Screen® CCHF Double-Antigen Multi-species – CCHFDA" ELISA-Kit). The results showed that the incidence of CCHF in the examined sheep and goats was 49.3% ($P \leq 0.01$). In sheep, the overall seroprevalence rate was 52.9%, while in goats it was 35.5%. The current investigation showed a substantial difference in seroprevalence rate between animals that had a high level of tick infestation and those without ticks. However, 57% ($P \leq 0.001$) of sheep and 29.2 % ($P \leq 0.05$) of goats with severe tick infestation showed a strong IgG positive reaction in the CCHF ELISA-test. As an interesting finding, it has been found that older goats and sheep had a higher chance ($P \leq 0.008$) of being CCHF IgG positive than younger ones. Since older animals are more likely to be exposed to CCHFV-positive ticks and to a variety of diseases. It has been shown that the infection rate was high in grazing animals which were more likely to come into contact with animals from different regions. The seroprevalence of CCHF was higher ($P \leq 0.1$, and 0.03 for sheep and goats respectively) in females than in males. Since CCHF has a high prevalence among small ruminants and inadequate eradication efforts, the disease poses a public health and livestock threat in Iraq, especially in Thi-Qar.

Keywords | IgG, CCHF outbreak, Zoonosis, Tick born, Thi-Qar, Iraq

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CCHF vectors or identification of regional CCHF strains have been carried out in Iraq. To evaluate their possible role in recent human outbreaks in Thi-Qar governorate, southern Iraq, the present study screens sheep and goats against CCHF antibodies using an ELISA test.

MATERIAL AND METHODS

ANIMALS AND AREA OF STUDY

The study designed to evaluate the seroprevalence of CCHF in sheep and goats located in Thi-Qar, Iraq where the disease is endemic in this governorate. A total of 304 small ruminants (sheep $n = 242$) and (goats $n = 62$) were used in this study. Blood samples were collected according stratified sampling method from animals that had been in contact with people infected with CCHF as well as from areas adjacent to recent human CCHF foci. Since there were no clinical manifestations of CCHF in animals; tick infestation status, age, sex, and, grazing were considered during sampling.

ETHICAL STATEMENT

In order to fulfill the government mandate of conducting surveillance and control programs for veterinary and zoonotic pathogens, the University of Basrah CCHF Containment team sampled sheep and goats in accordance with all applicable national and international regulations as well as ethical principles followed by the Animal Care and Use Committee/College of Veterinary Medicine, University of Basrah/Iraq.

SAMPLES COLLECTION

The blood samples were collected according to the methods of (Jackson, 2013). Five milliliters of blood samples were collected aseptically from the jugular vein. The sera were then separated accordingly in the Clinical Pathology Laboratory, College of Veterinary Medicine/University of Basra, Iraq and then stored in deep freeze until the day of analysis.

SEROLOGICAL ANALYSIS

Serological analysis were done following our previous research (Dakhil *et al.*, 2024). Sheep and goat sera were analyzed to evaluate anti-CCHFV IgG antibodies using "ID Screen® CCHF Double Antigen Multi-species – CCHF-DA" ELISA KIT, [ID.Vet] company, France. (https://www.ambifood.com/fotos/downloads/insert_cchfda_ver0917_en_doc8702_860019475d23173b6c04d.pdf). Serological analysis was performed following the manufacturer's instructions and each microplate was read at an OD value of 450nm.

STATISTICAL ANALYSIS

The obtained data of the current study was statistically analyzed at ($P < 0.05$) using SPSS software. Chi Square Test and/or Microsoft Excel software were also used for non-parametric variables.

Crimean-Congo hemorrhagic fever (CCHF), a tick-borne zoonosis illness, is mostly caused by the virus known as CCHFV (genus *Orthonairovirus*, family *Nairoviridae*) (Nalca and Whitehouse, 2007). Africa, the southern and eastern Europe, the Middle East, and Asian countries are endemic for the disease (Hawman and Feldmann, 2023). Ixodid ticks, mostly of the *Hyalomma* genus, are the vectors by which the virus is spread to humans and animals; in Europe, *H. marginatum* is the most significant vector (Hoogstraal, 1979). With a 50° North latitude restriction, the disease's geographic distribution is closely associated with that of *Hyalomma* ticks (WHO, 2017). The geographic range of CCHF and the range of the hard tick vector, primarily *Hyalomma marginatum*, which is Africa, Asia, and Europe, coincide (Maltezou and Papa, 2011; Messina *et al.*, 2015). Direct contact between human and animal bodily fluids, such as blood, is another way for the virus to spread to humans. Thus, the most vulnerable to infection are those who work in agro-pastoral or animal husbandry fields and come into touch with fresh animal meat and blood (Nasirian, 2019). Unlike human disease, CCHFV can spread undetected in many domestic and wild animals because the infection does not produce noticeable symptoms (Dakhil *et al.*, 2024). Specifically, in Europe, a high frequency of antibodies against CCHFV has been found in the sera of hares as well as domestic animals such cattle, horses, goats, and sheep. This implies that these creatures have a significant impact on the CCHF epidemic (Fanelli *et al.*, 2022; Spengler *et al.*, 2016). Since the first report of CCHF in Iraq in 1979 (Al-Tikriti *et al.*, 1981), There have been irregular disease outbreaks punctuated by stretches of time without any cases reported (AL-Shauwreed *et al.*, 2023). There has been a spike in instances over the past two years, according to data from the Iraqi Ministry of Health, with 19 laboratory-confirmed cases in 2021 and 108 during the first half of 2022 (Alhilfi *et al.*, 2023). Turkey and Iran, which lie to the north and east of Iraq, are endemic for CCHF (Gürbüz *et al.*, 2021; Sisman, 2013) and there have been reports of recent outbreaks and an increased number of cases (Ince *et al.*, 2014; Keshtkar-Jahromi *et al.*, 2013; Messina *et al.*, 2015; Tartar *et al.*, 2019). In 2016, a seroepidemiologic study on CCHF in sheep, camels, and cattle revealed prevalence rates ranging from 20 to 30 percent in Turkey, Iraq, and Iran. Comparatively, in all three nations, the prevalence of CCHF in goats was greater at 50% (Spengler *et al.*, 2016). There have been intermittent illness outbreaks punctuated by intervals without documented cases of CCHF in Iraq since the disease was first reported four decades ago. However, most of the cases and deaths of CCHF outbreaks were in Thi-Qar Governorate, southern Iraq. To our knowledge, the source of infection in the recent outbreaks in Iraq, particularly in Thi-Qar, has not been identified. As far as we are aware, no nationwide survey of

The virus that causes Crimean-Congo hemorrhagic fever (CCHF) is a severe tick-borne zoonosis that was initially identified in 1944–1945 during an outbreak among Soviet military men stationed in Crimea (Temur *et al.*, 2021). Since the initial instances appeared in Crimea, more than 30 nations have reported cases of CCHF, which is widely prevalent in both Africa and Eurasia (Blair *et al.*, 2019). But since 1979, CCHF has been widespread in Iraq, with nearly yearly outbreaks during that time, particularly in the last several years (Al-Rubaye *et al.*, 2022; AL-Shauwreed *et al.*, 2023; Al-Tikriti *et al.*, 1981; Alhilfi *et al.*, 2023; Atwan *et al.*, 2024; S Al-Yabis *et al.*, 2005). A number of infectious illnesses have recently been linked to livestock in southern Iraq (Naji *et al.*, 2019; Naji *et al.*, 2021; Saleh, 2019; W. Saleh *et al.*, 2019; WMM Saleh *et al.*, 2019). However, CCHF is not one of them. As far as we are aware, this document is the first to offer a current summary of the particular IgG antibodies that are now in circulation against CCHFV in sheep and goats in the Thi-Qar governorate of Iraq. On the other hand, not much is known regarding CCHFV's distribution and prevalence in Iraq.

Here, we used the detection of IgG antibodies in sheep and goats to examine the seroepidemiological status of small ruminants against CCHFV in the Thi-Qar governorate and the far south of Iraq. [Since the southern governorates have been the focus of the worst CCHF outbreak in Iraq (Alhilfi *et al.*, 2023)]. Significant ($P \leq 0.01$) higher (49.3%) seroprevalence rate has been discovered in small ruminants grown in the current study's target areas surrounding all previously verified human CCHF cases. Figure 1 shows the overall geographical distribution of CCHF seropositive cases in small ruminants in the Thi-Qar Governorate. Likewise, inadequate public health initiatives, substandard medical and veterinary treatment, and intimate contact between farmers and butchers and their livestock present significant risk factors for the transmission of diseases between humans and animals in the latter years of the Iraqi situation. Humans can become infected with CCHF from animals or patients who expose them to infected blood, tissue, or other bodily fluids. Iraq's Ministry of Health recorded 212 human cases of CCHF between January 1 and May 22, of which 115 were suspected and 97 confirmed, based on the greatest human outbreak of the disease. Thirteen lab-confirmed deaths bring the total number of deaths to 27. Most of the patients were butchers or livestock breeders who worked closely with animals (Islam, 2022).

Due to their vast distribution and close proximity to humans, small ruminants were used in this investigation, which raises the prospect of employing these species as an indicator for CCHFV seroepidemiological screening. In order to ascertain if CCHFV is present or absent in a

certain area, small ruminants are employed as indicator animals in CCHFV seroepidemiological monitoring investigations (Schuster *et al.*, 2016). In our study, sheep had a significantly ($P \leq 0.01$) higher prevalence rate than goats; 52.9% of sheep and 35.5% of goats were affected (Table 1). However, in a previous related study conducted in a neighboring Iraqi governorate, the seropositivity rate for CCHF was lower than our findings, reaching 20% and 37% in sheep and cattle, respectively (S Al-Yabis *et al.*, 2005). In the current study, the higher adaption rate of goats in this area compared to sheep and the variations in the kind and quality of each breed's hair coat condition can be blamed for this discrepancy. If not, the variations might be artifacts brought about by inadequate sampling or by variations in the samples that were obtained (Schuster *et al.*, 2016). When we studied the same kind of animals in a nearby region (Basra Governorate), we also found a comparable variation in seroprevalence rates between sheep and goats. But in that study, the seroprevalence rate was lower (Dakhil *et al.*, 2024). It's interesting to note that, in this study, sheep and goats showed a significant ($P \leq 0.008$) rise in CCHFV-specific IgG antibodies relative to age (Table 2). Older sheep and goats have a higher frequency of CCHF than younger ones. It is evident that in endemic locations, older ages are associated with higher exposure to a variety of diseases and CCHFV-positive ticks, which can lead to CCHFV infection (Schulz *et al.*, 2021; Wilson *et al.*, 1990).

Table 1: Points out the total seroprevalence rate of CCHF infection.

Animal	Number	Seroprevalence rate	
		CCHF+	CCHF-
Sheep	242	128	114
Goats	62	22	40
Total	304	150	154

$X^2 = 5.984$; p -value ≤ 0.01 .

Table 2: The seroprevalence rate of CCHF infection related to age group.

Age group	Number	Seroprevalence rate	
		CCHF +	CCHF -
3months-1 year	62	20	42
1-2 years	80	37	43
2-3 years	58	33	25
3-4 years	58	37	21
4-5 years	46	23	23
Total	304	150	154

$X^2 = 13.723$; p -value ≤ 0.008 .

Subsequent analysis in the current study showed that the CCHF seroprevalence rate was greater in sheep (57%) ($P \leq 0.001$) and in goats (29.2%) ($P \leq 0.05$) with severe tick

infestation (Tables 3 and 4). Furthermore, Figures 2 and 3 Show tick infestation on the ears of the CCHF-seropositive sheep and goats in the current study. As ticks primarily belonging to the species *Hyalomma* comprise the majority of CCHF reservoirs and vectors (Schulz *et al.*, 2021; Whitehouse, 2004). Poor husbandry practices and excessive exposure to ticks carrying the CCHFV virus could be risk factors contributing to the high seropositivity rate (Kasi *et al.*, 2020; Schulz *et al.*, 2021). The bulk of CCHF cases that have been confirmed have happened in the summer, which is consistent with the transmitting vector's ongoing activation from late spring through early October in temperate regions (Maltezou and Papa, 2010). Tick control efforts in Iraq led to a decline in the yearly number of cases of CCHF in 1996. The annual number of cases varied from 0 to 6 between 1998 and 2009, however due to noncompliance with tick control measures, the number of cases rose to 11 in 2010. This reflects the actual requirement for routine laboratory testing to determine whether animals are infected, as well as the regular monitoring of livestock tick infestation levels and the implementation of appropriate, timely preventive measures, such as dipping sheep and goats and spraying cattle and barns (Majeed *et al.*, 2012). Nonetheless, the rise in hard tick infestations of farms and animals in Iraq in 2022 can account for the rise in the incidence of CCHF. The absence of pest control measures during the coronavirus disease 2019 (COVID-19) pandemic in 2020 and 2021 may have contributed to this increase (AL-Shauwreed *et al.*, 2023; Alhilfi *et al.*, 2023). Furthermore, the seroprevalence rate of CCHF was unaffected ($P \leq 0.1$) by sex in sheep (Table 5) in contrast to goats ($P \leq 0.03$) (Table 6) since both sexes can be exposed equally to infected ticks.

Table 3: The seroprevalence rate of CCHF infection in sheep related to tick infestation.

Tick infestation status	Total Number	Seroprevalence rate	
		CCHF+	CCHF-
Positive	207	118	89
Negative	35	10	25
Total	242	128	114

$X^2 = 9.714$; p -value ≤ 0.001 .

Table 4: The seroprevalence rate of CCHF infection in goats related to tick infestation.

Tick infestation status	Total Number	Seroprevalence rate	
		CCHF+	CCHF-
Positive	48	14	34
Negative	14	8	6
Total	62	22	40

$X^2 = 3.706$; p -value ≤ 0.05 .

All areas surrounding the focal points of confirmed human CCHF infection in Thi-Qar Governorate were covered in

this record. All regions had different seroprevalence rates of IgG antibodies specific to CCHFV. Grazing animals, which are more likely to come into contact with animals from different regions, had a high seroprevalence rate. Small ruminants roam largely free in Thi-Qar, resulting in large grazing areas, which may pose an increased risk of exposure to reservoirs and/or vectors. There was a significant increase in CCHF seropositivity in grazing areas among cattle and goats (Atim *et al.*, 2022). Similarly, the bulk of recent instances in southern Iraq might be the result of illicit cross-border animal trafficking, the high infection rate in some areas is due to the number of animals taken and illegal trade between different areas and governorates, and the incidence of tick infestation was high. Controlling illegal trafficking is necessary to stop the spread of zoonotic illnesses, such CCHF (Alhilfi *et al.*, 2023). As a final recommendation, public health awareness should be raised for all individuals who deal with/use animals or their products. It is vital to evaluate an effective national program to eliminate ticks in order to reduce the spread of the disease between animals and to humans.

Table 5: The seroprevalence rate of CCHF infection related to sex in sheep.

Criteria	Animal	Number	Male	Female
CCHF+	Sheep	128	40	88
CCHF -	Sheep	114	47	67
Total		242	87	155

$X^2 = 2.607$; p -value ≤ 0.1 .

Table 6: The seroprevalence rate of CCHF infection related to sex in goats..

Criteria	Animal	Number	Male	Female
CCHF +	Goats	22	7	15
CCHF -	Goats	40	15	25
Total		62	22	40

$X^2 = 0.2$; p -value ≤ 0.03 .

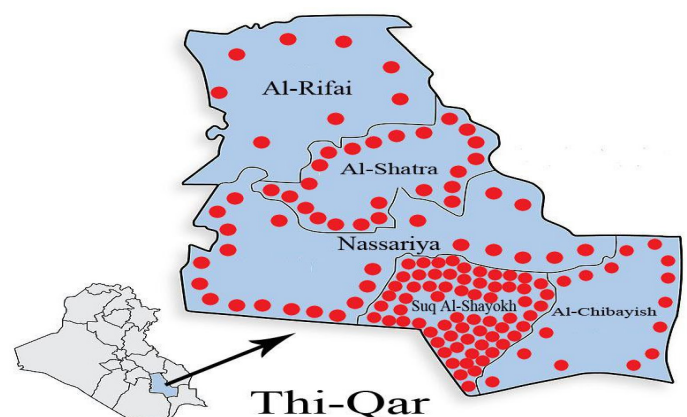


Figure 1: Showing the geographic distribution of CCHF infection in Thi-Qar Governorate, Iraq. • = positive CCHF case.



Figure 2: Showing tick infestation on the ear of CCHF-seropositive sheep in Thi-Qar Governorate, Iraq.



Figure 3: Showing tick infestation on the ear of CCHF-seropositive goat in Thi-Qar Governorate, Iraq.

CONCLUSIONS AND RECOMMENDATIONS

Our data shows that small ruminants in Thi-Qar, southern Iraq, have circulating CCHFV-specific IgG antibodies. This indicates that these animals are suitable as index animals for CCHFV seroepidemiological surveillance, which aims to ascertain if CCHFV is present or absent in this area. However, because of its high seroprevalence rate in

small ruminants and inadequate eradication efforts, CCHF poses a hazard to both public health and livestock in Iraq, especially in Thi-Qar. Additional research identifying the regional strains of CCHFV is desperately needed, in addition to a nationwide vector eradication effort that works.

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NOVELTY STATEMENTS

Since the outbreak of CCHF four decades ago, there have been intermittent outbreaks in Iraq, but most cases and deaths have occurred in Thi-Qar Governorate in southern Iraq without any trace of the outbreak's source. Therefore, in Thi-Qar where the diseases impact humans, our findings provide fundamental data regarding the possible role of small ruminants as sources of infection in recent outbreaks.

AUTHOR'S CONTRIBUTIONS

Wessam Monther Mohammed Saleh, Saad Shaheen Hamadi Al-Taher, and Afrah Ali Dakhil: Development of the Methodology, preparing and writing the initial draft, review and editing the manuscript and analyze the data. Wessam Monther Mohammed Saleh, Afrah Ali Dakhil: Collection of sheep and goats samples. Afrah Ali Dakhil, Khazaal Abbas Khazaal Alqaisi, Mazin Mahdi Naji and Layth Mohammed Salih AbdulRasool: Preparing samples and laboratory analysis. Wessam Monther Mohammed Saleh and Saad Shaheen Hamadi Al-Taher: Looked over the document, provided feedback, and approved the final version.

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CONFLICT OF INTEREST

There are no conflicts of interest, according to the authors.

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