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SEROEPIDEMIOLOGICAL SURVEY OF CRIMEAN-CONGO HEMORRHAGIC FEVER (CCHF) IN SMALL RUMINANTS AFTER A RECENT HUMAN OUTBREAK IN BASRA GOVERNORATE, SOUTHERN IRAQ IN 2023

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic tick-born disease transmitted by a lethal virus with a high case mortality rate and widespread geographic distribution. Human cases of CCHF infection were reported in Africa, the Middle East, Asia, and Southern and Eastern Europe, however, the disease is endemic in Iraq since 1979. Therefore, the present study was designed to evaluate the sero-epidemiological status of CCHF virus infection in small ruminants after a recent human outbreak in Basra Governorate, southern Iraq in 2023. Small ruminants (sheep and goats) were used as an experimental model in the current study. Serum samples were obtained from sheep (n=287) and goats (n=121) located in rural and urban areas around recent focal cases of CCHF infection in Basra, Iraq and then screened using the "ID Screen® CCHF Double Antigen Multi-species – CCHFDA" ELISA Kit. Our results showed that 13.23% of sheep and 4.2% of goats had high CCHFV seropositivity. Furthermore, 76.9% of sheep and 23.1% of goats with severe tick infestation had higher CCHFV seropositivity than those without tick infestation. The high seropositivity rate for CCHFV among small ruminants suggests that tick infestations influence the infection and may contribute to the high endemicity rate in this region, which is subsequently responsible for the high number of reported cases of CCHF infection in Basra in 2023. CCHF, however, must be screened nationally and an eradication program must be implemented to control and prevent it.

1. INTRODUCTION

Crimean- Congo Hemorrhagic Fever Virus (CCHFV); family of *Nairoviridae*, genus *Orthonairovirus* is the causative agent of a tick-borne zoonosis disease (Crimean–Congo hemorrhagic fever - CCHF) which is endemic in Africa, the Balkans, the Middle East, and Asian countries [1]. The virus is transmitted to humans and animals by Ixodid ticks, mainly belonging to *Hyalomma* genus, with *H. marginatum* as the most relevant vector in Europe [2]. As a result of the global climate change in recent decades, the geographical boundary of 50° N latitude has ceased to be the main limiting factor for the distribution of *Hyalomma* spp. Ticks [3]. Another transmission route to humans is through direct contact with blood and other body fluids of viremic patients and animals. Therefore, people working in the agro-pastoral or animal husbandry fields and in contact with fresh flesh and blood from animals are most at risk of infection [4]. Humans who become infected with CCHF acquire the virus from direct contact with blood or other infected tissues from infected persons or livestock or they may become infected from a tick bite [5], [6]. The majority of cases have occurred in those involved with the livestock industry, such as agricultural workers, slaughterhouse workers and veterinarians [6]. Furthermore, the virus penetrates the bloodstream and affects the majority of organs, including the lungs, liver, and lymph nodes [7]. CCHF was first reported in 1979 in Iraq, then in 1992, 1996, 2012, 2005, then appear in focal areas till 2023 [8- 10]. Since the first report of CCHF in Iraq four decades ago, there have been sporadic outbreaks of disease interspersed with periods of no registered cases. The largest CCHF outbreak was reported in 2022 [11]. According to the official reports of the Ministry of Health of Iraq for the year 2023, among 1827 suspected human cases of CCHF reported in 18 governorates in Iraq, 511 cases were confirmed by RT-PCR. The overall case fatality rate (CFR) was 12.7 (65 deaths out of 511 confirmed cases). However, in Basra Governorate alone, 83 out of 273 suspected CCHF reported cases were confirmed by RT-PCR, and the CFR was 12 (10 deaths out of 83 confirmed cases) [8], [12]. Since the first report of CCHF in Iraq four decades ago, there have been sporadic outbreaks of disease interspersed with periods of no registered cases. To our knowledge, no identification of local CCHF strains and no national survey of CCHF vectors were done in Iraq. Therefore, this study was designed to assess the sero-epidemiological status of CCHF in the susceptible small ruminants in southern Iraq, particularly Basra governorate.

2. MATERIALS AND METHODS

Animals and area of study: The study designed to evaluate the seroprevalence of CCHF in sheep and goats located in Basra, Iraq where the disease is endemic in this governate. A total of 408 small ruminants (sheep n = 287) and (goats n = 121) were used in this study from rural and urban areas focusing on recent focal cases of CCHF infection, tick infestation, age, and sex. Adults and growing animals, males and females, and animals with and without ticks' infestation in different areas of Basra governorate were used in the current study.

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.

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Samples collection: The blood samples were collected according to the methods of [13]. 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: Sheep and goat sera were analyzed to evaluate anti-CCHFV IgG antibodies using "ID Screen® CCHF Double Antigen Multi-species – CCHFDA" 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. Student t-Test was used for comparison the parametric variables. Chi Square Test and/or Microsoft Excel software were also used for nonparametric variables.

3. RESULTS

The overall seroprevalence anti CCHFV antibodies rate for small ruminants tested by ID Screen® CCHF Double Antigen Multi-species-CCHFDA" ELISA Kit in Basra Governorate was 17.4%. In sheep, an overall seroprevalence rate was 13.23%. For goats, the seroprevalence rate was found 4.2% (Table 1). In the present study, a significant difference in seroprevalence was demonstrated between animals with severe tick infestation and those without ticks. However, 76.9% of sheep and 23.1% of goats with severe tick infestation showed a strong seropositive reaction in the CCHF ELISA test (Table 2). As an additional finding, we observed that older sheep and goats were more likely to be seropositive than those younger (Table 3), while CCHFV status was unaffected by sex (Table 4).

4. DISCUSSION

Crimean-Congo hemorrhagic fever (CCHF) is a severe tick-borne zoonosis caused by Crimean-Congo hemorrhagic fever virus, it was first described during an outbreak among Soviet military personnel stationed in Crimea in 1944–1945 [14]. CCHF is broadly endemic in both Africa and Eurasia, with more than 30 countries having reported cases since the first cases emerged in Crimea [15]. However, CCHF has been endemic in Iraq since 1979 with recurrent outbreaks occurring almost annually over the past four decades, especially in the last years [8- 12], [16]. Recently, several infectious diseases have been reported in small ruminants in the Basra governorate [17- 21], but CCHF is not among them. To our knowledge, this record is the first to provide an updated overview of the circulating specific IgG antibodies against CCHFV in sheep and goats in Basra governorate/Iraq. However, very little is known about the prevalence and distribution of CCHFV in Iraq.

Here, we investigated the seroepidemiological status of small ruminants in Basra governorate/far south of Iraq against CCHFV by detecting IgG antibodies in sheep and goats (Since the largest outbreak of CCHF in Iraq has been concentrated in the southern governorates [11]. In the current study, significant seroprevalence rates have been found in sheep and goats raised around all focal areas of recently confirmed human CCHF cases. Likewise, poor public health measures, poor medical and veterinary care, and close contact between farmers/butchers and their animals pose serious risk factors for the spread of disease between animals and humans during the last years in Iraq. Viremic animals or patients can infect humans by exposing them to infectious blood, tissue, or other body fluids. Moreover, *Hyalomma* ticks are the main reservoir and transmission vector of CCHFV, and most virus infections result from bites from these ticks
[22]. Based on the largest outbreak of CCHF, Iraq's Ministry of Health reported 212 cases of CCHF between January 1 and May 22, of which 115 were suspected and 97 confirmed. The death toll stands at 27, with 13

lab-confirmed deaths. A majority of patients were livestock breeders or butchers who had direct contact with animals [23].

The use of small ruminants as an experimental model in the current study as they are widespread and in close contact with humans highlights the possibility of using these animals as an indicator for CCHFV seroepidemiological screening. Thus, in a certain region, small ruminants are used as indicator animals for CCHFV seroepidemiological surveillance studies to determine the presence or absence of CCHFV [24]. Prevalence rate in sheep is quite higher than goats within our study. It was 13.23% in sheep vs 4.2% in goats. This difference can be attributed to the higher adaptation rate of goats than sheep in this region and the difference in the quality and nature of the hair coat condition for each breed. Otherwise, the differences could be artifact caused by impaired sampling and/or differences in collected samples [24]. Interestingly, in the present study, an increase in CCHFV-specific IgG antibodies was recorded in sheep and goats, concerning the age group. The prevalence of CCHF was higher in aged sheep and goats than in younger. It is clear that the higher age correlates with increased exposure to a wide range of pathogens and CCHFVpositive ticks in endemic areas, resulting in CCHFV infection [25], [26].

Further investigation revealed that 76.9% of sheep and 23.1% of goats in the current study with severe tick infestation had a higher CCHF seroprevalence rate. Since most reservoirs and vectors of CCHF are ticks mainly of the genus *Hyalomma* [25]. Potential risk factors influencing the high seropositivity rate are overexposure to CCHFV-positive ticks with non-elimination of ectoparasites and a poor husbandry system [25], [27]. The majority of confirmed CCHF cases occurred in summer and this is in accordance with activation of transmitting vector in temperate areas in late spring and continuous activation through summer to early autumn [28]. In 1996, tick control campaigns in Iraq resulted in a decrease in annual number of CCHF cases. Between 1998 and 2009 the annual number of cases ranged from zero to six, while in 2010 they increased to 11 due to lack of compliance with tick control activities. This reflects the actual need for regular laboratory investigation for the presence of infection in livestock and the frequent checking for the extent of livestock tick infestation with application of the suitable immediate preventive measures such as cattle and barn spraying with sheep and goat dipping [29]. However, the increase in the occurrence of CCHF in Iraq in 2022 can be explained by the increase in hard tick infestations of animals and farms. This increase may have occurred due to the absence of insect control activities in 2020 and 2021 during the coronavirus disease 2019 (COVID-19) pandemic [8], [11].

In this record, all areas of Basra Governorate were covered, especially around focal areas of confirmed CCHF infection. The seroprevalence of CCHFV-specific IgG antibodies was distributed throughout all regions. Likewise, the fact that the majority of recent cases occurred in southern Iraq, especially in provinces bordering Iran such as Basra, may be due to illegal cross-border animal trafficking. There is a need to control illicit trade activities to reduce the spread of zoonotic diseases, including CCHF [11].

5. CONCLUSIONS

Our record demonstrates the circulating status of CCHFV-specific IgG antibodies in small ruminants in Basra/southern Iraq, highlighting their suitability as index animals for CCHFV seroepidemiological surveillance to determine the presence or absence of CCHFV in this region. However, CCHF is threatening livestock as well as public health in Iraq particularly in Basra since it records a significant seroprevalence rate in small ruminants combined with poor eradication programs. Further studies demonstrating the local CCHFV strains are urgently needed together with national effective program for vector eradication.

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

AUTHORS CONTRIBUTIONS
WMMS, SSHA, and AAD –Development of the Methodology, preparing and writing the initial draft, review and editing the manuscript and analyze the data. WMMS, AAD –Collection of sheep and goats samples. AAD, KAKA, MMN and LMSA –Preparing samples and laboratory analysis. WMMS and SSHA looked over the document, provided feedback, and approved the final version.

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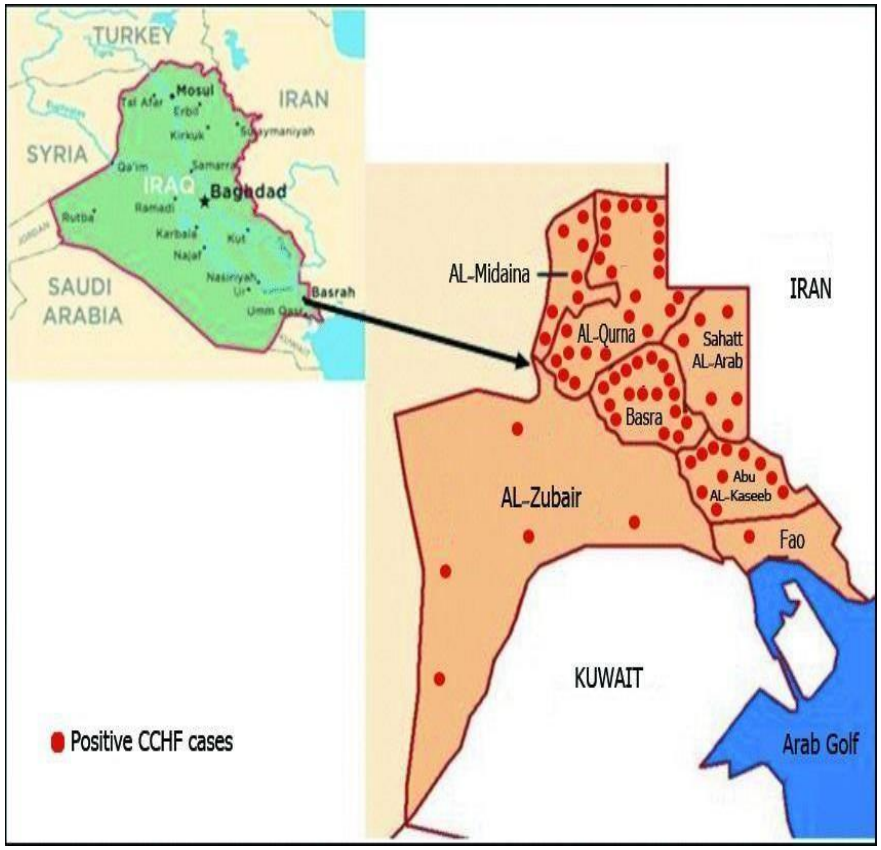

Figure 1; shows geographic distribution 0f CCHF in Basrah Governorate

Table 1: points out the total seroprevalence rate of CCHF infection in Basra governorate/Iraq regarding to the total number (n = 408) of animals.

		Seroprevalence Rate	
Animal	No	CCHF+	CCHF-
Sheep	287	54 (13.23%)	233 (57.1%)
Goats	121	17 (4.2%)	104 (25.5%)
Total	408	71 (17.4%)	337 (82.6%)

Table 2: The seroprevalence rate of CCHF infection related to tick infestation.

Criteria	Animal	Percentage
CCHF+	Sheep	50 (76.9%)
	Goats	15 (23.1%)
	Total	65
CCHF -	Sheep	10 (40%)
	Goats	15 (60%)
	Total	25

Table 3: The seroprevalence rate of CCHF infection related to age group regarding to the total number (n = 408) of animals.

Age group	CCHF +	CCHF -
3months-1 year	16 (3.9%)	92 (22.5%)
1-2 years	10 (2.5%)	87 (21.3%)
2-3 years	17 (4.2%)	70 (17.2%)
3-4 years	14 (3.4%)	54 (13.2%)
4-5 years	14 (3.4%)	34 (8.3%)

Table 4: The seroprevalence rate of CCHF infection related to sex.

Criteria	Animal	Percentage	
		Male	Female
CCHF+	Sheep n=54	16 (22.5%)	38 (53.5%)
	Goats n= 17	5 (7%)	12 (16.9%)
	Total =71	21 (29.8%)	50 (70.4%)
CCHF -	Sheep n=233	72 (21.4%)	161 (47.8%)
	Goats n=104	42 (12.5%)	62 (18.4%)
	Total n=337	136 (40.4%)	245 (72.7%)