Research Article

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Seroprevalence of Crimean–Congo Hemorrhagic Fever in Cattle in Basrah Province, Iraq

Douaa. A. Hashim, Tamadhir A. Al-Hamed

Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Corresponding Author Email Address: Tamadhir.abd-hafid@uobasrah.edu.iq

ORCID ID: https://orcid.org/0000-0002-6294-3683

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Abstract

The study was conducted in the prominent districts of Abu Al-Khasib, Al-Zubair, Al-Qurnah, Karamak Ali, Bani Sakīn, the College of Agriculture, and Al-Harithah in Basrah Governorate. The study aimed to detect *Crimean-Congo hemorrhagic feve*r as a result of a virological and epidemiological study of the types of calves, buffaloes, cows, races, and ages. The indirect site ELISA test detected antibodies against the CCHF virus using IgG antibodies. Fresh blood samples were collected from buffaloes, cows, and calves, starting from June until the end of September 2023. There were 42.44% of 172 cattle (73). IGg titers were higher among females compared to males. The number of infected females was 56, and the number of males was 17. A higher percentage of antibodies was also recorded in those aged 2–3 years compared to those aged less than two years. According to the regions of Abu Al-Khasib, the number of cases of virus infection was extremely high, with 33 cases (64.7%) the results were obtained. Hard and soft ticks were collected from Basrah Governorate, from the same animals that were collected blood samples and they were classified into tick family *Ixodidae*, known as three species: *Rhipicephalus sanguineus, Hyalomma anatolicum*, and *Hyalomma truncatum*. These results indicate that CCHV is widely spread in cattle in southern Iraq. 42.44% and found that the type of tick *Hyalomma* is clearly widespread in areas of Basrah.These results indicate that CCHV is widely spread in cattle areas in southern Iraq in Basrah Governorate.

Keywords: Crimean -Congo hemorrhagic, Hemorrhagic fever virus, Basrah.

Introduction

Crimean–Congo hemorrhagic fever virus (*CCHFV*) represents a globally prevalent vector-borne pathogen with a zoonotic nature. It exhibits a broad geographical distribution, encompassing vast regions of Africa as well as the southern and southeastern zones of Europe, extending into the Asian continent (1). The organism in question is classified within the *Nairoviridae* family and the *Orthonairovirus* genus. The primary transmission vectors are ticks from the *Hyalomma* genus, which also serve as reservoirs for the virus. Transmission of the *Crimean–Congo hemorrhagic fever virus* (*CCHFV*) can occur through tick bites or direct exposure to the infectious biological matter, including tissues and fluids, from both humans and animals (2). Individuals diseased with the virus may present with a clinical syndrome akin to Ebola, characterized by acute *hemorrhagic fever*, significant hemorrhage, and shock.Howover, , such human cases predominantly manifest as isolated occurrences, with full-scale epidemics being an infrequent phenomenon (1,3). Moreover, while a wide array of domesticated and wild mammalian species is vulnerable to infection, they typically exhibit only transient viremia and do not display overt clinical signs post-infection (4,5). The principal vectors for the virus are domesticated ruminants such as bovines, ovine, porcine, and caprine, which become infected through the parasitic activity of *ixodid* ticks in their mature stage. It was hypothesized that the virus spreads among domestic and wild animals and infects people when a tick bites them. When vectors, specifically ticks infected with Crimean-Congo Hemorrhagic Fever (CCHF), travel over long distances, the disease spreads. Host species, for example, rodents and hedgehogs, along with the larval nymphet phases of ticks, serve as agents in this process (6). Agriculturalists, abattoir personnel, and veterinary professionals operating within endemic zones are predominantly susceptible to *Crimean-Congo Hemorrhagic Fever (CCHF).* Additionally, intensive care settings have documented instances of nosocomial infections that affect individuals undergoing medical treatment.

The mortality rate can fluctuate from 5 to 40%. The disease is prevalent within and throughout Asia, Africa, and certain regions of Europe are affected (8, 1, 9). The case death ratio ranges from 10% to 40% (10).
Historically, the World Health Organization (WHO) designated a region or country as endemic for the *Crimean–Congo hemorrhagic fever virus (CCHFV)* based on documentation of positive serological or polymerase chain reaction (PCR) results in human, tick, or animal populations within those territories (11). The virus may be obtained from serum or plasma specimens acquired at the time One can obtain the virus from serum or plasma specimens obtained during fever or viremia, or from the hepatic tissue of affected fauna.

Traditional and quantitative reverse transcription polymerase chain reaction (RT-PCR) are utilized for the identification and

mapping of the virus. methodologies are applicable. Animal infections often remain asymptomatic, significantly reducing the probability of viral isolation from a viremic host (8,12- 15). Assays that use indirect immunofluorescence or a mix of IgG-sandwich and IgM-capture enzyme-linked immunosorbent assays (ELISAs) can find antibodies that are specific to a species.

Material and Methods

172 blood samples were collected from livestock, including46 males and 126 females from public areas in Basrah Governorate/Iraq. Samples were also collected from animals infected with the disease during the period from July 2023 to September 2023. Five Milliliters of blood was drawn, using a sterile device, are single-use and then transferred to a separator tube devoid of any leak-proof insulation. Blood sample centrifuged at the laboratory; at a speed of 3000 rpm for a period time. 5 minutes. which was later divided into marked Sterile tube 1.5ml. Then kept frozen until the time of comprehensive analysis.

Tick Screening Methods

Tick samples were collected from the Basrah governorate and diagnosed at the Natural History Museum in Basrah. We classified multiple tick types and identified various genera, with the *hyalomma* type endemic to the Basrah governorate being the most prominent.

Serological Testing

Hemorrhagic fever: In accordance with the prescribed protocol provided by the manufacturer, ID Vet, FRANCE, developed an ELISA test using indirect enzyme-linked immunosorbent assays. employed. This assay utilized a kit pre-coated with a specific antigen pertinent to the hemorrhagic fever virus. The primary objective was to facilitate the detection and subsequent quantification of the target immunoglobulin G (IgG) within the serum specimens collected from the cattle in this study. The procedural steps included the meticulous preparation, dilution, and incubation of the reagents and serum samples as per the kit's guidelines. The reaction was stopped by adding a sStop sSolution, and the sample's optical density was measured at 450 nanometers using a Microplate ELISA reader to assess the presence and concentration of specific IgG antibodies.For interpretation of OD's results, For each sample uses these formula

calculate the S\P percentage (S\P %).

$$S/P\ \% = \frac{OD_{sample}}{OD_{PC}} \times 100\ \%$$

Samples presenting a s\p percentage(s/p):

-Less than or equal to 30% are consider negative.

-More than 30% are considered positive.

Result	Status
s/p % ≤ 30%	Negative
s/p % > 30%	Positive

Statistical analysis.

Data was statistically analyzed by one-way ANOVA with multiple comparison tests and an independent sample test using a statistical software program (SPSS for windows version 22, USA). Differences were considered significant at ($P \leq 0.05$).

Results

A total of 172 serum samples were subjected to analysis using an indirect enzyme-linked immunosorbent assay (ELISA) to ascertain the presence of specific immunoglobulin G (IgG) antibodies. The test showed that 73 samples (42.44%) had antibodies against the Crimean-Congo Hemorrhagic Fever (CCHV) virus, which is called a seropositive status.Within the cohort of seropositive specimens, 46 samples (36.9%) were derived from male subjects, while 126 samples (44.4%) were obtained from female subjects. Upon statistical evaluation, no significant differences (P-value) were observed in the seropositivity rates between the male and female groups.

Table (1). Total Seropositive samples in Basrah

Governorate	Total	Positive	Negative	Rate
Basrah	172	73(42.44%)	99 (57.56%)	42.44%

Table **(2). Infected animals according to gender in Basrah.**

Gender	Positive	Negative	Total	Rate
Male	17 (36.96%)	29(63 %)	46	36.9%
Female	56* (44.44%)	70(55.55 %)	126	44.4%
$X^2 = 0.773$	73	99	172	

*** Significant (P≥ 0.05).**

Table (3) Showed infected animals high significant (P≥ 0.05) in2 ages compared with another ages (1,2)in Basrah Governorate.

Age	Positive	Negative	Total
1	4(28.57%)	10(71.42%)	14
2	59*(42.44%)	80(57.55%)	139*
3	10(52.63%)	9(47.37%)	19
$X^2 = 1.91$	73	99	172

significant P≥ 0.05.

Table (4). Results according to regions in Basrah.

Region	Positive	Negative	Totally	Type
Abu-Alkaseeb	33* (64.7%)	24(42.1%)	57	Cow+ buffalo
Agriculture collage	5(13.89%)	36 (87.8%)	41	C ow
Qurna	8(61.54%)	5(38.46%)	13	Cow
Bany skeen	9(36%)	16(64%)	25	Cow+calves
Zubair	7(70%)	3(30%)	10	Calves
Garmat-Ali	11 (64.7%)	6 (35.29%)	17	Cow
Haritha	---0%	9	9	Cow
$X^2 = 39.721$	73	99	172	

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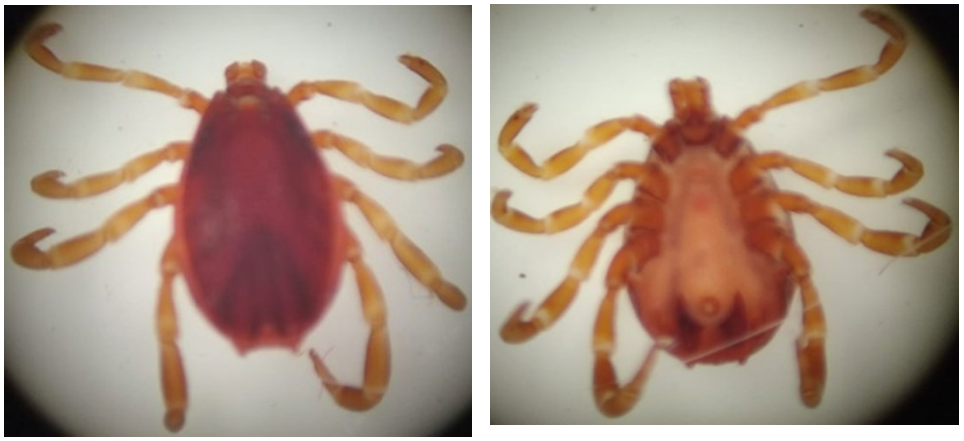
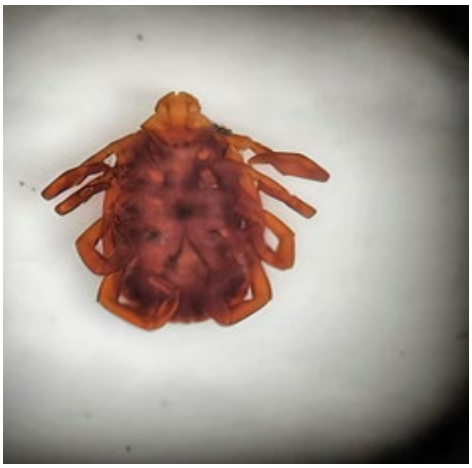


Figure (1). ***Hyalomma truncatum*** **(dorsal and ventral), 40x Koch, 1844**



Figure(2). ***Rhipicephalus venteral*** **(right) ,40x Koch, 1844.**

Figure (3). ***Hyalomma anatolicum*** **40x, (Koch,1844).**

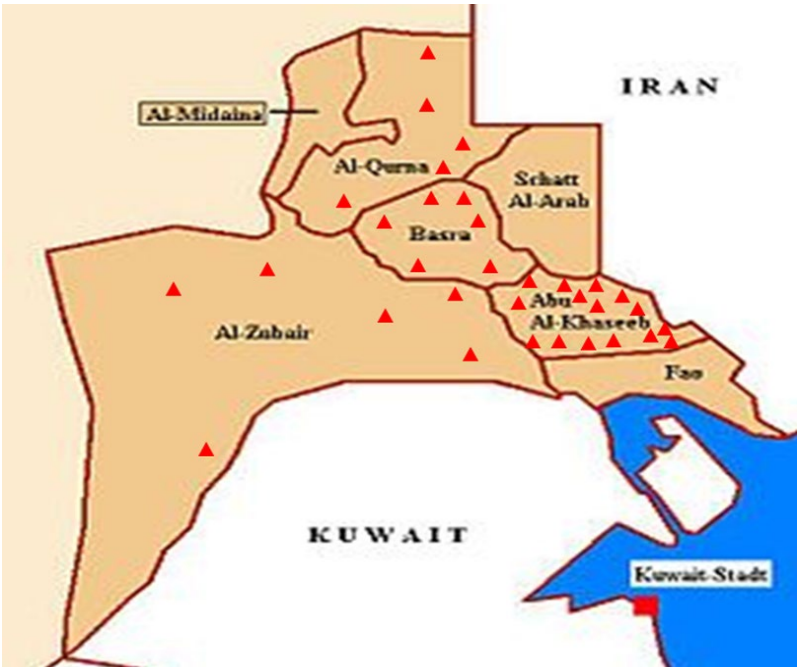


Figure (4). Basrah map explain distribution of disease according to regions.

Table (5). A showing the classification of tick types that cause of (CCHFV) disease.

Ticks' bio-typing Bio- type	Sample	No
Rhipicephalus- sanguineus Koch,1844	1	1
Rhipicephalus- sanguineus	6	2
Hyalomma- truncatum Koch, 1844	3	3
Hyalomma- truncatum	7	4
Hyalomma- truncatum	10	5

Discussion

Crimean-Congo Hemorrhagic Fever (CCHF) outbreaks pose a significant risk to human life and public health due to several critical factors. Epidemic Potential CCHF has the capacity to spread rapidly within a population, leading to widespread outbreaks. CCHF often causes severe illness, leading to high mortality rates and nosocomial outbreaks. Currently, there are only limited therapeutic options available for managing CCHF-infected individuals (16,17). Mild Febrile Syndrome Early signs include fever, fatigue, and weakness. Severe hemorrhagic disease in severe cases leads to bleeding from the nose, eyes, gums, or vagina, as well as severe vomiting or diarrhea (18,19). This study marks the first attempt to investigate the presence of CCHFV antibodies in cows and buffalo in Basrah. Basrah, a large southern province in Iraq, is home to highly cultivated cattle and buffaloes, and it shares a geographical border with other countries that have reported cases of the disease, including Turkey, Saudi Arabia, Kuwait, the Arab United Emirate, and Iran.Certainly, in the present study, the seroprevalence of *Crimean-Congo Hemorrhagic Fever* (CCHF) infection was determined by detecting IgG antibodies in cattle serum samples out of 172 total 72 seropositive (42.44%) in Basrah. The study found that seropositive in female rises than male ,126 female 56 (44.44%) seropositive also reported 10 (52.63%) seropositive in older animal that higher than in younger.

In comparison to studies conducted in other countries, 37 (64.8%) of the twenty-four cattle in Uganda tested positive for CCHFV(20). In comparison to studies conducted in other countries, 37 (64.8%) of the twenty-four cattle in Uganda tested positive for CCHFV (21).

This is also consistent with a study conducted in Iran, which found that the incidence rate of the disease varied between 9.5% and 40% (22,23, 24,25). Another study conducted in Afghanistan found that the seroprevalence in cattle was 22.4% (146 out of 768) 19%, which is a lower percentage compared to the current study. On the other hand, Keny's study (1) reported CCHFV seropositivity of 31.08% in 148 samples(1). The study in Keny (1) reported a CCHFV seropositivity of 31.08% from 148 samples. In Turkey, researchers conducted a prevalence study on sheep and goats. The enzyme-linked immunosorbent assay (ELISA) is used. The study found that seroprevalence rates were 31.8% in sheep and 66% in goats. Additionally, when using indirect ELISA, 14% of the animals tested positive, with 19.16% of sheep and 6.25% of goats showing seropositivity. The prevalence of antibodies in cattle was approximately 13.3%in Corsica (26). Other studies in Iran recorded from 200 tick's it was observed that. The viral genome was detected in 4.5% of the analyzed tick population, specifically in 9 samples. The species of the infected ticks were determined to be *Hyalomma marginatum, Hyalomma anatolicum*, and *Rhipicephalus sanguineus. Hyalomma marginatum:* The same species of ticks that were diagnosed in this study in Basrah also included *Rhipicephalus sanguineus, Rhipicephalus sanguineus*, and *Hyalomma truncatum*. The presence of

disease vectors prompted an investigation into the disease in the cities of Basrah.

Conclusions

The disease is endemic, has a high prevalence in Basrah Governorate, and is popular among various regions. We recorded the highest rate of infection with the disease in Basrah Governorate, at 42.44%, and the infection rate was highest in Abu Al-Khasib district compared to the rest of the districts. The types includedcalves, buffalo, and cows. We discovered that the type of tick widely spread in Basrah's districts and regions is hyalomma.

Acknowledgements

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Novelty Statement

The aim of the study was to detect Congo hemorrhagic fever through a viral and epidemiological study of the disease, which is endemic in the southern governorates, including Basrah Governorate. Additionally, the study aimed to identify the causative and most widespread type of tick .

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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الانتشار المصلي لحمى القرم _الكونغو النزفية *Hemorrhagic fever* في الماشية في محافظة البصرة ، العراق

دعاء امير هاشم، تماضر عبد الحامد.

فرع الطب البيطري الباطني والوقائي، كلية الطب البيطري، جامعة البصرة، البصرة، العراق.

الخلاصة

تم إجراء هذه الدراسة في محافظة البصرة. بهدف الكشف عن الاجسام المضاده لحمى القرم -الكونغو النزفية.تم جمع عينات الدم من الجاموس والابقار والعجول ابتداء من يونيو حتى نهاية سبتمبر 2023.تم استخدام اختبار الاليزا غير المباشرللكشف عن الاجسام المناعية المضادة IgG ضد فايروس CCHFV جمعت العينات من مناطق ابو الخصيب ,الزبير,القرنة , كرمة علي , بني سكين , كلية الزراعة والهارثة .يتم الكشف عن الأجسام المضادة للفيروسات. كان هناك 42.44% من عدد 172 رأس ماشية (73).حاملة لل IGg وسجلت نسبة اعلى بالاناث (ن=56) من المصابين مقارنة بالذكور (ن=17). كما سجلت نسبة عالية في الأعمار تتراوح بين 2-3 سنوات مقارنة مع اولئك الذين تقل اعمارهم عن عامين,وكان عدد حالات الاصابة بالفيروس مرتفعه جدا في ابو الخصيب مقارنه مع بقية المناطق بواقع 57 حالة، 33 (64.7%). تم جمع القراد الصلب والناعم من محافظة البصرة، من نفس الحيوانات التي تم جمع عينات الدم منهاوتم تصنيفها الى عائلة القراد Ixodidae، والمعروفة بثلاثة أنواع: Rhipicephalus sanguineus، Hyalomma anatolicum، و Hyalomma truncatum. تشيرهذه النتائج إلى انتشار فيروس CCHV على نطاق واسع في الأبقار في جنوب العراق في محافظة البصرة هو 42.44%، . ووجد أن نوع القراد المنتشر بشكل واضح في مناطق البصرة هو الهيالوما.

الكلمات المفتاحية: حمى - القرم النزفية، فيروس النزفية ، البصرة.